NEUROPHARMACOLOGICAL EFFECTS OF CERTAIN AGENTS WHICH INTERFERE WITH CELL METABOLISM

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ABSTRACT

A study has been made of the neuropharmacological effects of two classes of compounds which can interfere with cell metabolism.

Following their injection into the cerebral ventricles of either the conscious mouse or rat, both 2,4-dinitrophenol and pentachlorophenol, which uncouple oxidative phosphorylation, and the cardiac glycosides ouabain and digitoxin, which inhibit the sodium pump, produce marked changes in general behaviour and in the ability of the animals to regulate body temperature.

After central administration the phenolic uncouplers produce a loss of righting reflex similar to that seen after pentobarbitone given by the same route of injection. This leads to a re-examination of an earlier hypothesis that a possible mechanism of hypnotic action of the barbiturates involves uncoupling oxidative phosphorylation. The pharmacological profiles of the phenols and the barbiturates do, however, differ in several respects.

A biphasic effect is apparent after central administration of the cardiac glycosides. Ouabain produces a central nervous depression in small doses, whereas higher doses are excitatory, convulsant and lethal. The depressant effects show a number of similarities to those seen after peripheral administration of several drugs which interfere with aminergic systems, although the effect on brain amine levels of these compounds, which have similar pharmacological activity, is variable. Whereas the central excitant actions of the cardiac glycosides may involve noradrenergic mechanisms, their depressant actions seem to involve a dopaminergic component.

In view of the ability of both the phenolic uncouplers and the cardiac glycosides to produce effects on the central nervous system and to interact with a number of other drugs, the possible therapeutic implications of these findings are discussed. Although not postulated as a mechanism of action common to all centrally acting drugs, a primary interference in cellular energy metabolism may provide explanations for a number of hitherto inexplicable drug actions.

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PREFACE

Any compound capable of interfering with cell metabolism would be expected to produce diverse effects in the living organism. Such effects would depend to a large extent on the areas to which the particular compound gained access and could be both centrally and peripherally mediated. More specific effects would be produced by restricting the distribution of such a compound. The pharmacology of these agents on the central nervous system could be studied by injecting them directly into the cerebral ventricles of conscious animals. Problems would then arise in interpreting the observed effects as a result of (1) a primary change in cell metabolism or (2) a secondary change in transmitter function, for example, produced by this primary change, especially since marked changes in behaviour can be associated with comparatively small changes in brain biochemistry.

The development of an understanding of the pharmacodynamic effects of the psychotropic drugs has been slow and still remains unsatisfactory, primarily because of the complexity and multiplicity of their actions. Consequently, the relationships between the central biochemical and pharmacological effects of these psychotropic drugs on the one hand, and their behavioural manifestations on the other, remain obscure.

Certain drugs used in the treatment of mood disorders are capable of producing a primary effect upon cell metabolism, and it is therefore logical to investigate the neuropharmacological effects of compounds known to interfere with cell metabolism by decreasing the capacity of the cell to either produce or utilise energy.

This work was undertaken in the hope that some basic mechanisms involved in the production of mood disorders might be brought to light.

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I Biochemical basis of mood disorders

In reviewing the biochemistry of the affective disorders Coppen (1967) has presented evidence for disturbances in three main areas: adrenal cortical activity, electrolyte distribution and amine metabolism.

A number of reports on adrenal function have suggested that blood levels of glucocorticoid steroids are elevated in depression (Gibbons, 1965; Rubin & Mandell, 1966; Coppen, 1967; Fawcett & Bunney, 1967). However, other recent papers have indicated that this correlation may not be as close as originally thought (Brooksbank & Coppen, 1967; Sachar, 1967; Coppen, 1968).

An interference in electrolyte distribution could have several causes including effects on transport processes within the brain, which could occasion secondary changes in neurotransmitter activity, for example, as well as a primary interference in function.

The concept of a relationship between the brain monoamines and disorders of mood had its origin in the important discovery that certain drugs which excessively affect emotional tone in man also alter levels of one or more of the brain amines in animals. By 1956, reserpine, a drug which caused severe depression and reduced levels of the monoamines, and iproniazid, a drug which elevated mood in depressed patients and increased levels of the monoamines, had both been isolated. These findings provided the impetus for an extensive amount of psychopharmacological research, against which background there emerged several concepts regarding the rôle of monoamines in affective disorders. Since these drugs altered the metabolism of monoamines within the brain, the question arose as to which monoamines were most important in altering mood.

The first reports that a functional deficiency of the catecholamines might be involved in depression were those of Jacobsen (1964) and Rosenblatt, Chanley, Sobotka & Kaufman (1960), whose views have become known as the "catecholamine hypothesis of affective disorders". It has been stated as follows by Schildkraut (1965):

> ".....some, if not all, depressions are associated with an absolute, or relative deficiency of catecholamines, particularly noradrenaline, at functionally important adrenergic receptor sites in the brain. Elation, conversely, may be associated with an excess of such amines."

Part of the supporting evidence for this hypothesis was obtained with an animal model of human depression - reserpine-induced behavioural depression. This has been used in procedures for the depletion of amines, followed by their selective replacement by administering the appropriate amino-acid precursor. In the case of the catecholamines, dopa (3,4-dihydroxyphenylalanine) was found temporarily to reverse reserpine-induced depression in animals (Carlsson, Lindqvist & Magnusson, 1957) and in man (Degkwitz, Frowein, Kulenkampff & Mohs, 1960). However, in studies in which dopa has been administered to depressed patients (Pare & Sandler, 1959; Persson & Roos, 1967), little change in mood has been observed. Bunney, Janowsky, Goodwin, Davis, Brodie, Murphy & Chase (1969) have suggested that there may be two classes of depression, the first with a catecholamine deficit in which patients respond to dopa, the second with a 5-hydroxytryptamine deficit in which patients respond to tryptophan.

There is recent evidence from animal studies that a reversal of reserpine-induced depression by dopa may be the result of the formation of dopamine rather than noradrenaline. Havliček & Sklenowsky (1967) have reported that the administration of 3,4-dihydroxyphenylserine, an amino-acid which can be decarboxylated to noradrenaline without dopamine being formed, results in synchronisation of the electroencephalogram and behavioural depression. Creveling, Daly, Tokuyama & Witkop (1968) have de-

monstrated that behavioural depression in reserpinised animals is reversed by dopa but not by 3,4-dihydroxyphenylserine, although noradrenaline levels had been restored to normal by the latter amino-acid.

A study in which an inhibitor of catecholamine synthesis, α-methyl-paratyrosine, was administered to 20 patients, 11 of whom had phaeocromocytoma, demonstrated sedation in 16, but the authors do not mention the occurrence of altered mood (Sjoerdsma, Engelman, Spector & Udenfriend, 1965). Charalampous & Brown (1967) also observed that α-methylparatyrosine induced a marked sedative effect in mentally ill patients, most of whom were schizophrenic. No anti-psychotic effect was found. An attempt to lower brain noradrenaline levels in manic patients by means of α-methyldopa (Mosher, Klerman & Greaney, 1966) did not alleviate their condition.

It has also been suggested that an altered metabolism of tryptophan is associated with affective disorders (Coppen, 1967). There is disturbed tryptophan metabolism in certain types of depression, as shown by decreased excretion of tryptamine (Coppen, Shaw, Malleson, Eccleston & Grundy, 1965) and decreased levels of 5-hydroxyindoles in cerebrospinal fluid (Ashcroft & Sharman, 1960). The recent hypothesis of Lapin & Oxenkrug (1969) postulates that a deficiency of brain 5-hydroxytryptamine, resulting from an altered metabolism of tryptophan, occurs in depression.

The rôle of noradrenaline in the aetiology of affective disorders is further obscured by the divergence of opinion regarding the behavioural effects of this amine. The catecholamine hypothesis is based on the view that noradrenaline is a behavioural excitant and several reports have suggested such a rôle. Systemic administration of noradrenaline sometimes results in an electroencephalographic alerting response, but Goldstein & Munos (1961) found that only 20 % of the animals tested developed an arousal

pattern. Drugs, such as amphetamine, which release noradrenaline in the periphery and also in the brain (Carr & Moore, 1969), and dopa can cause an alerting response. However, there is a growing body of opinion that noradrenaline or adrenaline, administered in such a way as to cross the blood-brain barrier, (i.e. intracerebroventricularly, intracisternally or to animals with an immature blood-brain barrier) produces behavioural depression (Mandell & Spooner, 1968).

Direct actions of noradrenaline or adrenaline on the central nervous system, ranging from sedation and lethargy to unconsciousness, have been reported in various mammalian species - cat, dog, rat, sheep, ox, man - and also in young chickens. Dewhurst (1968) has presented an hypothesis regarding the action of noradrenaline and other amines in the central nervous system. Amines were classified into two main functional groups: type A amines, fat-soluble compounds with planar hydrocarbon structures (eg. indole and phenyl), had an excitant effect on behaviour. Tryptamine and phenylethylamine were the most active amines in this group. Type C amines, water-soluble (eg. catechols), had depressant effects on behaviour. A third group, type B amines, had biphasic actions, i.e. they could excite and then depress. The activity of members of this group depended primarily on lipid solubility and not on whether the substance was indoleamine, catecholamine or phenylethylamine. Dopamine and 5-hydroxytryptamine were classified with this intermediate B group.

The interpretation of the action of psychotropic compounds and the delineation of the possible rôles of brain amines in the aetiology of mental disorders would be advanced by definitive information concerning the effects of those amines, regarded as synaptic transmitters, on postsynaptic neurones.

An important consideration in the study of the biochemical basis of

mood disorders is the differentiation between cause and effect - is the observed alteration in brain biochemistry a result of the alteration in mood, or does it represent an integral factor in bringing about this change? The difference between the two is not always obvious.

II Mechanism of action of centrally acting drugs

Biochemical investigation of the sites of action of centrally acting drugs has a long history and, even before 1950, quite a considerable literature. However, it is only in recent years, following the rapid development of new drugs and the growth of neurochemistry as a distinct branch of biochemistry, that the subject has undergone a major expansion.

Information on the modes of action of psychotropic drugs is limited by the tendency for more intense investigation of certain drugs in certain fields than others - the general depressants in the field of energy metabolism, for example, and the monoamine oxidase inhibitors in that of biogenic amines. They can be considered to interfere with cerebral biochemistry in a number of ways, including (1) energy metabolism, (2) general aspects of membrane function, (3) biogenic amines. All these more or less specific interferences could, however, be brought about by a number of common mechanisms of action.

Smith & Williams (1965a,b) speculated that some drugs, rather than reacting with receptors, may alter biochemical processes in the vicinity of the receptors, with consequent changes in physiological function. Although purely conjectural, this concept is based firmly on well known chemical reaction mechanisms and may serve to explain drug action where more popular interpretations fail.

Laborit (1963) suggested that the reaction of a given tissue to drugs depends on its "metabolic structure", i.e. whether the Embden Meyerhof pathway or the pentose monophosphate shunt is predominant. In essence, the concept states that drugs which block, directly or indirectly, any stage in the Embden Meyerhof pathway will cause depression of those tissues or organs in which this system operates, and, conversely, stimulation of the

pathway leads to activation. The pentose monophosphate shunt, on the other hand, is not an energy-yielding pathway and drugs which influence it principally change the inter-conversion between NADP and NADPH. Laborit & Weber (1965) have interpreted the neurotropic actions of strychnine, thiopentone, ether and chloroform, and of leptazol on the basis of this theory.

In considering the effects of psychotropic drugs on cerebral energy metabolism, it is important to distinguish the energy-yielding from the energy-consuming processes occurring in the brain. The former comprise reactions involved in converting the energy of the chemical bonds of foodstuffs into a form which can be stored in the cell and made readily available for function. The mechanisms by which the brain metabolises its principal substrate, glucose, are in general similar to those that occur in other tissues. They involve coupled enzyme reactions which eventually result in the complete breakdown of glucose to carbon dioxide and water, with an accompanying synthesis of adenosine triphosphate (ATP) and phosphocreatine. These compounds retain the energy of the original glucose molecule in labile high energy bonds. In the brain, as elsewhere, the initial stages of glucose metabolism occur in the cytoplasm with the formation of pyruvic and lactic acids by glycolysis, and continues in the mitochondria by a cyclical series of enzymic reactions known as the tricarboxylic acid cycle. It is here that the major yield of consumable energy in the form of ATP occurs, through a complex process known as oxidative phosphorylation. Although incompletely understood, this process is intimately linked with the transfer of electrons from the intermediate substrates of the tricarboxylic acid cycle to molecular oxygen to form water. This transfer occurs along the structurally orientated series of electron carriers - nicotinamide adenine dinucleotide (NAD or NADH), flavoproteins and cytochromes, together with their associated enzymes - which constitute the

electron transport or respiratory chain. At three sites in the chain electron transfer is coupled to the synthesis of a molecule of ATP from adenosine diphosphate (ADP) and inorganic phosphate, this being the final stage in the energy-yielding process. The energy contained in the ATP is in part reversibly stored in phosphocreatine.

In contrast to the extensive investigation of energy-yielding processes, the reactions in neural tissues which consume energy in the course of function are less well documented. They may be divided into two categories: (1) a wide variety of synthetic processes essential for nervous activity, which at some stage require ATP, eg. synthesis and uptake of amines, (2) the maintenance of the gradients of sodium and potassium across the neuronal membrane, which involves a transport mechanism driven by the energy of ATP, generally believed to be a major energy-consuming process in the brain. The transport mechanism is only partly understood, but is known to involve a membrane-located adenosine triphosphatase (ATPase) enzyme, which breaks the terminal energy rich bond of ATP to yield ADP and inorganic phosphate.

It appears that the locus of action of drugs which act on mechanisms utilising energy in the cell is at the cell membrane and involves some aspect of cation transport. It should be noted that, in many cases, there is no evidence to suggest that the drugs act directly on the energy-consuming reactions involved in this process. The membrane-bound ATPase enzyme, believed to be an essential component in cation transport, is only inhibited by relatively high concentrations of depressant drugs, and then in a non-specific manner. Nor is there any involvement of the creatine phosphokinase enzyme, which is closely linked with the events at the membrane - concentrations of phenobarbitone as high as 1×10^{-3} M failed to induce inhibition (Shepherd, Lader & Rodnight, 1968a).

The effects of drugs on cerebral energy metabolism have been widely investigated *in vitro*, both in cell-containing systems, where the cell structure remains largely intact, and in mitochondrial systems derived from mechanically disrupted tissue. Cell-containing preparations of neural tissue have also been studied during electrical excitation, as well as in the resting state. Early work carried out before 1950 established a characteristic pattern of drug-induced changes in the levels of phosphates and lactic acid in the brain. The administration of depressant drugs, including several barbiturates in anaesthetic doses, resulted in a marked increase in the level of phosphocreatine in the brain, a small inconsistent increase in ATP, and a fall in inorganic phosphate and lactic acid. On the other hand, in both conscious and anaesthetised animals, convulsant and excitant drugs, as well as electrical excitation *in vivo*, led to general changes in the opposite direction (McIlwain, 1966). However, later work has shown that the major changes in the levels of tissue phosphates observed in anaesthesia do not occur following the administration of doses of depressant drugs which do not impair consciousness. Rats sedated with barbiturates were found to have normal levels of phosphocreatine and ATP (Minard & Davis, 1962). Chlorpromazine in doses of 300 - 500 mg/kg, sufficient to cause severe depression and ataxia but no loss of consciousness, has likewise been found to have no effect on the levels of cerebral phosphates when adequate methods of tissue fixation are used (Minard & Davis, 1962; Weiner & Huls, 1961).

This absence of significant effect on phosphocreatine and inorganic phosphate in the brain of conscious animals also applies to many of the psychotropic drugs, with monoamine oxidase inhibitors, tricyclic antidepressants, the amphetamines, reserpine derivatives, diphenylmethanes and chlordiazepoxide among those examined (Kaul & Lewis, 1963a,b; Lewis & Patten, 1962; 1963). However, in these studies, a number of drugs was

found to induce small, but apparently significant, changes in the ATP/ADP ratio in the brain, without affecting the level of the total adenine nucleotide pool. Stimulant drugs tended to increase this ratio and the doses required to produce this effect were correlated with those required to produce behavioural stimulation (Lewis & Patten, 1962). Anti-depressants also increased the ratio, the monoamine oxidase inhibitors being more active than the tricyclic compounds (Lewis & Patten, 1963). On the other hand, certain drugs causing sedation reduced the ATP/ADP ratio, and here again a number of correlations with behavioural response were observed (Kaul & Lewis, 1963b), although cause and effect were not always clearly defined.

With regard to the major changes in tissue phosphates induced by anaesthetic and convulsant agents, for some years the findings have been interpreted as indicating that the primary action of these drugs is on the utilisation, rather than the production, of energy. The direction of the changes in respiratory rate induced by depressant and excitant drugs is also consistent with this point of view. The picture has emerged of these rather extreme examples of modified cerebral functioning - anaesthesia and gross excitation - being related to a drug-induced increase or decrease in the rate of chemical events directly involved in the mechanisms subserving function. In the light of *in vitro* evidence from stimulated cerebral metabolism, it appears reasonable to apply this conclusion to a number of other centrally acting drugs, although changes in energy requirements *in vivo* have usually proved too small to detect. However, in the case of depressant drugs, there remains an undercurrent of support for the view that a direct action on energy-yielding processes, including an interference with oxidative phosphorylation, may also be involved in normal as well as toxic doses. Furthermore, the small drug-induced changes in the ATP/ADP

ratio observed by Lewis have also been interpreted as suggesting that stimulant drugs tend to increase, and depressant drugs to decrease, the available energy supplies of the brain. Other workers have been impressed by the relatively low concentrations of depressant drugs (including phenothiazines) required to inhibit mitochondrial metabolism *in vitro*. Although such concentrations may be comparable to those found *in vivo* by analysis of the whole tissue, it should be noted that there is no evidence to suggest that depressant drugs reach the mitochondria of intact tissues in such quantities.

Agents which can specifically interfere with cellular energy metabolism, either by reducing energy production, (eg. 2,4-dinitrophenol, pentachlorophenol) or by reducing energy utilisation (eg. ouabain), can also produce secondary effects as a result of this interference. For example, the permeability of membranes can be impaired as a result of the inhibition of energy-dependent transport mechanisms (eg. sodium pump) in adrenergic neurones. Similarly, the synthesis of biogenic neurotransmitters can be altered either directly or via a feedback system caused by a blockade of amine re-uptake. Thus, providing the required concentrations of agents capable of interfering specifically with cell metabolism can be realised in neural tissue, profound effects might be expected which may be of use in the investigation of modes of action of clinically used drugs.

III The blood-brain barrier

Ehrlich (1885) first demonstrated that, when injected into the peripheral circulation, certain dyes stained all tissues, except those of the central nervous system. Subsequent work showed that this barrier phenomenon existed for a great number of substances (Winterstein, 1961). For many years it was thought to be exclusive to the central nervous system (Dobbing, 1961), but it is now realised that this is not so, and it is possible to find many examples of non-neural tissue incorporating different substances in smaller proportion than does the brain (see Levin & Scicli, 1969). Some early workers considered all exchange of substances between the blood and the central nervous system to take place via the cerebrospinal fluid (Monakow, 1921). Later work (Friedemann, 1942) considered that the choroid plexus, meningeal vessels and cerebrospinal fluid were not involved, but that the barrier was more likely to be connected with the phenomenon of capillary permeability.

There is a considerable variation in capillary permeability throughout the body and the cerebral capillaries are no exception. However, the rate of exchange of many substances across the walls of these capillaries is so slow that it seems justifiable to speak of a blood-brain barrier. This barrier is currently thought to be a summation of many factors and may be considered to be located at three principal sites: (1) the blood-cerebrospinal fluid boundary, (2) between the blood and central nervous tissue and (3) between the cerebrospinal fluid and central nervous tissue.

The bulk of the cerebrospinal fluid is probably produced from the blood at the choroid plexus. However, about 20 % is produced at the ependymal walls lining the ventricles (Pollay & Curl, 1967). Interferences with function at these sites could affect the permeability of the barrier, by altering the rates of transfer of substances from the blood into the cerebrospinal fluid.

The cerebral vessels have a number of unique anatomical features. In the choroid plexus there are gaps between the endothelial cells of the capillary wall, but the choroid epithelial cells are densely intermeshed. The capillaries in the brain substance have no such gaps and the junctions between the endothelial cells are especially well sealed. About 50 % of the capillary surface is covered in pericytes found in the basement membrane, and the end feet of the astrocytic perivascular glia cover 85 % of the vascular surface (Maynard, Schultz & Pease, 1957). It has also been shown that the neuronal surfaces are covered by similar glial processes (Elliott, 1969) and Curtis & Eccles (1958) have revealed the possibility of a synaptic barrier surrounding the axonal endings and synaptic areas of the post-synaptic neurone. It seems likely that the astrocytes are an important element in the blood-brain barrier, since they must be traversed by all substances entering and leaving nervous tissue. Electron microscopy has indicated that the space between cells in the central nervous system is only 150 - 200 Å. Thus substances may be excluded from the brain because of the small amount of extra-cellular fluid available for their dissolution (Davson, Kleeman & Levin, 1962; Edstrom, 1964).

The concept that the blood-brain barrier depends on anatomical barriers is not regarded as a complete explanation. A number of authors have shown that the blood-cerebrospinal fluid and blood-central nervous system barriers behave as lipoid membranes towards foreign organic compounds (Butler, 1942; Brodie & Hogben, 1957; Shanker, 1961; 1962). The rate of penetration into brain tissue has been correlated with the lipid/water partition coefficients of drugs at pH 7.4 (Mayer, Maickel & Brodie, 1959). The blood-cerebrospinal fluid membrane has been demonstrated to be permeable to the unionised form of weak organic acids and bases, and relatively impermeable to the ionised form (Rall, Stabenau & Zubrod, 1959). Increased ionisation at a physiolo-

gical pH will, therefore, decrease the fraction of unionised compound available for passive diffusion (Rall & Zubrod, 1962). The degree of ionisation also alters the distribution of weak organic electrolytes, weak acids being partly barred from areas of high pH and concentrated in areas of low pH (Milne, Scribner & Crawford, 1958).

The binding of a drug to plasma proteins can also affect its entry into the central nervous system. This is a probable explanation of the inability of certain dyes to enter the cerebrospinal fluid, since this fluid is protein free (Rall & Zubrod, 1962).

The cerebrospinal fluid is returned to the blood at the arachnoid villi and consequently dissolved substances are returned to the peripheral circulation, thereby creating concentration gradients within the brain. Woodward, Reed & Woodbury (1967) found that the blood and cerebrospinal fluid act as sinks into which substances can flow when reaching the nervous parenchyma. Those substances supplied to the parenchyma by the blood will diffuse into the cerebrospinal fluid, and those supplied by the cerebrospinal fluid will diffuse into the blood. A compound reaching the cerebrospinal fluid, however, may not diffuse freely into the blood because of the brain capillary barrier (Levin & Scicli, 1969).

In addition to passive diffusion, active transport has been proposed as a mechanism of brain permeation (Crone, 1965; Bidder, 1966). Active transport of substances from the cerebrospinal fluid to the blood occurs within the choroid plexus and has been demonstrated for a number of substances, including glucose, diodrast, quaternary ammonium compounds and penicillin. There is also some evidence that potassium, 5-hydroxytryptamine and noradrenaline (Glowinski, Kopin & Axelrod, 1965) are actively transported out of the cerebrospinal fluid. Thus, in certain cases, con-

centration gradients may be attributed to the active efflux of substances at the choroid plexus (Levin & Scicli, 1969).

A further important component of the blood-brain barrier may involve metabolic considerations. Failure to produce pharmacological effects in the central nervous system does not necessarily mean that entry of the substance has been impeded, particularly when it is known to be rapidly metabolised (Dobbing, 1961). The resistance to catecholamines and 5-hydroxytryptamine reaching the brain from the peripheral circulation is well established (Weil-Malherbe, Whitby & Axelrod, 1961) and a similar effect has been shown for dopamine. Recently the brain capillaries, but not those in the periphery except some in the kidney, have been shown to contain dopa decarboxylase (Bertler, Falck, Owman & Rosengren, 1966; Owman & Rosengren, 1967), which functions as an enzymic trapping mechanism for dopa. It may be that similar enzyme traps exist for other substances and this would again suggest that the permeability of cerebral capillaries (particularly to specific substances) differs from that of peripheral capillaries.

In order to study the pharmacological effects of compounds on the central nervous system, it is essential to overcome or avoid the blood-brain barrier. This may be approached in two ways: either the physico-chemical properties of the compound are such that it will gain ready access to neural tissue after peripheral administration, or the compound can be presented to the central nervous system by injecting directly into the cerebral ventricles. Although this latter method does not completely avoid the blood-brain barrier, it localises and increases the concentration of injected agent, so that the concentration gradient at the barrier is increased. Furthermore, it has the advantage of minimising any direct peripheral activity, since relatively minute quantities of drugs need to be used.

IV Local administration of drugs into the cerebral ventricles

Interest in the cerebrospinal fluid was slow to develop, and even fifty years ago it was considered to serve a hydrostatic function only and to play no part in the nutrition of the brain and spinal cord. The studies of Ehrlich (1885) with alizarin blue indicated the presence of a barrier to the transfer of material between blood and brain, and provoked investigations into the passage of compounds through this barrier. Together with earlier reports that subdural injections of morphine into pentobarbitone-anaesthetised dogs caused excitement and reversal of anaesthesia (Bernard, 1875), these findings produced numerous descriptions of injection techniques, and reports of the effects of compounds following administration via the cerebrospinal fluid.

Dixon & Halliburton (1916) compared the rapidity of action of atropine, nicotine and adrenaline after injection into the cisterna magna with that after intravenous injection, and found the effects to be similar in both cases. Dikshit (1934) described a method for injection into the cerebral ventricles of anaesthetised cats, the skull being trephined before the injection was made; he later extended the method to conscious animals (Dikshit, 1935). Three years later a technique of perfusing the cerebral ventricles was introduced (Adam, McKail, Obrador & Wilson, 1938) and in 1947, Lackey described experiments in which injections were made into the cisterna magna of conscious dogs. However, it was not until 1953, when Feldberg & Sherwood described a permanent cannula for intraventricular injections in conscious cats, that the first systematic examination of the pharmacological effects of centrally-administered compounds began.

The methods available for injecting into the cerebral ventricles involve inserting the needle either into a permanently indwelling cannula

guide or directly through the skull. They can be divided into three groups: (1) injection into anaesthetised animals, (2) injection into animals lightly anaesthetised with a short acting anaesthetic, with subsequent observation in the conscious animals and (3) injection into conscious animals.

Injection into anaesthetised animals is frequently used to study uptake of compounds from the cerebral ventricles. Since compounds injected into the lateral ventricles pass into the third and fourth ventricles, through the foramina of Magendie and Luschka, and so into the subarachnoid space around the brain stem, special techniques have been developed to localise the area perfused (Feldberg, 1963; Feldberg & Fleischhauer, 1965).

The effects of intracerebroventricular injection can be observed in conscious animals if the drug is administered while the animal is lightly anaesthetised and is then allowed to recover. This technique is again best reserved for uptake studies (Fuxe & Ungerstedt, 1966) and extrapolation of results to the conscious animal should be carried out with care, since, even after light anaesthesia, the electroencephalogram does not return to normal for some time after consciousness has been regained (Sayers, A.: personal communication, 1971).

Feldberg & Sherwood (1953) introduced the principle of permanently implanted cannula guides in cats. Injections could be made by introducing the needle through a rubber protective membrane into the ventricles of the conscious animal, causing it no distress. This method avoided interactions with other drugs, such as anaesthetics, and the behavioural effects of centrally-administered compounds could be studied, without the results being influenced by stressful injection procedures. Similar cannula guides have been designed for the monkey (Feldberg, Hellion & Lotti, 1967), the rabbit (Bannergee, Burks, Feldberg & Goodrich, 1968), the dog (Haley &

Weinberg, 1955), the sheep (Bligh, 1966), the goat (Andersson, Jobin & Olsson, 1966), the ox (Findlay & Robertshaw, 1967), the rat (Hayden, Johnson & Maickel, 1966), and the chicken (Grunden & Marley, 1970).

In 1957, Haley & McCormick described a method for injecting compounds directly into the cerebral ventricles of the conscious mouse without using a cannula guide. This method has been used by Greef & Kasperat (1961a,b) in the conscious rat, and was later modified by Brittain & Handley (1967) for use again in the mouse.

Following the injection of compounds into the cerebral ventricles, localisation of action to a given structure cannot be precisely specified and, in order to obtain local specificity, micro-iontophoretic applications must be used. Even without these, however, a general idea of the central effects of a compound, without concern for localisation, can be obtained, and the technique has the merit of being relatively simple.

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I Animals, animal husbandry and laboratory conditions

The majority of experiments reported in this thesis were carried out on male albino mice. The strains used were:

i) TO (Scientific Products Farms, Ltd, Ash, Canterbury, Kent).

ii) TO (Aston Strain).

iii) A_2G (Aston Strain).

No obvious differences were observed between the three strains, apart from variations in the degree of ossification of the skull. This necessitated using slightly younger TO than A_2G mice when performing icv injections. The need to change from one strain to another was determined largely by the quality and availability of animals. TO (SPF) mice were used in Results II and III, TO (Aston) in Results I, IV, V and VII and A_2G (Aston) in Results VI.

Experiments involving rats were carried out on male Wistar rats, 100 - 150 g in weight, supplied by Scientific Products Farms Ltd, Ash, Canterbury, Kent.

Female animals were not used in any experiments (except those involving chicks, where both sexes were used) in order to avoid changes in pharmacological sensitivity owing to the oestrus cycle.

After receipt, all animals, except chicks, were maintained in the animal house for a minimum of five days. Mice were used at a weight of 16 - 20 g (TO strains) or 18 - 22 g (A_2G strain). The rats were used at a weight of 180 - 300 g. Both mice and rats were fed a conventional 41B cube diet (Pilsbury's Ltd, Birmingham).

Several experiments were performed using Ranger chicks supplied by Pilsbury's Ltd. They were maintained on Heygate's baby chick crumbs (Pils-

bury's Ltd). All chicks were two days old on receipt.

Animals were allowed tap water *ad libitum* to drink. Mice were kept in groups of 50 in large opaque polypropylene cages (40 x 50 x 15 cm) until at least 12 h before commencing the experiment, when they were randomly divided into groups of between 5 and 10, placed in smaller cages (20 x 27 x 10 cm) and moved to the experimental air-conditioned room. Rats were kept in groups of 5 in opaque polypropylene cages (40 x 50 x 15 cm). After cannulation and recovery they were placed, either individually, or in groups of 3, in similar sized cages and moved to the experimental air-conditioned room. Chicks were housed in large wire cages, in groups of at least 24, immediately after receipt. They were maintained at a temperature of 33° - $34^{\circ}C$ by means of an electric fan heater until at least 2 h before experiment, when groups of 5 were transferred to opaque polypropylene cages (20 x 27 x 15 cm) and removed to the experimental air-conditioned room.

For all animals and in all experiments, food and water were withdrawn 2 h before and during the experiment. The animal house temperature was $21 \pm 2^{\circ}C$ and that of the air-conditioned room $21 \pm 1^{\circ}C$, unless otherwise stated. Relative humidity was maintained in both areas between 50 % and 60 %. A normal light/dark cycle was maintained. All experiments were performed in the air-conditioned, temperature-controlled room between 09.00 h and 20.00 h at a temperature of $21 \pm 1^{\circ}C$ unless otherwise stated in the Results.

II Injection techniques

1. icv injections in the conscious mouse

The method used for the icv injection of drugs into mice was a modification of that first described by Haley & McCormick (1957) and subsequently by Brittain & Handley (1967).

In the present experiments, the injection needle was a specially prepared 20 gauge needle, 0.32 cm long. After preliminary investigations, an injection site more rostral and medial than that described by the above workers was decided upon. The needle was inserted perpendicularly through the skull in the region of the bregma, on the mid-line (fig. 1). This site was chosen because it provides adequate penetration of the ventricular system, is easy to find and, in the strains of mice used, the needle can be easily introduced through the skull at this point. Puncture of the sagittal venous sinus, which runs beneath the point of entry described by the previous two groups of workers, is avoided.

Most injections were made in a volume of 10 µl, although some animals received volumes of 20 µl where stated in the Results. All injections were made using a Hamilton 50 µl syringe.

In order to check the areas of brain tissue reached after injection, a 5 % solution of pontamine sky blue in sterile apyrogenic glass-distilled water was injected into the cerebral ventricles as described above. The animals were then killed by decapitation under chloroform anaesthesia 5 min after injection and the brains dissected out. A sledge microtome (Measuring & Scientific Equipment Ltd, London) with a Pelcool freezing stage was used to section the brain, and the extent of the spread of the dye was examined by inspecting the cut surface of the tissue. Since pontamine sky blue is a

vital dye, it was taken up by the periventricular proteins, thus giving a definite indication of the spread of the injected solution. It must be emphasised, however, that the depth of penetration into the periventricular tissues seen after injection of the dye cannot be extrapolated to indicate the areas of penetration achieved after injection of a particular drug, since in all cases transport mechanisms, which will differ with individual agents, account for the distribution of the injected substance. The injection of dye was simply a method of checking histologically that penetration of the ventricular system had been achieved using the described injection co-ordinates.

2. icv injections in the conscious rat

A method was used based on that described by Hayden, Johnson & Maickel (1966) and more recently by Sparkes & Spencer (1971). A cannula guide of 20 gauge stainless steel tubing was embedded in a perspex block 7 x 6 x 6.35 mm deep, so that the steel tubing protruded from the underside of the block by 4 mm (fig. 2). The needle for injection was made from a 26 gauge needle cut to a length of 11.30 mm and given a flat bevel (45°) by grinding on a wet-stone. A stilette of the following dimensions was inserted into the lumen of the cannula: 20 gauge stainless steel wire was cut to lengths of 14.35 mm, and the ends rounded off. The wire was then bent to an angle of 90°, 4 mm from one end. The longer arm was inserted into the lumen of the cannula guide and only removed during injection.

The cannulae were implanted in male Wistar rats, 180 - 200 g in weight, anaesthetised with a mixture of halothane in nitrous oxide (80 %) and oxygen (20 %), 3 % halothane being used for induction and 1 % for maintenance of anaesthesia. Each rat was then placed in a stereotaxic frame and injected subcutaneously at the operation site with 0.1 ml of a solution

containing 1 % procaine and 0.004 % adrenaline, to inhibit capillary bleeding and minimise discomfort on recovery. A mid-sagittal incision was made from just caudal to the eyes to just rostral to the base of the ears, the underlying tissues were cleared to one side and the skull cleaned with 1 % chlorhexidine (Hibitane, I.C.I.) in 70 % industrial methylated spirits. Using a No. 2 round dental burr, a hole was drilled at a point 2.5 mm lateral and 0.9 mm caudal to the bregma (fig. 1), taking care not to damage the dura. Three holes were drilled around this point to take small stainless steel screws (1.4 mm diameter) which tapped themselves into the bone. The cannula guide, previously soaked in 1 % aqueous chlorhexidine, was then lowered into position and dental acrylic built up around the guide and the securing screws. After the cement had hardened, the incision was sutured and the animal received intraperitoneally 48 mg sodium benzylpenicillin plus 40 mg streptomycin base (Crystamycin Forte, Glaxo).

The animals were subsequently used not sooner than 24 h and up to 28 days after recovery. All injections were made in a volume of 10 µl into the right lateral ventricle. At the end of each animal's useful experimental life, the accuracy of placing of the cannula guide was confirmed histologically by the method described in Methods II 1. The distribution of dye within the ventricular system was similar to that observed in mice after icv injection, penetration to all cerebral ventricles being achieved.

Trial experiments involving the injection of 10 - 20 µl 0.9 % sterile apyrogenic saline at pH 7 in both mice and rats produced no behavioural changes suggesting acute brain damage. All solutions were injected at a temperature of 35°C unless otherwise stated in the Results. Control animals, which had received an icv injection of vehicle alone, were always included in each experiment involving central administration.

3. icv perfusions in the conscious rat

In certain experiments it was necessary to give drugs icv without disturbing the animal. In these cases the following technique was employed.

The previously described cannula guide was used. In place of a 50 µl Hamilton syringe plus needle, a length of 26 gauge needle was cut from a No. 20 hypodermic needle and fixed to the end of a length of PP25 tubing, so that a length of 11.30 mm of needle was left protruding from the tubing. The polypropylene tubing was filled with the drug solution, as was the Hamilton 50 µl syringe, the latter being attached to the opposite end of the tube through a No. 20 hypodermic needle.

Prior to experiment, the 26 gauge needle was inserted into the cannula guide and the animal placed in the apparatus shown in fig. 3. The icv administration could now be given in the form of a short perfusion, 10 µl/10 sec, without disturbing the animal.

4. Conventional injections at peripheral sites

Subcutaneous (sc) injections were made into the skin of the nape of the neck, unless otherwise stated. Intravenous (iv) injections were made into the lateral tail vein. Intraperitoneal (ip) injections were made by inserting the needle obliquely and upwards through the lower abdominal wall.

In all cases the injection volume was 10 ml/kg.

III Pharmacological testing methods

1. Measurement of body temperature

i) Measurement of core temperature in the conscious mouse

Oesophageal temperatures were used as a measure of core temperature of the animals. Using the method of Brittain & Spencer (1964), a thermistor attached to a graduated electric thermometer (Light Laboratories Ltd, Brighton) was inserted into the oesophagus of the mouse to a depth of about 2 cm, so that its tip lay just above the cardia of the stomach.

ii) Measurement of skin temperature in the conscious mouse

Skin temperatures were determined in still air by placing a flattened thermistor between the digits on the ventral surface of the right hind paw, with the mouse standing on a sheet of expanded polystyrene foam insulation to minimise heat loss by conduction. Skin temperatures were measured using the calibrated electric thermometer described above. The sensitive window of the thermistor was situated on the flat end of the probe, which was held against the hind paw until the needle of the thermometer became stationary. The mice were housed individually for the duration of the experiment. Under these conditions skin temperature provides an index of skin blood flow.

iii) Measurement of core temperature in the conscious rat

Rectal temperature was used as an indication of core temperature since experience showed that repeated measurements of oesophageal temperature made the rats difficult to handle. Placing a probe in the rectum had considerably less disturbing effect, and after two or three measurements the procedure was well tolerated by the animals.

The thermistor used for mouse oesophageal temperatures was employed and, after lubricating with liquid paraffin, was inserted into the rectum to a depth of at least 6 cm (see Lomax, 1966). With repeated insertions in one animal to this depth, constant readings of body temperature were obtained. However, repeated insertions in one animal at depths up to 5 cm produced variations of about 0.5°C.

iv) Measurement of core temperature in the conscious chick

Rectal temperature was again used as an indication of core temperature, using the lubricated oesophageal thermistor described in Methods III 1. iii), and inserted into the rectum to a depth of 2 cm. Experience showed that this depth allowed constant readings of body temperature to be obtained after repeated insertions in one animal, and without causing any apparent discomfort.

In all experiments involving measurement of body temperature close age and weight ranges of animals were used to minimise variation between animals. This is particularly important during a fall in temperature, since heat loss is essentially a passive process and would be influenced by marked weight differences and variations in body structure and surface area to body weight ratio.

2. Convulsant activity in the mouse

In order to investigate the anti-convulsant or pro-convulsant activity of a number of agents, it was necessary to induce experimental convulsions in mice. These were of two types:

i) chemical

ii) electrical.

i) Convulsions were induced chemically by the ip injection of leptazol. Following injection the animals were housed individually in opaque polypropylene cages (20 x 27 x 10 cm) and observed continuously for 30 min. The time of onset of the first convulsion, the type of convulsion (whether tonic or clonic), and the number of animals dying were also noted.

ii) Electrically-induced convulsions were produced by a modification of the method of Cashin & Jackson (1962), using a constant voltage square-wave stimulator (Scientific and Research Instruments Ltd, London). For administration of the shock the external auditory meati of the mouse were filled with 0.9 % $^{w}/v$ NaCl solution (wetted with 0.5 % $^{v}/v$ Tween 80) and the mouse held with the electrodes placed in the ears. After preliminary experiments involving the application of a range of voltages, it was found that tonic extensor spasm could be induced in 100 % of control TO mice at 80 V using a pulse rate of 100 Hz, a pulse width of 2 msec and shock duration of 0.3 sec.

3. Nociceptive sensitivity in the mouse

i) Tail clip test

The method used was a modification of that described by Bianchi & Franceschini (1954). An artery clip with the branches enclosed in a thin rubber tube was applied to the root of the tail, about 5 mm from the body, for 15 sec. The mouse made continuous attempts to remove the noxious stimulus by biting the clip. All experimental animals were exposed to the stimulus before drug treatment, and any not responding within a 15 sec exposure period were rejected.

After a 3 h rest period the animals were injected with the drug or 0.9 % saline, and re-tested at various intervals after injection. Groups of 10 mice were used for each dose level. The results are expressed as the percentage of mice showing sensitivity to the noxious stimulus after a given dose of drug.

ii) Tail flick test

The method of D'Amour & Smith (1941) was modified, so that the light produced by a pre-focused projector bulb (supplied by 6 V D.C. current at 4.5 A, ≡ 27 W) was directed onto the tail of the mouse, the animal being restrained in a glass container with a close fitting lid through which the tail projected (fig. 4). This painful stimulus caused the mouse to flick its tail away, the end-point being taken as the first escape response. A cut-off time of 10 sec was employed. The stimulus intensity under the above conditions was such that the reaction time of both untreated and control animals lay consistently between 3.5 and 4.5 sec in the strains of mice used.

Groups of 10 mice were used for each dose level and the reaction times determined at intervals after administration of the drug. Results are expressed as group mean reaction times $\pm$ S.E. The significance of inter-group differences was assessed by student's "t" test, provided not more than 50 % of the animals in a group exhibited reaction times greater than the cut-off time.

4. Apomorphine-induced gnawing in the rat

Initial experiments were performed in mice using doses of apomorphine up to 10 mg/kg sc. The doses used, however, produced only very weak gnawing

behaviour. This, together with earlier work (Scheel-Krüger, 1970), which suggested that doses of apomorphine from 10 to 60 mg/kg sc induced only weak, variable gnawing behaviour, made it necessary to continue the investigations using a different species. Consequently, all further experiments were continued using rats, since low doses of apomorphine in this species produced consistently reproducible results.

Groups of 4 male Wistar rats weighing 180 - 200 g were implanted with cannulae into the right lateral ventricle, as described in Methods II 2. At least 10 days after this operation the rats received apomorphine by sc administration (in addition to icv injection of other agents under investigation). The rats were then housed individually in opaque polypropylene cages (40 x 50 x 15 cm), with corrugated paper covering the floor and side walls. The animals were observed continuously for at least 60 min after injection of apomorphine, and examined for gnawing behaviour at 5 min intervals. Results are expressed as the number of animals showing compulsive gnawing behaviour during observation periods of 1 min.

5. Behavioural tests

i) Measurement of spontaneous locomotor activity (S.L.A.) in the mouse

In a number of experiments, the assessment of changes in locomotor activity and general central nervous activity was made by direct visual comparison of test and control groups. However, when a more quantitative evaluation was desirable, S.L.A. was measured using a Faraday Electronics Animal Activity Recorder. Two groups of 5 mice (test and control) were placed in opaque polypropylene cages (20 x 27 x 10 cm). Each cage had two capacitor plates attached vertically to the outsides and at right angles to one another. A high frequency radio signal was fed into a steel mesh grid under

each of the two cages. Any movement inside an individual cage changed the capicitance of the system and activated an integrating amplifier and digital counter, so that a digital assessment of locomotor activity was displayed for a group of mice in a cage. Prior to experiment the cages were balanced electronically against each other so that the digital records were the same for similar degrees of movement. Naive animals were normally used when a decrease in S.L.A. was anticipated, so that the reduction of normal initial exploratory activity in a new environment could be seen. The experiments were repeated reversing the test and control groups in the two cages, to obviate any residual between-channel differences in the activity recorder.

ii) Measurement of conditioned avoidance behaviour in the rat

The method used was based on that described by Jacobsen & Sonne (1955, 1956), using a commercially available twin-compartment cage (Ugo Basile, Milan, Automatic reflex conditioner Cap 4), the animals being trained to escape from a combined audio/visual conditioned stimulus (C.S.) and an electrical unconditioned stimulus (U.S.). Before each experiment the rats were left undisturbed in the cage for 10 - 15 min. The C.S. was given for a fixed period of 3 sec. If the rat did not react by running into the other compartment of the cage the electric shock was given for a maximum time of 5 sec. The stimuli were given at intervals of 20 sec, 20 stimuli being given per trial. The relation between conditioned avoidance response (C.A.R.) and unconditioned response (U.R.) was then determined. A training period of seven experiments at intervals of 24 h resulted in 60 % of the animals exhibiting C.A.R. for an average of 90 % of the stimuli they received. Subsequently the percentage C.A.R. and U.R. was determined in the test experiment and compared with those in control experiments made immediately before and at intervals after the trial of the same rat. Four

animals were used for each dose level of each drug tested, 20 stimuli being given per trial. An estimation was also made of the length of time during each trial for which the C.S. was administered and this was recorded as the latency.

iii) Assessment of catalepsy in the mouse

Catalepsy may be defined as the acceptance and retention of an abnormal posture, and an attempt to quantitate this effect was made in the following manner (see Zetler, 1968).

The apparatus consisted of a vertical rod 35 cm long and 7 mm diameter, supported at its base and covered with 20 cm of string whipping. The test was carried out by taking a mouse by the nape of the neck and placing it on the rod in a head upwards position, so that it could grip the string. Untreated animals immediately ran up or down, while cataleptic animals remained stationary where placed and assumed a characteristic posture (fig. 5). The end-point was well defined and taken when the animal began to move either up or down the rod. Duration of catalepsy was determined using groups of 10 mice per dose level at various times after injection. A cut-off time of 30 sec was employed.

iv) Assessment of hypnotic activity in the mouse

The duration of hypnotic activity induced by drugs administered either peripherally or by icv injection to groups of 10 mice was recorded as the duration of a complete loss of righting reflex, determined by direct visual observation of individual animals. The results were expressed as the group mean sleeping times $\pm$ S.E. with the statistical significance of any observed differences being calculated from student's "t" test.

6. Determination of blood pressure in the conscious unrestrained rat

Of the methods published for the recording of blood pressure in the conscious rat (Still, Pradhan & Whitcomb, 1956; Popovic & Popovic, 1960; Weeks & Jones, 1960; Weeks & Davis, 1964; Thuranszky, 1966; Dawson, 1968; Fujita & Tedeschi, 1968), those of Weeks entail extensive surgery, while the methods of Fujita and Dawson, using the tail artery, seem to produce variable results.

A modification of the method of Popovic & Popovic (1960) was decided upon. This method involves carotid artery cannulation, but recordings of blood pressure can only be made for about 24 h afterwards, owing to occlusion of the carotid artery. In order to overcome this a specially designed cannula was pushed down the left carotid artery, so that its tip was free-floating in the aortic arch.

Cannulae were made from PP25 tubing. This was cut to lengths of about 15 cm, one end being slightly sharpened and bent. The design of the cannulae is shown in fig. 6. The cannulae were sterilised by soaking in a solution of 1 % chlorhexidine in 70 % industrial methylated spirits. They were then washed and filled with sterile heparinised saline 1,000 i.u./ml (Pularin: Heparin Injection B.P.). One end of the tube was blocked by placing a tight fitting pin into the lumen.

Rats were anaesthetised with the halothane, nitrous oxide and oxygen mixture as described in Methods II 2. and a 2 cm long incision made on the ventral surface of the neck, lateral to the mid-line. The left common carotid artery was exposed and ligaturated and the cannula introduced downwards into the artery, so that the tip lay at the level of the aortic arch. It was then tied in securely with thread.

The cannula was exteriorised in the following manner: a stainless steel tube 2 mm in diameter was pushed under the skin from the back of the neck and the cannula passed down this tube. The tube was then withdrawn leaving the cannula protruding from the stab wound in the skin, caused earlier by the tube. Both incisions were sutured and the animal allowed to recover.

With this preparation, recordings of blood pressure could be made in the conscious rat for up to three weeks after operation, providing the cannulae were flushed daily with 0.1 ml sterile heparinised 0.9 % saline containing 1,000 i.u. heparin/ml. Failure to do this resulted in the formation of a localised inflammatory reaction at the tip of the cannula, ultimately occluding it. In order to prevent the cannulae from being pulled out of the aortic arch (this was possible despite it being securely tied into the tissues of the neck), a bubble was blown in the polypropylene tubing at the point where it was tied into the carotid artery.

The length of exposed cannula was kept short to prevent the animal from biting it and a headless pin was used to occlude the free end. Prior to recording, the PP25 tubing was clamped with artery forceps, the headless pin removed, and a length of PP60 tubing filled with heparinised 0.9 % saline (1,000 i.u./ml) pushed over the exposed end, making a blood-tight joint. The PP60 was connected to a blood-pressure transducer using a disposable nylon 3-way tap. A 1 ml syringe was fitted to one side arm of the transducer filled with heparinised saline. In the event of the cannula becoming blocked, or blood flowing back up the tube due to leakage in the system, flushing could be accomplished promptly using the syringe. The pressure was recorded on a pen recorder (Devices, using a DC.2C. and Sub. 1C. pre-amplifier).

During recording the animals were housed individually (fig. 3). Before experiment the animals were thoroughly familiarised with the experimental environment and were handled as much as possible. In practice the majority of animals remained placid during experiment, and a constant trace could easily be produced.

7. Acute toxicity (LD_{50}) determinations

i) Dexamphetamine crowded toxicity

Groups of 8 mice received ip injections of dexamphetamine at one of 5 dose levels. Immediately afterwards they were placed in standard two litre beakers lined with sawdust. The beakers were wrapped in brown paper to prevent light entering through the side walls. This gave a floor space of approximately 20 cm^2 per mouse. The experiments were performed in the early afternoon and the percentage mortality determined 20 h later. LD_{50} values were calculated by the method of Litchfield & Wilcoxon (1949). Differences between the test and control animals are quoted at 5 % significance levels. The experiments were repeated in test and control animals, the results pooled and compared.

ii) Pentobarbitone acute toxicity

Groups of 10 mice received ip injections of pentobarbitone at one of 6 dose levels, and were placed in opaque polypropylene cages (20 x 27 x 10 cm). After induction of hypnosis, the animals were placed close together to minimise heat loss, taking care that their respiratory passages were not obstructed. They were then left undisturbed in the laboratory at a constant environmental temperature of 25 $\pm$ 1°C. After 20 h the number of dead animals in each group was counted and the LD_{50} values calculated as above.

IV Biochemical testing methods

Whole-brain catecholamine and indoleamine levels were determined spectrophotofluorimetrically.

Groups of 8 mice were killed by cervical dislocation, and the brains dissected out, weighed, and homogenised in 4 ml 0.4N perchloric acid at $0^{o}C$. The homogenate was centrifuged at 15,000 g for 8 min at $0^{o}C$ and the supernatant stored at $0^{o}C$. A second homogenisation using a further 2 ml 0.4N perchloric acid was performed on the original sample and recentrifuged as before, the second supernatant being bulked with the first.

The total clear supernatants from two groups of brains were combined, shaken and divided into two equal portions, one for dopamine and noradrenaline determination, the other for the 5-hydroxytryptamine determination. Known amounts of the three amines were added to some extracts as a check on the recovery of these amines.

1. Estimation of whole-brain noradrenaline and dopamine

The aliquot of clear supernatant was titrated to pH 6.5 using 5N potassium carbonate at a pH meter, the potassium perchlorate precipitate thus produced removed by centrifugation at 15,000 g for 6 min at $0^{o}C$, and the clear supernatant passed on to a Dowex 50 W.X.8 resin (100 mg dry weight) column, which had been washed previously with: (1) 8 ml 2N HCl, (2) 10 ml distilled water, (3) 5 ml 0.5M phosphate buffer pH 6.5, (4) 10 ml distilled water, (5) two further 10 ml volumes of distilled water. The dimensions of the washed resin column were 4 mm diameter and 12 - 15 mm long.

The supernatant was passed through the resin at a flow rate not exceeding 1 ml/2 min. After adsorption of the amines, the columns were washed with 10 ml distilled water. Then, after passing 0.5 ml 0.4N HCl on to the column to displace residual water, the noradrenaline was eluted with 8 ml 0.4N HCl at a flow rate not exceeding 1 ml/2 min. The dopamine was then eluted with 8 ml 2N HCl at the same flow rate (having first displaced any 0.4N HCl with 0.5 ml 2N HCl). This procedure was a modification of that used by Bertler, Carlsson & Rosengren (1958).

The noradrenaline was assayed by a trihydroxyindole method evolved from those of Euler & Floding (1955) and Bertler, Carlsson & Rosengren (1958). Phosphate buffer was used instead of acetate buffer, and zinc sulphate was omitted. In the alkaline ascorbate, sodium borohydride stabilised the fluorescence (Gerst, Steinsland & Walcott, 1966), although it was necessary to use a concentration of sodium borohydride 10 times larger than that suggested by these workers. This stabilised the fluorescence of noradrenaline for at least 60 min. The fluorescence intensity of noradrenaline was read at the activation and emission wavelengths 395/500 mμ respectively in an Aminco Bowman Spectrophotofluorimeter.

Dopamine was assayed by the method of Carlsson & Waldeck (1958), with the modification of Carlsson & Linqvist (1962). However, only 0.05 ml iodine solution was used instead of 0.1 ml in the oxidation, and maximum fluorescence developed without the use of ultraviolet irradiation. The fluorescent principle produced by this procedure was unstable and faded rapidly when subjected to the activation light in the fluorimeter. However, if the tubes were immersed in a boiling water bath for 5 min immediately after the oxidation and then allowed to cool to room temperature, the dopamine fluorescence was stabilised at its maximum for at least 60 min. The fluorescence

intensity was then read at the activation and emission wavelengths 325/378 mμ respectively.

2. Estimation of whole-brain 5-hydroxytryptamine

The 5-hydroxytryptamine aliquot was neutralised with 5N potassium carbonate, centrifuged as for the catecholamine determinations and the clear supernatant passed on to a column of Dowex 50 W.X.8 resin (100 mg dry weight), previously prepared in the sodium form with: (1) 8 ml 1N NaOH, (2) 15 ml distilled water, (3) 15 ml 0.1N NaOH containing 0.2 % $^{w}/v$ EDTA, (4) 10 ml distilled water, (5) two further 10 ml volumes of distilled water. The clear supernatant was passed through this column at a flow rate not exceeding 1 ml/2 min. The 5-hydroxytryptamine was then eluted from the column with 15 ml 0.1N NaOH containing 0.2 % $^{w}/v$ EDTA into 1.5 ml sodium acetate buffer, pH 4.6, and read directly in the spectrophotofluorimeter at the activation and emission wavelengths 295/345 mμ respectively. This method is a modification of that used by Cox & Potkonjak (1967).

The recoveries of the three amines by the methods outlined were:

Noradrenaline:	38 %
Dopamine:	52 %
5-hydroxytryptamine:	75 %

All values are given corrected for recovery.

V Drugs and vehicles used

Drugs for peripheral administration were dissolved in 0.9 % $^{w}/v$ NaCl solution unless otherwise stated and administered in a dose volume of 10 ml/kg body weight.

Drugs for icv injection were dissolved in 0.9 % $^{w}/v$ sterile apyrogenic NaCl solution and administered in a dose volume of 10 µl, unless otherwise stated.

Doses are quoted in terms of the free acid or base, unless otherwise stated.

The following drugs and vehicles, listed together with their sources and vehicles, were used.

DRUG		SOURCE	VEHICLE
Dose calculated as:	used as:		
Adenosine 5'-diphosphate	sodium salt	Sigma	saline
Adenosine 5'-triphosphate	disodium salt	Sigma	saline
Apomorphine	hydrochloride	Allen & Hanbury's Ltd	saline
Caffeine	sodium iodide	May & Baker	saline
Chlorpromazine	hydrochloride	May & Baker	saline
Cocaine	hydrochloride	British Drug Houses	saline
Cyclic N^6-2'0-dibutyryl adenosine 3,5-monophosphate	→	Boehringer GmbH Mannheim	saline
Desmethylimipramine	hydrochloride	Geigy	saline
Dexamphetamine	sulphate	Ward Blenkinsop	saline
Digitoxin	→	British Drug Houses	Ethanol 45 % Glycerin 40 % Distilled Water to 100 % Dilute with sterile saline
2,4-dinitrophenol	→	British Drug Houses	Dimethylsulfoxide 50 % Saline 50 %
2,4-dinitrophenol	sodium salt	British Drug Houses	1) Dimethylsulfoxide 50 % Saline 50 % 2) Saline
Dopamine	hydrochloride	Sigma	saline

Halothane	→	Imperial Chemical Industries	-
Heparin	→	Evans Medical	saline
6-hydroxydopamine	hydrochloride	Kistner Labtjänst A.B.	saline
Leptazol	→	British Drug Houses	saline
α-methyl-meta-tyrosine		Sigma	saline
Morphine	hydrochloride	May & Baker	saline
Nialamide	→	Pfizer	Dissolve in 1 ml N/10 HCl make up to volume with saline and adjust to pH 7 with N/10 NaOH
L-Noradrenaline	bitartrate	British Drug Houses	saline
Ouabain	octahydrate	Sigma	saline
Pentobarbitone	→	British Drug Houses	Dimethylsulfoxide 50 % saline 50 %
Pentobarbitone	sodium salt	British Drug Houses	1) Dimethylsulfoxide 50 % saline 50 % 2) saline
Pentachlorophenol	sodium salt	British Drug Houses	1) Dimethylsulfoxide 50 % saline 50 % 2) saline
Phentolamine	methane sulphonate	Ciba	saline

Reserpine	⟶	British Drug Houses	1 drop lactic acid, 2 drops ethanol. Heat gently and make up to volume with saline
Sodium diethyldithio-carbamate	⟶	British Drug Houses	saline
Tetrabenazine	⟶	Hoffmann La-Roche	2 drops 95 % ethanol, 5 drops N/10 HCl. Heat gently. Make up to volume with saline and adjust to pH 7 with N/10 NaOH

VEHICLE	SOURCE
Saline 0.9 % (Sterivac)	Allen & Hanbury's
Dimethylsulphoxide	Hopkin & Williams
Glycerin	Procter & Gamble
Ethanol	British Petroleum Chemicals

VI FIGURES

FIG. 1

Skull, dorsal aspect, diagrammatical x 3, to show points of entry of icv injection needle in the mouse (1) and the rat (2).

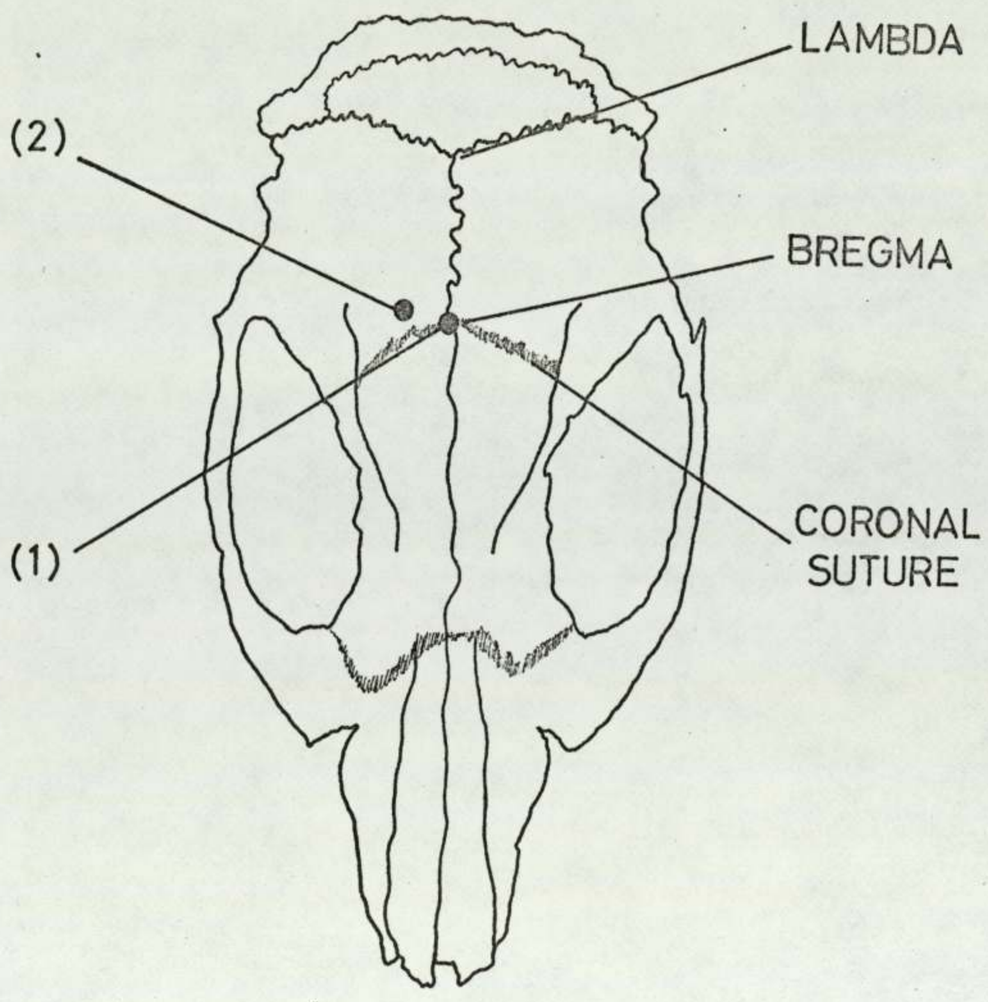


FIG. 2

Vertical section and plan view of the cannula guide implanted into the lateral ventricle of the conscious rat.

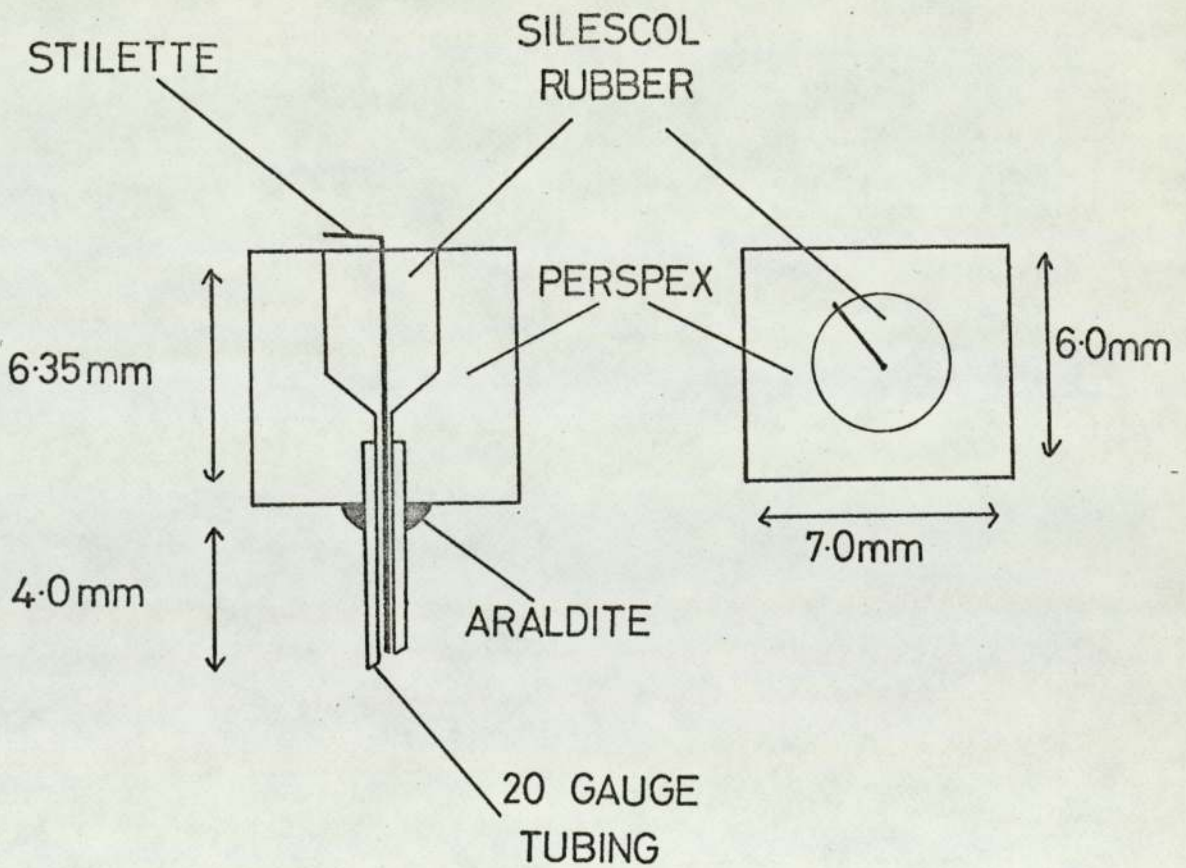


FIG. 3

Apparatus used for icv perfusion of the conscious unrestrained rat. The syringes were driven by a modified slow injection apparatus (Palmer Ltd, London). During blood pressure measurement the cage in the foreground contained one rat per compartment.

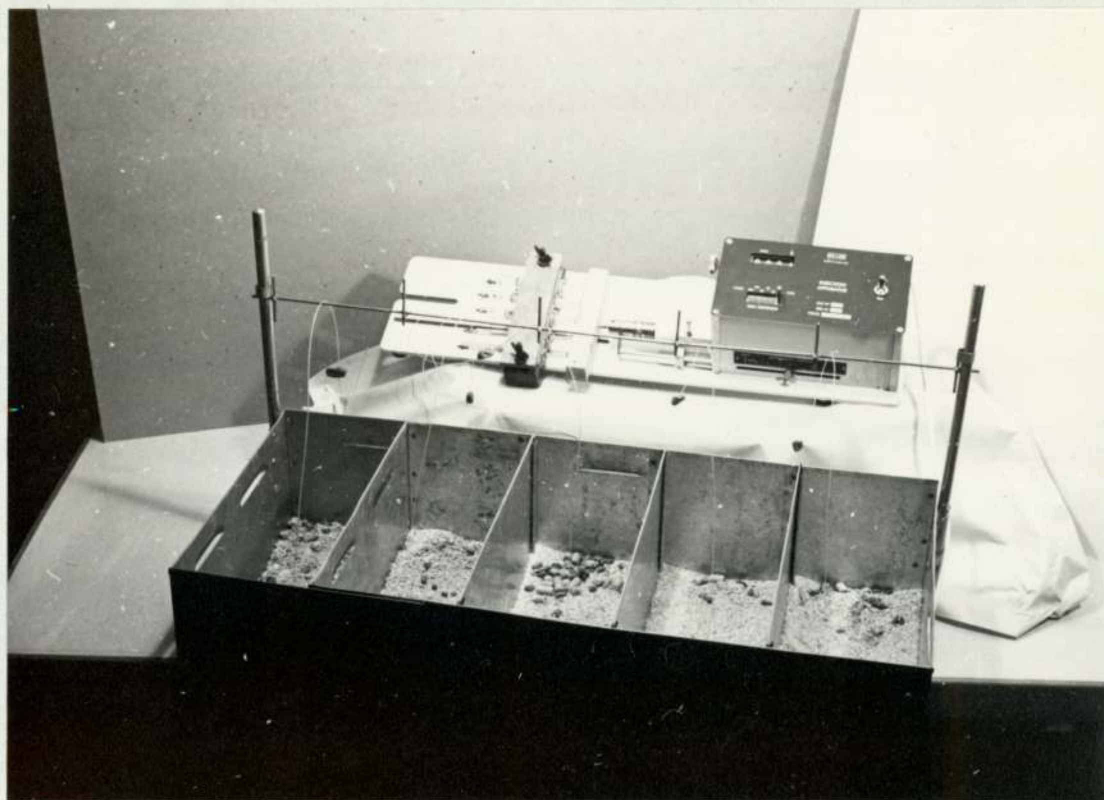


FIG. 4

Apparatus used for testing nociceptive sensitivity of the mouse by the tail-flick method.

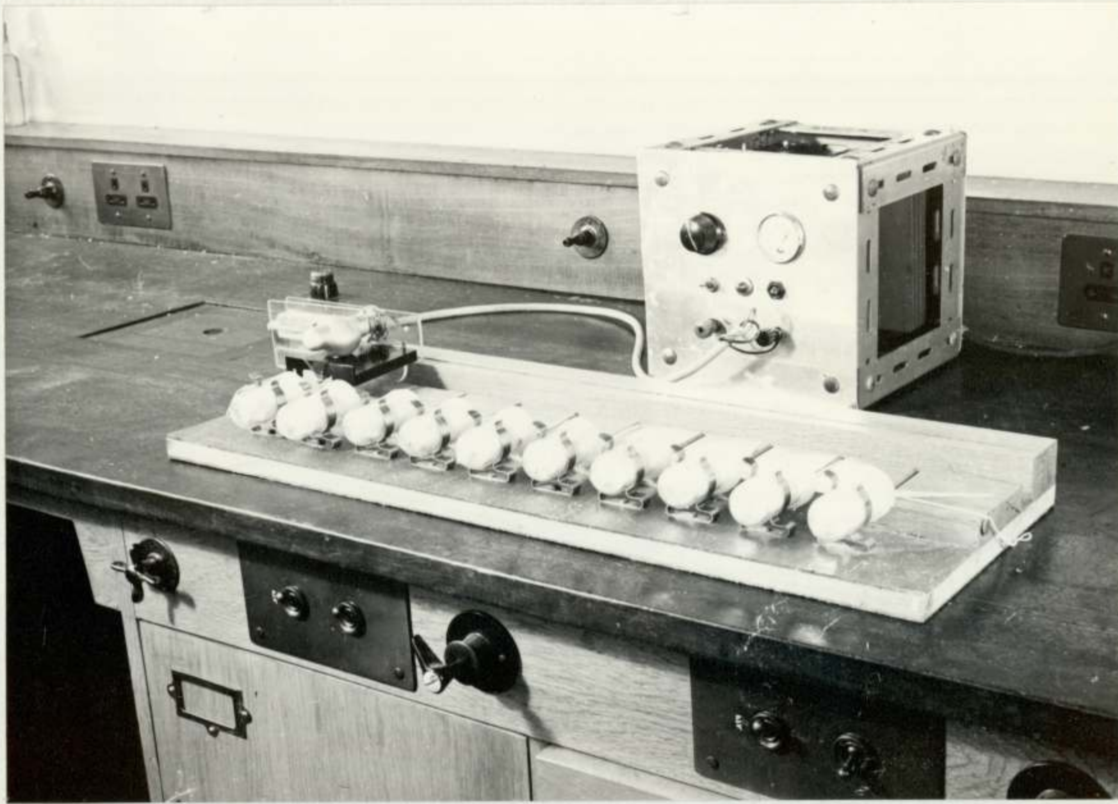


FIG. 5

Catalepsy in the mouse following centrally-administered ouabain. Animal received ouabain (0.3 μg icv) 30 min before the test.

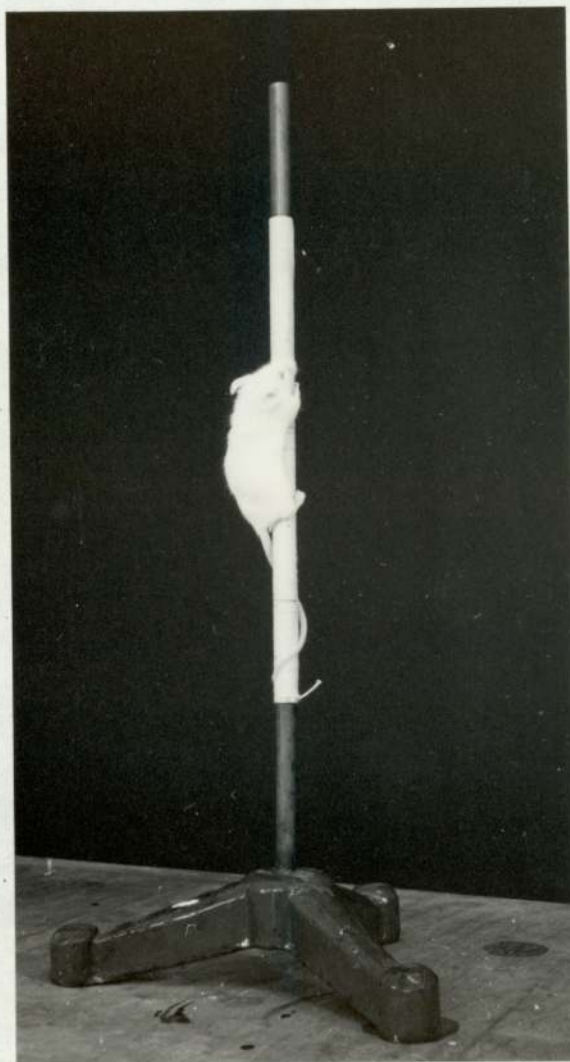
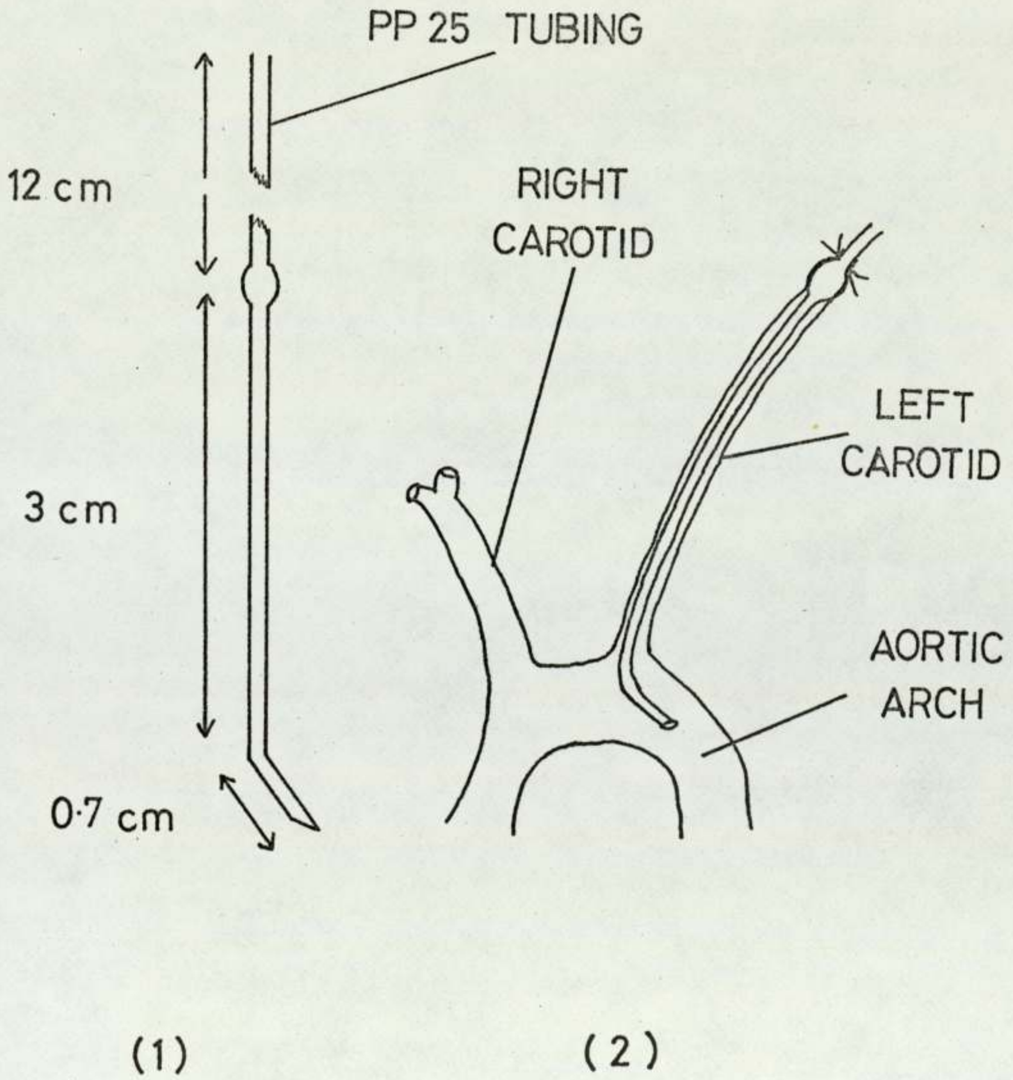


FIG. 6

Cannula used for continuous recording of blood pressure in the conscious, unrestrained rat. Diagram shows dimensions of cannula (1) and location of the tip of the cannula within the aortic arch (2).



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I PRELIMINARY EXPERIMENTS TO DETERMINE WHETHER ICV INJECTION AND/OR VENTRICULAR PUNCTURE *PER SE* IN THE MOUSE INDUCES CHANGES IN BEHAVIOUR AND SENSITIVITY TO BARBITURATE

I Preliminary experiments to determine whether icv injection and/ or ventricular puncture *per se* in the mouse induces changes in behaviour and sensitivity to barbiturate

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I In experiments involving the technique of injection of drugs directly into the cerebral ventricles, one cannot overemphasise the importance of stringent controls to eliminate artifacts produced by injection volume, temperature, tonicity and pH, particularly when the dose-volume, usually 10 μl, is greater than the volume of the cerebrospinal fluid in mice and comparable to that of rats. The injection of such volumes, or indeed the trauma of the injection itself, may be responsible for modifications in behaviour observed in the test animals, or may so affect the pharmacological responses to the drugs under investigation that direct comparison with untreated animals is impossible.

Accordingly, preliminary investigations were carried out in mice to determine the extent of these effects. The experiments were also designed to give an idea of the extent to which substances were distributed throughout the ventricular system, following injection into the cerebral ventricles by the technique described in Methods II 1.

1. Distribution of pontamine sky blue following icv injection

Groups of 5 mice received 10 μl or 20 μl of a 5 % solution of the dye (in Water for Injection B.P.) by icv injection and were killed 5 min later by decapitation under chloroform anaesthesia. The brains were then carefully dissected out from the skulls, sectioned in a coronal plane, using a freezing microtome, and the extent of spread of the dye examined. Inspection of the brain *in situ* after removing the interparietal, parietal and frontal bones revealed no superficial staining of the dorsal surfaces of the cerebral hemispheres. Coronal sections of the brain revealed the presence of dye throughout the ventricular system (fig. 7). No evidence of dye was found in the superficial cerebral vasculature. There was no apparent difference in distribution produced by the two dose-volumes of dye solution injected.

2. Effect of icv saline

i) Dose-volume

Insertion of the needle or injection of 5 - 20 µl 0.9 % saline at pH 7 had a slight effect on the mice. Immediately after removal of the needle the animals were hyperexcitable for a period of 10 - 15 sec and frequently shook their heads and groomed their vibrissae, after which they remained quiet for about 60 sec. Their normal activity resumed immediately afterwards, whereupon they were no longer distinguishable from untreated mice. Only minor, short lived changes of body temperature (usually a hypothermia) occurred. None of the animals showed any residual or detrimental effects from the procedure.

ii) Temperature of injected solution

Solutions of drugs and control vehicles were normally injected at 35°C and at pH 7 unless otherwise stated. The injection of 10 µl 0.9 % saline at this temperature and pH to a group of 6 mice produced a slight transient hypothermia, possibly due to the simultaneous decrease in heat production associated with a reduction in spontaneous locomotor activity, causing the animals to lose heat rapidly in the low environmental temperature (21 ± 1°C). The icv administration of a similar volume of saline at higher temperatures, 42°C and 45°C, to two further groups of mice was followed by a more exaggerated hypothermic response (fig. 8). All three groups were behaviourally indistinguishable.

iii) Tonicity and pH of injected solution

Because certain experiments involved the use of concentrated basic solutions, it was important to examine the effects of icv injections of these solutions.

Following the icv administration of 10 µl 5 % saline at pH 7 to groups of 8 mice, the animals initially showed a tendency to writhe, and a number would begin to groom the tops of their heads. They quickly became quiet, showing no tendency to aggregate in groups, but remained isolated in their cage. There were no convulsions, either clonic or tonic, and all the animals survived these injections, resuming normal activity after about 15 min. When the pH of the hypertonic saline was raised to 9.5, similar effects were observed, but in addition there was a marked tendency for the animals to writhe. It was found that, within 10 sec of injection, 20 % of the animals exhibited a tonic extensor spasm resulting in death. After injection both groups exhibited a slight transient hypothermia, slightly greater than that seen after injection of 0.9 % saline at pH 7 or 9.5 (fig. 9).

3. Effect of icv saline on pentobarbitone hypnosis

Preliminary investigations suggested that icv saline, injected immediately after the peripheral administration of pentobarbitone, produced a potentiation of sleeping time, as measured by duration of the loss of righting reflex, induced by the barbiturate. The following experiment was performed to confirm this observation. A group of 10 mice received pentobarbitone (20 mg/kg ip), this being a threshold dose which just failed to produce a loss of righting reflex. A further group received pentobarbitone (20 mg/kg ip) followed 2 min later by saline (10 µl icv). In this case a definite loss of righting reflex was observed in all animals (table 1). It was not known at this stage

whether the potentiation of pentobarbitone sleeping time was due to the physical effects of an icv injection of a comparatively large volume of saline (presumably with a consequential interference in the dynamics of the cerebrospinal fluid), or whether these observations were the direct result of ventricular puncture. In order to clarify this further, the experiment was repeated without injecting any saline, but merely puncturing the cerebral ventricles.

4. Effect of simple penetration of the cerebral ventricles on pentobarbitone hypnosis

Ventricular puncture alone might cause an alteration in the permeability and function of the blood-brain barrier. Alternatively, the trauma of injection could cause an increase in the sensitivity to the barbiturate.

Groups of 10 mice received either pentobarbitone (20 mg/kg ip) alone, or this dose of pentobarbitone followed 2 min later by insertion and withdrawal of the icv injection needle. Again the group receiving pentobarbitone alone showed no loss of righting reflex with this sub-threshold dose, but those animals receiving a needle insertion in addition to pentobarbitone showed a loss of righting reflex in all cases (table 1). Furthermore, the duration of loss of righting reflex was significantly longer following pentobarbitone and simple needle insertion than it was following pentobarbitone and a proper icv saline injection ($P < 0.001$), although there was no significant difference between the two group mean onset times.

5. Discussion

An icv-injected agent could reach almost all parts of the brain and it is important to examine the chances of this occurring. The distribution of pontamine sky blue, after its injection into the cerebral ventricles of mice, shows that substances given by icv injection could be expected to influence the activity of those vital centres located in the walls of the cerebral ventricles. Neuronal links between these and other centres in the brain might modify the responses observed. There is also the possibility that, if the injected substance leaked out of the foramina of Magendie and Luschka and passed over the external surface of the brain, it might activate many other sites of nervous activity by a direct action. Similarly, leakage of drug back through the needle track, followed by absorption from the sub-arachnoid space into the systemic circulation, could take place. Normally the doses of drugs given by icv injection are so small as to mitigate the possibility of significant peripheral activity, particularly considering the dosage differential required to produce pharmacological effects after peripheral administration.

In the experiments reported above, no dye was observed on the dorsal surfaces of the cerebral hemispheres, and this suggests that, at most, only small quantities of injected solutions reached the sub-arachnoid space. Nevertheless, only small quantities of potent compounds may be necessary to produce profound effects, and it appears from previous work in cats (Borison, 1959; Feldberg, 1963) that substances injected into the cerebral ventricles can reach a large part of the brain, both via the ventricular walls and the sub-arachnoid space. The actual areas penetrated by a particular substance would be extremely variable and would depend largely on its physico-chemical properties and its method of transport across cell-membranes.

The injection of 5 - 20 μl 0.9 % saline produced minimal effects on the experimental animals, and it seems that the icv injection technique described can be usefully employed under the experimental conditions used, providing that the test groups are always compared with properly controlled animals.

The effect of localised warming of the ventricular system brought about by the injection of warm saline at 42°C or 45°C resulted in a hypothermia greater than that observed after saline at 35°C, possibly due to an activation of heat loss mechanisms. It is generally agreed that thermoregulation depends on the integrity of the anterior hypothalamic region of the brain, into which information is fed by afferent pathways from central and peripheral thermosensors, and from which appropriate control is exerted over the thermoregulatory effectors. Thus it is possible that the warm saline affects the central thermosensors causing an activation of heat loss mechanisms, with a resulting decrease in core temperature (see Cremer & Bligh, 1969). The marginal hypothermia produced by saline at 35°C is possibly due to a decrease in heat production, associated with the observed reduction in locomotor activity.

The deleterious effects of hypertonic and basic solutions may be related to changes in the composition of the extracellular fluid. It is known that experimental seizures can easily be initiated in normal brain tissue by chemical agents, and that changes in pH, total osmotic pressure and electrolyte composition of the fluid environment of brain cells are capable of precipitating convulsions (see Goodman, Toman & Swinyard, 1949). Even small groups of neurones, variously injured, may give rise to rapid and uncontrolled bursts of discharges affecting normal neighbouring cells and related centres. The entire brain may then be involved in the production

of a tonic/clonic seizure by such a self-propagating process. The proconvulsive effects of hypertonic basic saline explains subsequent observations of convulsions following immediately after the icv injection of alkaline solutions of drugs in high concentration (see Results VII 1. i)).

Of particular interest is the alteration in the sensitivity of mice to the hypnotic effects of pentobarbitone, following either the icv injection of saline or simple ventricular puncture. There is no reason to suppose that this variation in sensitivity should be restricted to pentobarbitone. It may also occur with other drugs whose pharmacological action depends upon them gaining access to the central nervous system and interacting with central processes. The observed effects may be explained by two possible mechanisms: (1) an alteration in the permeability, integrity or function of some part of the blood-brain barrier, (2) a change in the sensitivity of central neurones to pentobarbitone. The alteration in permeability of the blood-brain barrier could be brought about by the procedure involved in ventricular puncture and also, in view of the immediate, short-lived excitation in mice following this procedure, might be associated with an increased activity of many central neurones, which may result in their changed sensitivity to the barbiturate. There may also be a change in local blood flow, which could impair removal of barbiturate from brain tissue.

Furthermore, it is interesting to note that the potentiation of pentobarbitone hypnosis is greater following simple insertion and withdrawal of the icv injection needle than following the proper icv injection of 10 μl saline. This difference may be explained by an interference with cerebrospinal fluid dynamics, associated with the injection of such a relatively large volume of liquid, which decreases the rate of transfer of the barbiturate from the blood to the brain tissue and thus reduces its availability.

Further studies are needed in order to elucidate fully the mechanisms involved in the observed pentobarbitone potentiation. The effects following puncture of other areas of the brain could be examined, either with or without the injection of saline. A determination of barbituric acid content of the brain following ventricular puncture could be made, and compared with that of animals receiving the barbiturate without ventricular puncture.

However, it is not the purpose of this section to elucidate these mechanisms, but solely to draw attention to the existence of these interactions, and to emphasise the necessity for comparing all animals receiving drugs by icv injection with those which have received an equivalent volume of the appropriate vehicle.

6. FIGURES AND TABLES

FIG. 7

Photograph showing distribution of dye throughout the ventricular system in the mouse brain 5 min after icv injection of 5 % pontamine sky blue in a dose volume of 10 µl. (1) lateral ventricles; (2) lateral and third ventricles; (3) fourth ventricle.

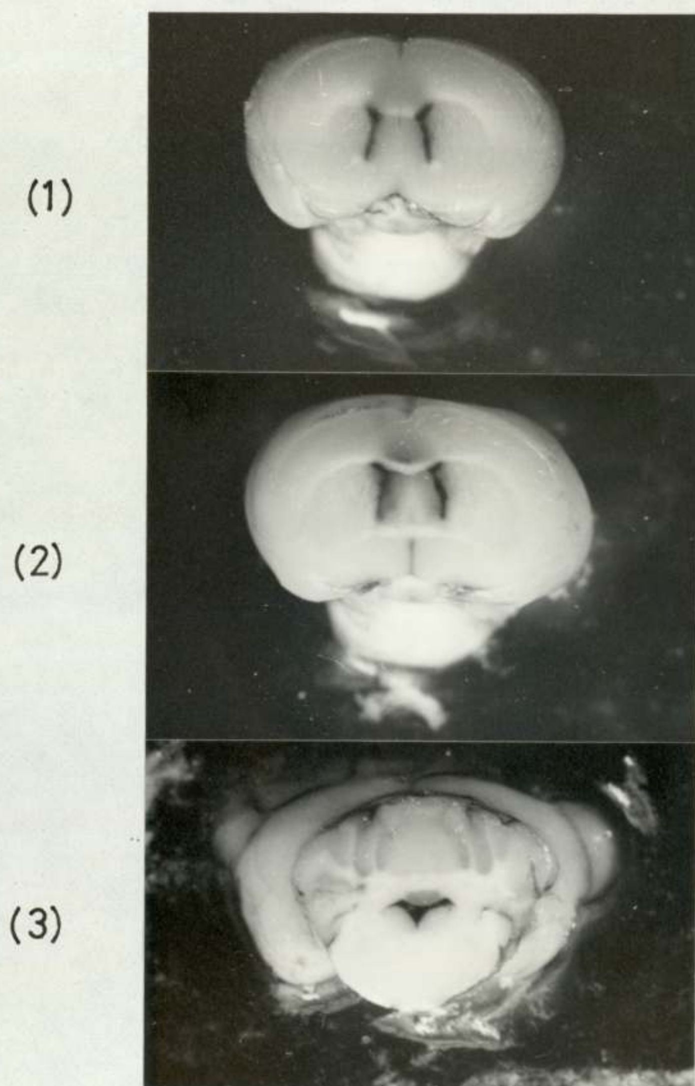

FIG. 8

Effect of icv injection of 10 µl 0.9 % saline at different temperatures on the body temperature of the mouse. (x——x) saline at 35° C; (o——o) saline at 42° C; (●——●) saline at 45° C.

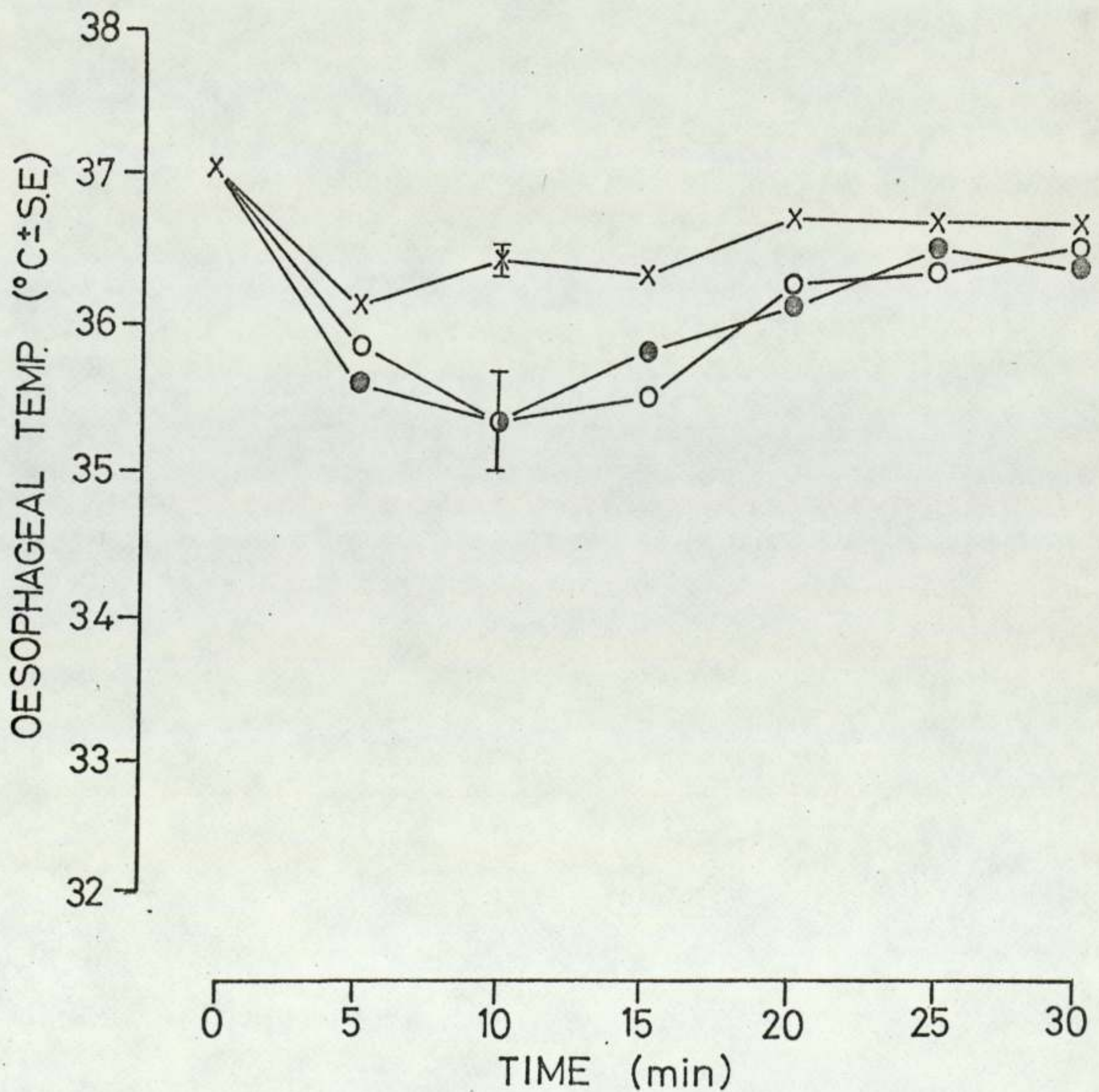

FIG. 9

Effect of icv injection of 10 μl saline at different tonicities and pH on the body temperature of the mouse.
(x——x) 0.9 % saline pH 7.0; (o——o) 0.9 % saline pH 9.5;
(●——●) 5 % saline pH 7.0; (Δ——Δ) 5 % saline pH 9.5.

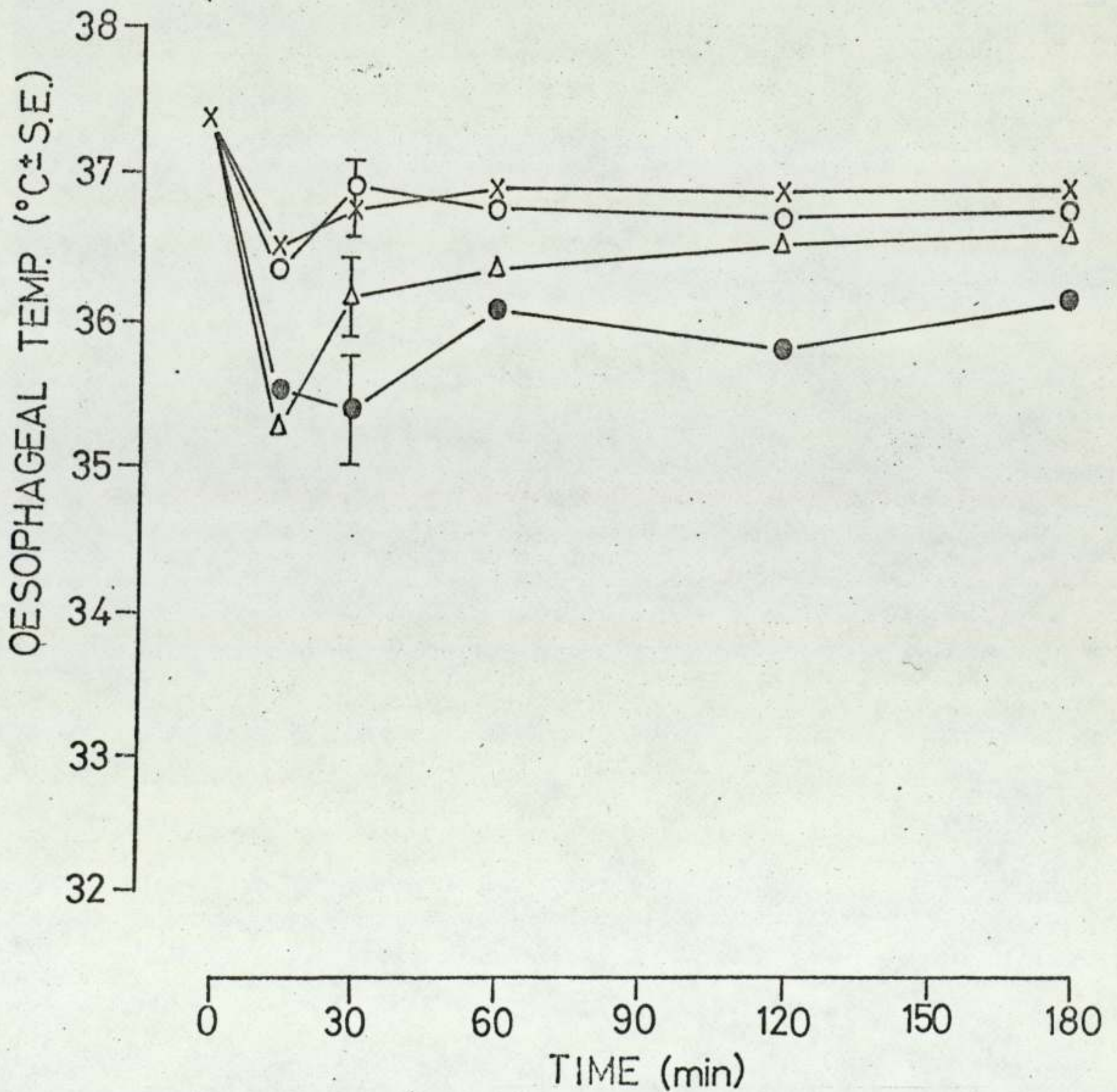

TABLE 1

Effect of icv saline or ventricular puncture on pentobarbitone sleeping time in the mouse. Animals received saline (10 µl icv) or ventricular puncture 2 min after pentobarbitone (20 mg/kg ip). (n = 10).

PRETREATMENT	ONSET TIME OF SLEEP GROUP MEAN (sec ± S.E.)	DURATION OF SLEEP GROUP MEAN (sec ± S.E.)
Pentobarbitone	-	-
Pentobarbitone + saline	317 ± 29	505 ± 54
Pentobarbitone + needle insertion	307 ± 26	1021 ± 64

II PHARMACOLOGICAL PROPERTIES OF BARBITURATE AND AGENTS INTERFERING WITH OXIDATIVE PHOSPHORYLATION WHEN GIVEN BY ICV INJECTION

II Pharmacological properties of barbiturate and agents interfering with oxidative phosphorylation when given by icv injection

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II Remarkably little is known about the precise mechanism of action of the barbiturates. Whereas a number of authors have pointed to a simple physico-chemical effect on the neuronal membrane (Heinbecker & Bartley, 1940; Schoepfle, 1957; Doran, 1959; Larsen, Van Dyke & Chenoweth, 1968), other workers have outlined a more specific biochemical action involving an uncoupling of oxidative phosphorylation (Brody & Bain, 1954; Brody, 1955), or, alternatively, a more direct interference with tissue respiration, involving an inhibition of the electron transfer chain at the flavoprotein stage (Aldridge & Parker, 1960; Reading & Wallwork, 1969). In recent years there has been a growing opinion that the primary action of the barbiturates is on energy utilisation rather than energy production (Shepherd, Lader & Rodnight, 1968a). Certainly there is an overall decrease in tissue oxygen demands which supports this view (McIlwain, 1953).

There have been several reports in the literature that the hypnotic and toxic effects of various barbiturates are potentiated in hyperthyroidism (Conney & Garren, 1961; Prange, Lipton, Shearin & Love, 1966) and this has been confirmed by recent findings in these laboratories (Spencer & Waite, 1970). Under certain circumstances thyroxine is a selective inhibitor of phosphate uptake during the process of oxidative phosphorylation (Lardy & Feldott, 1951; Martius & Hess, 1951; Hoch & Lipman, 1953). The possibility exists, therefore, that the observed increase in barbiturate potency is brought about, at least partially, by a thyroxine-induced increase in sensitivity of the central nervous system, caused by an interference in oxidative phosphorylation. This possibility is supported by later results (see Results III) and by the observation that pretreatment with 2,4-dinitrophenol (DNP) potentiates the hypnotic effect of a number of barbiturates, without influencing the rates at which they are metabolised (Brody & Killam, 1952).

In 1951 Brody & Bain reported that thiopentone, pentobarbitone and amylobarbitone uncoupled oxidative phosphorylation *in vitro* in concentrations of 1 - 5 x 10^{-4} M, concentrations which, the authors claimed, were similar to those attained *in vivo* during barbiturate hypnosis. They postulated that this uncoupling phenomenon may be partially responsible for the pharmacological effects of the barbiturates. Later the same workers (Brody & Bain, 1954) confirmed their original findings and showed furthermore, that other hypnotic barbiturates effectively uncoupled oxidative phosphorylation. They also observed that brain tissue was more susceptible than liver preparations to this uncoupling action. Hulme & Krantz (1955) compared the uncoupling potencies of a number of barbiturates and found that those compounds which are hypnotic uncouple, whereas those which are not hypnotic do not uncouple. Furthermore, the same authors demonstrated that the hypnotic activity of the five barbiturates tested: 5-ethyl-5-(hexan-1-yl)barbituric acid, 5-ethyl-5-(1,1-dimethylpropionyl)barbituric acid, 5-ethyl-5-(1,1-methylethylpropionyl)barbituric acid, barbitone and pentobarbitone, correlated with their ability to uncouple.

Peripherally-administered nitrated and halogenated phenols produce hyperthermia and an increased metabolism quite unlike the effects seen after barbiturate administration. However, these compounds exhibit very poor penetration into the central nervous system (Deichman, Machle, Kitzmiller & Thomas, 1942), in contrast to the barbiturates, and Brody (1955) suggested that the inability of the phenolic uncoupling agents to produce hypnosis may be due, in part, to this poor penetration.

In view of the number of studies linking an uncoupling effect with hypnosis, together with reports of a thyroxine-induced increase in barbiturate potency, experiments were performed to compare the pharmacological

properties of pentobarbitone with two uncouplers of oxidative phosphorylation, DNP and pentachlorophenol (PCP) after icv injection, thereby permitting direct comparison of the barbiturate and the uncouplers *in vivo*. The icv route of administration largely overcame the blood-brain barrier by presenting a higher concentration of the phenolic compounds to neural tissue than would be possible after peripheral administration.

1. Effect of icv pentobarbitone in the mouse

The icv injection of pentobarbitone in doses ranging from 80 - 200 μg in groups of 10 mice produced a short-lasting, but definite, dose-dependent loss of righting reflex of 150 - 354 sec duration (fig. 10). The pentobarbitone dose-response curve was not extended further, since with doses in excess of 200 μg, there was severe respiratory depression associated with a mortality of greater than 50 %. Typically the hypnosis was preceded by an increased rate and depth of respiration and a short period of intense excitement, during which the animals ran about the cage, became increasingly disorientated and ataxic and finally lost their righting reflex. Following these effects the animals regained consciousness, appeared normal and 24 h later were behaviourally indistinguishable from untreated mice.

2. Effect of icv 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP) in the mouse

Following icv administration of DNP (20 - 200 μg) and PCP (30 - 600 μg), identical effects to those seen after injection of pentobarbitone were observed. As with pentobarbitone, the loss of righting reflex was preceded by a marked excitatory phase and after recovering from the hypnosis the animals appeared normal. The hypnotic potency was about 3 times greater in

the case of both DNP and PCP than in the case of pentobarbitone. Like pentobarbitone, their effects were dose-dependent (fig. 10). Furthermore, the log. dose-response curves for both the phenolic uncouplers appeared almost identical and that of pentobarbitone was parallel to them. The highest doses of DNP and PCP produced a similar proportion of deaths to that seen after the highest dose of pentobarbitone (table 2).

Elevation of the ambient temperature to $31 \pm 1^{o}C$ produced no significant alteration in the duration of hypnosis produced by DNP, PCP, or in that produced by pentobarbitone.

The pH of the DNP and PCP solutions was 8, and that of pentobarbitone was 9. The injection of 0.9 % saline at pH 9 was without marked behavioural effects on the control animals.

3. Effect of solvent and dose-volume on the pharmacological properties of pentobarbitone, DNP and PCP given by icv injection in the mouse

The physico-chemical properties of both DNP and PCP are those of polar acidic molecules and they are relatively lipid insoluble at physiological pH. Penetration into brain tissue is restricted (Deichman *et al.*, 1942) and this restriction may also apply after direct injection into the cerebral ventricles. In aqueous solution the ionised moieties of sodium dinitrophenate, sodium pentachlorophenate and sodium penobarbitone represent the most abundant species present. In an endeavour to increase the proportion of unionised molecules, and so facilitate penetration into the lipid phase, experiments were performed to compare the potencies of both pentobarbitone and the two phenols, following dissolution of the free acids in 50/50 dimethylsulphoxide/0.9 % saline solvent, with those of the sodium salts in

the same solvent. Injection of the dimethylsulphoxide/0.9 % saline solution alone produced repeated grooming of the injection site, but otherwise no behavioural disturbance.

At doses of 130μg and 200 μg of pentobarbitone, no significant difference in hypnotic potency could be produced by the solvent change (fig. 11). In the case of DNP (40 μg), however, the hypnosis produced by injection of the sodium salt was significantly greater than that produced after injection of the free acid ($P < 0.01$). However, at a higher, although still submaximal, dose (80 μg), no significant difference could be observed (fig. 12). PCP, free acid, was not sufficiently soluble in the dimethylsulphoxide/0.9 % saline solvent system to allow a comparison to be made. Preliminary experiments, performed to investigate the effect of the solvent *per se*, showed that the potency of the salts in 0.9 % saline was not significantly different from the potency of the salts in the mixed solvent (fig. 13).

Attempts to increase the area of distribution within the ventricular system by increasing the injection volume in which the agents were administered failed to produce any significant alteration in hypnotic potency of either pentobarbitone, DNP or PCP (fig. 14).

4. Effect of icv pentobarbitone and DNP on body temperature in the mouse

In these experiments the effect of icv administration of the barbiturate and the uncoupling agent were compared. Doses were chosen that were equipotent in their production of loss of righting reflex. Thus the effects of pentobarbitone (120 μg icv) and DNP (40 μg icv) on core temperature were determined (fig. 15). It can be seen that at an ambient temperature of $21 \pm 1^{\circ}C$ both agents produced a hypothermia, which was maximal 10 min

after injection. The duration of hypothermia was about 30 min in the case of pentobarbitone, but with DNP it had not fully recovered within 60 min. Whereas the effect of icv pentobarbitone is similar to that seen after peripheral administration, the production of hypothermia by icv DNP is directly opposed to its peripheral effect where it is known to give rise to a hyperthermia (Bianchetti, Pugliatti & Jori, 1967).

Repetition of these experiments at $32 \pm 1^{\circ}C$ showed that no significant hypothermia was produced by either icv pentobarbitone or icv DNP, but it was noted that a number of DNP-treated animals showed salivation at this temperature.

5. Effect of icv pentobarbitone and DNP on conditioned avoidance behaviour in the rat

Pentobarbitone is capable of producing overall central nervous depression with abolition of both conditioned avoidance response (C.A.R.) and unconditioned response (U.R.) in sub-hypnotic doses. After injection of pentobarbitone (300 µg icv) the animals were immediately hyperexcitable, this being followed by ataxia, with both C.A.R. and U.R. abolished. The animals recovered from the ataxia within 5 min, the U.R. returning first, followed by the C.A.R., which returned 4 - 5 min after injection. Normal gait reappeared 6 - 10 min after injection. With smaller doses (100 µg icv), all animals exhibited a heightened awareness of the environment, with neither C.A.R. nor U.R. affected (table 3).

Following a sub-hypnotic dose of DNP (150 µg icv) the animals were again hyperexcitable and showed hyperventilation and vocalisation for 30 - 60 sec. Abolition of the C.A.R. followed immediately after injection and lasted

about 4 min. There was no marked effect on the U.R.. Similar effects (table 4) were observed after a lower dose of DNP (40 µg icv). The animals took considerably longer to recover from the effects of DNP than from pentobarbitone, and 10 min after injection had still not resumed their normal exploratory activity.

6. Effect of icv pentobarbitone and DNP on whole-brain amine levels in the mouse

In order to compare further the barbiturates with the uncoupling agents, the effects of pentobarbitone and DNP on whole-brain amine levels were studied.

Three groups of 24 mice were given icv injections of 10 µl 0.9 % saline, 150 µg pentobarbitone or 50 µg DNP. (These doses of pentobarbitone and DNP were equipotent in producing loss of righting reflex - see fig. 10). The animals were killed before recovery, 3 min after the onset of sleep in the drug treated groups. The time of sacrifice of the controls was obtained by averaging the times from injection to loss of righting reflex in both of the drug treated groups (pentobarbitone, 45 $\pm$ 5 sec; DNP, 47 $\pm$ 3 sec) and killing at 3 min plus this value (i.e. 226 sec after injection). Amine levels were estimated spectrophotofluorimetrically.

It was found that pretreatment with pentobarbitone significantly increased whole-brain levels of dopamine ($P < 0.01$) and decreased those of 5-hydroxytryptamine ($P < 0.05$), but had little effect on noradrenaline. In contrast, DNP pretreatment significantly decreased noradrenaline levels ($P < 0.001$), but had no effect on either dopamine or 5-hydroxytryptamine (table 5).

7. Discussion

An impressive number of compounds has been found to interfere with the process of oxidative phosphorylation in isolated animal mitochondria (Brody, 1955; Weinbach & Garbus, 1969), among them the barbiturates (Brody & Bain, 1951). It has been suggested that this uncoupling process causes a reduction in available cell energy levels, and is at least partially responsible for the hypnotic activity of the barbiturates seen *in vivo*. Although the specific inhibitory action of the barbiturates on oxidative phosphorylation does not appear to be shared by all central nervous depressants (Wolpert, Truitt, Bell & Krantz, 1956), it may be that those depressants which show no uncoupling activity *in vitro* may require an intact cell system to exert this type of pharmacological action.

The classical uncouplers of oxidative phosphorylation, such as DNP and PCP, are not hypnotic when given by conventional routes of administration. In view of their highly polar physico-chemical properties and poor lipid solubility one would expect little, if any, of either agent to cross the blood-brain barrier. The present technique of direct injection into the cerebral ventricles has made it possible to raise the concentration in the brain, without a concomitant increase in plasma concentration, so that a substantial proportion of the phenols should gain access to brain tissue. For the first time the central pharmacological properties of the uncouplers have been examined *in vivo*, and they possess a marked dose-dependent central nervous depressant action, similar to that produced by the barbiturates.

After icv injection both DNP and PCP were hypnotic, the observable characteristics of this hypnosis being identical to that produced by pentobarbitone. Despite their lower lipid solubility, the phenols were three

times more potent than pentobarbitone and the log. dose-response curves for all three agents were remarkably similar. The phenols were less toxic than pentobarbitone, and this may be explained either by the fact that they possess a more specific depressant action on the cerebral cortex and/or ascending reticular formation, or by a variation in the inherent sensitivity of the responding structures to these agents. Equally it may be a reflection of the difference in solubility of the two classes of compound, penetration of the barbiturate to all parts of the cerebrospinal axis being facilitated, whereas the phenols should be very much restricted in distribution because of their physico-chemical properties. A generalised widespread depression of all neural tissues, including the medullary centres, is more likely to occur after barbiturate administration.

Attempts to increase the potency of both barbiturate and phenolic uncouplers by increasing their penetration into lipids, using a dimethylsulphoxide/saline solvent were largely unsuccessful, and it appears that, following icv administration, conversion into the unionised form is not the rate-limiting step involved in the observed pharmacological actions. Furthermore, no alteration in activity could be observed by increasing the injection volume. However, experiments involving protein-bound dyes have shown that there appears to be little difference in area of penetration after icv injection of 10 µl or 20 µl of solution. Also, increasing injection volume does lower the effective concentration of agent in solution, which might impair its pharmacological effect.

Following peripheral administration it is known that the phenolic uncouplers can cause a hyperthermia, whereas the barbiturates cause a hypothermia. The results presented here show that both DNP and pentobarbitone produce a fall in body temperature after icv injection. It is well known

that thermal stimulation of various regions of the hypothalamus can produce changes in body temperature. The earliest attempt to cool and warm the basal ganglia in anaesthetised dogs was by Barbour (1912), who found that heating the region with water at 51°C caused a hypothermia, and cooling with water at 11°C caused a hyperthermia. Prince & Hahn (1918) confirmed these results in cats, and since then a series of investigators have heated or cooled various parts of the hypothalamus. It is now accepted that this region is sensitive to both heating and cooling: the area just ventral to the anterior commissure probably contains warmth receptors, and it is possible that cold receptors are also present there. Euler (1964) has heated the preoptic and supra-optic regions of the hypothalamus and found large falls in rectal temperature (1.6°C) for small increases in hypothalamic temperature (0.2°C).

Previous experiments have shown that an icv injection of warm 0.9 % saline (42°C and 45°C) in mice causes hypothermia (see Results I 2. ii)). It therefore follows that the introduction of any compound capable of producing an increase in cell metabolism and an associated hyperthermia into the region of the hypothalamus may be capable of producing a fall in body temperature by activating heat loss mechanisms, and this is a possible explanation of the hypothermia produced by icv DNP. These mechanisms involve vasodilatation at low ambient temperatures and thermal salivation at higher ambient temperatures. It was found that the administration of icv DNP at 32 $\pm$ 1°C caused salivation in the absence of whole-body hypothermia. This is additional evidence that icv DNP can affect the central thermosensors. The duration of hypothermia produced by icv DNP is significantly longer than that produced by icv pentobarbitone, and this is consistent with the greater potency of DNP as an uncoupler.

Experiments involving a comparison of pentobarbitone and DNP on con-

ditioned behaviour in rats indicate that there are differences between the two classes of compounds. These differences may again reflect the more widespread non-specific depression of the central nervous system produced by pentobarbitone. It will be noted that icv DNP resembles both icv ouabain (see Results IV 4.) and icv chlorpromazine (see Results VII 4. i)).

The present experiments show that there is no similarity of action on endogenous brain amine levels following icv injection of pentobarbitone or DNP. Although difficult to elucidate the mechanism of action of either agent by studying its effect on whole-brain amine levels, these experiments were useful in order further to compare and contrast pentobarbitone and the uncouplers. Pentobarbitone produced an increase in dopamine and no effect on noradrenaline. This latter finding confirms previous work (Fuxe & Hökfelt, 1966) using pentobarbitone and H 44/68. These authors also showed that dopamine neurones are in a lower state of activity than normal during pentobarbitone sleep, a finding which may explain the observed elevation in whole-brain dopamine levels. They could not, however, produce changes in dopamine levels following pentobarbitone alone, given peripherally. Anderson & Bonnycastle (1960) found that, following peripheral administration of pentobarbitone, there was an elevation in whole-brain 5-hydroxytryptamine, and this could be correlated with the depth of central nervous depression produced. This depression was, however, found immediately to precede the rise in 5-hydroxytryptamine, which thus appears to be the result rather than the cause of the depression. Furthermore, the observed decrease in brain 5-hydroxytryptamine, following icv administration of pentobarbitone, suggests that any elevation of 5-hydroxytryptamine levels might be peripherally mediated.

The decrease in noradrenaline levels produced by DNP may be explained by an effect on the activity of noradrenergic neurones, since Godfraind, Krnjevic & Pumain (1970) have demonstrated that the application of DNP leads to a profound decrease in electrical excitability of cortical neurones, associated with a tendency to hyperpolarisation and a lower resistance of the membrane. It may be that the uncoupler also blocks the synthesis of noradrenaline.

Because icv injections of the two uncouplers of oxidative phosphorylation produce hypnosis, it is tempting to suggest that the uncoupling phenomenon is a component in barbiturate-induced sleep. It must be remembered, however, that high concentrations of the uncouplers can cause a suppression of tissue respiration (Wilson & Merz, 1969). A study of the concentrations of these drugs in the lipid phase of brain tissue after central administration would provide useful information in this respect. The data suggests that centrally-administered uncouplers of oxidative phosphorylation are hypnotic in mice, but a number of differences between them and the barbiturates makes it likely that they produce their effects by predominantly different mechanisms. The differences will be further enumerated in Results III.

8. FIGURES AND TABLES

FIG. 10

Variation of sleeping time with dose of icv administered drug in the mouse. (o——o) pentobarbitone; (●——●) 2,4-dinitrophenol; (Δ——Δ) pentachlorophenol.
Ambient temperature 21 ± 1° C.

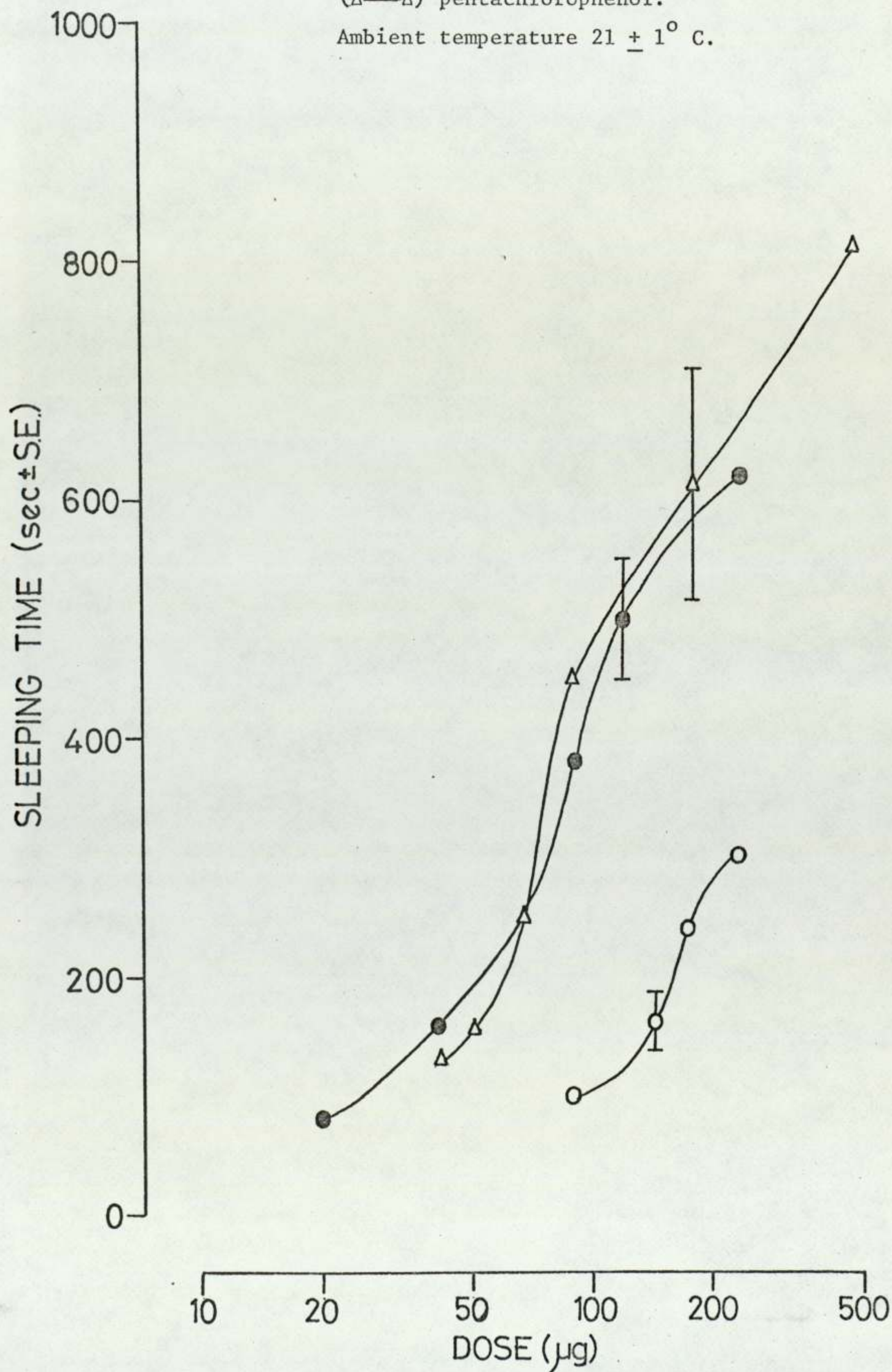

FIG. 11

Effect of solvent on the hypnotic potency of icv pentobarbitone in the mouse. Hatched columns, base in 50/50 dimethylsulphoxide/0.9 % saline; open columns, sodium salt in 0.9 % saline. (n = 10).

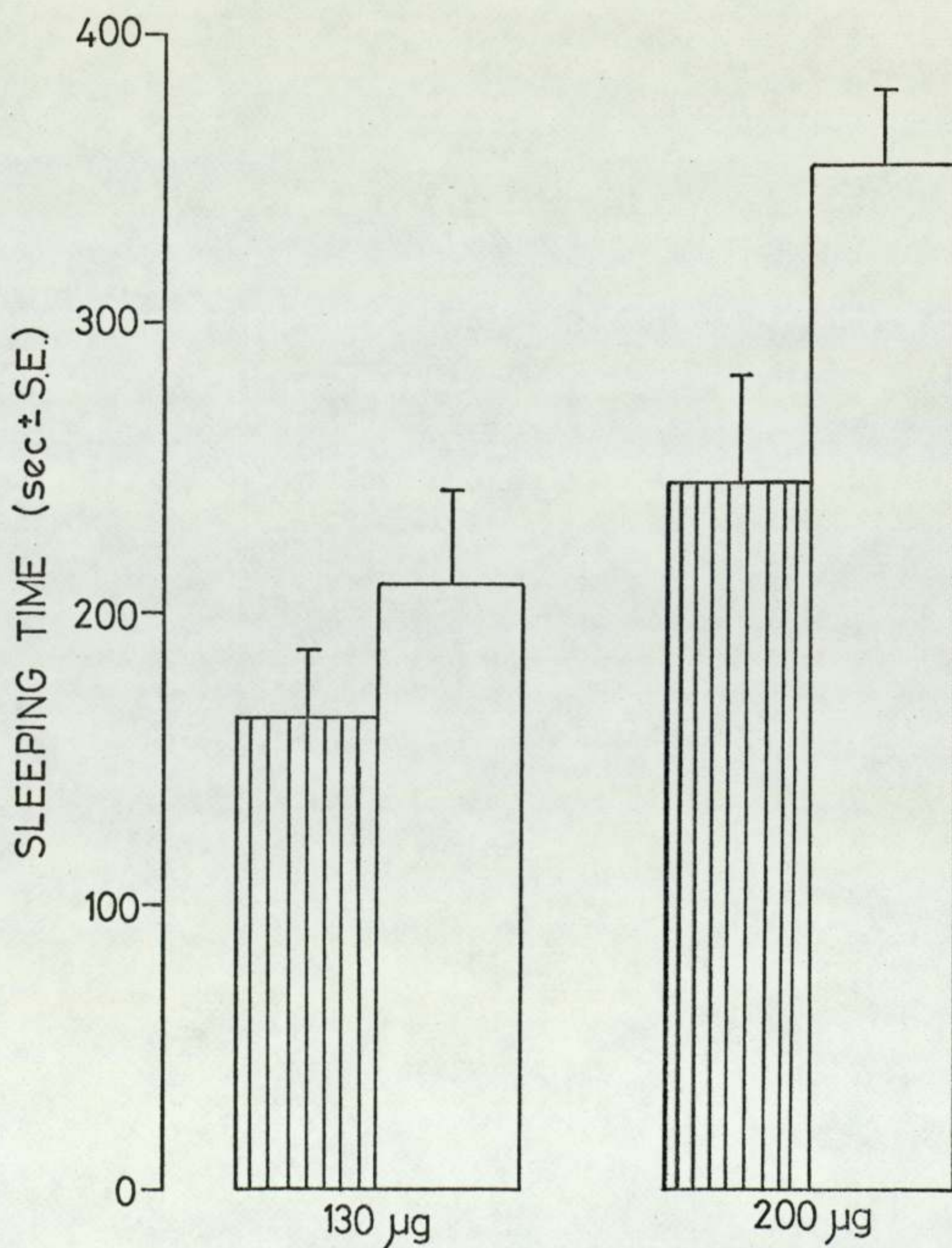

FIG. 12

Effect of solvent on the hypnotic potency of icv 2,4-dinitrophenol in the mouse. Hatched columns, base in 50/50 dimethylsulphoxide/0.9 % saline; open columns, sodium salt in 0.9 % saline. (n = 10).

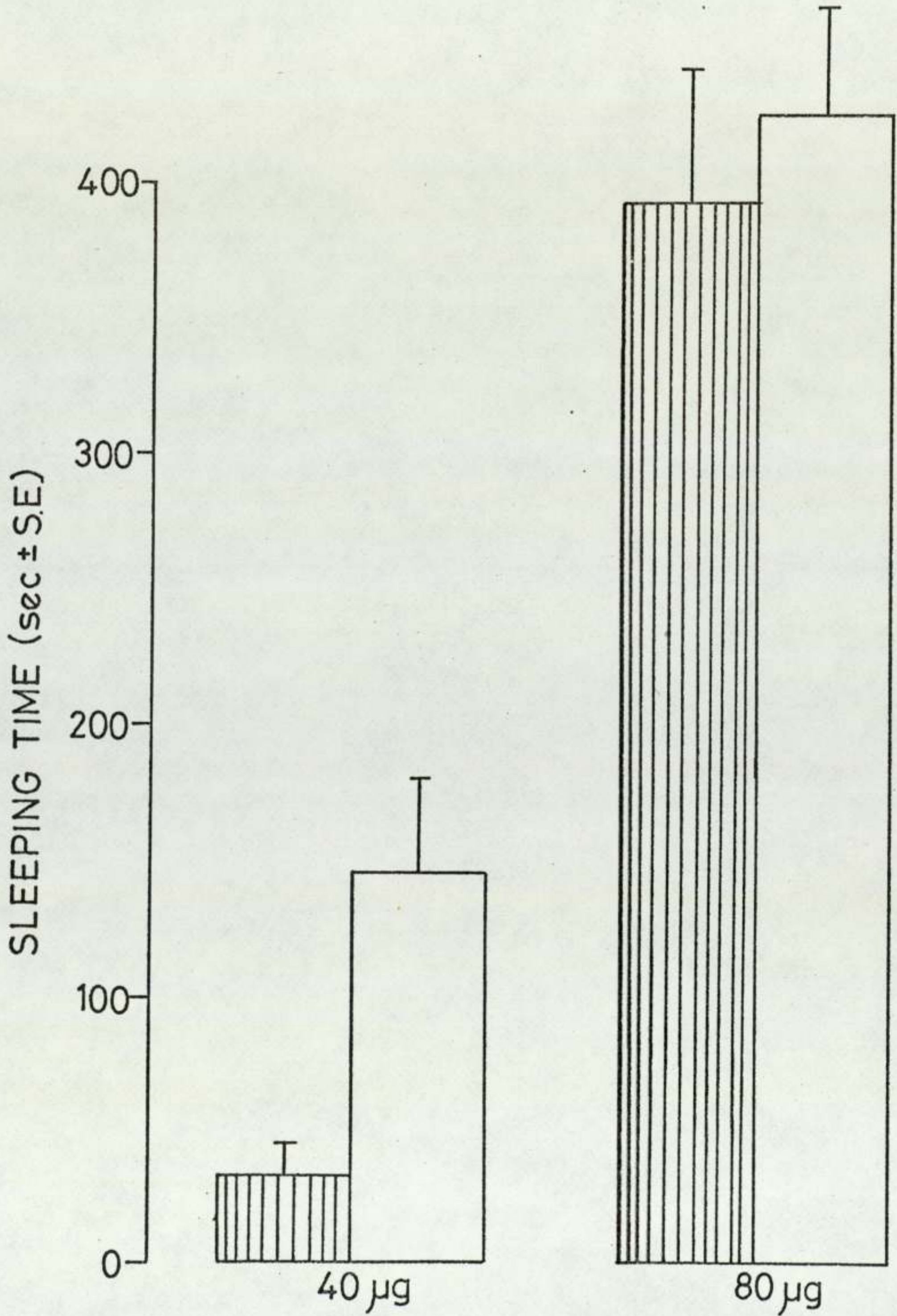

FIG. 13

Effect of solvent on the hypnotic potency of the sodium salts of pentobarbitone (B5) and 2,4-dinitrophenol (DNP) given by icv injection in the mouse. Hatched columns, sodium salt in 50/50 dimethylsulphoxide/0.9 % saline; open columns, sodium salt in 0.9 % saline. (n = 10).

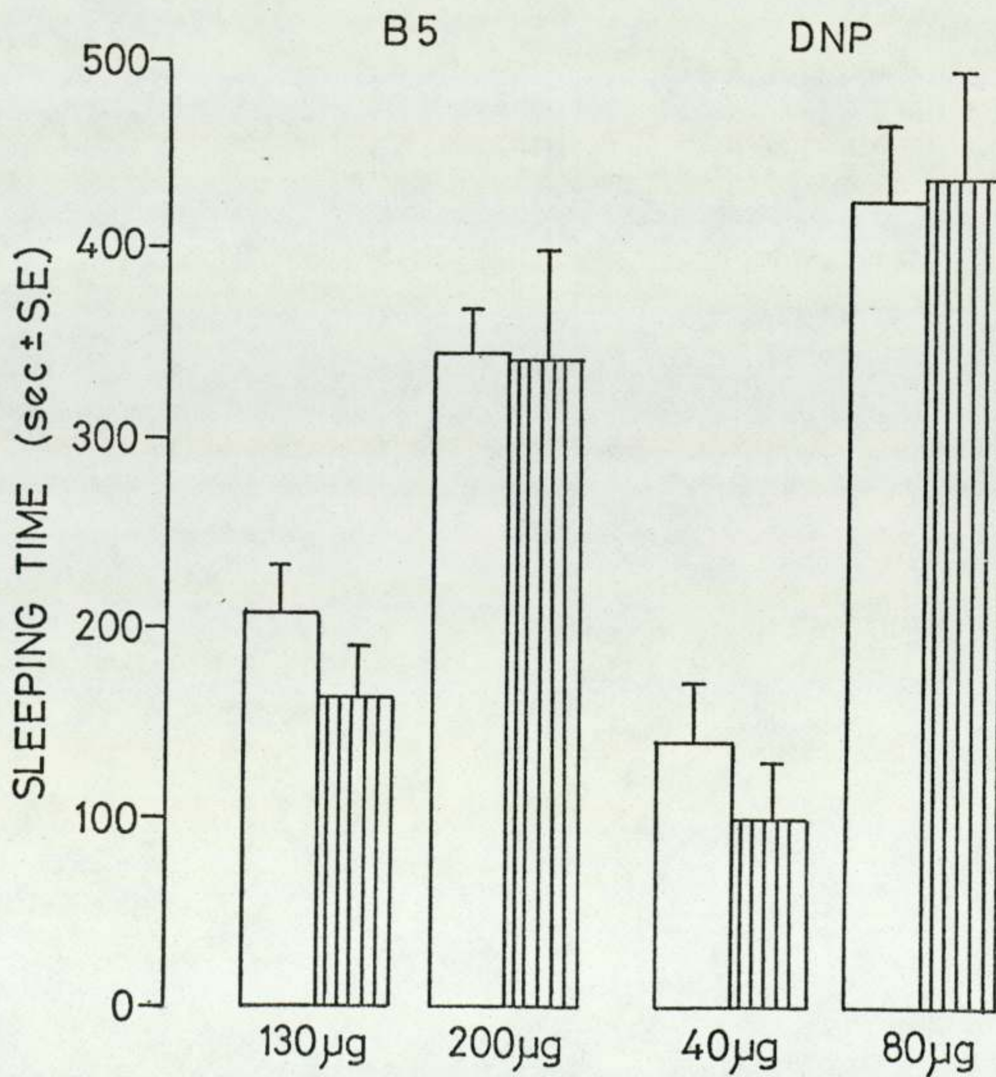

FIG. 14

Effect of dose-volume on the hypnotic potency of icv pentobarbitone (B5), 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP) in the mouse. Dotted columns, 20 µl; open columns, 10 µl. (n = 10).

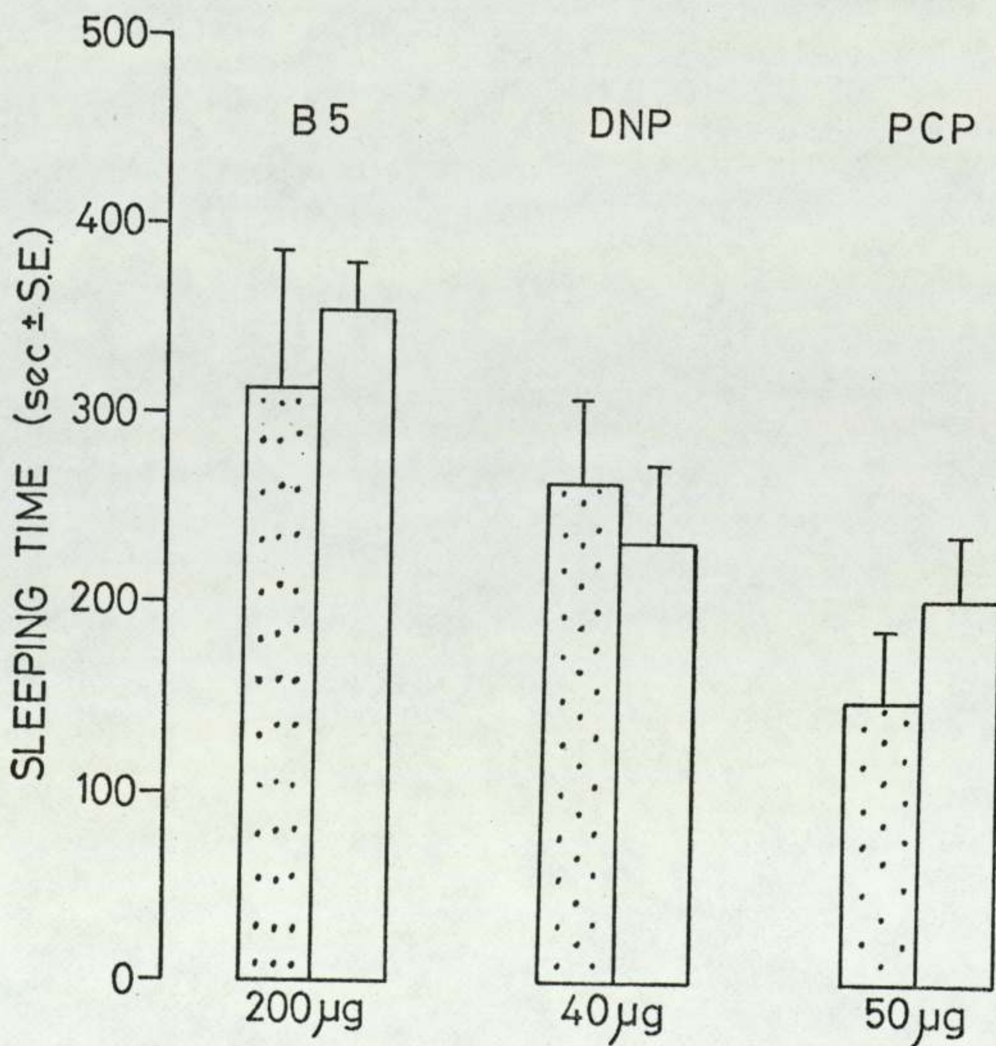

FIG. 15

Effect of icv injection of pentobarbitone and 2,4-dinitrophenol on the body temperature of the mouse. (x——x) 10 µl saline; (o——o) 120 µg pentobarbitone; (●——●) 40 µg 2,4-dinitrophenol. Ambient temperature 21 ± 1° C.

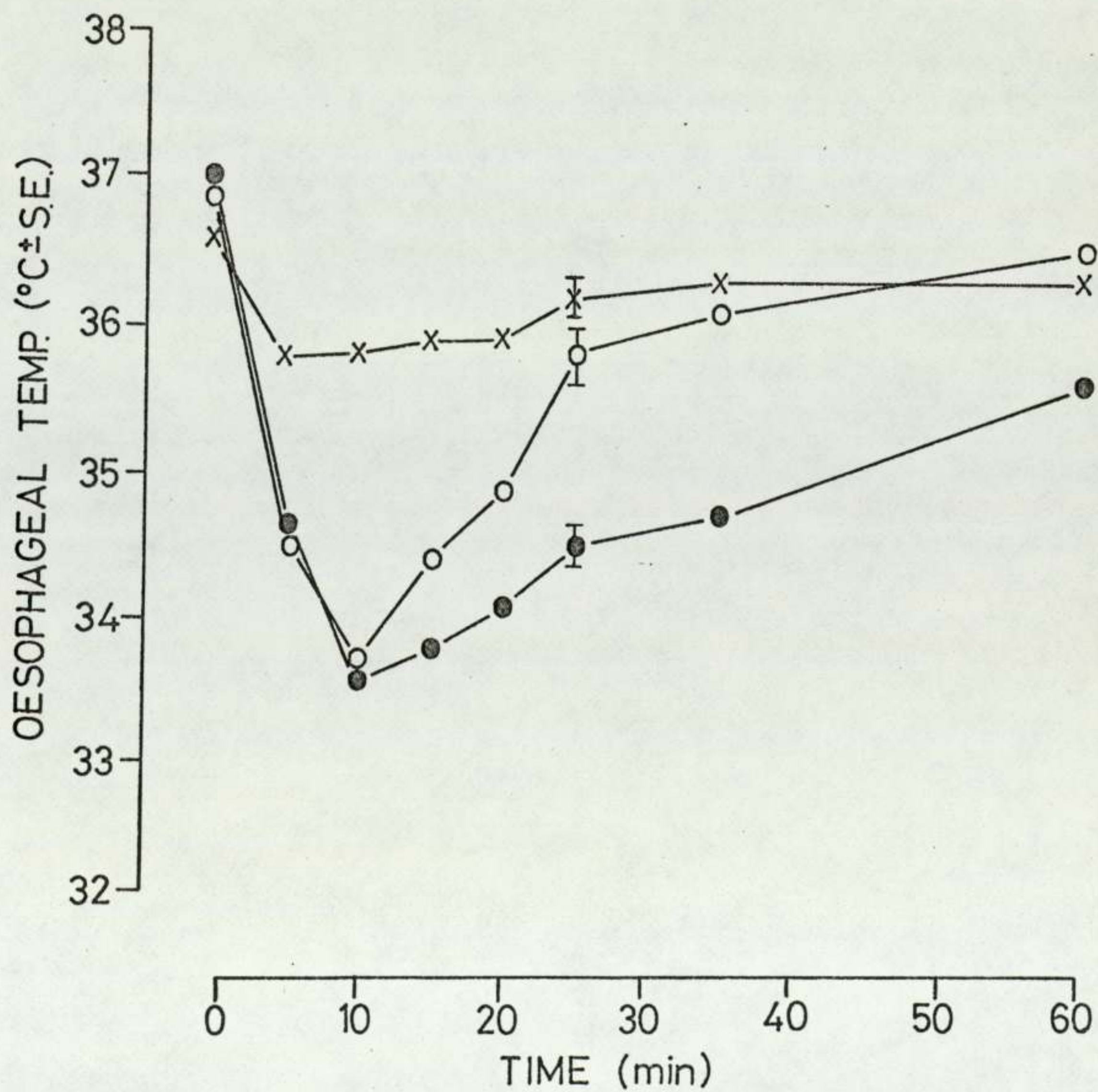

TABLE 2

Lethality of pentobarbitone, 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP) in the mouse following icv injection. (n = 10).

PENTOBARBITONE		D N P		P C P	
Dose (μg)	% Mortality	Dose (μg)	% Mortality	Dose (μg)	% Mortality
80	0	20	0	40	0
130	30	40	0	50	0
150	40	80	10	60	0
200	50	100	20	80	0
220	100	200	50	160	10
300	100	400	50	200	20
-	-	-	-	400	60
-	-	-	-	600	50

TABLE 3

Effect of pentobarbitone given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Controls received saline (10 μl icv) immediately before trial.

DOSE (μg)	PARAMETER MEASURED	MEAN RESPONSES (% ± S.E.)		
		Control	Immediately after Inj.	30 min after Inj.
100	% avoid	98 ± 2	98 ± 2	100
	Escape failure	0	0	0
	Latency	257 ± 12	294 ± 52	320 ± 10
300	% avoid	100	70 ± 5	100
	Escape failure	0	23 ± 3	0
	Latency	251 ± 18	1119 ± 239	261 ± 19

TABLE 4

Effect of 2,4-dinitrophenol given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Controls received saline (10 μl icv) 2 min before trial.

DOSE (μg)	PARAMETER MEASURED	MEAN RESPONSES (% ± S.E.)			
		Control	Immediately after Inj.	2 min after Inj.	30 min after Inj.
40	% avoid	100	48 ± 7	33 ± 18	100
	Escape failure	0	3 ± 3	16 ± 10	0
	Latency	236 ± 26	676 ± 84	928 ± 243	279 ± 21
150	% avoid	100	27 ± 9	18 ± 15	93 ± 2
	Escape failure	0	0	20 ± 10	0
	Latency	249 ± 26	684 ± 71	1044 ± 196	353 ± 26

TABLE 5

Effect of 150 μg pentobarbitone icv and 50 μg 2,4-dinitrophenol (DNP) icv on whole-brain amine levels in the mouse. See text for details. The figures in parenthesis indicate the number of determinations made; data are expressed as mean ± S.E. * indicates significant difference from control.

PRETREATMENT	NORADRENALINE (ng/g)	DOPAMINE (ng/g)	5-HYDROXYTRYPTAMINE (ng/g)
Saline (10 μl)	287 ± 20 (9)	652 ± 27 (8)	884 ± 32 (8)
Pentobarbitone	237 ± 15 (8)	848 ± 53 (7)*	789 ± 32 (7)*
DNP	180 ± 16 (7)*	619 ± 45 (7)	790 ± 40 (8)

III INTERACTION OF CENTRALLY-ADMINISTERED BARBITURATE AND UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION WITH OTHER AGENTS IN THE MOUSE

III Interaction of centrally-administered barbiturate and uncouplers of oxidative phosphorylation with other agents in the mouse

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III In view of the findings of the previous section, further experiments were performed to compare and contrast the central nervous depressant actions produced by pentobarbitone and the two phenolic uncoupling agents. Consequently, a comparison was made of the interactions of pentobarbitone, DNP and PCP with a number of other agents, namely adenosine triphosphate (ATP) and adenosine diphosphate (ADP), dexamphetamine and leptazol, together with a study of the effects of both types of compounds on electrically-induced convulsions. Finally, the interaction of a centrally-administered uncoupling agent with peripherally-administered pentobarbitone was investigated.

1. Anti-convulsant activity of centrally-administered pentobarbitone, DNP and PCP

Groups of 10 mice received various doses of pentobarbitone, DNP, PCP or vehicle by icv injection 1 min before leptazol (80 mg/kg ip) or 5 min before the application of electroshock (2 msec pulses, 100 Hz for 0.3 sec at 80 V). The activity of all three agents as anti-convulsants was investigated against both chemically and electrically induced seizures and the results are summarized in table 6.

Following icv injection, pentobarbitone antagonised leptazol convulsions, showing substantial anti-convulsant activity in sub-hypnotic doses. The threshold for the production of tonic extensor spasm by electroconvulsive shock was also elevated, although pentobarbitone was considerably less potent against the electrical stimulus. Neither DNP nor PCP was able to protect against electroshock convulsions even in high doses (160 μg icv and 400 μg icv respectively), although a degree of protection was afforded against leptazol in substantially lower doses (10 μg icv and 20 μg icv res-

pectively). This latter effect against leptazol was not, however, dose-dependent.

2. Interaction of pentobarbitone, DNP and PCP with adenine nucleotides

If barbiturate hypnosis is due to an uncoupling of oxidative phosphorylation, there should be a reduction in intracellular levels of energy-rich compounds, including ATP. By increasing the level of endogenous ATP, one might obtain a reversal of the loss of righting reflex produced by the barbiturate and the uncouplers. Consequently, the effects of injected ATP and ADP upon the depressant activity of both the barbiturate and the uncouplers were next studied (fig. 16).

Because of the labile nature of these two nucleotides, it seemed likely that only a small proportion of any peripherally given dose would attain an intracellular site in the brain. Therefore, doses up to 200 mg/kg of both ATP and ADP were given intraperitoneally, in an attempt to raise the intracellular levels of these agents. Groups of 10 mice received ATP or ADP (20 and 200 mg/kg ip) 20 min before administration of hypnotic doses of pentobarbitone (130 μg icv), DNP (20 μg icv) or PCP (40 μg icv). Instead of reducing the effect, both nucleotides greatly enhanced the hypnotic effect of icv pentobarbitone. However, there was no significant effect on PCP sleep. ATP pretreatment significantly prolonged DNP sleep ($P < 0.05$), although prior administration of ADP produced no effect. The only observation after pretreatment with a lower dose (20 mg/kg ip) of the nucleotides was that ATP produced a small but significant increase in pentobarbitone sleeping time ($P < 0.05$).

Peripheral administration of either ATP or ADP alone (200 mg/kg ip) produced a slight depressant effect, characterised chiefly by a reduction in spontaneous locomotor activity. No such behavioural effects were apparent

following 20 mg/kg ip of either nucleotide. In order to investigate further this depressant activity, both nucleotides were next given by icv injection.

3. Pharmacological effect of centrally-administered ATP and ADP

ATP and ADP (200 - 800 μg icv) produced a dose-dependent, reversible loss of righting reflex, the appearance of which was consistent with hypnosis (fig. 17). Immediately after injection there was an initial hyperventilation, followed by hyper-activity similar to that seen after icv injection of pentobarbitone, DNP and PCP. Extension of the hind limbs was a characteristic feature of the first few minutes after injection. With both nucleotides, the higher doses also caused muscular weakness and respiratory depression, associated with dyspnoea, sometimes followed by death. The sleep-like state produced by the icv injection of adenine nucleotides is supported by previous work in cats (Feldberg & Sherwood, 1954).

After recovery from the loss of the righting reflex, all animals remained quiet for several hours before resuming a normal level of activity; at 24 h they were indistinguishable from controls.

4. Interaction of pentobarbitone, DNP and PCP with dexamphetamine

It is known that the central stimulant sympathomimetics can lessen the degree of depression caused by moderate doses of hypnotics (Innes & Nickerson, 1965), and earlier workers (White & Boyajy, 1959) have shown that large doses of amphetamine antagonise the synchronous E.E.G. pattern produced by pentobarbitone, and can concomitantly awaken the animal. Consequently, experiments were performed to investigate the interaction of dexamphetamine with pentobarbitone and the two uncouplers of oxidative phos-

phorylation given by icv injection.

Dexamphetamine (5 and 10 mg/kg ip) was injected 20 or 60 min before injections of DNP (20 and 40 μg icv) or PCP (40 and 60 μg icv). No significant change in the duration of their hypnotic acitivity was observed (table 7). However, dexamphetamine (5 mg/kg ip), given 60 min before icv administration of pentobarbitone, markedly attenuated the hypnotic effect of the barbiturate, and allowed considerable extension of the pentobarbitone log. dose-response curve by antagonising the respiratory depression and high mortality attending the use of the higher doses of pentobarbitone alone (fig. 18). No effect of dexamphetamine on DNP or PCP toxicity was observed.

In another experiment two groups of 8 mice each received dexamphetamine at one of five dose levels (5 - 75 mg/kg ip), and the effect of DNP (15 μg icv) on the LD_{50} of dexamphetamine under crowded conditions was investigated. Results showed that DNP did not alter the toxicity of dexamphetamine under these conditions (table 8).

5. Interaction of pentobarbitone with DNP

i) Effect on duration of loss of righting reflex and acute toxicity

Previous workers (Brody & Killam, 1952) have reported that mice given DNP peripherally and then challenged with barbiturate were anaesthetised, even though sub-anaesthetic doses of amylobarbitone were used. They further showed that DNP (5 - 30 mg/kg) produced progressive increases of 80 - 300 % in the duration of sleep induced by quinolbarbitone in mice.

In the experiments described in this section, pentobarbitone (20 mg/kg ip) was given, and the duration of sleep measured following saline (10 µl icv) or various doses of DNP (15 - 100 µg icv), given 2 min after the pentobarbitone. DNP, given by icv injection, produced a whole-body hypothermia, which could be prevented by elevating the ambient temperature. Such an elevation of ambient temperature, however, had no effect on the hypnotic effect of icv DNP (see Results II 2.). Since it is known that the duration of barbiturate hypnosis can be prolonged by a decrease in body temperature (Raventós, 1938; Fuhrman, 1947), the experiment was repeated at $32 \pm 1^{o}C$, in order to prevent the DNP-induced hypothermia from potentiating the pentobarbitone, perhaps by a change in the metabolism of either agent, or by a change in tissue sensitivity.

It was found that icv DNP could produce a dose-dependent potentiation of pentobarbitone hypnosis. Repeating the experiment at $32 \pm 1^{o}C$ significantly reduced ($P < 0.05$), but did not abolish, the DNP-induced potentiation of pentobarbitone hypnosis (table 9). Following a peripherally-administered dose of DNP (100 µg sc) 2 min after pentobarbitone (20 mg/kg ip), there was no potentiation of the barbiturate hypnosis at either temperature.

In a further experiment, six groups of 10 mice received pentobarbitone at one of six dose levels (80 - 130 mg/kg ip), and the effect of icv DNP on the LD_{50} of pentobarbitone was investigated. It was shown that DNP (15 µg icv) produced no significant potentiation of the acute toxicity of pentobarbitone if given either immediately before or 30 min after the barbiturate (table 10).

ii) Effect on body temperature

Pentobarbitone (20 mg/kg ip) produced no significant change in body temperature of mice at laboratory temperatures of either 21 $\pm$ 1°C or 32 $\pm$ 1°C. Although icv DNP produced hypothermia at 21 $\pm$ 1°C (see Results II 3.), there was no effect on body temperature at the higher ambient temperature. If the pentobarbitone injection was followed 2 min later by DNP (100 μg icv), a hypothermia was produced, maximal at 15 min, if the ambient temperature was 21 $\pm$ 1°C. There was no effect at the higher ambient temperature (fig. 19).

Following a peripherally-administered dose of DNP (100 μg sc) 2 min after pentobarbitone (20 mg/kg ip), there was no significant change in body temperature produced at an ambient temperature of either 21 $\pm$ 1°C or 31 $\pm$ 1°C.

6. Discussion

In the previous section (Results II), both pentobarbitone and the two phenolic uncouplers, given by icv injection, were shown to be hypnotic, the observable characteristics of this sleep being similar. The two classes of compounds did differ in some respects, however, particularly as regards their toxicity and effect on endogenous brain amine levels. It was postulated that pentobarbitone may have a more widespread depressant effect than DNP or PCP. This might explain the ability of pentobarbitone to elevate the threshold to electroconvulsive shock, against which both phenols were inactive. Pentobarbitone, given by icv injection, was also active against leptazol convulsions, where it was ten times more active than against electroshock. The phenols also afforded some protection against leptazol, al-

though this effect was not dose-related. It could be that rapid diffusion out of the cerebrospinal fluid into the blood may act as a complicating factor in the dose relationship of the phenols.

The adenine nucleotides ATP and ADP were administered in anticipation of the fact that they might antagonise the hypnotic effects of pentobarbitone and the two phenols. Previous work (Gomahr, 1957; Lessin & Parkes, 1957; Mathieu, 1960; Mathieu-Levy, 1967; 1968) had shown that peripherally-administered ATP could in fact potentiate experimental hypnosis produced by barbiturates. The present results show that this is also true of ATP and ADP given by icv injection. Yet, in contrast, both nucleotides were inactive against the two phenols. It seems likely that this potentiation of pentobarbitone was mediated indirectly, since a recent report shows that large doses of peripherally-administered ATP do not increase brain levels of this nucleotide (Mathieu-Levy, 1967). It has been suggested (Lessin & Parkes, 1957) that, since ATP lowers body temperature (Green & Stoner, 1950), this may be its mechanism of potentiation. However, this has since been disputed (Mathieu-Levy, 1968). Furthermore, ATP does not affect the absorption or catabolism of barbiturates (Mathieu-Levy, 1967). That the phenols were not potentiated suggests, therefore, that their mechanism of hypnotic action is different from that of the barbiturates.

Following icv injection ATP and ADP themselves produced a loss of righting reflex, and this is in agreement with the findings of Feldberg & Sherwood (1954), who demonstrated that ATP (200 - 400 µg icv) could induce sleep in cats. They also showed that these effects are not due to the phosphate in the molecule, since icv injections of sodium pyrophosphate had little effect on behaviour. They do point out, however, that it was difficult to assess the relative importance of muscular weakness and of light anaesthesia.

Whereas there is no information about the amount of nucleotide that gained access to intraneuronal or intrasynaptic sites within the central nervous system, it seems likely that the nucleotides exert their effects within the brain, since, although slightly depressant when given intraperitoneally, they did not produce sleep. Assuming that a significant amount of this icv-administered material did reach an intracellular site in the brain, it is possible that its hypnotic action was due to some feed-back mechanism inhibiting energy utilisation or the production of energy in an available form in the neurone.

A further difference between the phenols and pentobarbitone was the inability of dexamphetamine to antagonise the hypnotic or toxic action of the former, whereas marked antagonism to both the hypnotic and toxic action of pentobarbitone was observed. This observation may reflect the difference in effect of the two classes of compounds on brain amine levels, but further work is needed before any conclusions can be drawn concerning the specificity or otherwise of this antagonism. Nevertheless, it is further evidence of an inherent difference in the mechanisms of hypnotic action of pentobarbitone and the phenols.

The potentiation of pentobarbitone hypnosis produced by centrally-administered DNP can be resolved into two components: (1) a direct action involving interaction at a central site, (2) an indirect effect presumably produced by a delay in the metabolism of pentobarbitone, caused by a decrease in body temperature (see Fuhrman, 1947). This latter effect only becomes apparent at low ambient room temperatures. It appears that higher doses of DNP are needed to potentiate the toxic effects of pentobarbitone than are necessary to potentiate the hypnotic effects.

In summary, although the hypnosis produced by icv injection of DNP or PCP is qualitatively similar to that produced by icv injection of pento-barbitone, the former does not involve a dexamphetamine-sensitive component, nor a substantial anti-convulsant action. Even though the present results show an association between the barbiturates, uncouplers of oxidative phos-phorylation and adenine nucleotides, further work involving detailed bio-chemical investigations is needed to elucidate the mechanisms by which each type of agent produces its observed depressant effects on the central nerv-ous system.

7. FIGURES AND TABLES

FIG. 16

Effect of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) on the hypnotic activity of icv pentobarbitone (B5), 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP) in the mouse. Animals received ip nucleotide or saline 20 min before icv agent. Solid columns, saline (10 ml/kg ip); open columns, nucleotide (200 mg/kg ip); hatched columns, nucleotide (20 mg/kg ip). (n = 10).

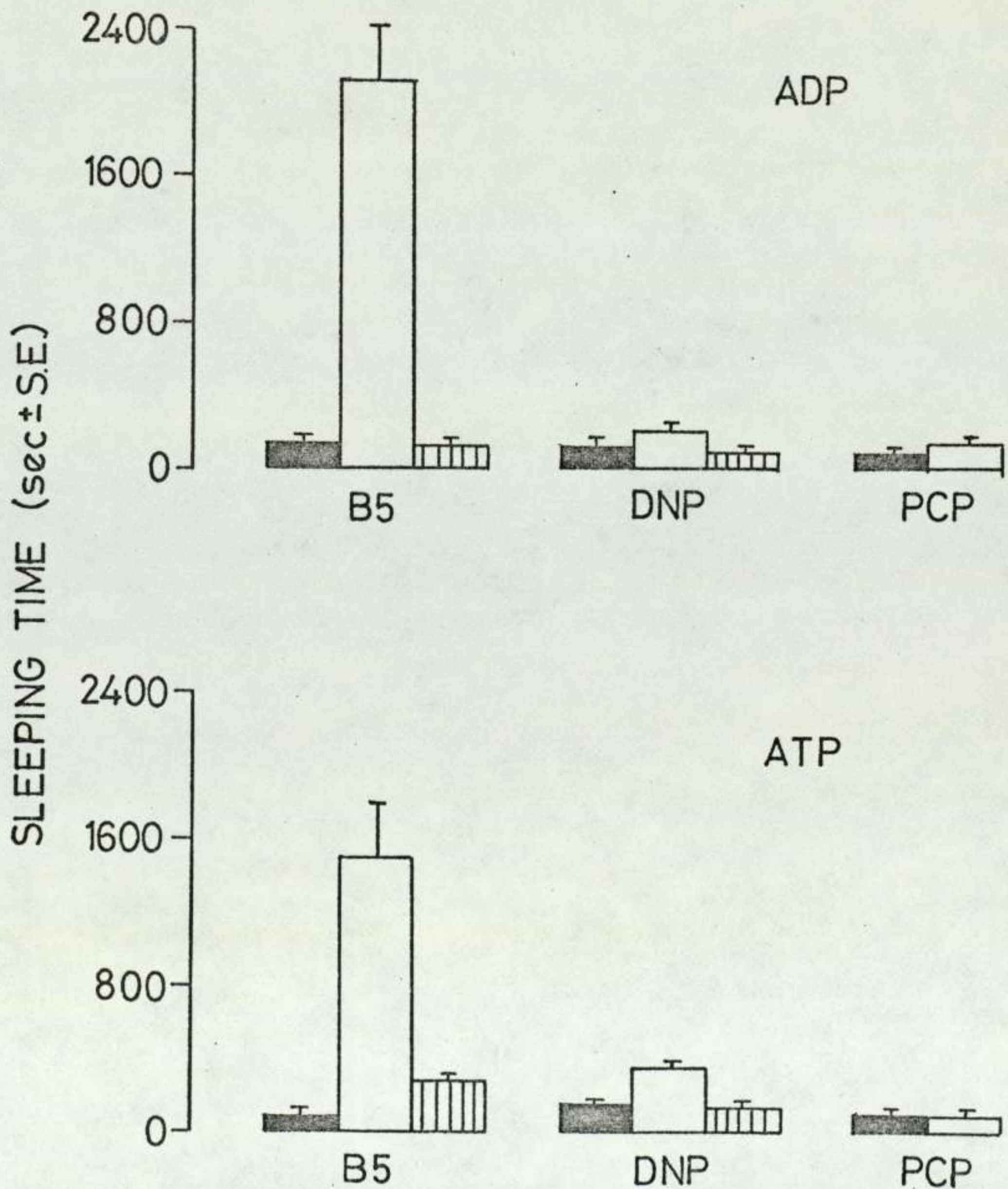

FIG. 17

Variation of sleeping time with dose of adenine nucleotides given by icv injection in the mouse. (o—o) ATP; (●—●) ADP.

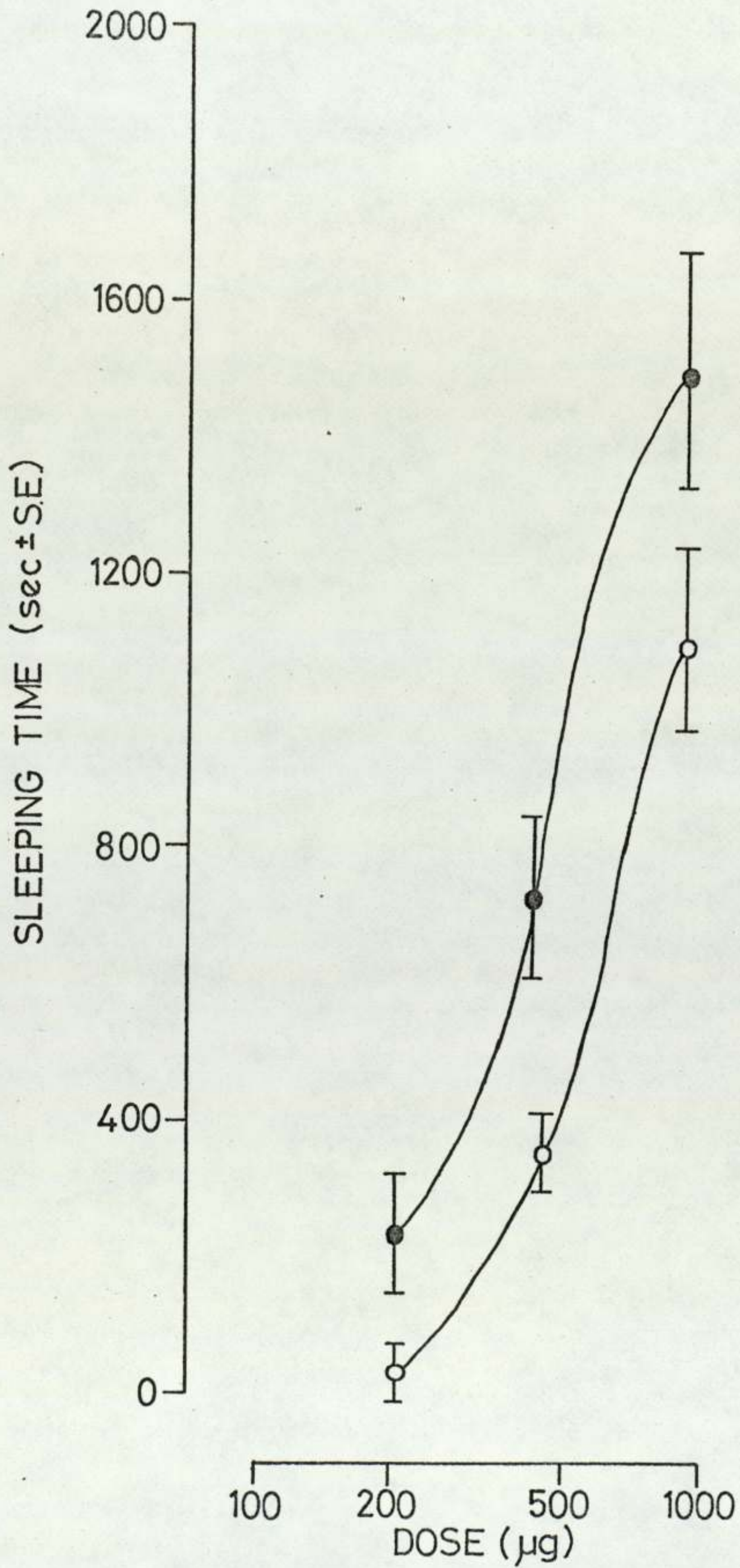

FIG. 18

Effect of dexamphetamine pretreatment on the hypnotic activity of icv pentobarbitone in the mouse. Animals received (o——o) 5 mg/kg dexamphetamine ip, or (●——●) 10 ml/kg saline ip 60 min before icv pentobarbitone.

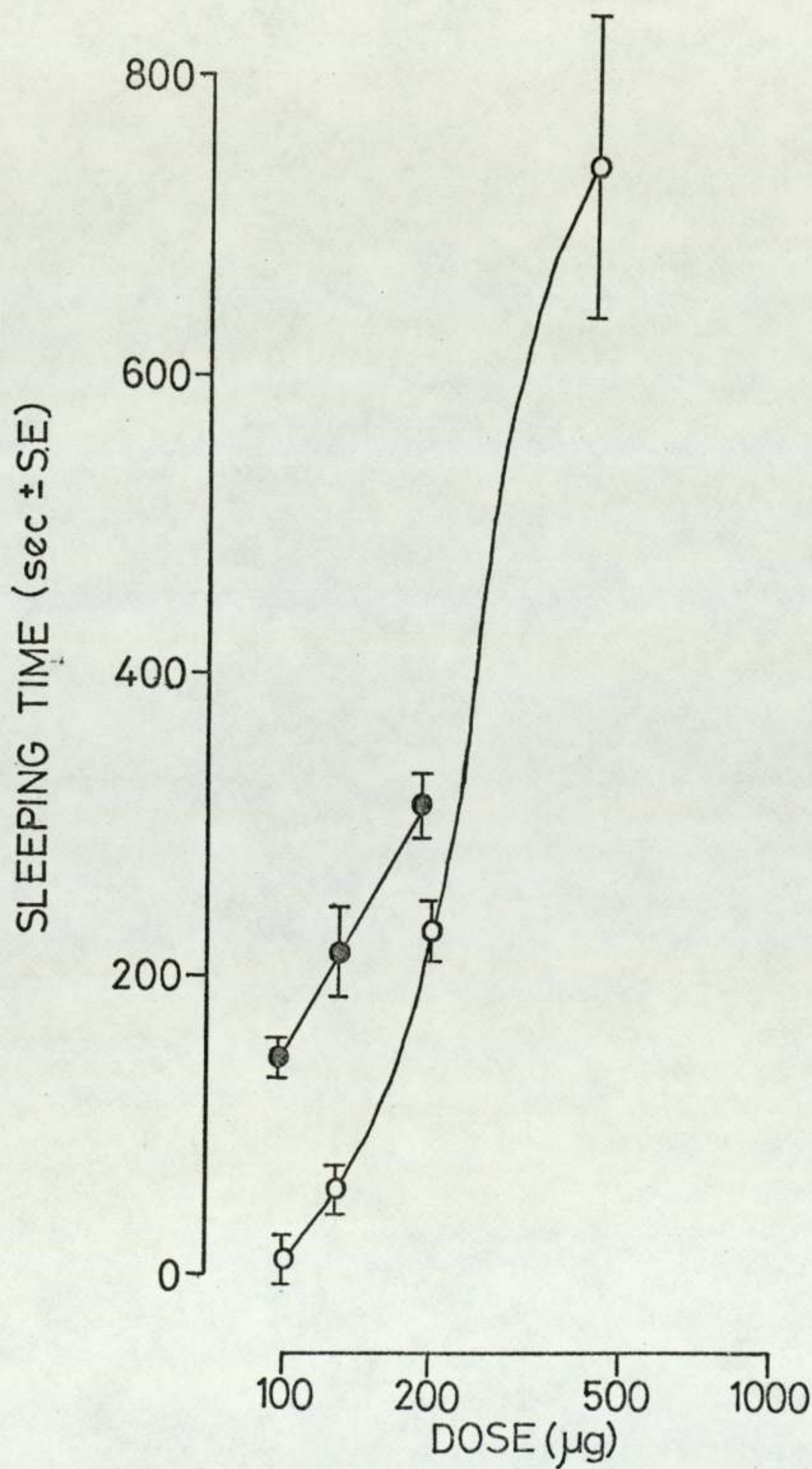

FIG. 19

Effect of peripherally-administered pentobarbitone on the body temperature of the mouse. Animals received 20 mg/kg pentobarbitone ip 2 min before (o——o) 10 µl saline icv, or (●——●) 100 µg 2,4-dinitrophenol icv.

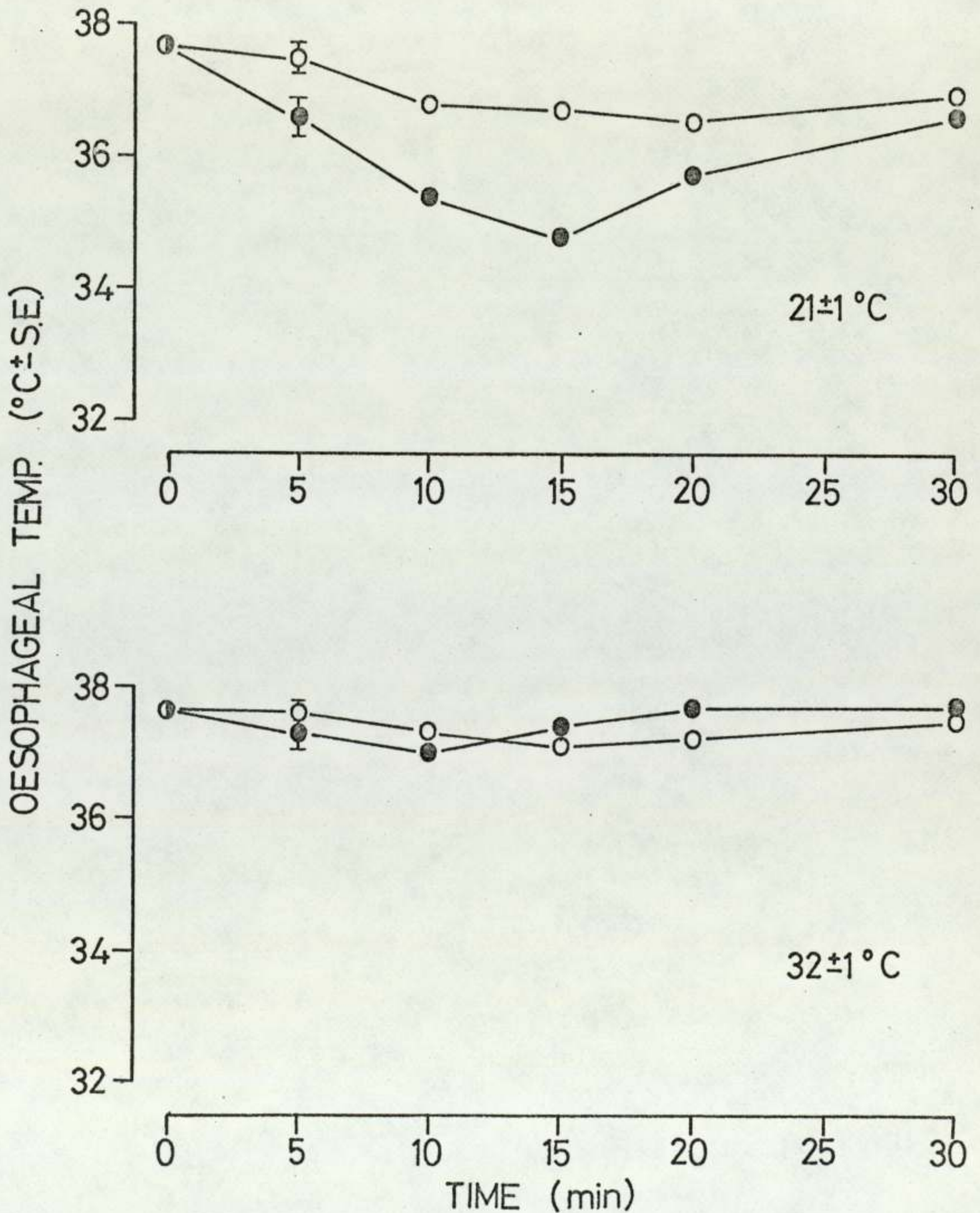

TABLE 6

Incidence of leptazol and maximal electro-shock convulsions (M.E.S.) in mice pretreated with pentobarbitone, 2,4-dinitrophenol (DNP) or pentachlorophenol (PCP). Mice received various doses of pentobarbitone, DNP, PCP or saline (controls) by icv injection 1 min before leptazol (80 mg/kg ip), or 5 min before application of electroshock 2 msec pulses, 100 Hz for 0.3 sec at 80 V. Percentage convulsing in leptazol treated animals refers to those showing clonic or tonic convulsions at twice mean onset time for controls. (n = 10).

	PENTOBARBITONE DOSE (μg)	% CONVULSING	DNP DOSE (μg)	% CONVULSING	PCP DOSE (μg)	% CONVULSING
LEPTAZOL	0	90	0	90	0	90
	5	70	2.5	50	5	50
	10	60	5	40	10	70
	20	40	10	20	20	40
	50	10	20	50	40	40
	80	0	80	40	80	40
M.E.S.	0	100	0	100	0	100
	50	90	10	90	40	100
	80	70	40	90	160	100
	100	60	80	90	200	100
	150	50	160	80	400	90

TABLE 7

Mean sleeping times (sec ± S.E.) of mice given 5 and 10 mg/kg dexamphetamine ip 20 or 60 min before 20 and 40 μg 2,4-dinitrophenol (DNP) icv or 40 and 60 μg pentachlorophenol (PCP) icv. Controls received 10 ml/kg saline ip. (n = 10).

ip PRETREATMENT	PRETREATMENT TIME (min)	MEAN SLEEPING TIME (sec ± S.E.)			
		DNP 20 μg	DNP 40 μg	PCP 40 μg	PCP 60 μg
Saline	20	145 ± 31	-	-	113 ± 27
Saline	60	174 ± 32	233 ± 44	117 ± 21	-
Dexamphetamine (5 mg/kg)	20	133 ± 20	-	-	146 ± 21
Dexamphetamine (5 mg/kg)	60	149 ± 16	243 ± 24	132 ± 16	-
Dexamphetamine (10 mg/kg)	60	187 ± 21	-	131 ± 14	-

TABLE 8

Effect of 2,4-dinitrophenol (DNP) on acute LD_{50} of dexamphetamine in the mouse, measured under crowded conditions. Animals received DNP (15 μg icv) or saline (10 μl icv) immediately before dexamphetamine ip, and the results obtained 20 h later.

DOSE OF DEXAMPHETAMINE (mg/kg)	ALIVE / TESTED	
	Saline icv	DNP icv
5	16/16	16/16
15	11/16	6/16
35	2/16	6/16
55	2/16	4/16
75	2/16	0/16

PRETREATMENT	LD_{50} (mg/kg)	19/20 CONFIDENCE LIMITS
Dexamphetamine + Saline	21.5	12.7 —— 36.6
Dexamphetamine + DNP	21.0	13.3 —— 33.2

TABLE 9

Effect of icv 2,4-dinitrophenol (DNP) on pentobarbitone hypnosis in the mouse. Animals received pentobarbitone (20 mg/kg ip) 2 min before saline (10 µl icv) or the appropriate dose of DNP icv. (n = 10).

AMBIENT TEMP. (° C)	icv INJ.	ONSET TIME OF SLEEP GROUP MEAN (sec±S.E.)	DURATION OF SLEEP GROUP MEAN (sec±S.E.)
21 ± 1° C	Saline	317 ± 29	505 ± 54
	DNP 15 µg	390 ± 33	869 ± 109
	DNP 30 µg	361 ± 26	981 ± 102
	DNP 100 µg	350 ± 22	1421 ± 50
32 ± 1°C	Saline	301 ± 21	491 ± 41
	DNP 15 µg	342 ± 29	628 ± 20
	DNP 30 µg	286 ± 34	750 ± 29
	DNP 100 µg	240 ± 25	913 ± 50

TABLE 10

Effect of 2,4-dinitrophenol (DNP) on acute LD_{50} of pentobarbitone in the mouse. Animals received DNP (15 µg icv) or saline (10 µl icv) either (1) immediately after, or (2) 30 min after pentobarbitone ip, and the results obtained 20 h later.

DOSE OF PENTOBARBITONE (mg/kg)	ALIVE / TESTED			
	Saline icv		DNP icv	
	(1)	(2)	(1)	(2)
80	10/10	10/10	10/10	10/10
90	10/10	10/10	9/10	8/10
100	7/10	7/10	6/10	8/10
110	1/10	1/10	1/10	8/10
120	3/10	3/10	1/10	3/10
130	0/10	0/10	0/10	0/10

PRETREATMENT	LD_{50} (mg/kg)		19/20 CONFIDENCE LIMITS	
	(1)	(2)	(1)	(2)
Pentobarbitone + Saline	105	105	97.2-113.4	97.2-113.4
Pentobarbitone + DNP	101	112	89.4-114.1	100 -125.4

IV THE PHARMACOLOGICAL PROPERTIES OF CARDIAC GLYCOSIDES WHEN GIVEN BY ICV INJECTION

IV The pharmacological properties of cardiac glycosides when given by icv injection

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IV Weinberg & Haley (1955) recognised that the possible importance of centrally-mediated effects in the control of the myocardium had not been considered. Although the effects of cardiac glycosides on the heart were well documented, there was evidence to suggest that one of their principal sites of action may be elsewhere than the heart itself. The situation is largely the same today.

The involvement of the central nervous system in digitalis intoxication has been recognised and frequently reported in the literature since the observations of Withering in 1785. Santesson & Strindberg (1917) observed that subdural injections of 1 mg/kg gitalin (0.1 % solution) into the frontal region of the brain of unanaesthetised rabbits induced tremor, tetanus and clonic twitching, compulsive postures, mydriasis, paralysis of the neck muscles and death from asphyxia. Peripherally-administered gitalin (2 - 5.8 mg/kg iv) also produced central effects including tremor, dyspnoea, miosis and convulsions with localised muscular weakness and paralysis, followed by asphyxial convulsions and death. Whereas centrally-administered gitalin potentiated certain spinal reflexes, the authors found that these reflexes were attenuated or abolished after peripheral administration. Rizzolo (1929) showed that, following direct application of a 2 % solution of strophanthin, there was an initial increase, followed by a decrease, in the excitability of the cerebral cortex in dogs. Weiss (1932) stated that digitalis acted directly on nervous structures, although the mechanism of action was not very well understood. More recently Ri & Schmidt (1960) demonstrated that injections of K-strophanthin into the carotid artery of rats produced a transitory central respiratory depression. Following toxic doses of digitalis, degenerative changes have been found in the cortex, basal ganglia, cerebellum, pons and spinal cord of several species (Hueper & Ichniowski, 1941; Dearing, Barnes, Kernohan & Essex, 1943).

The various neurological toxicities which have been reported range from excitement, restlessness and irritability to depression, weakness and fatigue. They have been summarised by Batterman & Gutner (1948). These symptoms may occur regardless of the degree of cardiotoxicity, and in the absence of any other manifestations of intoxication. Since these effects can be observed after as little as 5 μg/kg digitoxin, it appears that the glycosides must indeed be potent agents to produce these central nervous symptoms, particularly when one considers that the low lipid solubility of these agents might contribute to a low permeability of the blood-brain barrier. The question that obviously arises is by what mechanism do the cardiac glycosides produce these effects, and why do the manifestations of an effect on the central nervous system fall between two extremes of behavioural activity?

Many reports have appeared implicating catecholamines in both the therapeutic and toxic effects of the cardiac glycosides on the heart. In some species it has been shown that the cardiac glycosides can release endogenous catecholamines which act on the myocardium to produce their characteristic inotropic effects (Tanz, 1967). More recently, Hermansen (1970) has shown that the cardiotoxic effects of ouabain in guinea-pigs are also, at least partially, brought about by a liberation of endogenous catecholamines. Furthermore, Butterbaugh & Spratt (1970a,b) have suggested that intact brain amine levels have a permissive rôle in the development of digitoxigenin toxicity, and they have also postulated a predominance of central mechanisms in the toxicity and lethality of digitoxigenin (Butterbaugh & Spratt, 1970c).

A number of recent studies have examined the pharmacological actions of substances given by icv injection. In the mouse, for example, noradren-

aline produces marked depression of central nervous activity when given by this route (Brittain & Handley, 1967). Clearly, if the cardiac glycosides are capable of releasing noradrenaline from its storage sites within the brain, one would expect their central actions to resemble, at least partially, those of noradrenaline. Yet, observations to date, at least at toxic doses, have shown that they cause death by convulsions when given by icv injection (Greef & Kasperat, 1961a,b).

Accordingly a study has been made of the pharmacological actions of ouabain given by icv injection in the mouse and the rat. A rodent was the species of choice in view of the relative resistance of the heart to the effects of cardiac glycosides, the effects on the central nervous system being much more pronounced (Megges & Repke, 1964; Seager, Murphee & Monroe, 1965).

Since the biochemistry of ouabain has been extensively documented, this glycoside has been chosen as representative of its class. Initial experiments involving a comparison with digitoxin indicated that differences between the cardiac glycosides are quantitative rather than qualitative.

1. General pharmacological properties of icv-administered ouabain and digitoxin

i) Effect of icv ouabain and digitoxin in the mouse

Under normal laboratory conditions ($21 \pm 1^{\circ}C$ and relative humidity of 50 - 60 %) groups of 5 mice were given icv injections of ouabain (0.1 - 0.4 μg/mouse) and the animals observed continuously for the next 5 - 6 h for changes in motor activity, body temperature and general behaviour.

Ouabain produced an initial slight hypersensitivity to external stimuli, followed quickly by a profound, dose-related reversible depression of central nervous activity, characterised by a decrease in locomotor activity (fig. 20), hypothermia (fig. 21), ptosis, lowered body posture and tone, and failure to respond to external stimuli such as sound, touch or tail-pinch. At 30 min after doses of 0.2 - 0.4 μg, the animals were cataleptic (fig. 22). The whole-body hypothermia was preceded by a sudden transient increase in skin temperature (fig. 23), indicative of peripheral vasodilatation.

When the experiments were repeated at a raised environmental temperature ($32 \pm 1^{\circ}C$) there was an initial slight hypothermia, followed by hyperthermia developing within 1 ½ h (fig. 24), but there was still marked central nervous depression, including a decrease in locomotor activity (fig. 25). All these effects were completely reversible and could be reproduced in the same animals by a second injection of ouabain 24 h after the first (fig. 26).

At doses of 0.1 - 0.4 μg, icv ouabain possessed no anti-convulsant activity against electro-shock convulsions, but was instead marginally pro-convulsant (table 11). At doses in excess of 0.4 μg, icv ouabain alone caused tonic or clonic convulsive phases in a proportion of mice; above 1.0 μg convulsions and death were the predominant behavioural effects of icv-administered ouabain in all mice.

Since ouabain is the most water soluble cardiac glycoside it was considered of interest to compare its effects with digitoxin, the most lipid soluble. Digitoxin (2.5 μg icv) produced qualitatively similar results to ouabain, but was considerably less potent (fig. 27). It was in-

jected in ethanol/glycerin/water solvent which itself produced no gross behavioural changes following injection (beyond scratching and grooming of the injection site).

ii) Effect of icv ouabain in the rat

In order to confirm the findings in the mouse, the effect of ouabain in the rat was studied. Following an icv injection of ouabain (1 μg) there was an initial period of hyperactivity and hypersensitivity, followed by a behavioural depression similar to that seen in the mouse. Although some catalepsy was apparent, the animals did not exhibit ptosis. A marked peripheral vasodilatation was seen, particularly in the tail veins, and the animals showed signs of diarrhoea. They became hypothermic at an ambient room temperature of $21 \pm 1^{o}C$, the fall in body temperature being maximal at about 1 h after injection (fig. 28).

2. Effect of peripherally-administered ouabain in the mouse and chick

In order to confirm that the effects of ouabain described above resulted from an action on the central nervous system, and were not mediated indirectly through a peripheral action, experiments were also performed in which ouabain was administered peripherally.

Doses of ouabain up to 1μg/mouse iv and 20 μg/mouse sc produced no signs of central nervous depression. Occasionally the largest doses produced a small degree of hypothermia (up to $3^{o}C$ at 30 min).

Further evidence of a potent central action, independent of peripheral activity, was obtained with experiments involving young chickens. During embryonic or young life the blood-brain barrier is either absent

altogether or its effectiveness is markedly deficient, and the blood-brain characteristics in the chicken are not present until about the fourth week of life (Waelsch, 1955). Whereas the administration of ouabain peripherally to mice produced little effect in doses up to 20 μg/mouse sc, ouabain (0.2 - 1 mg/kg ip) given to young chickens produced effects similar to those seen after icv injection in mice. These effects were dose-dependent, and it was also found that the magnitude of the response varied with the age of the chick. Ouabain at a dose of 0.5 mg/kg ip (= 20 μg/40 g chick) produced a profound hypothermia, accompanied by muscular weakness, incoordination and ptosis in chicks two days old. Vomiting developed 1 h after injection. The chicks assumed a squatting posture with the wings and beak touching the floor (fig. 29), and were not easily aroused by sensory stimuli, including handling. Ouabain consistently stopped vocalisation. Smaller doses produced similar but less pronounced effects. The dose-dependent nature of the effect of ip ouabain on the rectal temperature of chicks three days old is shown in fig. 30. The sensitivity of the chicks to this central depressant action of ouabain decreased rapidly with age, until, at ten days after hatching, no effects either on behaviour or body temperature could be produced at doses up to 0.7 mg/kg ip (fig. 31).

3. Effect of icv ouabain on blood pressure of the conscious rat

In view of the known cardiovascular actions of the cardiac glycosides, it seemed necessary to study the effects of icv ouabain on the blood pressure and heart rate of the conscious unrestrained rat, in order to eliminate any possible effects on the myocardium, which might influence or contribute to the effects described so far. The blood pressure was recorded and the ouabain administered in such a way that the animal was not disturbed during the experiment (see Methods II 3.).

Doses of ouabain up to 1 μg icv produced no effect on blood pressure or heart rate, neither immediately nor within 1 ½ h of injection (fig. 32).

4. Effect of icv ouabain on conditioned avoidance behaviour in the rat

In order to characterise further the effects of icv ouabain, its ability to modify the behaviour of rats conditioned to avoid an electric shock by means of an audio/visual conditioned stimulus was investigated.

Ouabain (0.3 μg icv) produced no effect on either conditioned avoidance reaction (C.A.R.) or unconditioned reaction (U.R.) for up to 20 h after injection. Higher doses (0.5 - 1 μg icv) abolished the C.A.R. from 10 min until more than 2 h after injection. The U.R. was unaffected. All animals recovered fully within 20 h (table 12).

Thus icv ouabain resembles icv chlorpromazine (see Results VII 4. ii) and differs from pentobarbitone, which, in doses of 300 μg icv, appears to have a non-specific depressant action, abolishing both C.A.R. and U.R. (see Results II 5.).

5. Effect of icv ouabain on whole-brain amine levels in the mouse

There have been a number of reports that, both *in vitro* and *in vivo*, ouabain may interfere with the synthesis (Anagnoste & Goldstein, 1967; Goldstein, Ohi & Backstrom, 1970) and uptake (Berti & Shore, 1967a; Blackburn, French & Merrills, 1967; Bogdanski, Tissari & Brodie, 1968) of catecholamines and indolalkylamines. Consequently, the effects of icv ouabain on whole-brain amine levels were investigated in an attempt to explain some of the previously observed pharmacological effects produced by ouabain when given by this route.

Whole-brain levels of dopamine, noradrenaline and 5-hydroxytryptamine were determined in mice killed 90 min after the injection of ouabain (0.3 μg icv). Control mice received an equivalent amount of vehicle. The results are summarised in table 13.

The level of 5-hydroxytryptamine remained unchanged, but there was a highly significant increase in whole-brain dopamine levels after ouabain pretreatment (103 % increase; $p < 0.01$), together with a small non-significant decrease in the level of noradrenaline.

6. Discussion

The icv injection of small doses of ouabain into conscious mice produced a profound central nervous depression, characterised by a marked loss of spontaneous locomotor activity, catalepsy, ptosis and poikilothermia. These effects could also be produced following icv injection of digitoxin in the mouse. Similar effects were observed after icv injection of ouabain in the rat. In both species, the effects are reminiscent of those seen after peripherally-administered reserpine or chlorpromazine, which suggests that ouabain too may interfere in some way with central aminergic function.

The observation that spontaneous locomotor activity could be reduced and catalepsy induced by ouabain, without producing hypothermia (experiments conducted at $32 \pm 1^{o}C$), confirms that, although inter-related, temperature changes and behavioural changes are essentially separately induced effects. In this respect the present observations with ouabain confirm earlier work in these laboratories, using chlorpromazine and reserpine (Spencer & Waite, 1968). The icv injection of large doses of ouabain

(above 0.4 μg) was excitatory and caused death by convulsions, thus confirming the earlier observations of Greef & Kasperat (1961a,b).

In contrast, ouabain given peripherally to mice was not accompanied by any change in behaviour: there was no catalepsy, no measurable reduction in locomotor activity and no ptosis. A slight hypothermia was limited to $3^{o}C$ at 30 min. This general lack of depressant or excitatory activity after peripheral injection is taken to indicate that the effects of small doses of icv ouabain are mediated centrally. The lack of effect of ouabain on blood pressure and heart rate of rats, following icv injection, indicates that the doses used do not cause deleterious effects on the heart.

Further evidence for a potent central action of ouabain is furnished by experiments showing that the effects produced by icv ouabain in mice and rats can be reproduced following peripheral administration to young chickens, whose blood-brain barrier is very poorly developed. Of particular interest here is the rapidity with which insensitivity to these effects develops in the chicken with age. The possible reasons for this include: (1) rapid changes in blood-brain barrier permeability to ouabain, (2) changes in endogenous catecholamine levels within the brain, (3) variations in the ability of the chick to regulate body temperature with age.

Ouabain differs from reserpine in one important aspect - it does not reduce the whole-brain levels of the amines dopamine, noradrenaline and 5-hydroxytryptamine at doses which produce marked central nervous depression, but in fact causes a marked increase in whole-brain dopamine levels. A difference in effect on amine levels is also apparent between ouabain and chlorpromazine, which, as a phenothiazine, has no effect on the levels of endogenous catecholamines in the brain (Ehringer, Hornykiewicz &

Lechner, 1960; Gey & Pletscher, 1961).

Despite the apparent difference between reserpine, chlorpromazine and icv ouabain, there are a number of similarities in their effects on behaviour, including those on conditioned avoidance behaviour (see Results VII 4. ii)). This prompted a study of the interactions of icv ouabain with agents known to alter the effects of reserpine and chlorpromazine; the findings will be discussed in the following section.

It is known that thymoleptics which interfere with amine uptake mechanisms, for example protryptyline (Persson & Waldeck, 1968) and desmethylimipramine (Nyback & Sedvall, 1968), also enhance the synthesis of dopamine, and reduce that of noradrenaline in the central nervous system. It is also known that, both *in vitro* and *in vivo*, ouabain can block the sodium pump through an action on the Na-K ATPase activity (Elshove & Rossum, 1963; Berti & Shore, 1967a; Elsmore & Withrow, 1968; Williams, Withrow & Woodbury, 1969), and Tarve & Brechtlova (1967) have shown that imipramine too can inhibit the Na-K ATPase system in guinea-pig brain microsomes. Recently Ebadi & Carver (1970) have demonstrated that chlorprothixene has similar effects in the rat brain. These observations, together with the knowledge that the re-uptake of neuronally-released catecholamines is inhibited by blockade of the sodium pump (Berti & Shore, 1967a), indicate that behavioural changes may be brought about by an interference with amine re-uptake in the brain. The pharmacological effects of icv ouabain may involve such a mechanism.

Experiments involving icv injections of sodium diethyldithiocarbamate (DDC) support the view that an interference with dopamine metabolism may be an important factor in the mechanism of action of icv ouabain (see Re-

sults VII 1.). Recently Kleinrok, Zebrowska & Wielosz (1970) have observed that DDC-induced motor inactivity and hypothermia in rats coincides with maximum decreases in noradrenaline, and increases in dopamine levels in the brain. Anagnoste & Goldstein (1967) have demonstrated that large doses (25 - 75 μg icv) of ouabain can enhance the synthesis of dopamine, and reduce that of noradrenaline, in different areas of the rat brain. Whereas this might confirm the increase in whole-brain dopamine levels observed above, their work with convulsant doses of ouabain might also predict an associated reduction in noradrenaline levels, not prominent after smaller depressant doses. The absence of any effect on noradrenaline levels following these depressant doses suggests that noradrenergic systems may be involved predominantly in the production of the excitatory effects of ouabain. It should be emphasised, however, that the actions of ouabain may not adequately be interpreted from observations on whole-brain amine levels, since the behavioural effects witnessed above may be dependent upon other effects produced only within the periventricular tissue (at the hypothalamic level, for example), and not detected by static whole-brain determinations. There is also the possibility that the variations in amine levels are the result rather than the cause of the hypothermia. Further work is indicated here.

The data presented so far does no more than outline the pharmacological effects of ouabain in central nervous tissue. In an endeavour to elucidate further the systems on which ouabain operates, a study was made of its interactions with a number of other drugs. The results will be discussed in Results V.

7. FIGURES AND TABLES

FIG. 20

Decrease in locomotor activity produced by icv ouabain in the mouse at an ambient temperature of 21 ± 1° C. Open columns, saline (10 µl); solid columns, ouabain (0.3 µg). Results are means of two experiments. (n = 10).

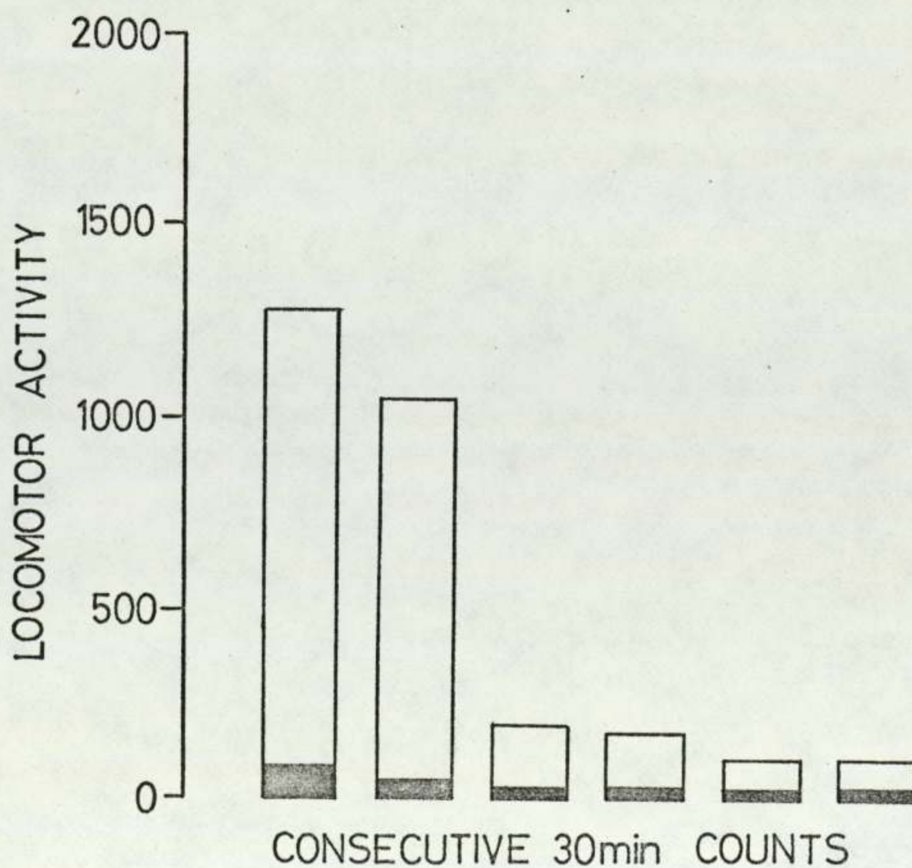

FIG. 21

Effect of icv ouabain on the body temperature of the mouse. (o——o) 10 µl saline; (●——●) 0.1 µg ouabain; (Δ——Δ) 0.2 µg ouabain; (▲——▲) 0.4 µg ouabain. Ambient temperature 21 ± 1° C.

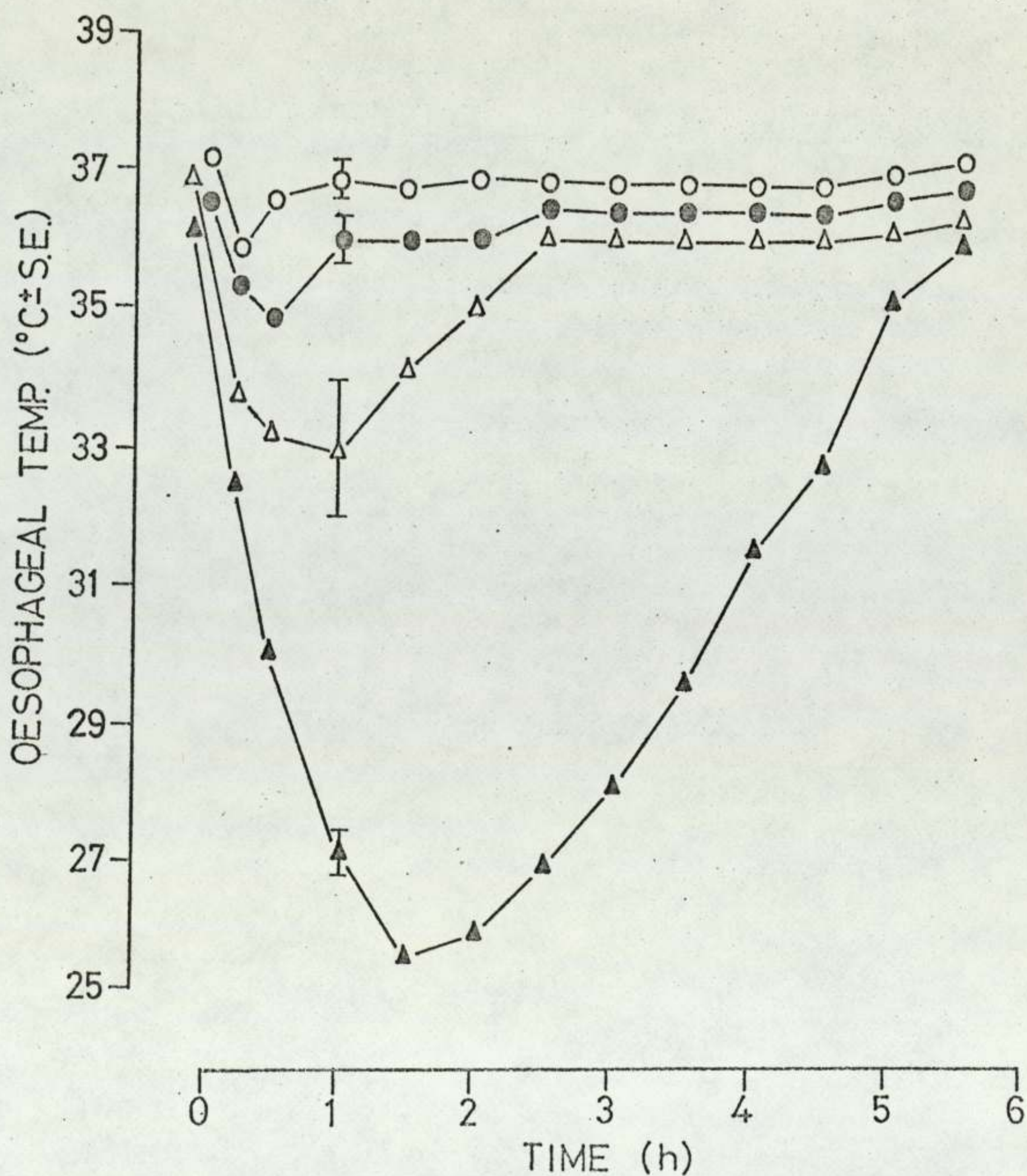

FIG. 22

Production of catalepsy by icv ouabain in the mouse. (x——x) 0.2 µg ouabain; (o——o) 0.25 µg ouabain; (•——•) 0.3 µg ouabain.

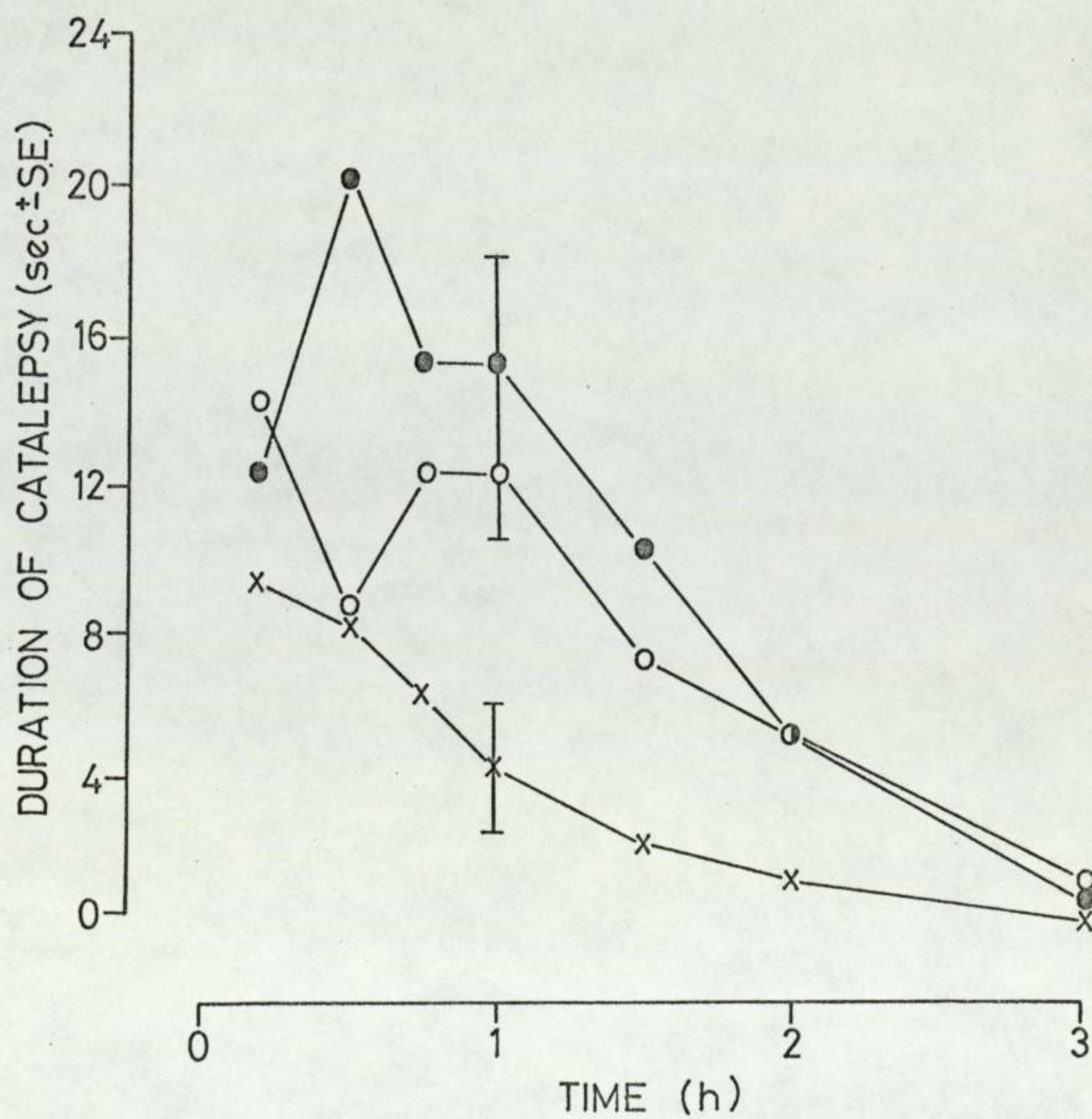

FIG. 23

Effect of icv ouabain on the skin temperature of the mouse. (o—o) 10 µl saline; (●—●) 0.4 µg ouabain. Ambient temperature 21 $\pm$ 1° C.

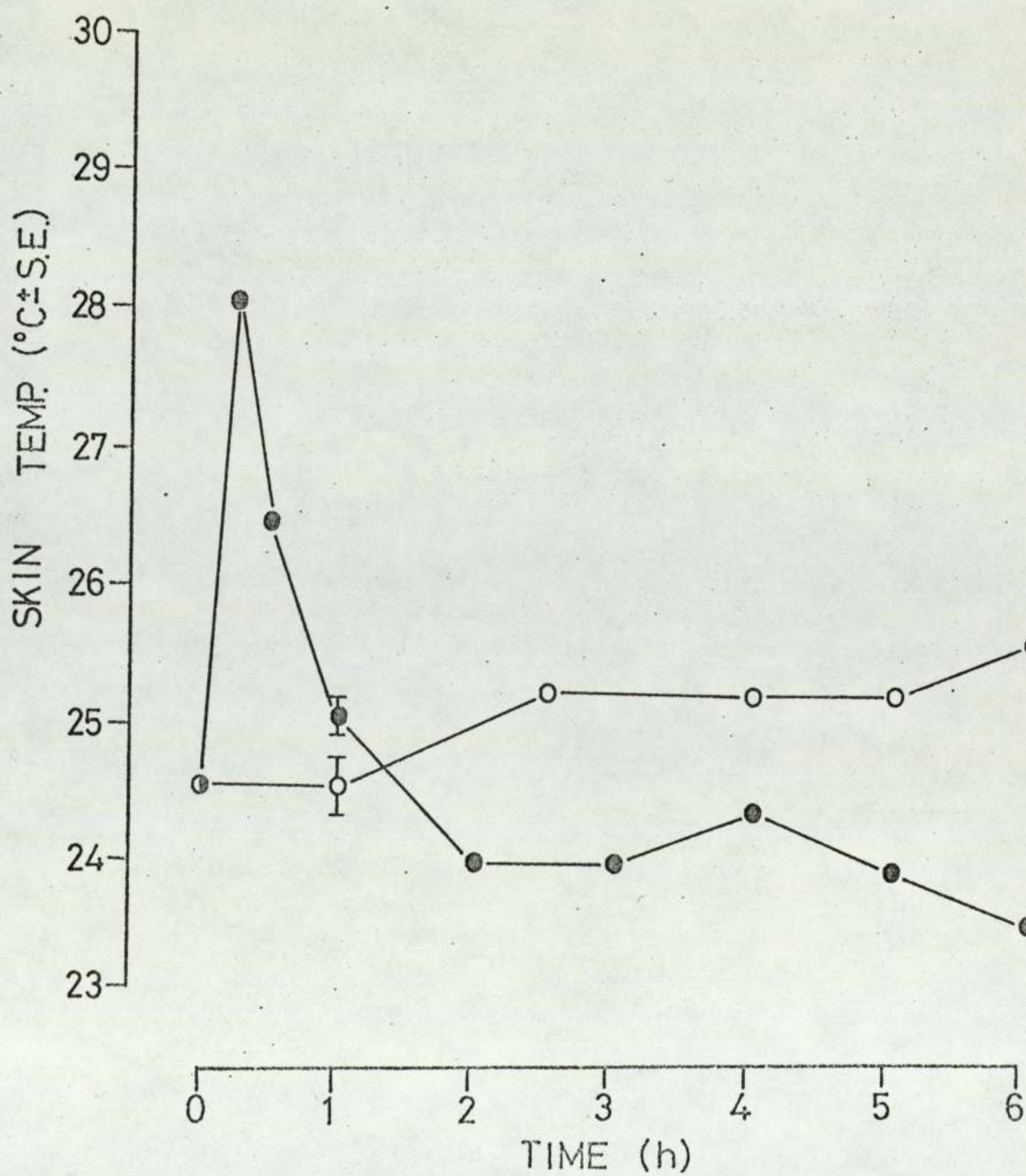

FIG. 24

Effect of icv ouabain on the body temperature of the mouse. (o—o) 10 µl saline; (x—x) 0.3 µg ouabain. Ambient temperature 32 ± 1° C.

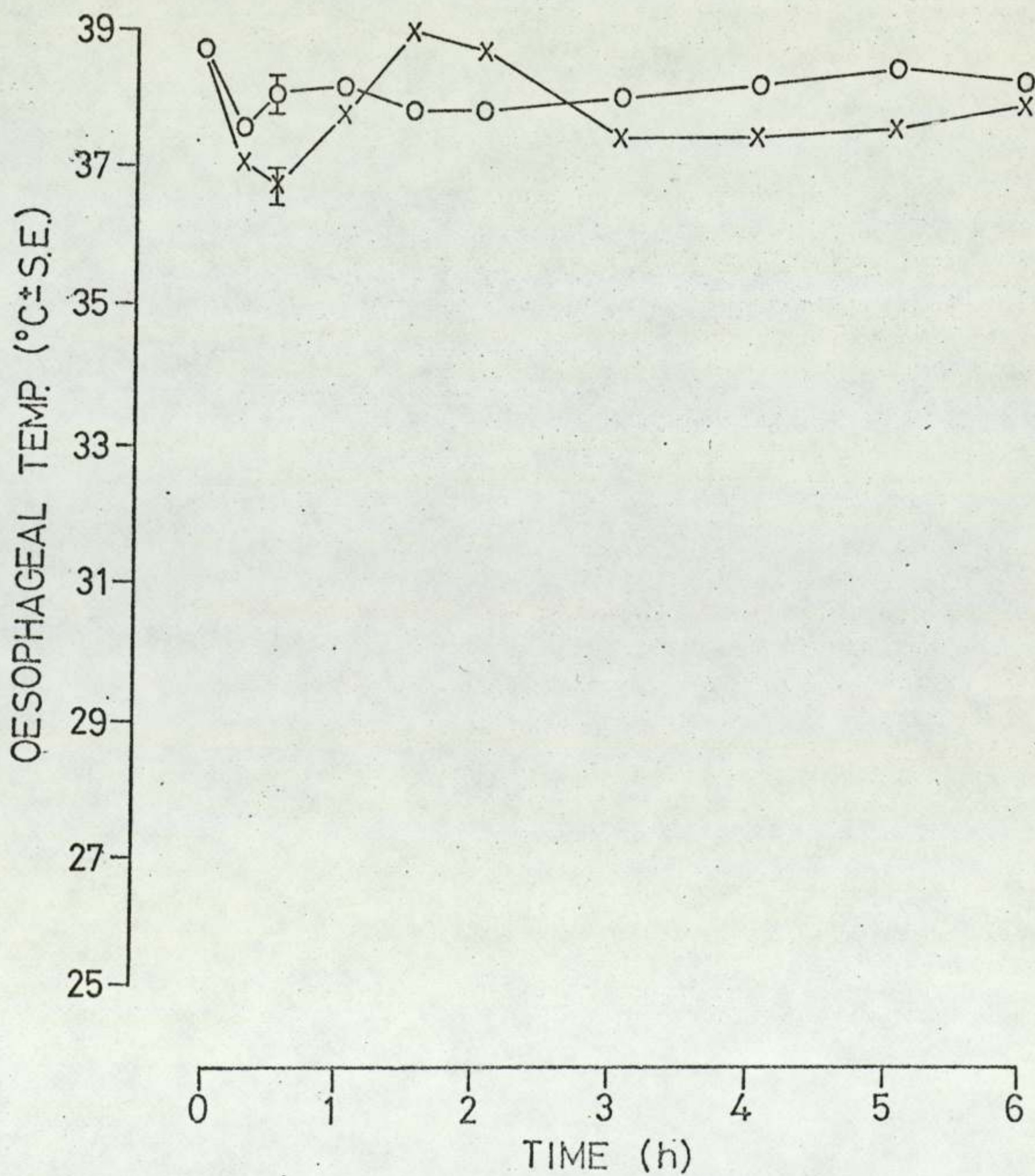

FIG. 25

Decrease in locomotor activity produced by icv ouabain in the mouse at an ambient temperature of 32 $\pm$ 1° C. Open columns, saline (10 μl); solid columns, ouabain (0.3 μg). Results are means of two experiments. (n = 10).

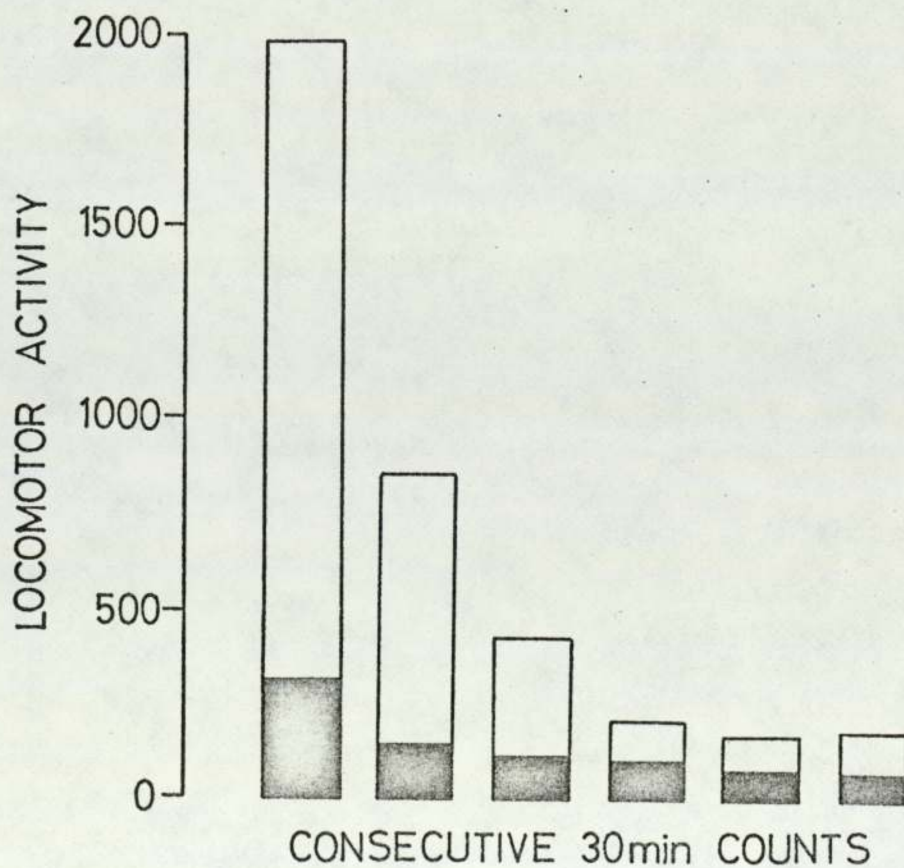

FIG. 26

Reproducibility of the effect of icv ouabain on the body temperature of the mouse. On day 1 animals received (o——o) 10 µl saline or (Δ——Δ) 0.2 µg ouabain, and 24 h later on day 2 (●——●) 10 µl saline or (▲——▲) 0.2 µg ouabain.

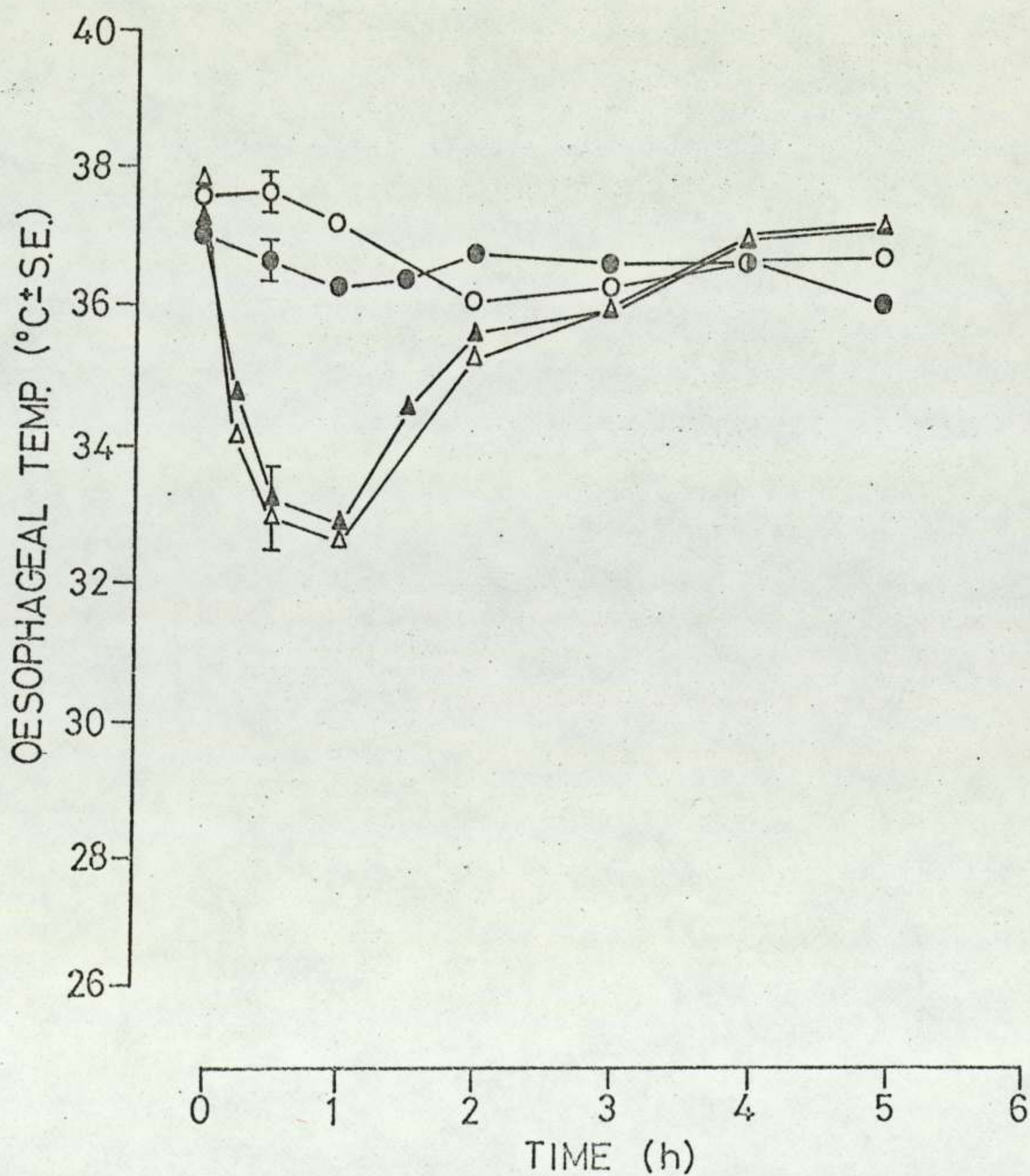

FIG. 27

Effect of icv digitoxin on the body temperature of the mouse.
(x——x) 10 μl saline; (o——o) 2.5 μg digitoxin.

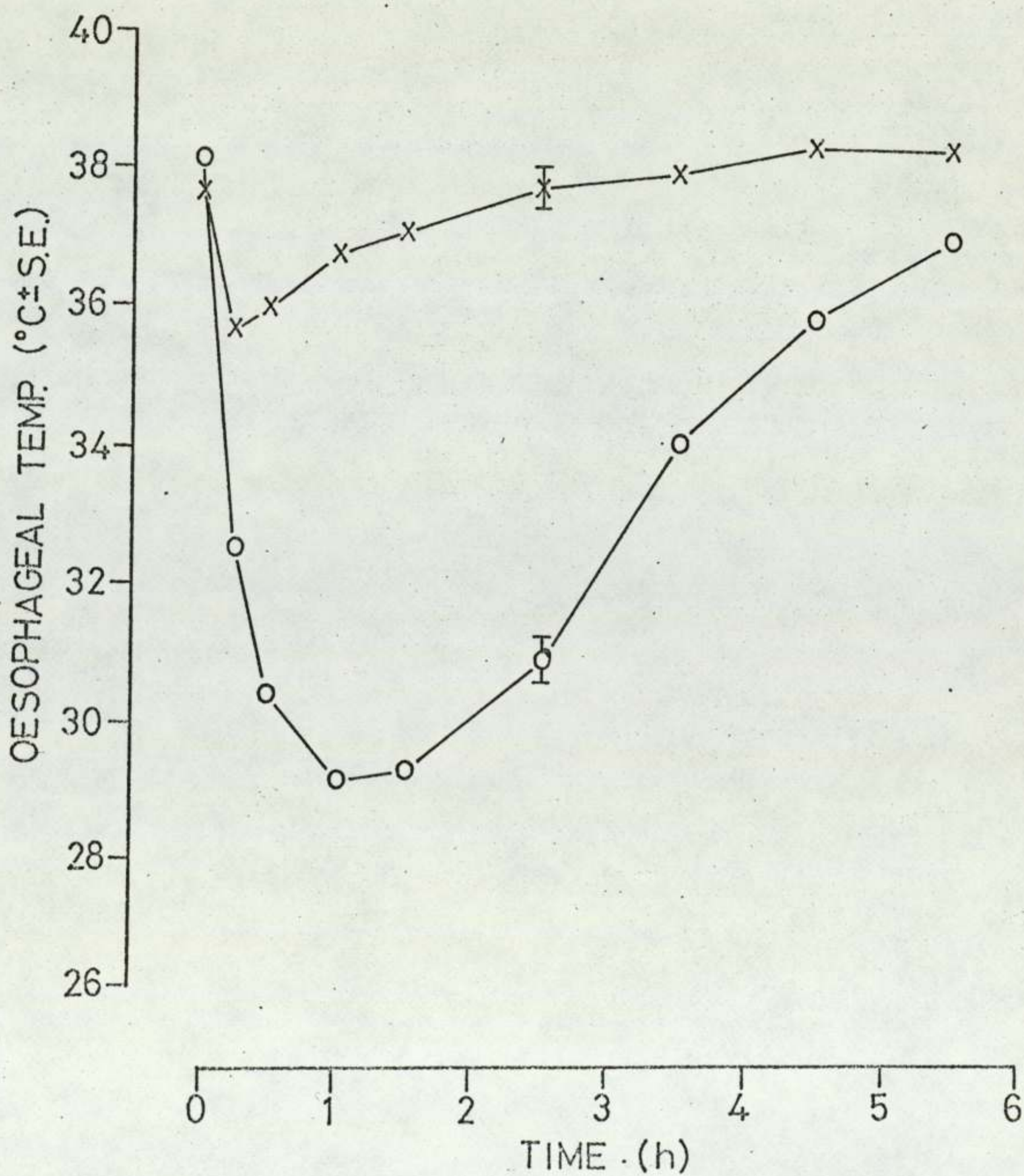

FIG. 28

Effect of icv ouabain on the body temperature of the rat.
(x——x) 10 µl saline; (o——o) 1.0 µg ouabain.

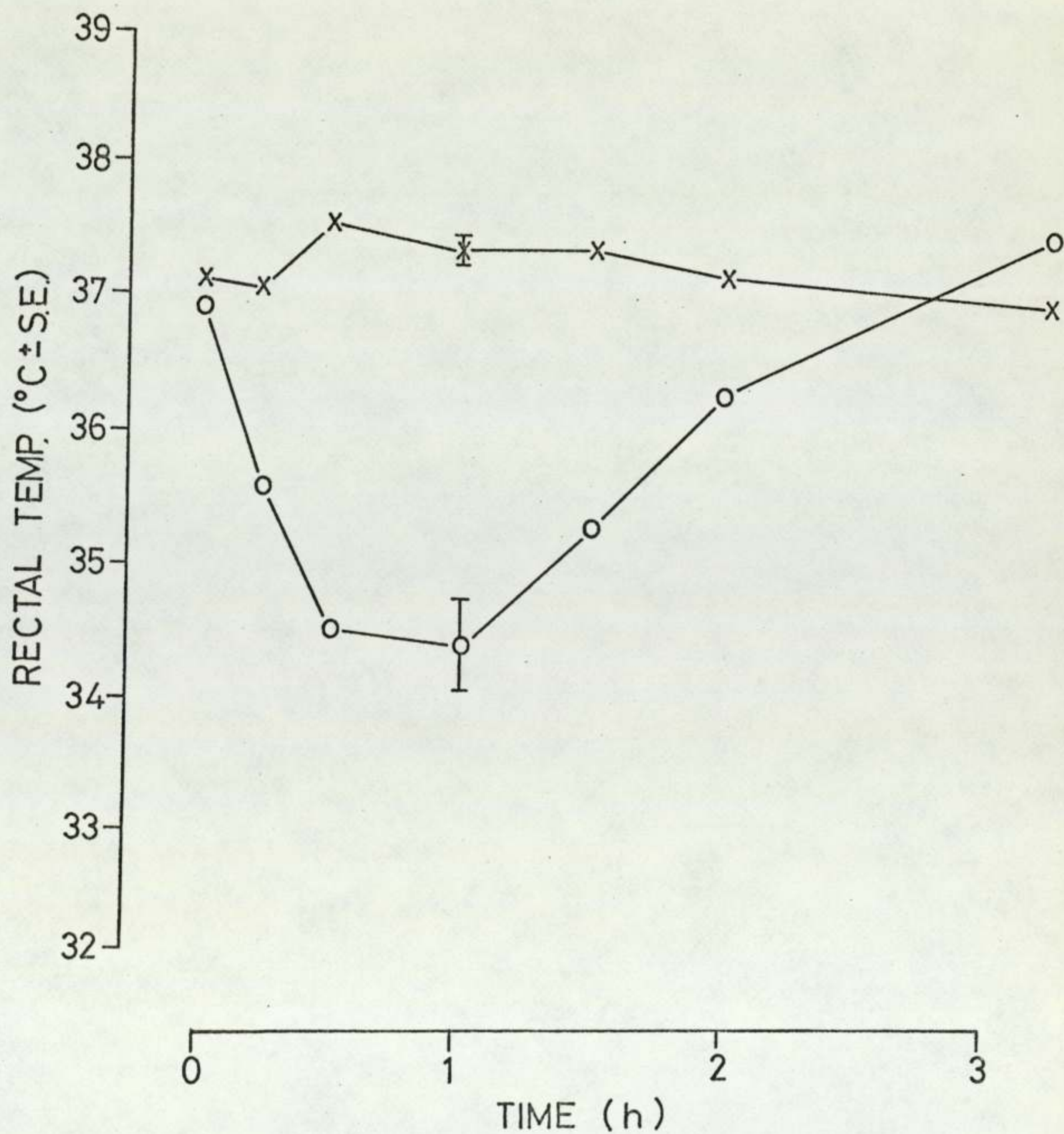

FIG. 29

Characteristic posture assumed by young chicken after peripherally-administered ouabain. Chick (48 h after hatching) received 0.7 mg/kg ouabain ip 30 min before photograph was taken.

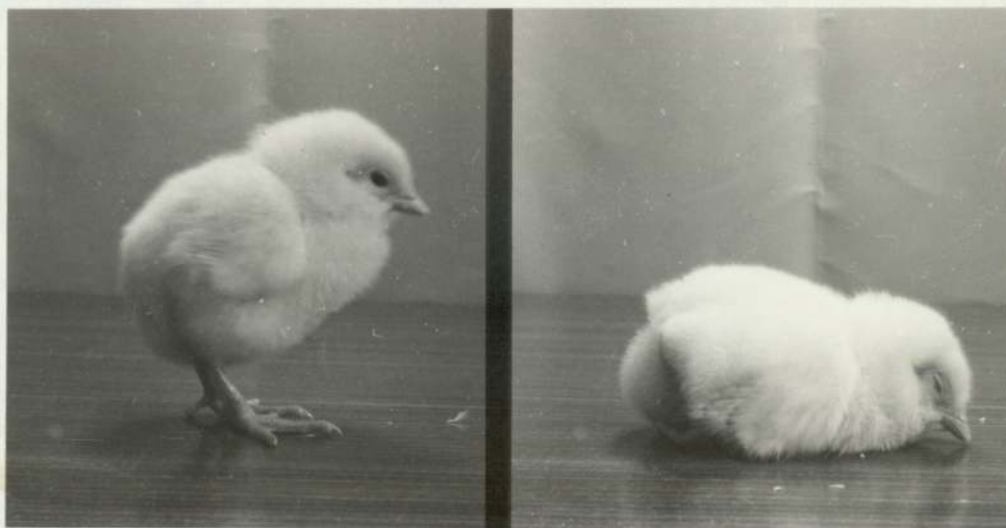

FIG. 30

Effect of peripherally-administered ouabain on the body temperature of young chickens. Chicks (72 h after hatching) received (x——x) 10 ml/kg saline ip; (o——o) 0.2 mg/kg ouabain ip; (●——●) 0.3 mg/kg ouabain ip; (Δ——Δ) 0.5 mg/kg ouabain ip.

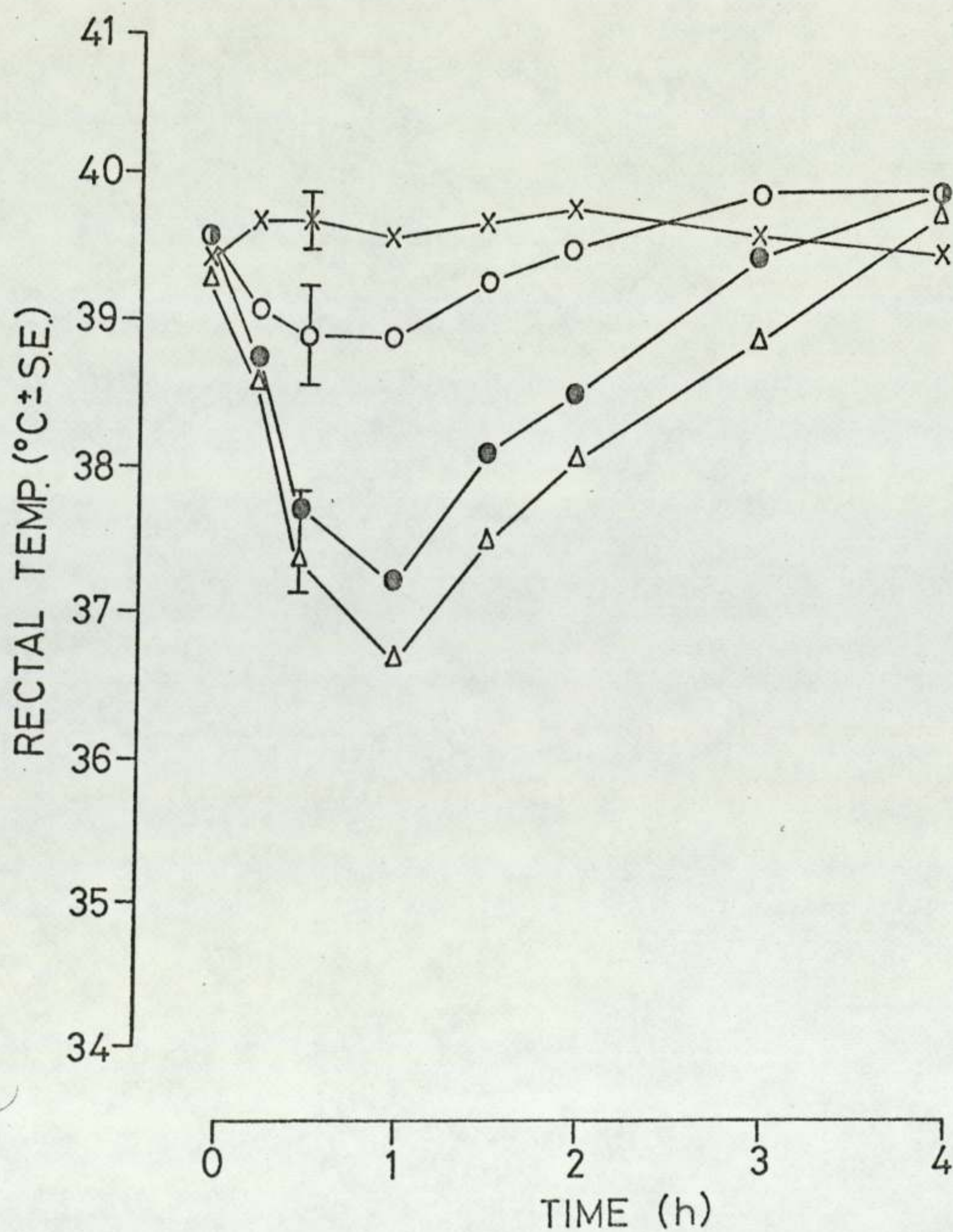

FIG. 31

Effect of age of young chickens on the response to peripherally-administered ouabain. Chicks received 0.7 mg/kg ouabain ip (x——x) 2 days; (o——o) 3 days; (●——●) 4 days; (Δ——Δ) 5 days or (▲——▲) 10 days after hatching. In each case controls received 10 ml/kg saline ip.

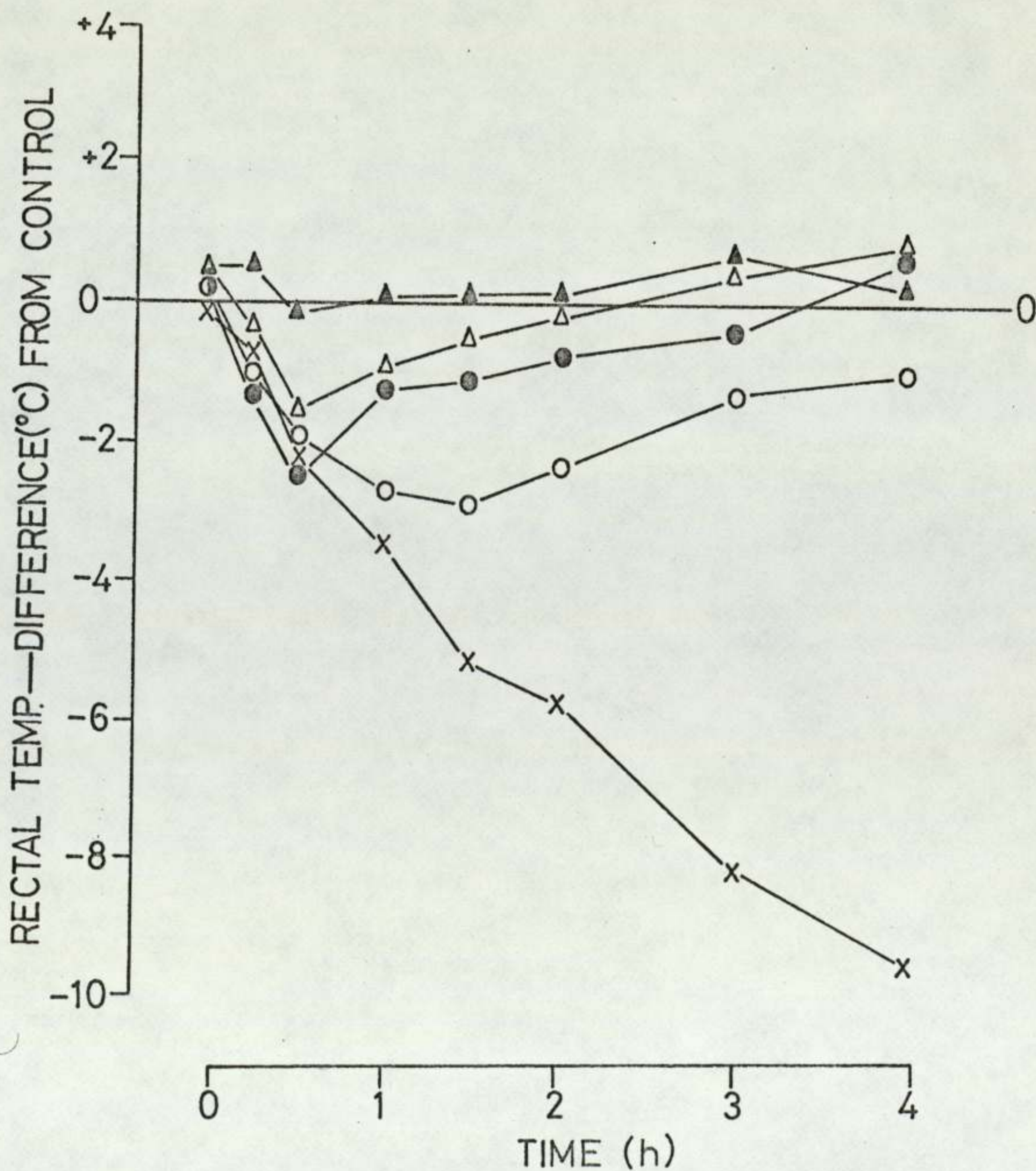

FIG. 32

Effect of icv ouabain on the blood pressure of the conscious unrestrained rat. Time marker 1/min.

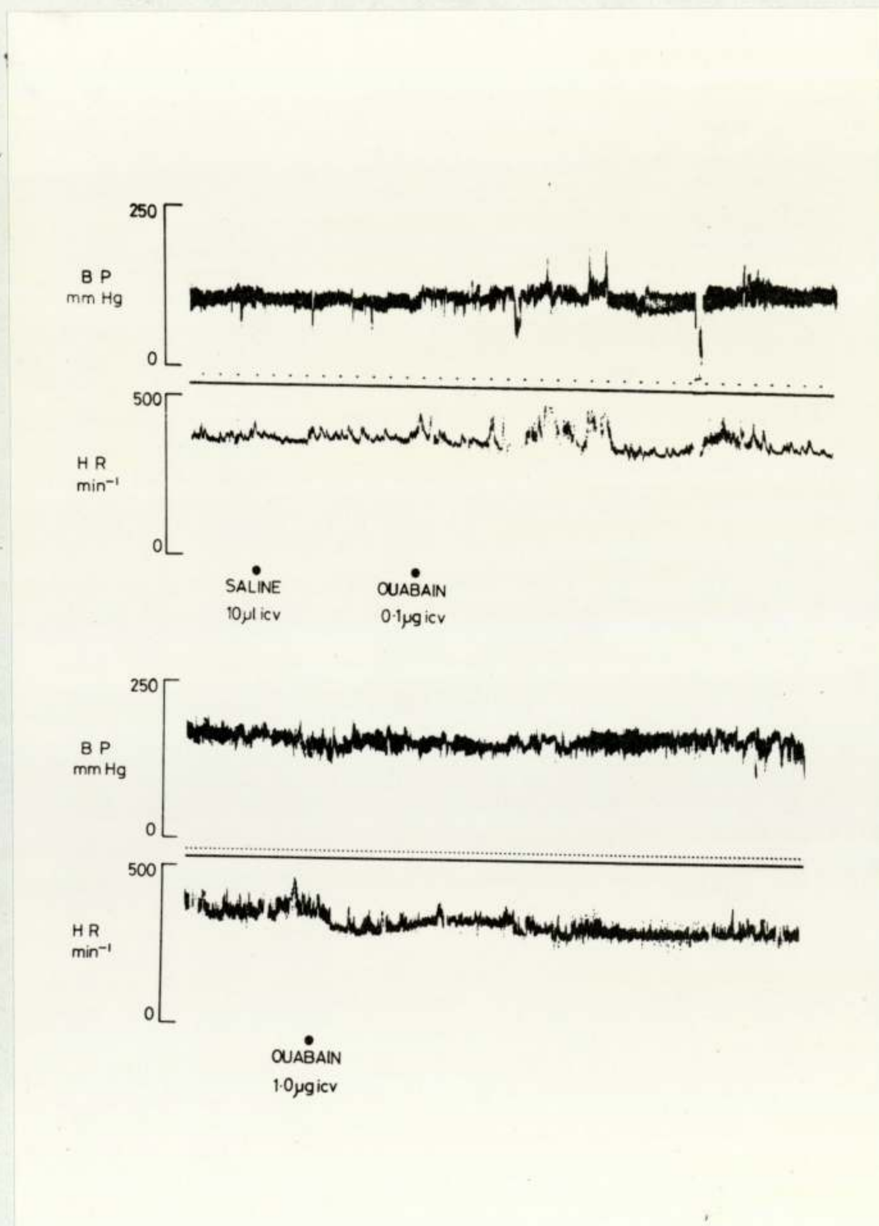

TABLE 11

Proconvulsive activity of icv ouabain following electro-shock. Animals received saline (10 μl icv) or ouabain 30 min before the application of electro-shock (2 msec pulses, 100 Hz for 0.3 sec at 65 V). (n = 20).

DOSE OF OUABAIN (μg)	% SHOWING CLONIC EXTENSOR SPASM	% SHOWING TONIC EXTENSOR SPASM	% MORTALITY
0	70	30	10
0.05	70	30	0
0.1	40	60	20
0.2	10	90	60
0.4	20	80	60

TABLE 12

Effect of ouabain given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Controls received saline (10 µl icv) 10 min before trial.

DOSE (µg)	PARAMETER MEASURED	MEAN RESPONSES (% ± S.E.)				
		Control	10 min after Inj.	30 min after Inj.	2 h after Inj.	20 h after Inj.
0.3	% avoid	100	95	100	98 ± 2	100
	Escape failure	0	0	0	0	0
	Latency	268 ± 38	276 ± 15	251 ± 11	260 ± 23	325 ± 12
0.5	% avoid	87 ± 6	36 ± 9	33 ± 19	40 ± 5	100
	Escape failure	0	0	5 ± 5	0	0
	Latency	438 ± 32	805 ± 29	794 ± 170	638 ± 54	301 ± 14
1.0	% avoid	85 ± 4	27 ± 12	22 ± 9	20 ± 0.3	91 ± 6
	Escape failure	0	5 ± 2	2 ± 2	13 ± 8	0
	Latency	446 ± 43	932 ± 124	724 ± 38	1064 ± 227	409 ± 39

TABLE 13

Effect of ouabain (0.3 μg icv) on whole-brain amine levels in the mouse. Animals received saline (10 μl icv) or ouabain 90 min before sacrifice. The figures in parenthesis indicate the number of determinations made; data are expressed as mean ± S.E. * indicates significant difference from control.

PRETREATMENT	NORADRENALINE (ng/g)	DOPAMINE (ng/g)	5-HYDROXYTRYPTAMINE (ng/g)
Saline	287 ± 20 (9)	652 ± 27 (8)	884 ± 32 (8)
Ouabain	265 ± 61 (4)	1326 ± 199 (5)*	884 ± 24 (5)

V INTERACTION OF CENTRALLY-ADMINISTERED OUABAIN WITH OTHER AGENTS

V Interaction of centrally-administered ouabain with other agents

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V The pharmacological effects of icv ouabain in the conscious mouse show marked similarities to the effects produced by peripherally-administered reserpine and chlorpromazine, although the whole-brain amine determinations suggest that ouabain elicits its effects through a mechanism different from those of the two tranquillizers.

Centrally-administered catecholamines produce central depressant effects, including hypothermia, in chickens (Grunden & Marley, 1970), mice (Brittain & Handley, 1967), rats (Grunden, 1969), cats (Feldberg & Myers, 1964), dogs (Feldberg, 1966), and human psychotic patients (Sherwood, 1955), together with several other species.

In view of the depressant effect produced by icv ouabain, together with its ability to interfere with the uptake and synthesis of catecholamines, it seemed important to examine the interaction of icv ouabain with other agents known to interfere with central aminergic function. Accordingly, a study was made of the pharmacological effects of icv ouabain in the presence of a number of these agents given by peripheral or concomitant icv injection.

1. Interaction with dexamphetamine, desmethylimipramine and nialamide in the mouse

Dexamphetamine (0.5 - 10 mg/kg ip) produced an immediate dose-related reversal of all of the previously described central nervous depressant effects of icv ouabain (0.4 μg), when given 30 min after the ouabain. The reversal of ouabain-induced hypothermia is illustrated in fig. 33. After complete reversal of the ouabain-induced depressant effects, the mice receiving dexamphetamine + ouabain were markedly more excitable than those

receiving dexamphetamine alone at the same dose. Furthermore, dexamphetamine (10 mg/kg ip), which produced no significant hyperthermia when administered alone, produced a significant elevation of body temperature (maximal 3°C) within 1 h of injection when given after ouabain. A similar sensitisation to the effects of dexamphetamine was produced by icv ouabain, which also potentiated the LD_{50} of dexamphetamine measured under crowded conditions. Two groups of 8 mice each received dexamphetamine at one of five dose levels (5 - 75 mg/kg ip), followed immediately by ouabain (0.1 μg icv). There was a definite sensitisation to the acute toxic effects of dexamphetamine produced by this small dose of ouabain (table 14), which was consistently reproducible.

In another experiment, desmethylimipramine (5 and 10 mg/kg ip) was given 5 min before injection of ouabain (0.3 μg icv). There was a marked reduction in all of the central nervous depressant effects of ouabain, associated with a definite increase in excitability and sensitivity to external stimuli such as handling. Fig. 34 summarises the effects of desmethylimipramine pretreatment on the icv ouabain-induced hypothermia.

In contrast, pretreatment with the monoamine oxidase inhibitor nialamide (10 and 20 mg/kg ip) 2 h before ouabain (0.3 μg icv) produced a small potentiation of the depth of the ouabain-induced hypothermia, followed by an enhanced rate of recovery (fig. 35).

2. Interaction with noradrenaline, phentolamine and α-methylmetatyrosine in the mouse

Groups of 5 mice received by icv injection one of the following: (1) phentolamine (2 μg), (2) noradrenaline (5 μg) or (3) ouabain (0.2 μg).

Further groups of mice received by icv injection in mixed solution: (1) noradrenaline (5 μg) + phentolamine (2 μg), (2) ouabain (0.2 μg) + phentolamine (2 μg) or (3) ouabain (0.2 μg) + noradrenaline (5 μg). Both noradrenaline alone and phentolamine alone, in the doses used, produced ptosis and hypothermia (fig. 36), although no other signs of central nervous depression were observed. Ouabain, however, produced ptosis, hypothermia and central nervous depression, which have been described in detail in Results IV 1. i).

Phentolamine potentiated the ouabain-induced ptosis and hypothermia and, to a lesser degree, the noradrenaline-induced ptosis and hypothermia (fig. 37). In contrast to the potentiating effects of phentolamine, it was found that noradrenaline partly reversed the ptosis and hypothermia (fig. 38) produced by ouabain.

Noradrenaline levels in the rat brain are depressed for several days following the injection of α-methylmetatyrosine (400 mg/kg ip), the noradrenaline being replaced almost stoichiometrically by the metabolite metaraminol. Dopamine and 5-hydroxytryptamine levels return to normal within 24 h of administration (Andén, 1964). Pretreatment of mice with α-methylmetatyrosine (400 mg/kg ip) 24 h before the injection of ouabain (0.25 μg icv) produced no significant change in the intensity of hypothermia (fig. 39) or behavioural effects induced by the ouabain.

3. Interaction with the adenyl cyclase system in the mouse

In a recent paper Abdulla & Hamadah (1970) claimed that the successful treatment of clinical depression with anti-depressant drugs was accompanied by in increase in urinary levels of cyclic 3',5'-adenosine monophos-

phate (cAMP). Since icv ouabain in the mouse produces central nervous depressant effects, which are also reminiscent of the effects of reserpine, and, since the above workers claim that reserpine ptosis can be reversed by dibutyryl cyclic 3',5'-adenosine monophosphate (dbcAMP), an attempt was made to antagonise the depressant effects of icv ouabain with concomitantly-adminstered dbcAMP. This agent was used because it can cross cell membranes more readily than its analogue cAMP (Butcher & Sutherland, 1962; 1967), and its rate of hydrolysis by phosphodiesterase is very much slower (Moore, Iorio & McManus, 1968).

Groups of 5 mice were given by icv injection either ouabain alone (0.3 μg), dbcAMP alone (25 μg), or a mixture of both these doses in the same injection. (Preliminary examination of this mixture by infra-red and ultra-violet spectroscopy had shown that there was no chemical interaction between these two agents). Compared with control animals the icv injection of dbcAMP alone produced no overt changes in behaviour or body temperature. Whereas icv ouabain alone produced the expected profound depression of central nervous activity, there was a clear reduction in the level of depression when animals were given the mixture of ouabain and dbcAMP. They were noticeably more mobile, were not cataleptic, were more sensitive to touch, sound and painful stimuli, and the level of hypothermia was reduced (fig. 40).

In view of the observed antagonism of the effects of ouabain by dbcAMP, two experiments were performed to study the effect of caffeine, an inhibitor of the enzyme phosphodiesterase in the brain (Cheung, 1967). In the first experiment, caffeine (20 or 40 mg/kg ip) was given at the same time as the injection of ouabain (0.25 μg icv). There was a substantial antagonism of the central nervous depressant effects of ouabain,

with ptosis, lack of sensitivity and hypothermia (fig. 41) all substantially less than that observed when icv ouabain was administered alone. In the second experiment, caffeine (75 mg/kg ip) was given 30 min before the ouabain (0.25 μg icv). There was a marked reduction, but not a complete prevention, of the central nervous depressant effects of icv ouabain. Maximum hypothermia (at 60 min) was 3.7°C, as opposed to 7.2°C in animals treated only with ouabain - a highly significant reduction ($P < 0.001$).

4. Interaction with pentobarbitone in the mouse

i) Effect on duration of loss of righting reflex and acute toxicity

Small doses of ouabain (0.1 and 0.2 μg icv) administered 30 min before a small sub-hypnotic dose of pentobarbitone (20 mg/kg ip) produced a marked potentiation of pentobarbitone sleeping time at an ambient temperature of 21 ± 1°C. Repeating the experiment at a raised ambient temperature (32 ± 1°C) significantly reduced ($P < 0.01$), but did not abolish, the ouabain-induced potentiation (table 15). When given peripherally, the same doses of ouabain and pentobarbitone produced no potentiation of the hypnotic effects of pentobarbitone at either temperature.

In a further experiment, six groups of 10 mice received pentobarbitone at one of six dose levels (80 - 130 mg/kg ip) and the effect of ouabain (0.1 μg icv) on the LD_{50} of pentobarbitone was investigated. In contrast to the marked potentiating effects on pentobarbitone hypnosis, ouabain (0.1 μg icv) produced no effect on the acute toxicity of pentobarbitone when administered either immediately after or 30 min after the barbiturate (table 16).

ii) Effect on body temperature

The administration of ouabain (0.1 and 0.2 μg icv), followed 30 min later by pentobarbitone (20 mg/kg ip), produced a whole-body hypothermia similar to that seen after these doses of ouabain given alone (fig. 42). Repeating the experiment at 32 $\pm$ 1°C produced no significant decrease in body temperature compared with controls. When given peripherally, these doses of ouabain and pentobarbitone produced no hypothermia at either ambient temperature.

5. Interaction with apomorphine in the mouse and the rat

It has been observed previously that ouabain given in small doses by icv injection produces behavioural effects similar to those produced by reserpine and chlorpromazine. Furthermore, icv ouabain can abolish C.A.R. in trained rats, a property which is also shared by centrally-administered (see Results VII 4. ii)) and peripherally-administered (Herz, 1960) chlorpromazine. Since it is known that neuroleptics, including chlorpromazine, can block dopamine receptors (Rossum, 1966; Pletscher & Da Prada, 1967), and also that chlorpromazine can increase cerebral dopamine turnover (Da Prada & Pletscher, 1966), it was necessary to examine the effect of icv ouabain on dopamine receptors, particularly since ouabain is also known to increase the formation of catecholamines (Goldstein, Ohi & Backstrom, 1970).

Apomorphine can produce compulsive gnawing in mice and rats, and this behavioural manifestation is produced by a stimulation of central dopamine receptors (Andén, Rubenson, Fuxe & Hökfelt, 1967; Ernst & Smelik, 1966; Ungerstedt, Butcher, Butcher, Andén & Fuxe, 1969) not affected by inhibit-

ion of dopamine synthesis (Ernst, 1967). Consequently, apomorphine appears to act directly on central dopaminergic receptors, and it was decided to use this drug as a tool in the investigation of the interaction of ouabain with dopamine receptors.

Preliminary experiments in mice failed to produce a consistent gnawing response to apomorphine at doses up to 10 mg/kg sc, thus confirming previous work (Scheel-Krüger, 1970). It was therefore necessary to use rats with cannula guides permanently implanted into the right lateral ventricles. It was found that compulsive gnawing could be induced in 100 % of animals following apomorphine (2 or 3 mg/kg sc) + saline (10 μl icv). It developed within 10 min of injection and lasted until 50 - 60 min after injection (table 17). Behavioural effects included the development within 5 min of injection of a spastic gait, slight ptosis and head drop, sniffing and hyperpnoea. The animals were hyperexcitable and exhibited a characteristic alternate tapping movement with the front paws.

Administration of ouabain (0.3 μg icv) immediately before apomorphine (2 mg/kg sc) produced no effect on the behaviour produced by apomorphine, including gnawing. Following a larger dose of ouabain (1 μg icv), all of the behavioural effects induced by apomorphine were blocked, including the gnawing (table 17), and after injection the animals remained quiet for 30 - 35 min, after which they became markedly more active.

Since ouabain produces catalepsy in the mouse, and dopamine is thought to play an important rôle in the antagonism of the cataleptic action of neuroleptics by dopa (Maj & Zebrowska, 1966), an attempt was made to reverse the ouabain-induced catalepsy by pretreatment with apomorphine. Two groups of 10 mice received ouabain (0.3 μg icv), or a

combination of this dose of ouabain followed immediately by apomorphine (20 mg/kg sc). The apomorphine produced a rapid reversal of ouabain-induced catalepsy (fig. 43), associated with a complete reversal of all central nervous depressant effects of ouabain, with the exception of ptosis. In contrast, apomorphine (20 mg/kg sc) given immediately before ouabain (0.2 μg icv) did not produce any effect on the ouabain-induced hypothermia (fig. 44).

6. Discussion

Despite the apparent differences in effect between reserpine, chlorpromazine and icv ouabain on whole-brain amine levels, there are a number of similarities in their effects on behaviour, and a study of the interactions of icv ouabain with agents known to alter the effects of reserpine or chlorpromazine was made. These agents included dexamphetamine, desmethylimipramine and the monoamine oxidase inhibitor nialamide. Like reserpine, icv ouabain appears to sensitise mice to the effects of dexamphetamine, small doses of the latter promptly reversing all of the behavioural changes induced by icv ouabain. Ouabain also sensitises the animals to the acute toxic effect of dexamphetamine under crowded conditions. Similarly, desmethylimipramine was able to antagonise the effects of ouabain. Yet nialamide was devoid of similar anti-ouabain activity at doses up to 20 mg/kg, when given 2 h before the ouabain, producing instead a biphasic response. This consisted of a small potentiation of the depth of ouabain-induced hypothermia, followed by an enhanced rate of recovery. Since icv oubain causes an increase in whole-brain dopamine, and dopamine is considered to be a good substrate for monoamine oxidase, nialamide should theoretically enhance ouabain-induced hypothermia, if the enhanced levels of dopamine within the brain are involved in the production of this hypothermia. It

may be that any interference with transmitter function by icv ouabain occurs partially at a cell membrane or extracellular site - two areas in which amine function is not likely to be markedly affected by inhibitors of monoamine oxidase. This, together with a possible effect on noradrenaline levels, may contribute to the small biphasic response produced by nialamide. It is interesting to note here that nialamide had similar effects on the hypothermia produced by sodium diethyldithiocarbamate (DDC), a substance which elevates whole-brain dopamine levels and produces comparable effects to ouabain when given by icv injection (see Results VII 1. i)).

Since desmethylimipramine does not affect dopamine uptake (Fuxe & Ungerstedt, 1968), the anti-ouabain effect of desmethylimipramine does not detract from the view that ouabain may be acting primarily through an effect on dopaminergic systems. It may be that the observed antagonism between these two agents involves a desmethylimipramine-induced effect on noradrenaline levels, particularly since icv noradrenaline produces a reduction in the hypothermia produced by icv ouabain.

Centrally-administered ouabain resembles centrally-administered noradrenaline in that it causes hypothermia, which, in the experiments described above, could not be blocked by phentolamine. Instead it was found that administration of phentolamine alone caused hypothermia, and administration of phentolamine plus ouabain or phentolamine plus noradrenaline potentiated the hypothermia produced by ouabain or noradrenaline alone. There have been no reports that icv ouabain is capable of causing an increase in brain noradrenaline, rather the available evidence suggests that the levels remain either substantially unchanged or decrease. This, together with the finding that noradrenaline can reduce ouabain hypothermia, and the suggestion that a depletion of brain noradrenaline causes hypo-

thermia (Reid, Volicer, Smookler, Beaven & Brodie, 1968), indicates that further work is needed here. Chemical or histochemical assays in specific brain structures following icv ouabain, instead of determinations on the whole brain, may help to solve this problem. It may be that the elevation in brain dopamine caused by ouabain is responsible for the hypothermia, since Barnett & Taber (1968) have suggested a possible rôle for dopamine in the central regulation of temperature in mice.

Pretreatment with α-methylmetatyrosine produced no significant changes in the pharmacological effects of icv ouabain. Metaraminol produced by decarboxylation and subsequent β-hydroxylation of α-methylmetatyrosine is an effective false transmitter, which is much more firmly bound to the tissues than noradrenaline (Andén, 1964). The absence of any antagonism between ouabain and α-methylmetatyrosine suggests that a displacement of tissue noradrenaline is not important in producing the observable effects of icv ouabain.

Both dbcAMP and caffeine can antagonise the effects of ouabain. Field, Plotkin & Silen (1968) have demonstrated an antagonism between the cardiac glycosides and cAMP on ion permeability. Thus the reduction of icv ouabain-induced central nervous depression by dbcAMP and caffeine may be the result of an antagonism between these agents and ouabain on ion transport in the cell membrane. An alternative explanation, at least for caffeine, stems from the observation that methylxanthines can release catecholamines from central nervous stores, an effect not attributable to an inhibition of phosphodiesterase (Berkowitz, Tarver & Spector, 1969). Thus, an alteration of endogenous catecholamine levels by caffeine may contribute to the observed antagonism.

Small doses of icv ouabain are capable of producing a marked potentiation of pentobarbitone sleeping time, which can be resolved into two components: (1) a direct effect involving an interaction at a central site, since peripherally-administered ouabain at the same dose level does not reproduce this potentiation, (2) an indirect effect, the decrease in body temperature produced by ouabain causing a potentiation of the barbiturate by decreasing its metabolism. In this respect the action of icv ouabain resembles that of DNP by the same route of administration (see Results III 5. i)), and both DNP and ouabain resemble chlorpromazine, which can prolong barbiturate-induced sleeping time in mice, guinea-pigs and dogs (Shepherd, Lader & Rodnight, 1968b). A further similarity between ouabain and DNP, following icv injection, is that neither agent potentiates the acute toxicity of pentobarbitone in doses which markedly potentiate its hypnotic activity.

The experiments involving apomorphine-induced compulsive gnawing in rats suggest that relatively large doses of ouabain (1 μg icv) can block central dopamine receptors, presumably in the caudate nucleus, whereas lower doses (0.3 μg icv) have no such effect. It is interesting to recall here that the higher dose (1 μg icv) can block C.A.R. in trained rats but the lower dose (0.3 μg icv) has no effect. It may be that a dopaminergic system is involved in these conditioned responses. Furthermore it would be expected that both these doses are capable of increasing dopamine synthesis, since 5×10^{-6} M ouabain inhibits the amine concentrating mechanism in adrenergic neurones (Berti & Shore, 1967b) and 1×10^{-5} M ouabain can increase the formation of C^{14}-catecholamines (Goldstein, Ohi & Backstrom, 1970). A dose of 1 μg/10 μl represents a concentration of 1.4×10^{-4} M and it may be that, while both 0.3 μg and 1 μg in the rat can increase dopamine synthesis, the higher dose can, in addition, block central dopamine receptors.

Apomorphine can also rapidly reverse the catalepsy induced by ouabain (0.3 μg icv) in the mouse and this suggests that ouabain produces its cataleptic effects through a reversible blockade of dopamine receptors, the dose required for an interaction with dopamine receptors after icv injection in the mouse being about one third of that required in the rat. Since no reversal of ouabain-induced hypothermia was produced by apomorphine, it is not the blockade of dopamine receptors which is responsible for this hypothermia. The earlier suggestion that the elevation of dopamine levels plays some part in the production of the hypothermia cannot be ruled out.

It is difficult to reconcile the observed pharmacological effects of centrally-administered ouabain with any one simple mode of action. The effects seen may, however, involve a dopaminergic component. Because of the known biochemical effects of ouabain and related glycosides, a number of tentative explanations of the observed central nervous depressant effects have been put forward. The interference with transmitter function may be pre- or post-synaptic, or a combination of both.

In view of the antagonistic effects of dexamphetamine and the thymoleptic desmethylimipramine, coupled with the overall similarity to reserpine in its behavioural effects (in the absence of peripheral action), icv ouabain might prove to be a satisfactory alternative tool to reserpine in the experimental evaluation of certain classes of anti-depressant drugs.

7. FIGURES AND TABLES

FIG. 33

Effect of dexamphetamine on ouabain-induced hypothermia in the mouse. (●——●) 10 µl saline icv + 10 ml/kg saline ip at the arrow; (▲——▲) 10 µl saline icv + 10 mg/kg dexamphetamine ip at the arrow; (Δ——Δ) 0.4 µg ouabain icv + 10 ml/kg saline ip at the arrow; (o——o) 0.4 µg ouabain icv + 10 mg/kg dexamphetamine ip at the arrow; (x——x) 0.4 µg ouabain icv + 5 mg/kg dexamphetamine ip at the arrow.

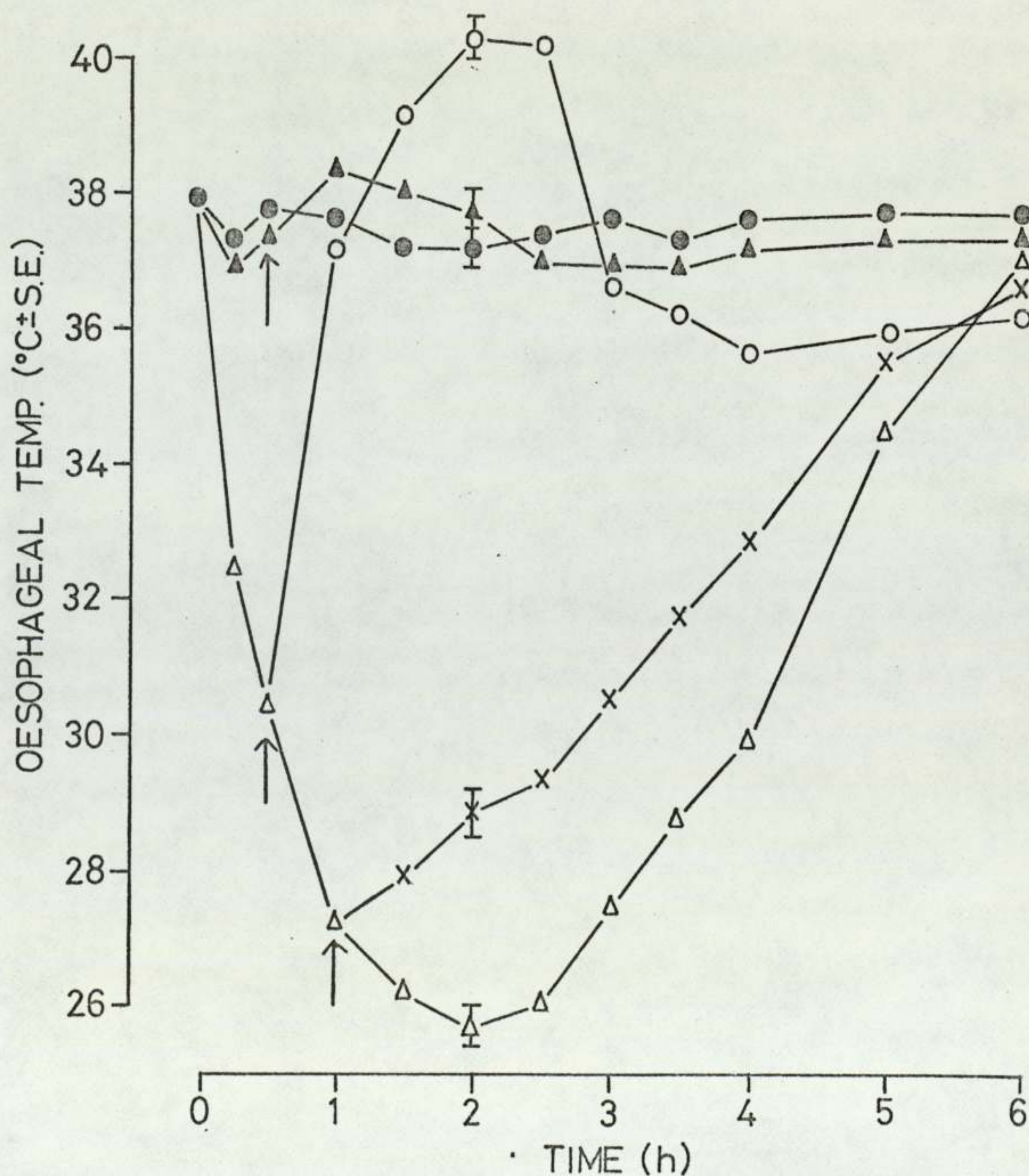

FIG. 34

Effect of desmethylimipramine pretreatment on ouabain-induced hypothermia in the mouse. (●——●) 10 ml/kg saline ip 5 min before 10 µl saline icv at time 0; (x——x) 5 mg/kg desmethylimipramine ip, (o——o) 10 mg/kg desmethylimipramine ip or (Δ——Δ) 10 ml/kg saline ip 5 min before 0.3 µg ouabain icv at time 0.

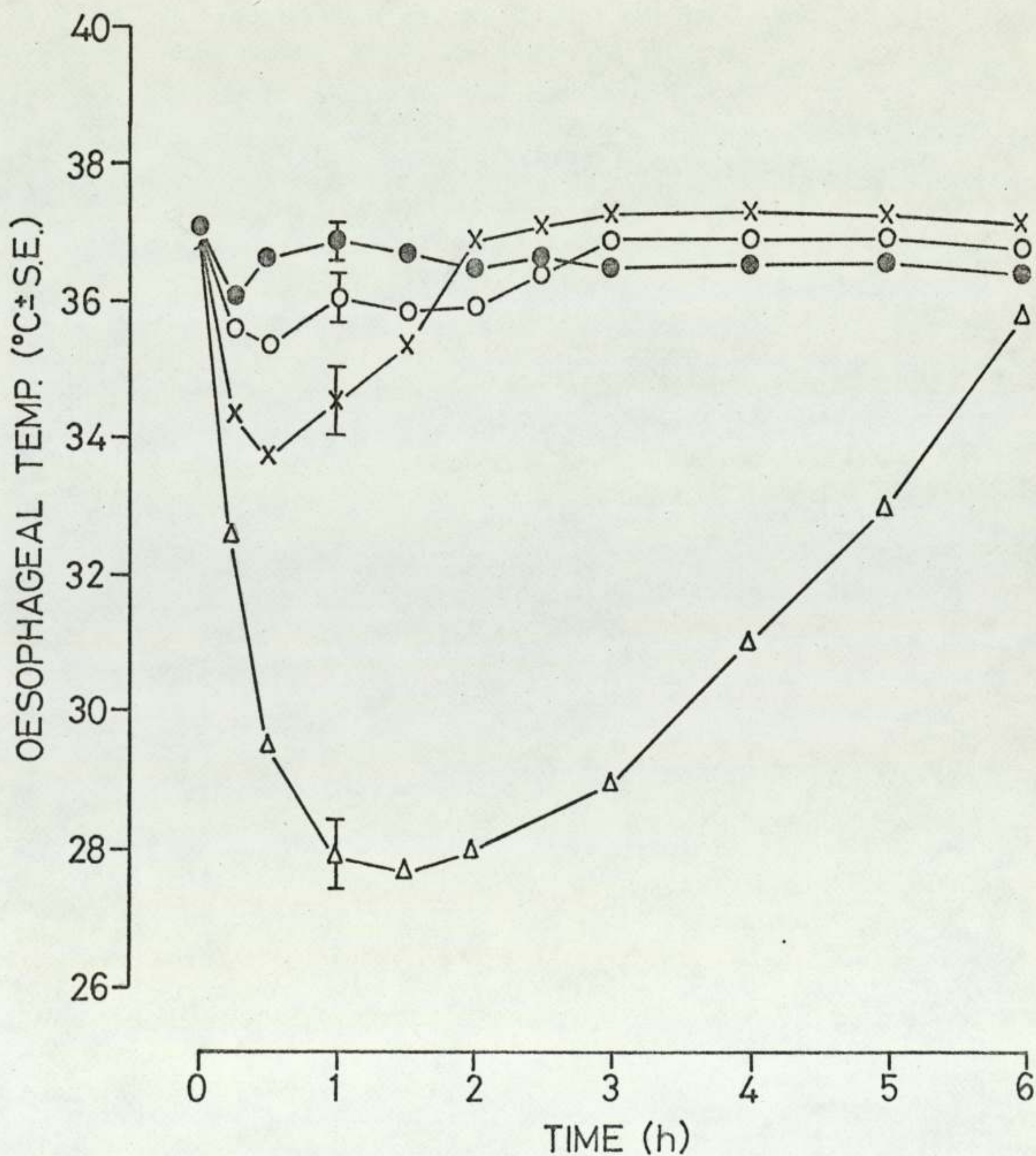

FIG. 35

Effect of nialamide pretreatment on ouabain-induced hypothermia in the mouse. (o——o) 10 mg/kg nialamide ip 2 h before 10 µl saline icv at time 0; (●——●) 10 mg/kg nialamide ip, (▲——▲) 20 mg/kg nialamide ip or (Δ——Δ) 10 ml/kg saline ip 2 h before 0.3 µg ouabain icv at time 0.

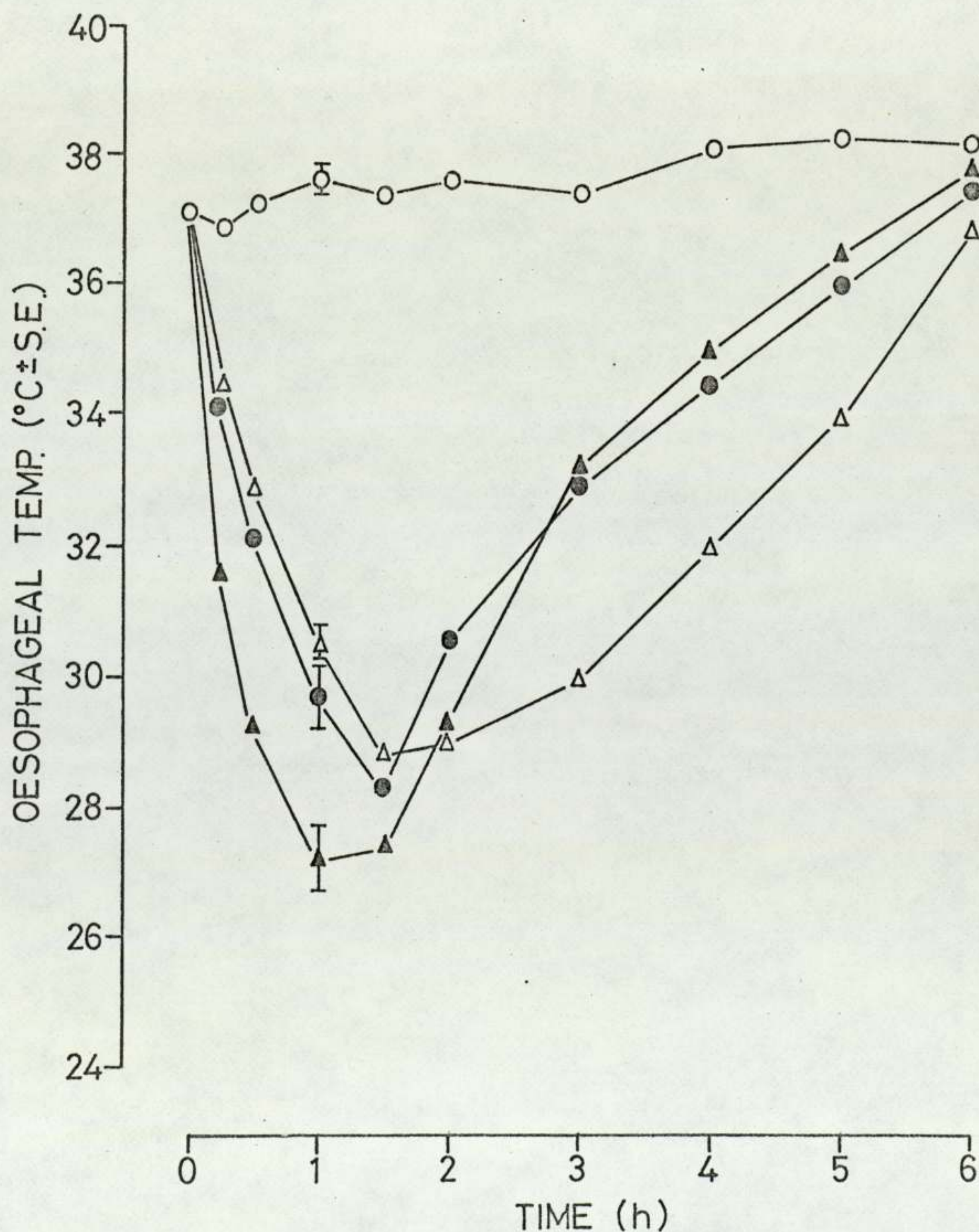

FIG. 36

Effect of icv noradrenaline, phentolamine and ouabain on the body temperature of the mouse. (o——o) 10 µl saline icv; (●——●) 5 µg noradrenaline icv; (x——x) 2 µg phentolamine icv; (Δ——Δ) 0.2 µg ouabain icv.

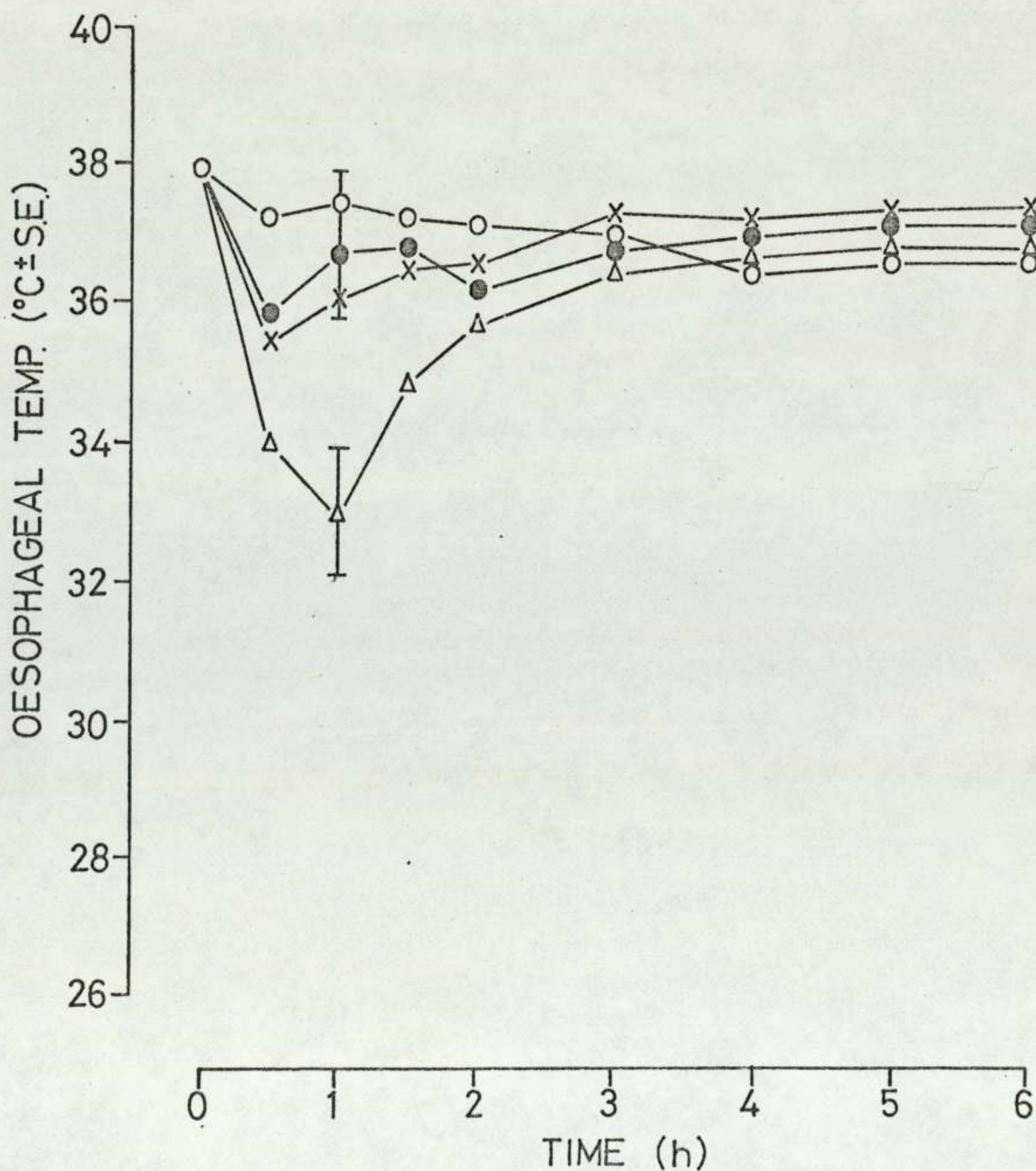

FIG. 37

Effect of concurrent administration of icv phentolamine on the hypothermia produced by either icv ouabain or icv noradrenaline in the mouse. (x——x) 2 μg phentolamine icv; (Δ——Δ) 0.2 μg ouabain icv; (●——●) 5 μg noradrenaline icv; (▲——▲) 2 μg phentolamine + 0.2 μg ouabain icv; (o——o) 2 μg phentolamine + 5 μg noradrenaline icv.

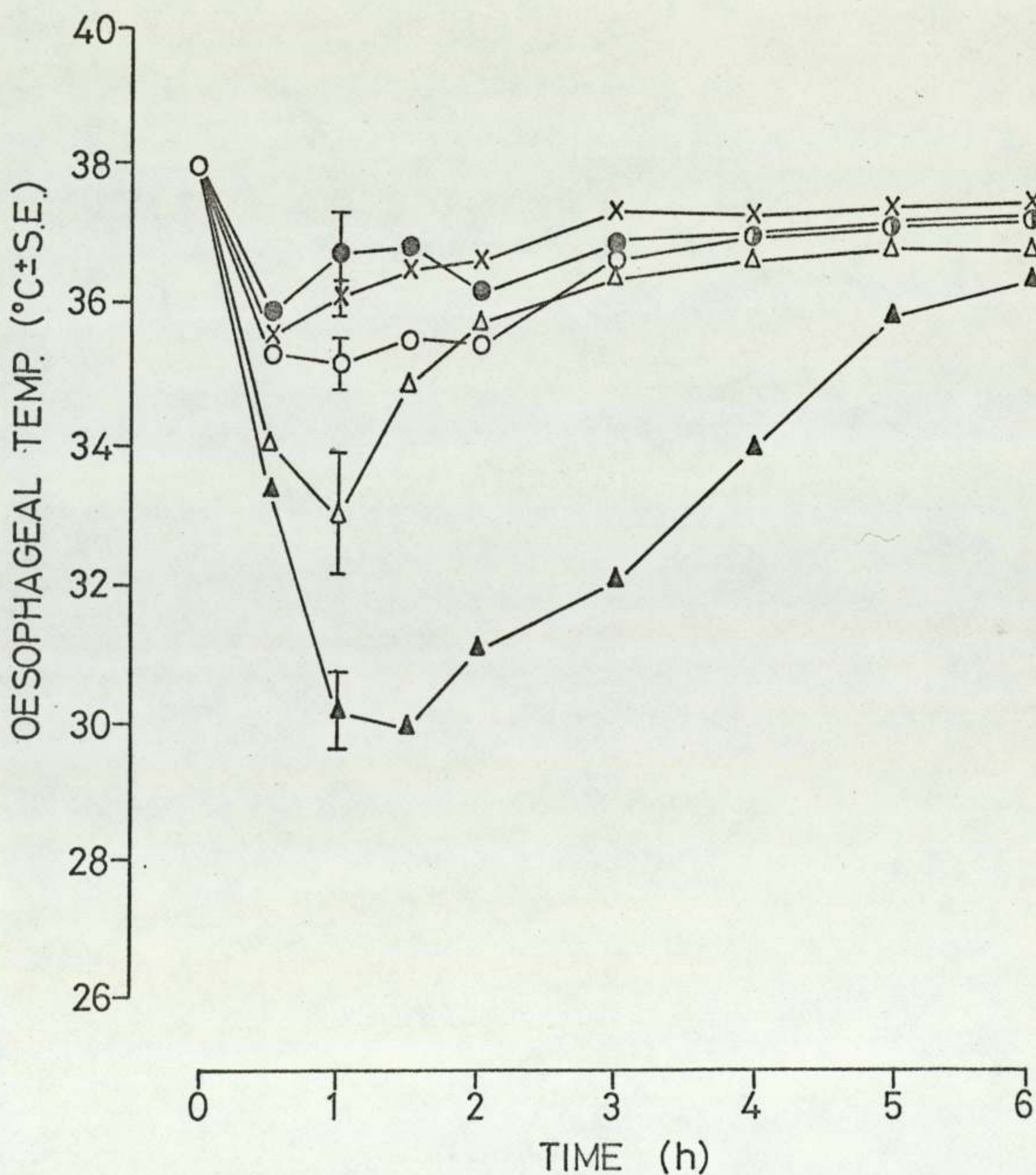

FIG. 38

Effect of concurrent administration of icv noradrenaline on the hypothermia produced by icv ouabain in the mouse. (o—o) 10 µl saline icv; (●—●) 5 µg noradrenaline icv; (Δ—Δ) 0.2 µg ouabain icv; (▲—▲) 5 µg noradrenaline + 0.2 µg ouabain icv.

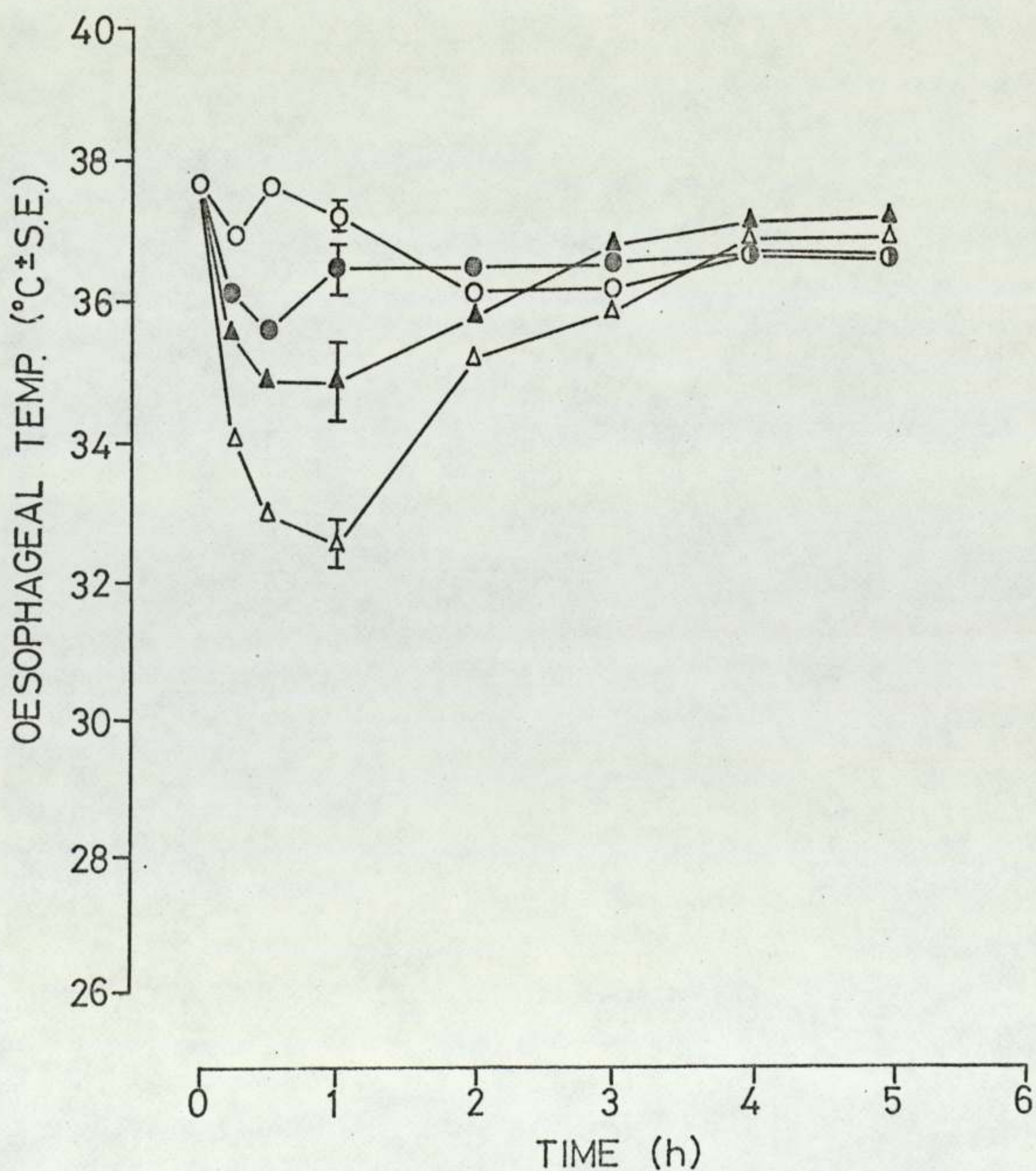

FIG. 39

Effect of α-methylmetatyrosine (α-MMT) pretreatment on ouabain-induced hypothermia in the mouse. (o——o) 400 mg/kg α-MMT ip 24 h before 10 μl saline icv; (▲——▲) 400 mg/kg α-MMT ip or (Δ——Δ) 10 ml/kg saline ip 24 h before 0.25 μg ouabain icv.

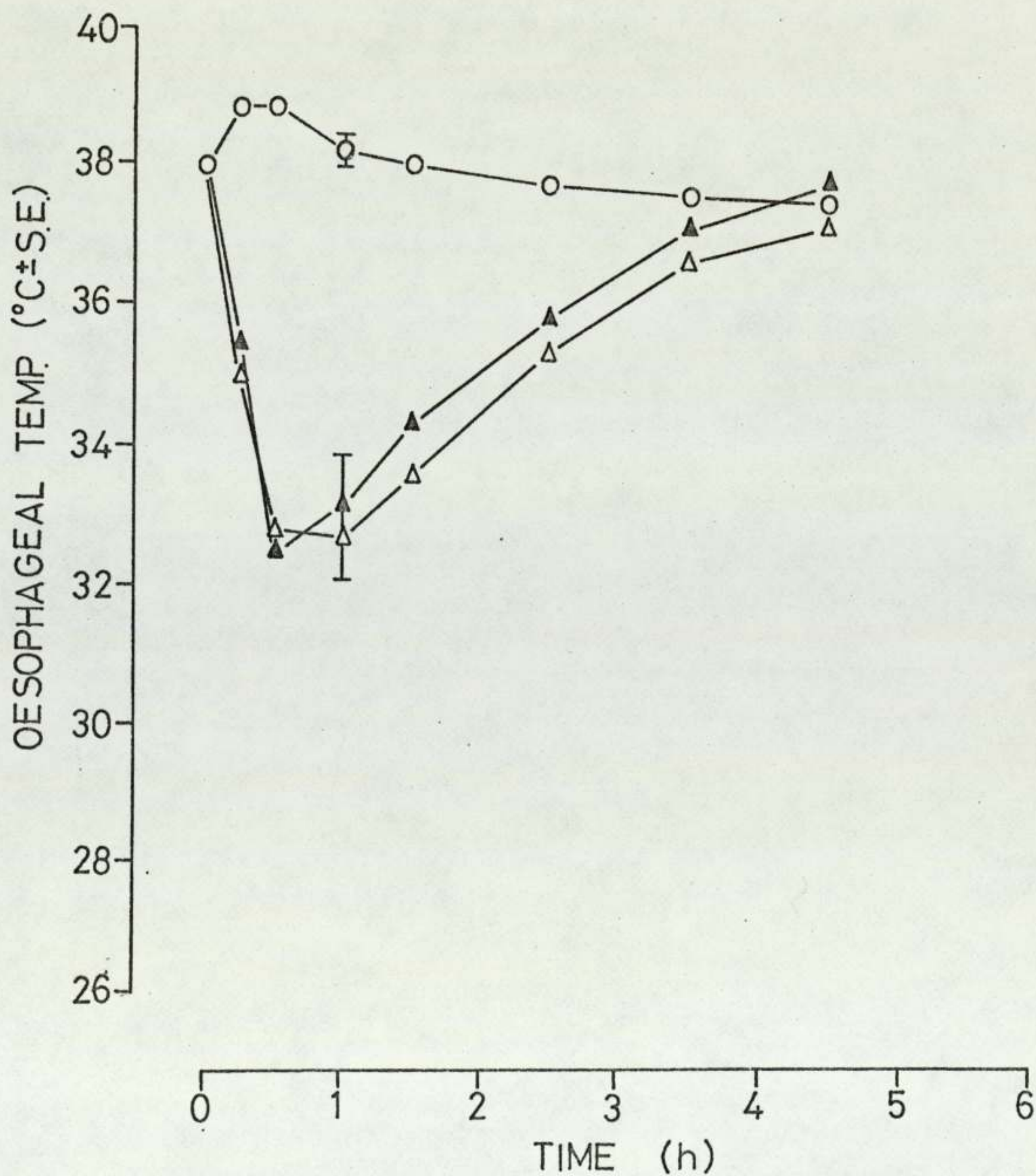

FIG. 40

Effect of concurrent administration of icv dibutyryl cyclic 3',5'-adenosine monophosphate (dbcAMP) on the hypothermia produced by icv ouabain in the mouse. (o——o) 10 μl saline icv; (●——●) 25 μg dbcAMP icv; (▲——▲) 25 μg dbcAMP + 0.3 μg ouabain icv; (Δ——Δ) 0.3 μg ouabain icv.

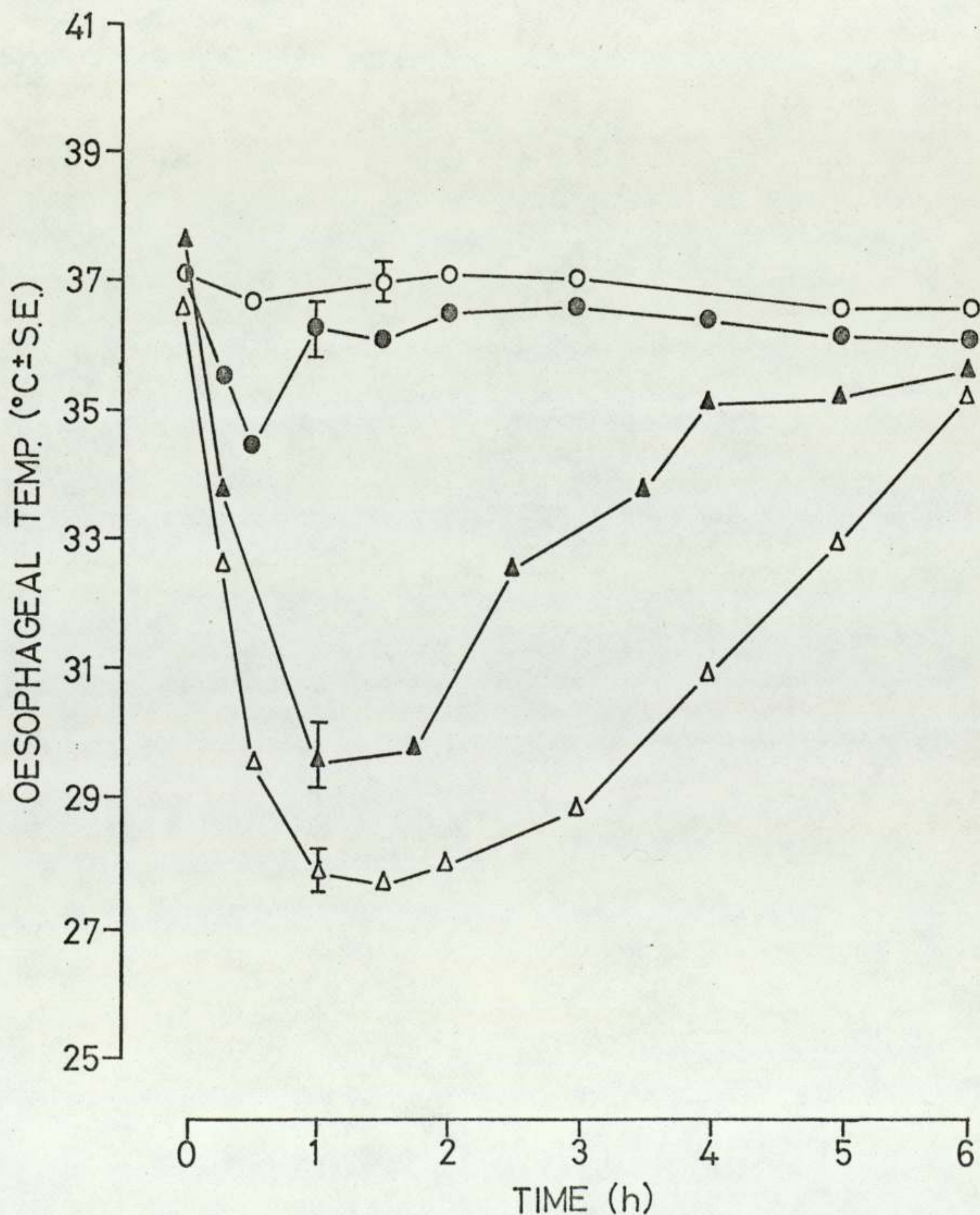

FIG. 41

Effect of caffeine on ouabain-induced hypothermia in the mouse. (o——o) 10 µl saline icv, 10 ml/kg saline ip; (●——●) 0.25 µg ouabain icv, 20 mg/kg caffeine ip; (▲——▲) 0.25 µg ouabain icv, 40 mg/kg caffeine ip; (Δ——Δ) 0.25 µg ouabain icv, 10 ml/kg saline ip.

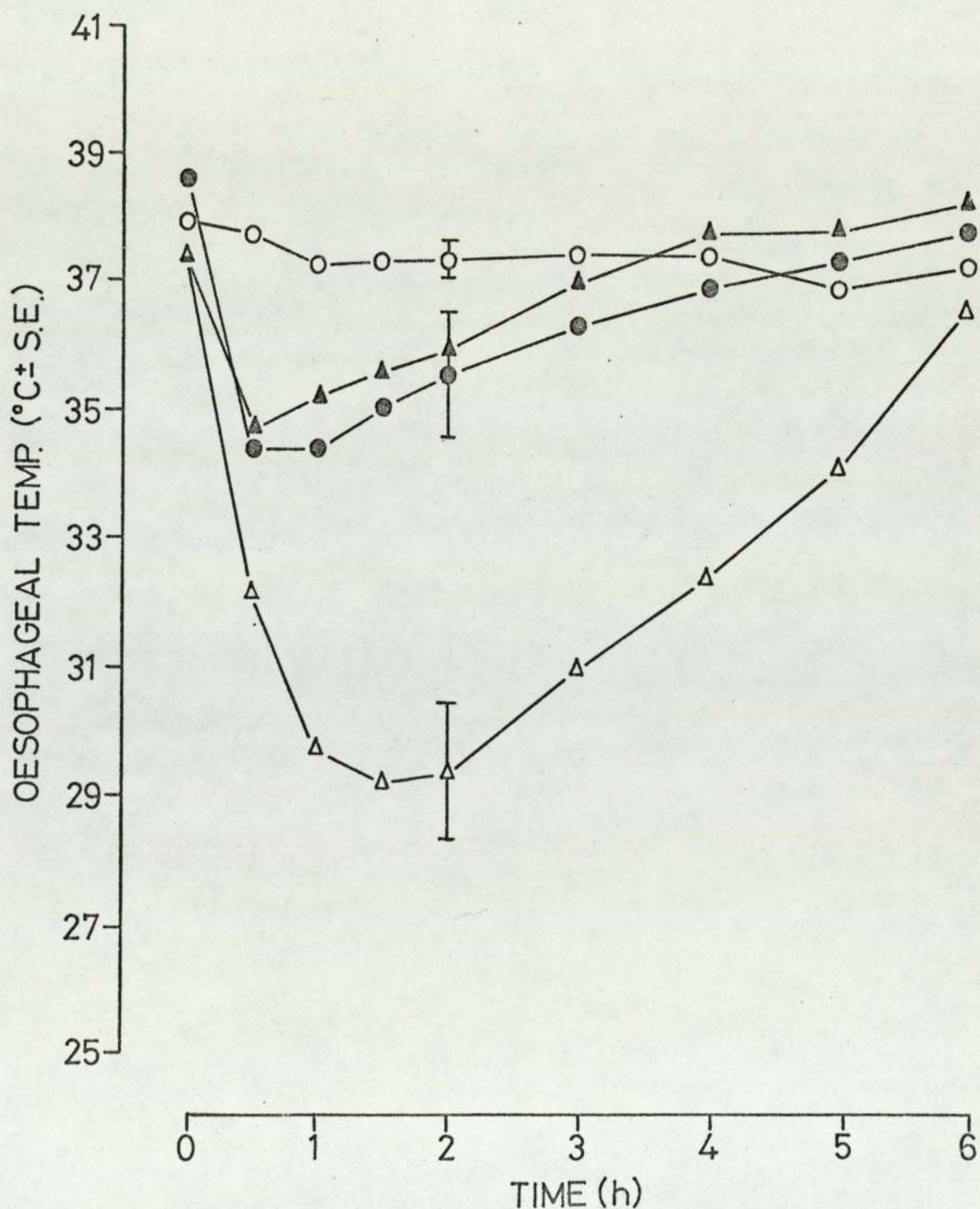

FIG. 42

Effect of peripherally-administered pentobarbitone on the hypothermia produced by icv ouabain in the mouse. Animals received 10 μl saline icv + (o——o) 10 ml/kg saline ip or (●——●) 20 mg/kg pentobarbitone ip at the arrow; (▲——▲) 0.1 μg ouabain icv + 20 mg/kg pentobarbitone ip at the arrow; (Δ——Δ) 0.2 μg ouabain icv + 20 mg/kg pentobarbitone ip at the arrow.

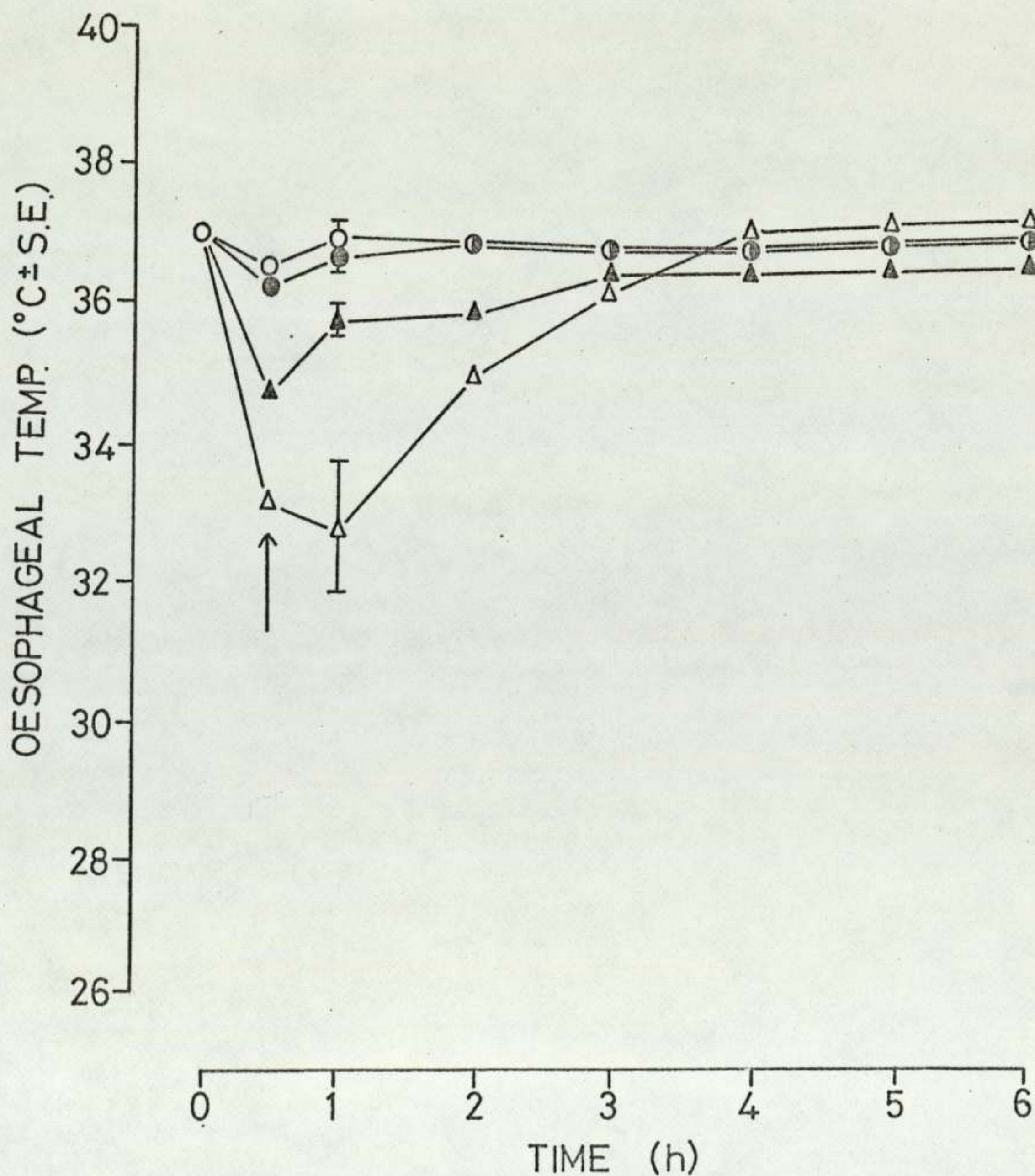

FIG. 43

Effect of apomorphine on ouabain-induced catalepsy in the mouse. (o—o) 0.3 μg ouabain icv, 10 ml/kg saline sc; (●—●) 0.3 μg ouabain icv, 2 mg/kg apomorphine sc.

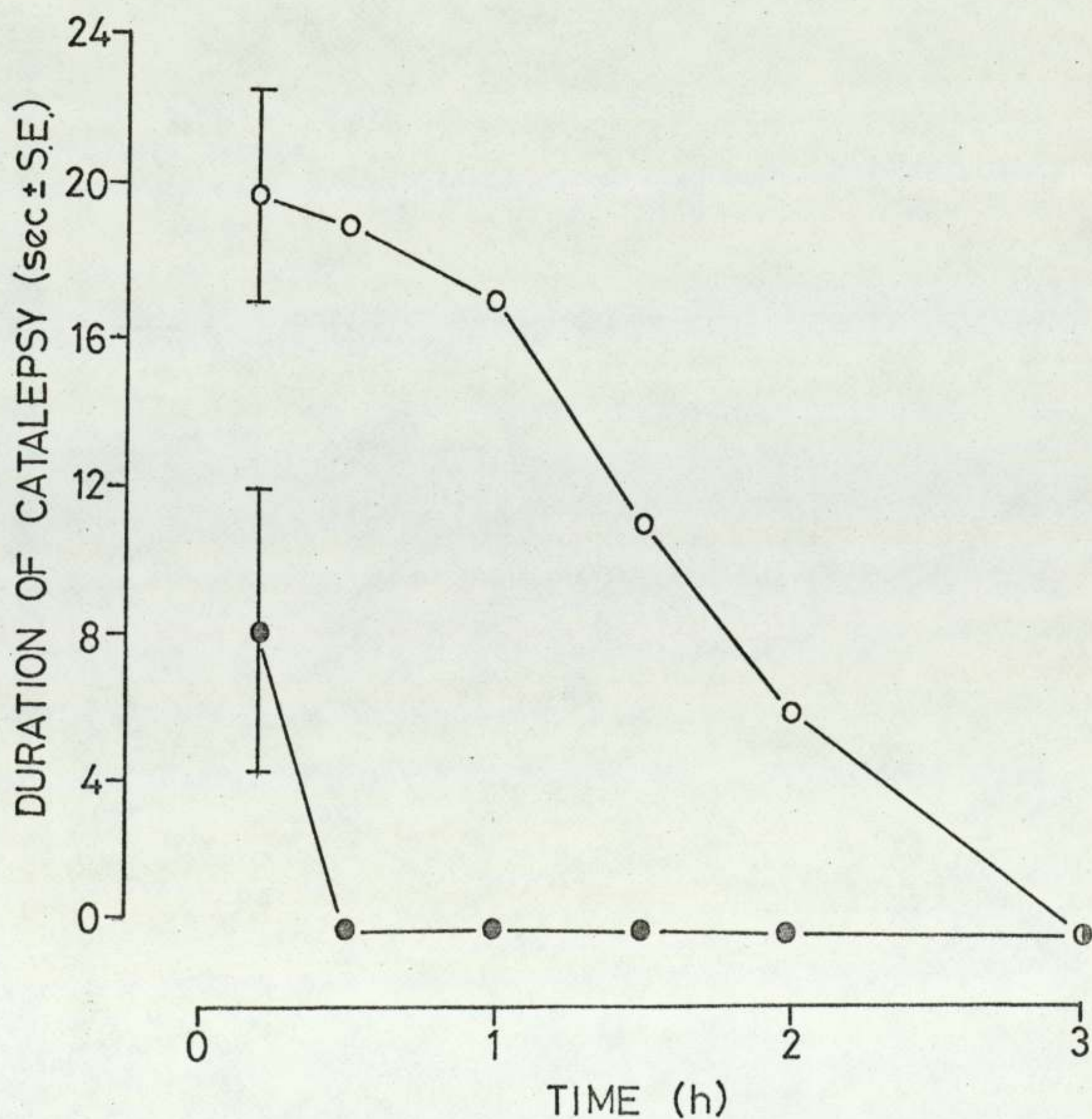

FIG. 44

Effect of apomorphine on ouabain-induced hypothermia in the mouse. (x——x) 10 µl saline icv, 10 ml/kg saline sc; (o——o) 0.2 µg ouabain icv, 10 ml/kg saline sc; (●——●) 0.2 µg ouabain icv, 20 mg/kg apomorphine sc.

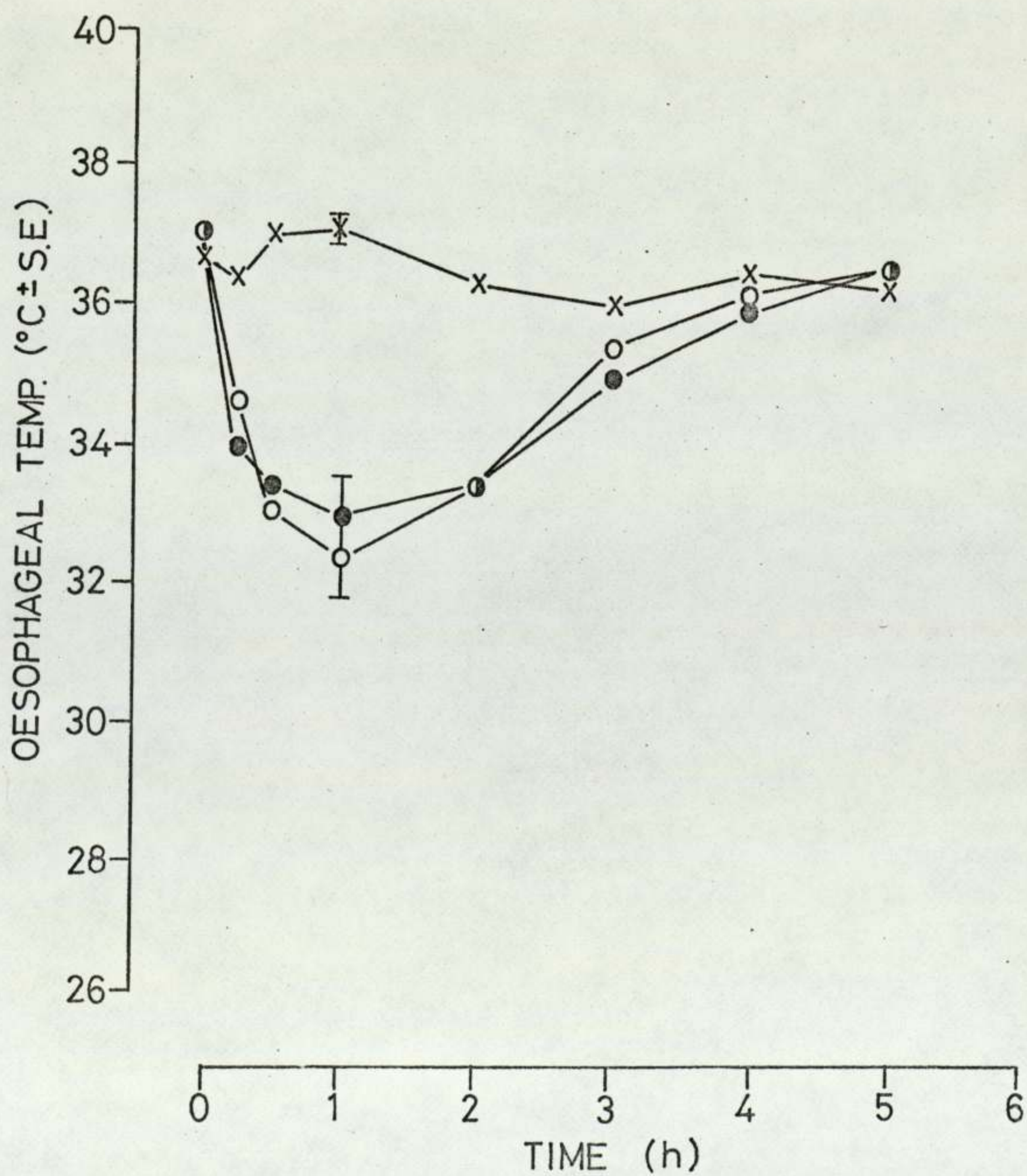

TABLE 14

Effect of ouabain on acute LD_{50} of dexamphetamine in the mouse, measured under crowded conditions. Animals received ouabain (0.1 μg icv) or saline (10 μl icv) immediately before dexamphetamine ip, and the results obtained 20 h later.

DOSE OF DEXAMPHETAMINE (mg/kg)	ALIVE / TESTED	
	saline icv	ouabain icv
5	16/16	14/16
15	11/16	3/16
35	2/16	2/16
55	2/16	1/16
75	2/16	1/16

PRETREATMENT	LD_{50} (mg/kg)	19/20 CONFIDENCE LIMITS
Dexamphetamine + Saline	21.5	12.7 —— 36.6
Dexamphetamine + Ouabain	14.0	7.8 —— 25.2

TABLE 15

Effect of icv ouabain on pentobarbitone hypnosis in the mouse. Animals received pentobarbitone (20 mg/kg ip) 30 min after saline (10 µl icv) or icv ouabain. (n = 10).

AMBIENT TEMPERATURE (^{o} C)	icv PRETREATMENT	ONSET TIME OF SLEEP GROUP MEAN (sec ± S.E.)	DURATION OF SLEEP GROUP MEAN (sec ± S.E.)
21 ± 1° C	saline	0	0
	ouabain 0.1 µg	318 ± 10	1018 ± 33
	ouabain 0.2 µg	486 ± 9	1794 ± 170
32 ± 1° C	saline	0	0
	ouabain 0.1 µg	230 ± 15	763 ± 21
	ouabain 0.2 µg	274 ± 10	1149 ± 69

TABLE 16

Effect of ouabain on acute LD_{50} of pentobarbitone in the mouse. Animals received ouabain (0.1 μg icv) or saline (10 μl icv) either (1) immediately after, or (2) 30 min after pentobarbitone ip, and the results obtained 20 h later.

DOSE OF PENTOBARBITONE (mg/kg)	ALIVE / TESTED			
	Saline icv		ouabain icv	
	(1)	(2)	(1)	(2)
80	10/10	10/10	10/10	10/10
90	10/10	10/10	8/10	9/10
100	7/10	7/10	6/10	8/10
110	1/10	1/10	0/10	4/10
120	3/10	3/10	1/10	0/10
130	0/10	0/10	0/10	0/10

PRETREATMENT	LD_{50} (mg/kg)		19/20 CONFIDENCE LIMITS	
	(1)	(2)	(1)	(2)
Pentobarbitone + saline	105	105	97.2-113.4	97.2-113.4
Pentobarbitone + ouabain	104	112	96.3-112.3	98.3-127.7

TABLE 17

Effect of icv ouabain on apomorphine-induced compulsive gnawing in the rat. Animals received ouabain (0.3 or 1.0 µg icv) or saline (10 µl icv) immediately before apomorphine (2 or 3 mg/kg sc).

TIME (min)	N$^{\underline{o}}$. OF ANIMALS GNAWING			
	Apomorphine (2 mg/kg sc)	Apomorphine (3 mg/kg sc)	Apomorphine (2 mg/kg sc) + ouabain (0.3 µg icv)	Apomorphine (2 mg/kg sc) + ouabain (1.0 µg icv)
10	4/4	4/4	5/6	0/4
15	4/4	4/4	6/6	0/4
20	4/4	4/4	6/6	0/4
25	4/4	4/4	6/6	1/4
30	4/4	4/4	6/6	1/4
35	4/4	4/4	6/6	0/4
40	4/4	4/4	6/6	0/4
45	4/4	4/4	3/6	0/4
50	2/4	3/4	0/6	0/4
55	0/4	3/4	0/6	0/4
60	0/4	2/4	0/6	0/4
65	0/4	0/4	0/6	0/4
70	0/4	0/4	0/6	0/4

VI MODIFICATION OF THE ANTI-NOCICEPTIVE ACTIVITY OF MORPHINE BY CENTRALLY-ADMINISTERED OUABAIN AND DOPAMINE IN THE MOUSE

VI Modification of the anti-nociceptive activity of morphine by centrally-administered ouabain and dopamine in the mouse

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VI There have been a number of attempts to explain the central pharmacological actions of morphine. Usually the experiments have involved a study of the interaction of morphine and its congeners with centrally occurring biogenic substances, such as noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine.

That variations in the brain levels of these endogenous substances and their metabolites can alter the anti-nociceptive activity of morphine is well established (see Harris, 1970). Previous investigations of the interaction between dopamine and morphine have yielded contradictory data. Most recently Calcutt & Spencer (1971) have shown that icv dopamine in large doses (for example 50 μg) antagonises the anti-nociceptive action of morphine in mice, whereas earlier work by Saarnivaara (1969) suggested that an elevation of central dopamine levels would enhance the activity of morphine.

Anagnoste & Goldstein (1967) found that large doses of ouabain (25 - 75 μg icv) reduce the formation of noradrenaline in the hypothalamus, brain stem and cerebellum, producing at the same time an increase in dopamine levels in these areas. Earlier (see Results IV) it was shown that much smaller doses of ouabain (0.1 - 0.4 μg) given by icv injection produce a marked depression of central nervous activity, associated with unchanged brain levels of noradrenaline and 5-hydroxytryptamine, together with a large increase in whole-brain dopamine levels. Consequently, it seemed of interest to examine the anti-nociceptive activity of morphine in the presence of small doses of ouabain or dopamine, given by icv injection in the mouse.

1. Anti-nociceptive activity of morphine and ouabain

Morphine possesses anti-nociceptive activity after either sc or icv injection, and this can be demonstrated by means of the tail flick test in mice (fig. 45). In contrast, ouabain exhibits significant anti-nociceptive activity in the tail flick test only after icv injection (fig. 46), and then only at dose levels also associated with marked central nervous depression and hypothermia. Yet, doses of ouabain up to 10 μg/mouse sc produced no significant increase in the tail-flick reaction time.

Experiments designed to test the anti-nociceptive activity of ouabain in the tail clip test revealed that the hypothermia produced by ouabain may itself play a part in the anti-nociceptive activity of this glycoside after central administration. Administration of ouabain (0.05 - 0.2 μg icv) at an ambient temperature of 21 $\pm$ 1°C produced anti-nociceptive activity only in doses previously shown to be accompanied by large falls in body temperature (fig. 47a). Repeating the experiment at 32 $\pm$ 1°C (which prevents marked hypothermia from developing - see Results IV 1. i)) showed that an anti-nociceptive effect was still apparent, although it was much reduced (fig. 47b). Thus, even though the hypothermia predisposes the animals to behavioural depression, accompanied by a decrease in sensitivity to this painful stimulus, the ouabain-induced anti-nociceptive activity may be mediated in part through a direct action independent of the fall in body temperature.

2. Potentiation of the anti-nociceptive activity of morphine by centrally-administered ouabain

Ouabain exhibited anti-nociceptive activity in the tail flick test at doses of 0.2 µg and above, when given by icv injection. At a lower dose (0.1 µg icv) ouabain produced no anti-nociceptive activity alone, although some depression of central nervous activity and slight hypothermia ($1^{o}C$) maximal ½ h after injection, were observed. Consequently, this dose of ouabain was chosen to investigate the interaction between ouabain and morphine. Ouabain (0.1 µg icv) markedly potentiated the anti-nociceptive activity of morphine (2.5 mg/kg sc), when given either 15 min before (fig. 48) or 15 min after morphine (fig. 49).

3. Potentiation of the anti-nociceptive activity of morphine by centrally-administered dopamine

Previous work showed that, after icv ouabain, there is a marked increase in whole-brain dopamine levels (see Results IV 5.), and a possible explanation of the ouabain-induced potentiation of morphine observed above is an increased availability of central dopamine. Since studies with dopamine itself are conflicting, further experiments were performed, in which the anti-nociceptive activity of morphine (2.5 mg/kg sc) was determined in the presence of small doses of dopamine (5 µg icv). Dopamine produced an immediate enhancement of morphine, which was maximal within 45 min of the dopamine injection (fig. 50).

4. Effect of centrally-administered ouabain and dopamine on morphine-induced hypothermia

Since icv ouabain can induce hypothermia, the possibility exists that its potentiation of the anti-nociceptive activity of morphine may be due to an indirect effect on the metabolism of morphine, brought about by this fall in body temperature. Morphine alone, at the dose level used in these experiments (2.5 mg/kg sc), induces an initial marginal level of hypothermia, which is also enhanced by ouabain (0.1 μg icv). These results are summarised in fig. 51. Whilst this small increase in hypothermic response may play a part in the later stages of the anti-nociceptive activity of the ouabain/morphine combination, that this is not the sole mechanism of potentiation is clear from the rapidity with which this potentiation appears, as well as the small difference in body temperature ($2^{o}C$) between the group receiving morphine alone and the group receiving morphine + ouabain. When the body temperatures of animals receiving morphine (2.5 mg/kg sc) alone and morphine (2.5 mg/kg sc) + dopamine (5 μg icv) were compared, there was no significant difference in the degrees of hypothermia observed (fig. 51). Thus an enhancement of hypothermia is also clearly not the mechanism by which dopamine potentiates the anti-nociceptive activity of morphine.

5. Discussion

Small doses of centrally-administered dopamine potentiate the anti-nociceptive activity of morphine in the mouse. Similarly, small doses of icv ouabain also enhance the action of morphine. Since icv ouabain produces an increase in whole-brain dopamine levels, it is tempting to explain the observed potentiation of morphine on this basis. However, it is important to note that the ouabain-induced potentiation of morphine occurs

immediately after the icv injection of ouabain, a time-course not entirely consistent with the suggestion that an increase in whole-brain dopamine levels is the mechanism by which this potentiation is brought about. Nevertheless, the length of time needed for a substantial change in dopamine levels at a specific localised site might be relatively short. It is also known that there is an immediate, short-lasting excitation in mice after ventricular puncture, which might be associated with an increased impulse flow in central neurones. In the case of a ouabain injection, this could lead to an immediate marked localised increase in dopamine levels.

It is known that sodium diethyldithiocarbamate (DDC) is an inhibitor of the enzyme dopamine-β-hydroxylase in the brain (Carlsson, Lindqvist, Fuxe & Hökfelt, 1966; Carlsson, Fuxe & Hökfelt, 1967), and its administration is associated with a decrease in noradrenaline and an increase in dopamine levels. In Results VII it is shown that small doses of DDC (20 μg icv) produce an immediate and marked potentiation of the anti-nociceptive activity of morphine in the mouse, a finding which has subsequently been confirmed by Zebrowska-Lupina, Kleinrok & Smolarz (1971). Earlier work (Watanabe, Matsui & Iwata, 1969) showed that DDC is capable of potentiating the anti-nociceptive activity of morphine soon after injection of the former in rats, when both noradrenaline and dopamine levels are "normal". Again, whereas the time-course of events is not entirely consistent with a mechanism of potentiation involving an elevation of dopamine levels, it is possible that important, immediate, localised changes in dopamine levels are induced by icv DDC (cf. icv ouabain), which in turn are responsible for the potentiation of the anti-nociceptive activity of morphine.

A recent paper (Asghar & Way, 1970) indicates that the active removal of morphine from the cerebral ventricles can be inhibited by ouabain, and

the authors have suggested that the major proportion of morphine is transported into the blood after its removal from the ventricular system by the choroid plexus. Other workers (Miller & Elliott, 1955) have already shown that morphine localises in the choroid plexus after peripheral administration. Thus, it may be that ouabain blocks the uptake of morphine into pharmacologically inactive sites, and this possibility must merit some consideration in the observed potentiation of morphine.

The present observations with small doses of icv dopamine are at variance with earlier studies (Calcutt & Spencer, 1971), which showed that large doses of dopamine (50 μg icv) are capable of antagonising the anti-nociceptive activity of morphine. It may be that these opposing effects reflect differences in distribution of the icv-administered dopamine within the brain. Equally, it may be that after large doses of dopamine, sufficient noradrenaline is formed to antagonise morphine. This latter hypothesis is supported by the findings of Glowinski & Iversen (1966), who have demonstrated that within 15 min of an icv injection of C^{14}-dopamine in the rat, C^{14}-noradrenaline could be detected in both the hypothalamus and medulla. Furthermore, Sparkes & Spencer (1969) and Calcutt & Spencer (1971) showed that icv noradrenaline antagonises the anti-nociceptive activity of morphine in both the rat and the mouse.

Although it is clear that small doses of ouabain and dopamine given by icv injection can potentiate the anti-nociceptive activity of morphine, the observed efffects of ouabain may not be produced solely by an effect upon central dopamine stores.

6. FIGURES

FIG. 45

Effect of morphine on the nociceptive threshold of the mouse in the tail flick test. (x——x) 10 µl saline icv; (●——●) 5 mg/kg morphine sc; (o——o) 1.5 µg morphine icv.

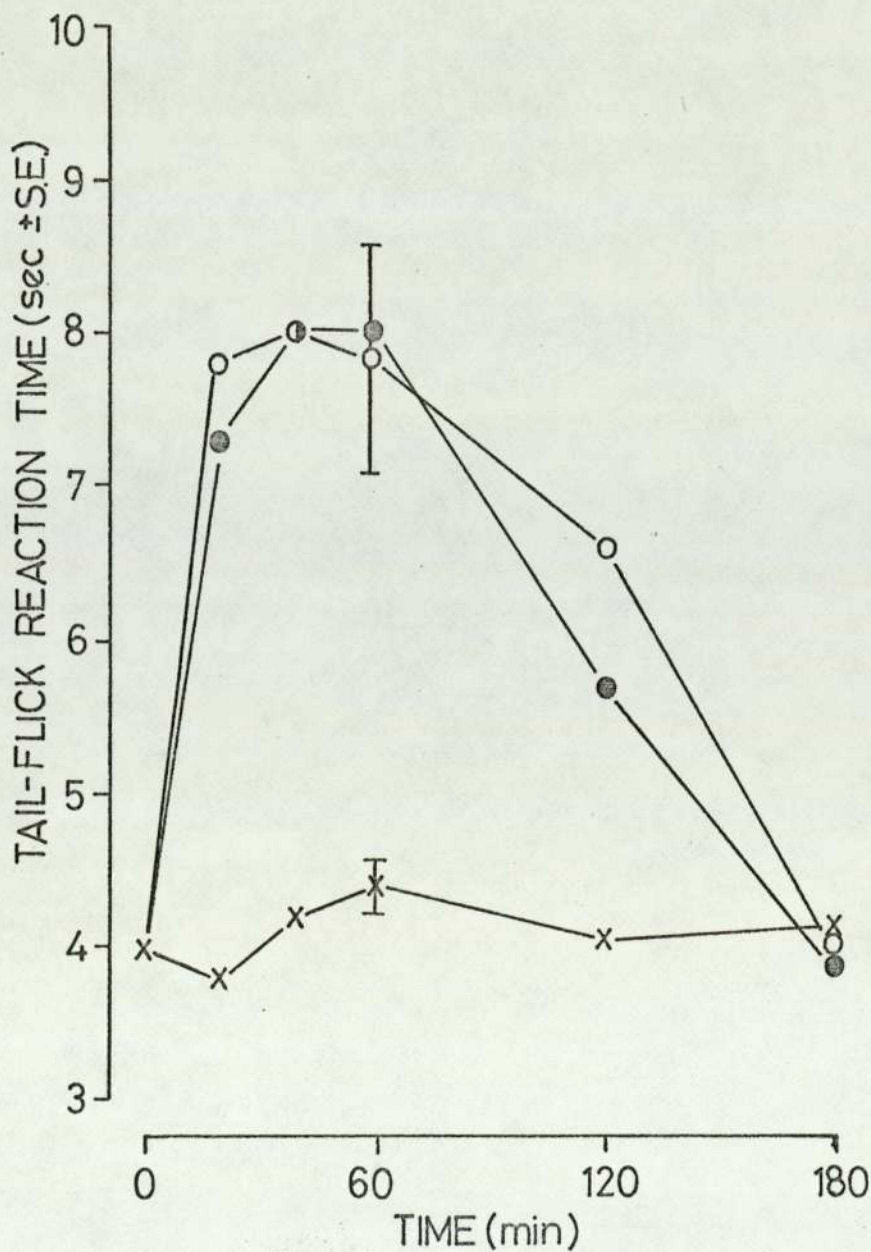


FIG. 46

Effect of icv ouabain on the nociceptive threshold of the mouse in the tail flick test. (Δ——Δ) 0.1 μg ouabain; (o——o) 0.2 μg ouabain; (●——●) 0.3 μg ouabain.

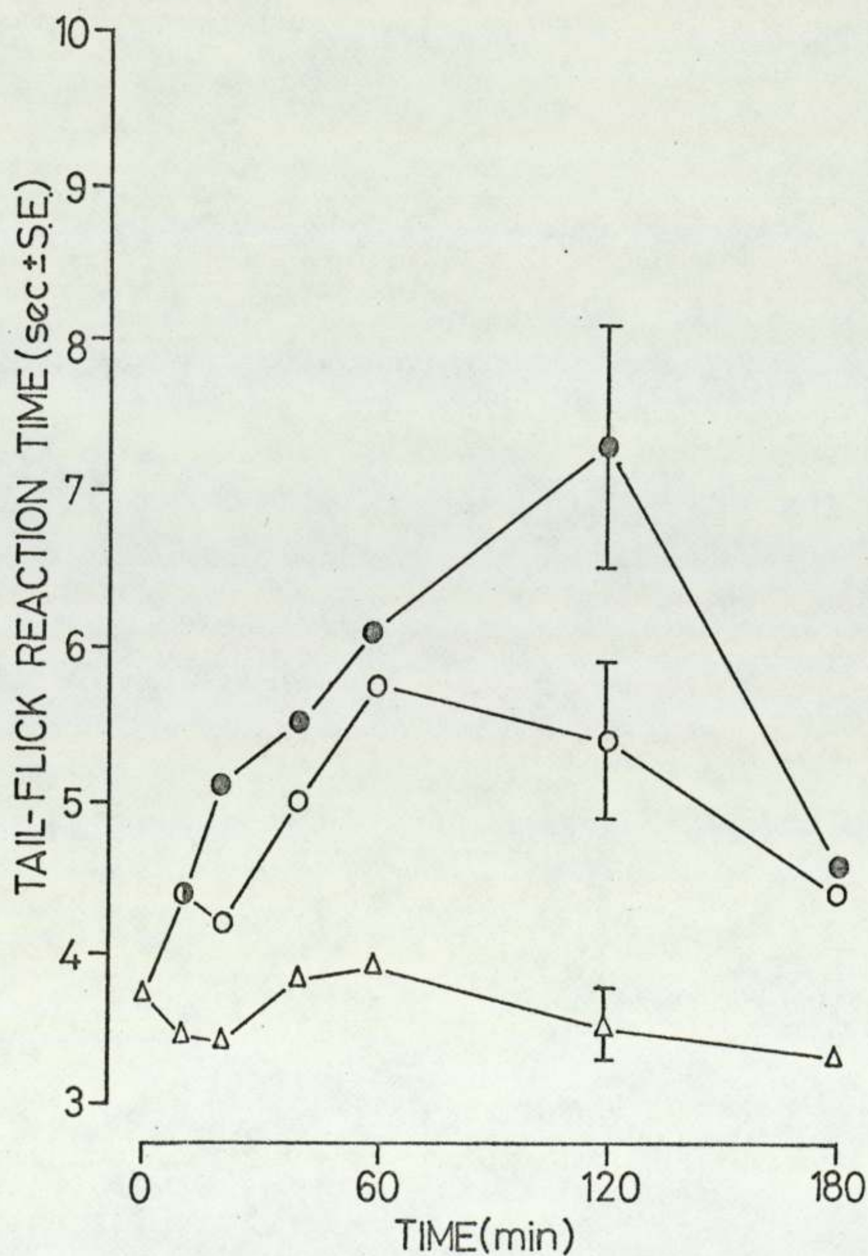


FIG. 47

Effect of icv ouabain on the nociceptive threshold of the mouse in the tail clip test at different ambient temperatures: (a) at 21 ± 1° C; (b) at 32 ± 1° C. Animals received (x——x) 10 μl saline; (o——o) 0.05 μg ouabain: (●——●) 0.1 μg ouabain; (Δ——Δ) 0.2 μg ouabain; (▲——▲) 0.3 μg ouabain.

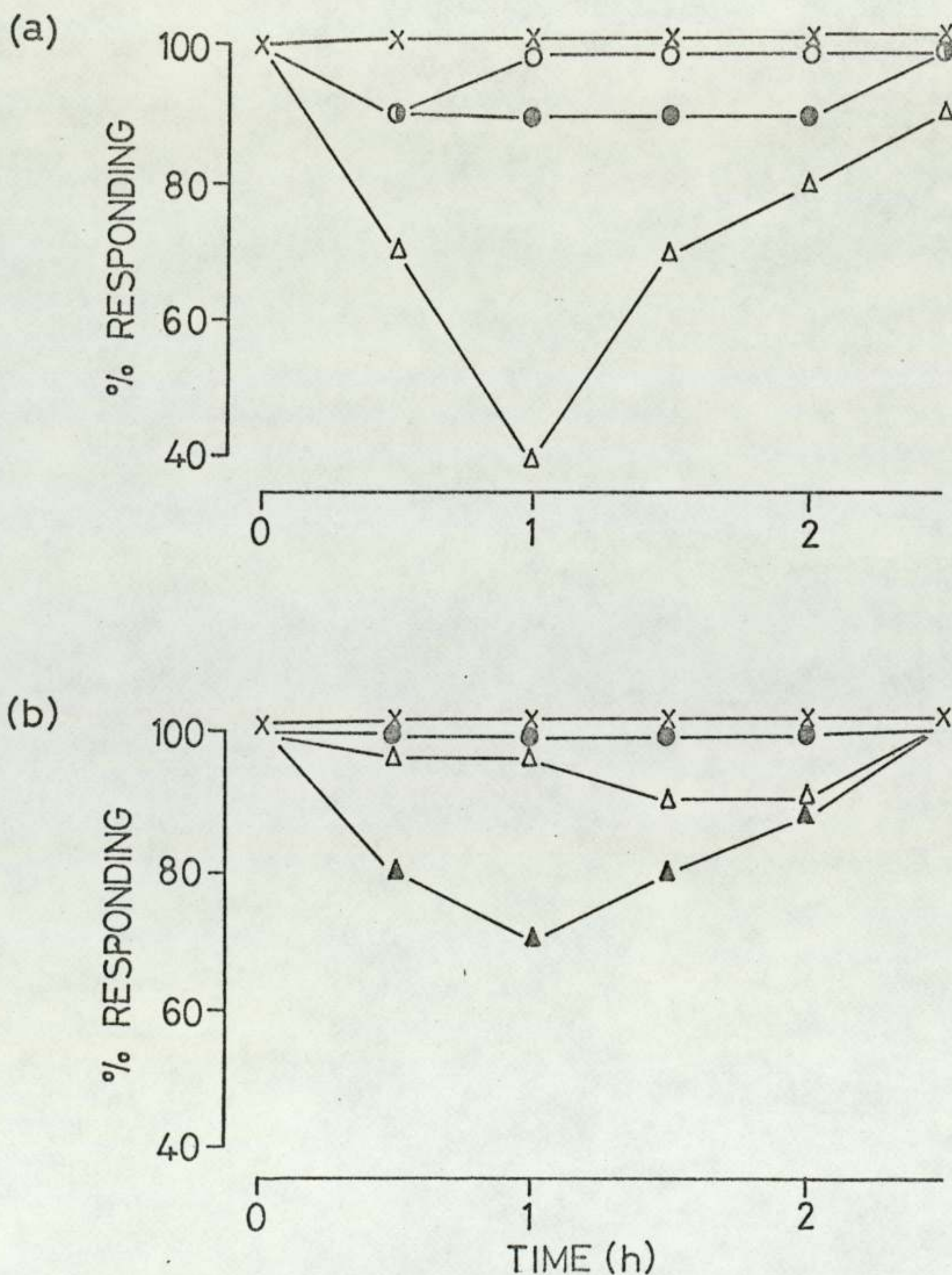


FIG. 48

Effect of icv ouabain on the anti-nociceptive activity of morphine in the tail flick test. Mice received (o—o) 10 µl saline icv or (●—●) 0.1 µg ouabain icv 15 min before 2.5 mg/kg morphine sc at time 0.

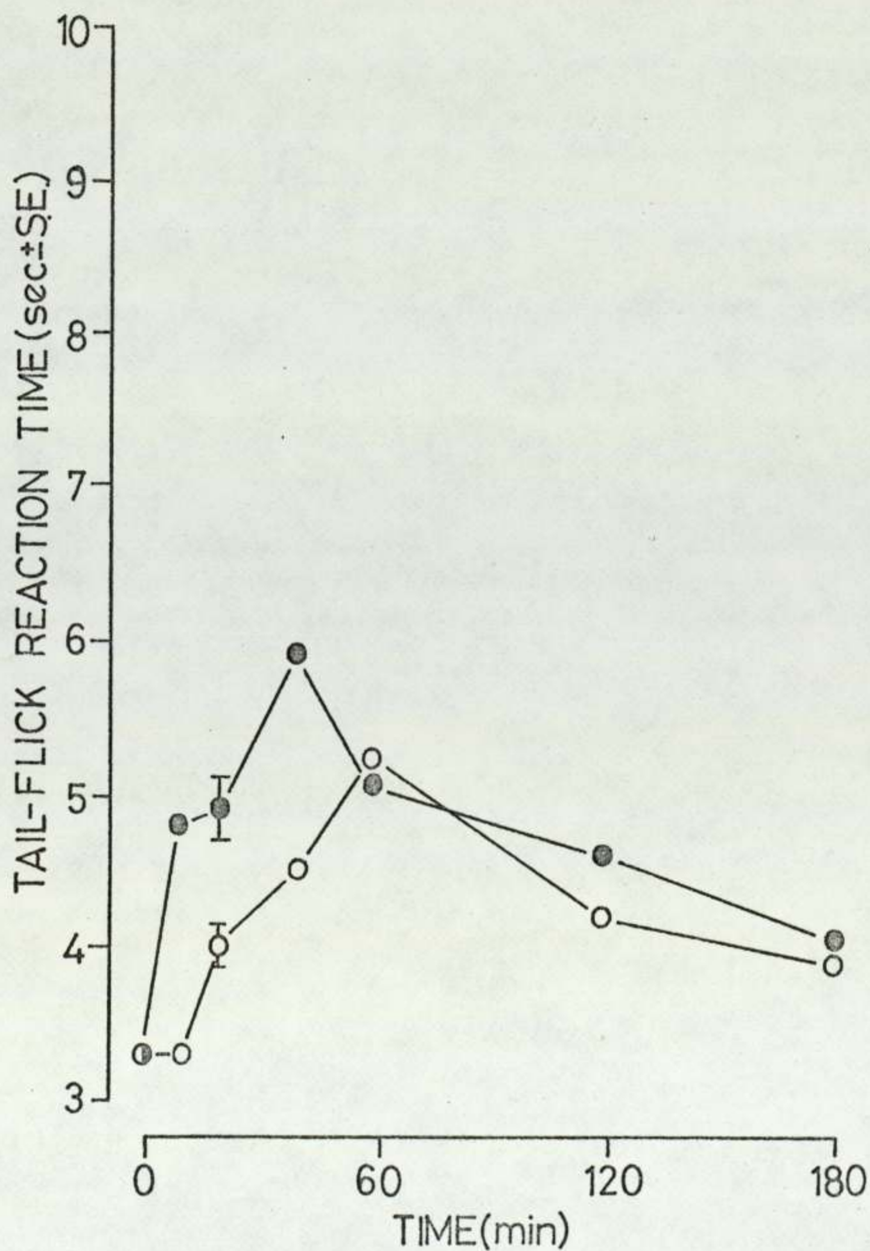


FIG. 49

Effect of icv ouabain on the anti-nociceptive activity of morphine in the tail flick test. Mice received 2.5 mg/kg morphine sc at time 0, 15 min before (o—o) 10 µl saline icv or (●—●) 0.1 µg ouabain icv at the arrow.

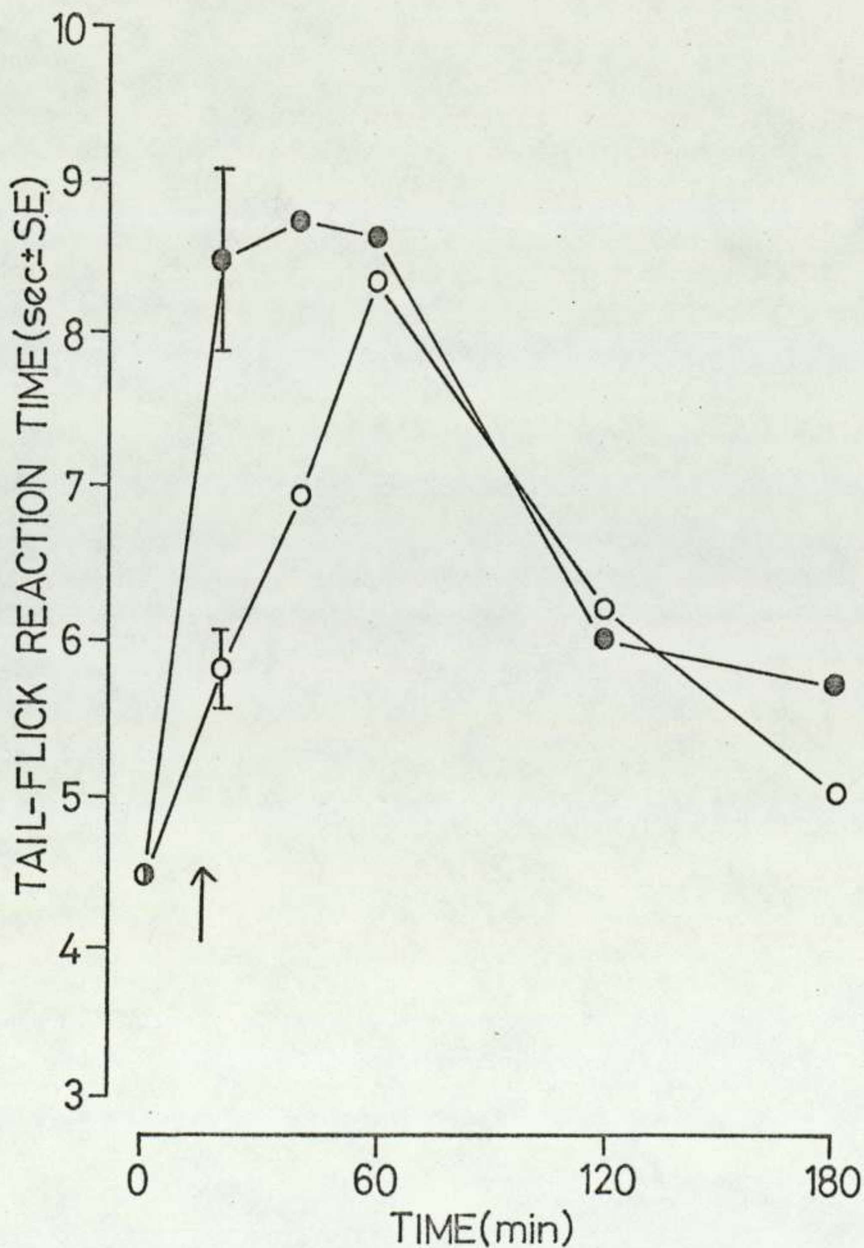


FIG. 50

Effect of icv dopamine on the anti-nociceptive activity of morphine in the tail flick test. (x——x) 10 ml/kg saline sc at time 0 + 5 μg dopamine icv at the arrow; (o——o) 2.5 mg/kg morphine sc at time 0 + 10 μl saline icv at the arrow; (●——●) 2.5 mg/kg morphine sc at time 0 + 5 μg dopamine icv at the arrow.

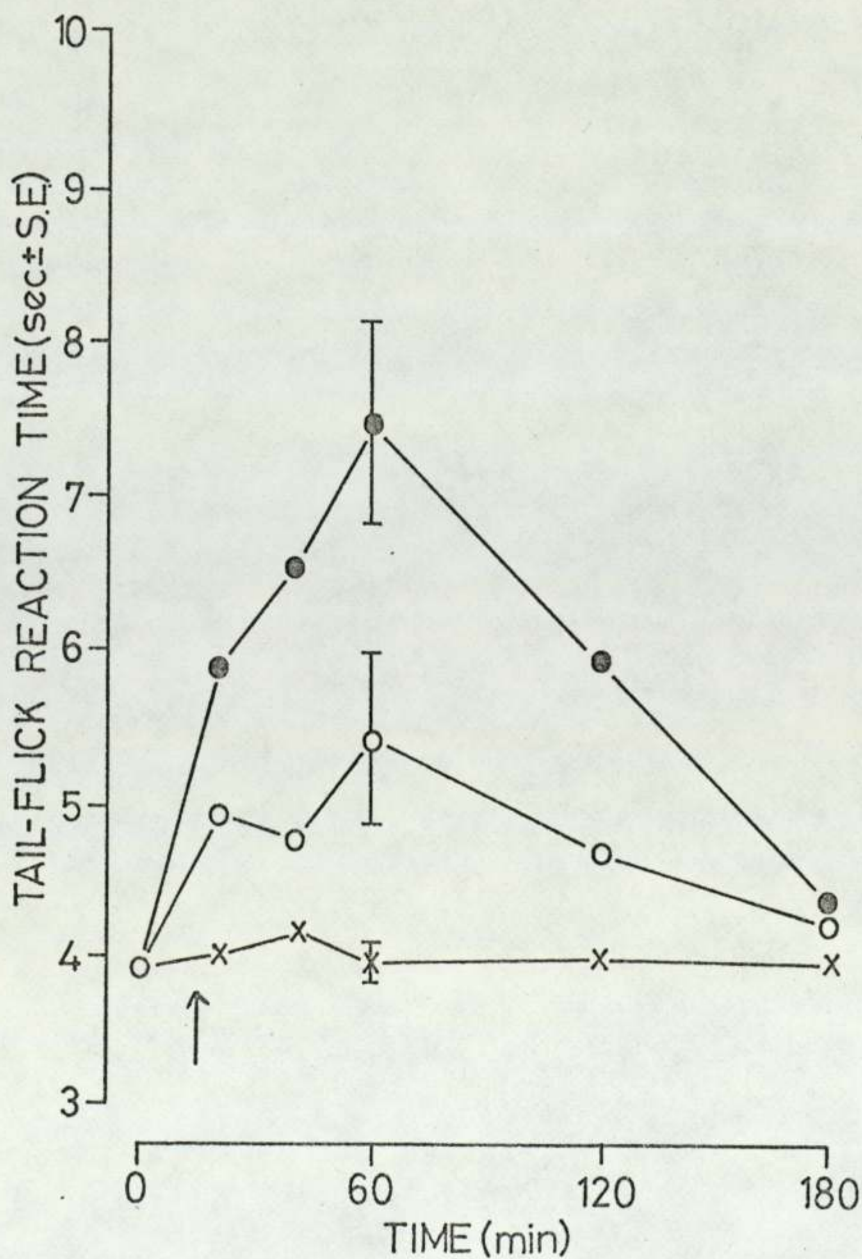
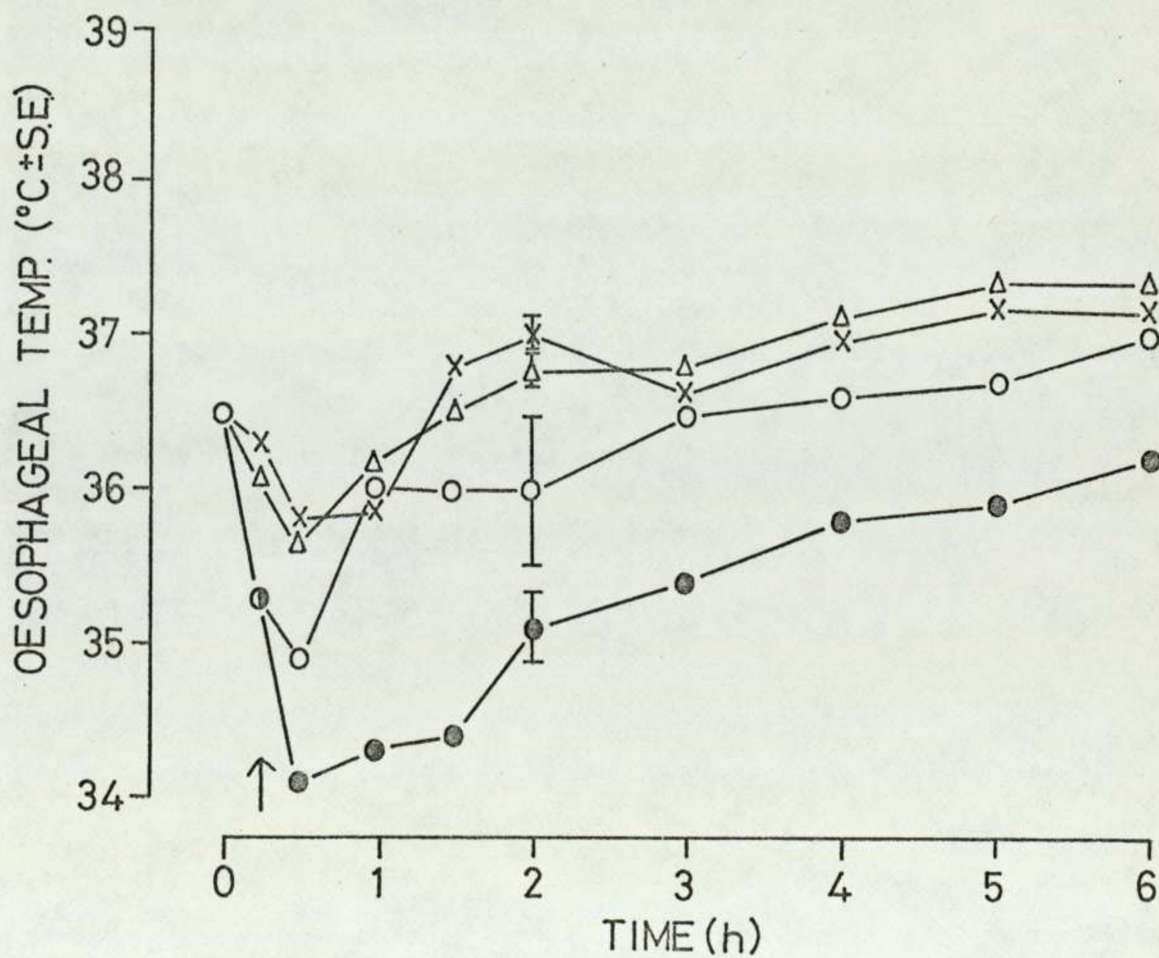


FIG. 51

Effect of icv ouabain or icv dopamine on morphine-induced body temperature changes in the mouse. Animals received (o——o) 0.1 μg ouabain icv at time 0 + 10 ml/kg saline at the arrow; (x——x) 10 μl saline icv, (●——●) 0.1 μg ouabain icv or (Δ——Δ) 5 μg dopamine icv at time 0 + 2.5 mg/kg morphine sc at the arrow.



VII COMPARISON OF THE PHARMACOLOGICAL EFFECTS OF CENTRALLY-ADMINISTERED OUABAIN WITH THOSE OF OTHER AGENTS INTERFERING WITH NEUROTRANSMITTER FUNCTION IN THE CENTRAL NERVOUS SYSTEM

VII Comparison of the pharmacological effects of centrally-administered ouabain with those of other agents interfering with neurotransmitter function in the central nervous system

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VII In order to investigate further the mechanism of action of centrally-administered ouabain, a comparison was made with a number of other drugs given by icv injection. Ouabain, given in small doses by this route of administration, is capable of markedly increasing dopamine levels and producing a small non-significant decrease in noradrenaline levels in the brain. Sodium diethyldithiocarbamate (DDC), given by icv injection in rats (Kleinrok, Zebrowska & Wielosz, 1970), causes an increase in dopamine levels and a decrease in noradrenaline levels in the brain; 6-hydroxydopamine causes a long lasting depletion of both dopamine and noradrenaline from the brains of rats (Uretsky & Iversen, 1970). Consequently a comparison was made of these two agents with icv ouabain.

Studies on the mechanism of amine accumulation by the adrenergic neurone have revealed the existence of two separate amine concentrating mechanisms, one operating as an amine pump at the neurone membrane, the other operating intracellularly at the levels of amine storage granules (Carlsson, 1966; Giachetti & Shore, 1966). The membrane pump is blocked by cocaine, desmethylimipramine (and its congeners), chlorpromazine and ouabain, whereas the granular mechanism is blocked specifically by reserpine and tetrabenazine. The effects of cocaine, desmethylimipramine, chlorpromazine and tetrabenazine were therefore studied following their icv injection. Since dibutyryl cyclic 3',5'-adenosine monophosphate (dbcAMP) can antagonise the effects of icv ouabain, and because icv ouabain produces similar effects to peripherally-administered reserpine, the effect of dbcAMP on the effects produced by reserpine were studied.

1. Effect of icv sodium diethyldithiocarbamate (DDC) in the mouse

i) General pharmacological effects

Many authors have described different behavioural changes in animals treated with DDC - an active metabolite of disulfiram. It has been suggested that these changes are dependent on the depletion of brain noradrenaline or on the increase of brain dopamine (Pfeifer, Galambos & György, 1966; Randrup & Scheel-Krüger, 1966; Scheel-Krüger & Randrup, 1967; Krantz & Seiden, 1968). The behavioural changes described include a reduction of locomotor activity and hypothermia.

In the present experiments four groups of 5 mice were given DDC (0.2 - 2 mg icv) and all animals exhibited a dose-dependent hypothermia which lasted for about 3 h (fig. 52), accompanied by catalepsy, ptosis and a reduction in locomotor activity. The animals did not group together, but remained isolated in the cage, unlike the controls. Within 10 sec of injecting the highest dose of DDC (2 mg icv) 40 % of the animals exhibited tonic convulsions, which were followed by death. The effects of icv DDC appeared to be qualitatively similar to those observed after icv ouabain. Furthermore, a dose of dexamphetamine (2 mg/kg ip) which produced no hyperthermia alone, immediately reversed the hypothermia (fig. 53) and behavioural depression induced by DDC (1 mg icv). Whereas dexamphetamine can reverse the depressant effects of icv DDC, injection of the latter does not sensitise the animals to dexamphetamine, unlike ouabain, which does so.

Desmethylimipramine (5 and 10 mg/kg ip) pretreatment was also capable of reversing both the hypothermic (fig. 54) and behavioural effects of DDC (1 mg icv). This pretreatment also resulted in death from convulsions

in mice following icv injection of 1 mg DDC, a dose which did not produce convulsions when administered alone. In contrast, nialamide (20 mg/kg ip), given 2 h previously, was unable to antagonise the effects of DDC (1 mg icv), producing instead a biphasic response similar to that seen after icv ouabain (fig. 55).

Thus the interaction of desmethylimipramine and nialamide with icv DDC parallels their interaction with icv ouabain, while that of dexamphetamine, though similar, is not identical.

ii) Effect on the anti-nociceptive activity of morphine

Four groups of 10 mice received morphine (2.5 mg/kg sc), followed by DDC (20 - 500 μg icv) or saline (10 μl icv) 15 min later. Two other groups received DDC (100 and 500 μg icv) alone. The animals were tested for nociceptive sensitivity by means of the tail flick test at intervals after the injection of morphine or DDC. It was found that icv DDC produced a rapid, marked potentiation of the anti-nociceptive activity of morphine in doses which, when administered alone, produced no significant increase in tail-flick reaction times (fig. 56).

2. Effect of icv 6-hydroxydopamine in the mouse

The injection of 6-hydroxydopamine (1 - 50 μg icv) into four groups of 5 mice was followed by a fall in body temperature of up to 4°C, which lasted for about 2 h, the degree of hypothermia increasing with increased dosage (fig. 57). No ptosis was observed and catalepsy was present only in those animals receiving higher doses (25 and 50 μg icv). All animals showed a decrease in locomotor activity compared with controls, an observ-

ation previously made in the rat by Laverty & Taylor (1970).

3. Effect of icv cocaine and desmethylimipramine in the mouse

Cocaine (1 - 100 μg icv) was injected into four groups of 5 mice. No significant effect on body temperature was observed, there was no decrease in spontaneous locomotor activity, no ptosis and no decreased responsiveness to external stimuli characteristic of central nervous depression. On the contrary, within the dose range investigated, the animals showed an increased locomotor activity and excitability and an increased responsiveness to external stimuli.

In a further experiment four groups of 5 mice received desmethylimipramine (1 - 10 μg icv). Again there was no significant hypothermia or central nervous depression, but an increased excitability and responsiveness similar to that seen after cocaine. No significant effects on locomotor activity were observed.

4. Effect of icv chlorpromazine and tetrabenazine

i) General pharmacological effects in the mouse

Centrally-administered chlorpromazine (1 - 10 μg icv) was injected into groups of 5 mice. A small dose-dependent hypothermia (fig. 58) accompanied by a decrease in locomotor activity was produced. Catalepsy was present for about 25 min after injection, although no ptosis was seen. The animals exhibited similar but more transient behaviour to that seen after peripheral administration of neuroleptics, and they remained isolated in the cage, not moving until touched.

In an experiment to investigate the pharmacological properties of a centrally acting reserpine-like drug after icv injection, tetrabenazine (1 - 50 µg icv) was given at five dose levels to groups of 5 mice. It produced no significant effect on body temperature. Within 1 min of injection marked ptosis appeared, together with a decrease in locomotor activity, and catalepsy developed after 5 min. Ptosis had disappeared within 90 min and catalepsy within 20 min of injection, the animals rapidly becoming indistinguishable from controls. Except for the lack of hypothermia, icv tetrabenazine produced similar effects to those produced by peripherally-administered reserpine, although they had a much shorter time-course.

ii) Effect of chlorpromazine on conditioned avoidance behaviour in the rat

In view of the marked similarity of icv ouabain to peripherally-administered neuroleptics on conditioned avoidance behaviour in the rat (see Results IV 4.), the activity of centrally-administered chlorpromazine on conditioned avoidance behaviour was next studied.

Groups of 4 rats received chlorpromazine (50 µg icv) and were tested 10 min after injection. This dose produced a selective abolition of C.A.R. with no effect on U.R. (table 18). A decrease in locomotor activity was apparent within 2 min of injection and catalepsy developed within 8 min. As in mice, no ptosis was observed. C.A.R. was returned to control levels within 30 min of injection.

5. Effect of icv dibutyryl cyclic 3',5'-adenosine monophosphate (dbcAMP) on reserpine-induced hypothermia and behavioural depression in the mouse

Earlier experiments (see Results V 3.) showed that icv dbcAMP can antagonise the hypothermia and behavioural depressant effects produced by icv ouabain. Since icv ouabain produces behavioural effects similar to those of peripherally-administered reserpine, and Abdulla & Hamadah (1970) have suggested that reserpine ptosis can be reversed by dbcAMP, the interaction between icv dbcAMP and peripherally-administered reserpine was next examined.

Two groups of 8 mice received reserpine (2 mg/kg ip) 20 h before the administration of dbcAMP (25 µg icv) or saline (10 µl icv). The body temperature of the mice fell to about 25° C at 20 h after reserpine. The dbcAMP produced a greater rate of recovery of body temperature towards normal levels, which became apparent after 1 h, and reached a maximum after 2 h (fig. 59). It may be that this delayed onset time reflects a metabolic effect of dbcAMP. There was, however, no observable reversal of the reserpine-induced ptosis produced by icv dbcAMP, nor any marked reversal of the associated behavioural depression.

6. Discussion

There is an overall similarity in the pharmacological effects of icv ouabain and icv DDC. The hypothermia produced by centrally-administered DDC was of a qualitatively similar nature to, and of approximately the same duration as, that reported after its peripheral administration (Barnett & Taber, 1968), although after icv injection DDC acted in doses approximately 600 times lower than those used peripherally by these authors.

The effects of both icv ouabain and icv DDC could be antagonised by dexamphetamine and desmethylimipramine, whereas nialamide had a biphasic effect against both ouabain and DDC. Unlike ouabain, DDC failed to sensitise the animals to dexamphetamine. Ouabain did sensitise them to both the behavioural and hyperthermic effects of dexamphetamine, and this probably reflects the ability of ouabain to block amine uptake in nerve terminals. Furthermore, Maj & Przegaliński (1967) demonstrated an interaction between disulfiram (of which DDC is an active metabolite) and amphetamine, showing that the former could block amphetamine-induced hyperactivity in mice, thus supporting an antagonism of, but not a sensitisation to, the effects of amphetamines by DDC.

Desmethylimipramine not only antagonised the hypothermia and behavioural effects produced by icv DDC, but also predisposed DDC-treated animals to convulsions. It was found that DDC (2 mg icv) caused tonic extensor spasm and death in 40 % of animals within 10 sec of injection. It seems likely that this may be caused by the high tonicity and pH of the DDC solution (see Results I 2. iii)). However, doses of DDC which do not cause convulsions when given alone (1 mg icv), do so if the animals have been pretreated with desmethylimipramine. Since tricyclic antidepressants in toxic doses do cause convulsions (Stille & Sayers, 1964), it may be that, at lower doses, desmethylimipramine may act as a predisposing factor in the production of the observed convulsions.

Barnett & Taber (1968) found that pargyline pretreatment did not significantly affect the hypothermia produced by peripherally-administered DDC, in contrast to the small biphasic response seen with centrally-administered DDC. The depletion of noradrenaline, as well as the elevation of dopamine levels, could play a part in the production of the pharmacol-

ogical effects following DDC administration, since Kleinrok, Zebrowska & Wielosz (1970) showed that, following icv administration in rats, a large reduction in body temperature and motility was observed during maximum decreases of brain noradrenaline and concomitant increases of brain dopamine. If this is so, desmethylimipramine may produce its effect by elevating the concentration of noradrenaline at the receptor sites after DDC injection. The biphasic effect of monoamine oxidase inhibition may also be explained by an alteration in both noradrenaline and dopamine levels, since both these may be elevated after nialamide pretreatment.

A further similarity between icv ouabain and icv DDC is their effect on the anti-nociceptive activity of morphine. Results presented in section VI showed that icv ouabain produced an immediate potentiation of morphine, in doses which had no anti-nociceptive activity when administered alone. Similarly, small doses of icv DDC produced an immediate potentiation of morphine. Watanabe, Matsui & Iwata (1969) also showed potentiation of the anti-nociceptive activity of morphine by DDC given peripherally to rats, and this has been confirmed in mice, again following peripheral administration of DDC (Maj, Sowinska, Boran & Durek, 1971; Zebrowska-Lupina, Kleinrok & Smolarz, 1971). In contrast, Vedernikov & Afrikanov (1969) found that disulfiram, which has a similar inhibitory effect on dopamine-β-hydroxylase (Carlsson, Lindqvist, Fuxe & Hökfelt, 1966), given peripherally, inhibited the anti-nociceptive activity of morphine in rats.

The possibility of an effect on the metabolism of morphine cannot be excluded in the case of DDC, in view of its known ability to interfere with enzymes other than dopamine-β-hydroxylase. It does seem unlikely to be an important mechanism following icv injection, particularly when one considers the rapidity of onset of the potentiation. As suggested in Re-

sults VI an elevation in brain dopamine levels may play a part in the interactions of icv ouabain and icv DDC with morphine.

Although icv 6-hydroxydopamine results in a depletion of noradrenaline and dopamine from rat brain (Ungerstedt, 1968; Uretsky & Iversen, 1969; 1970), it has been suggested (Simmonds & Uretsky, 1970) that 6-hydroxydopamine may release endogenous dopamine in an active form in the brain to cause the observed hypothermia. If this is so, support is given to the suggestion that the hypothermia produced by icv ouabain also involves a dopaminergic mechanism. Ouabain inhibits the membrane pump, and this appears to include an interference with dopamine as well as noradrenaline uptake (Goldstein, Ohi & Backstrom, 1970). Desmethylimipramine also blocks the uptake of noradrenaline, but with no effect on dopamine (Carlsson, Fuxe, Hamberger & Lindqvist, 1966; Ross & Renyi, 1967; Fuxe & Ungerstedt, 1968). Since desmethylimipramine does not produce depressant effects, it again appears that the depressant effects of icv ouabain involve a dopaminergic component. However, Ross & Renyi (1967) showed that cocaine blocks both noradrenaline and dopamine uptake in mouse brain slices, although it produced little effect *in vivo*. The fact that cocaine produced behavioural excitation supports the view expressed in Results V, that the depressant effects produced by icv ouabain may involve a blockade of dopamine receptors.

The behavioural depression and hypothermia produced by centrally-administered ouabain, DDC and 6-hydroxydopamine are similar to those produced by peripherally-administered reserpine. Furthermore, centrally-administered ouabain, DDC, 6-hydroxydopamine and chlorpromazine all produced similar effects (icv tetrabenazine also produced behavioural depression, but had little effect on body temperature). Centrally-administered ouabain,

in doses which cause central nervous depression, causes an elevation in dopamine and little effect on noradrenaline levels, with an inhibition of both dopamine and noradrenaline uptake. DDC causes an increase in dopamine and a decrease in noradrenaline levels, and 6-hydroxydopamine depletes both dopamine and noradrenaline. Chlorpromazine inhibits amine uptake in brain slices *in vitro* (Carlsson & Waldeck, 1965), but has no effect on monoamine levels in the brain (Gey & Pletscher, 1961), whereas tetrabenazine, like reserpine, also blocks amine uptake (Ross & Renyi, 1966), but depletes brain monoamine levels (Häggendal, 1968).

This apparent variation in effect on endogenous brain amine levels serves to emphasise the need for caution when interpreting the possible mechanisms by which these drugs interfere with behaviour and thermoregulation. However, an interference with dopaminergic systems within the brain may be a common factor involved in a number of types of drug-induced central nervous depression.

7. FIGURES AND TABLES

FIG. 52

Effect of icv sodium diethyldithiocarbamate (DDC) on the body temperature of the mouse. (o——o) 10 μl saline; (●——●) 0.2 mg DDC; (Δ——Δ) 0.5 mg DDC; (▲——▲) 1 mg DDC; (x——x) 2 mg DDC.

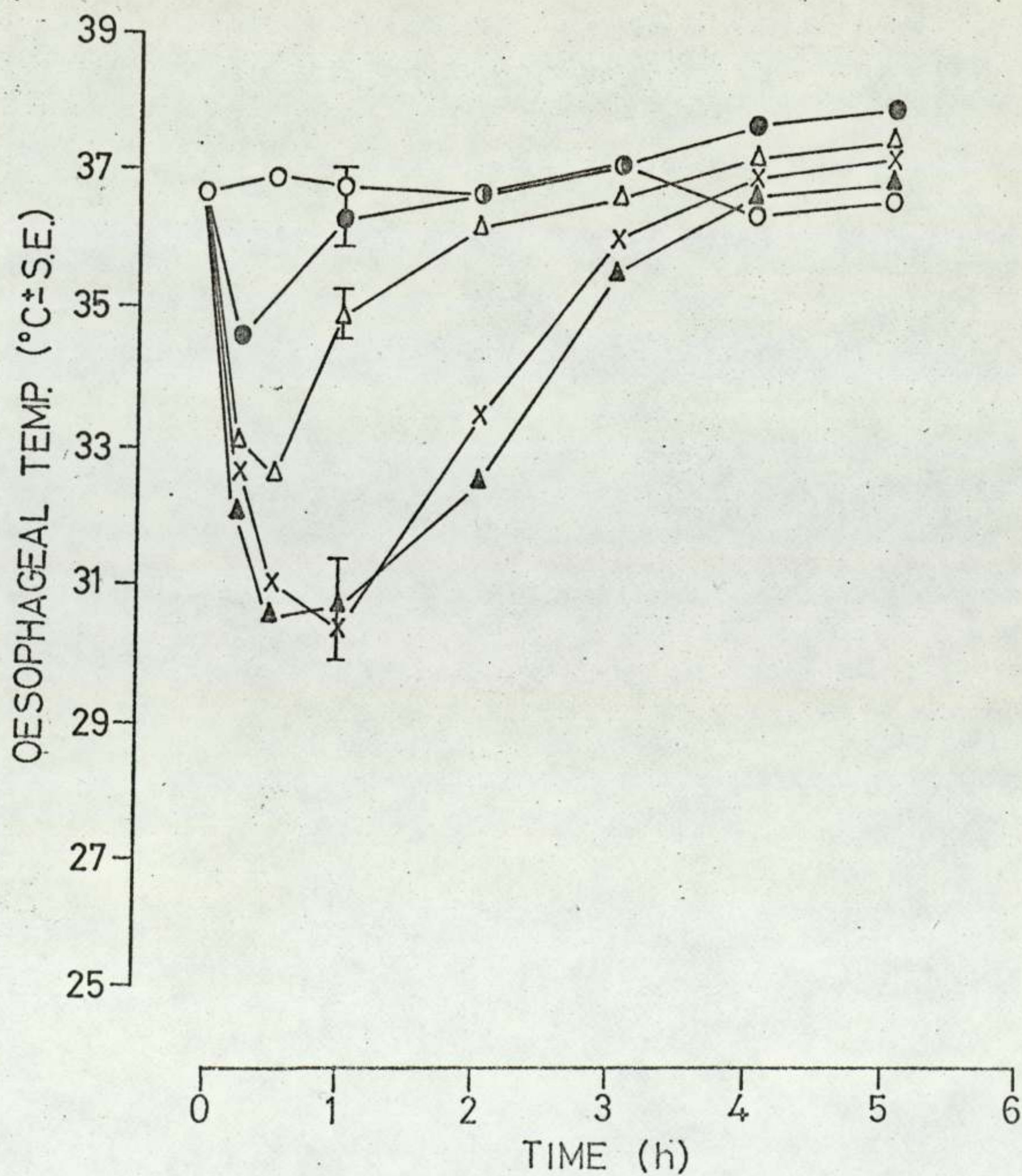

FIG. 53

Effect of dexamphetamine on the hypothermia produced by sodium diethyldithiocarbamate (DDC) in the mouse. (o——o) 10 µl saline icv + 10 ml/kg saline ip at the arrow; (●——●) 10 µl saline icv + 2 mg/kg dexamphetamine ip at the arrow; (Δ——Δ) 1 mg DDC icv + 10 ml/kg saline ip at the arrow; (▲——▲) 1 mg DDC icv + 2 mg/kg dexamphetamine ip at the arrow.

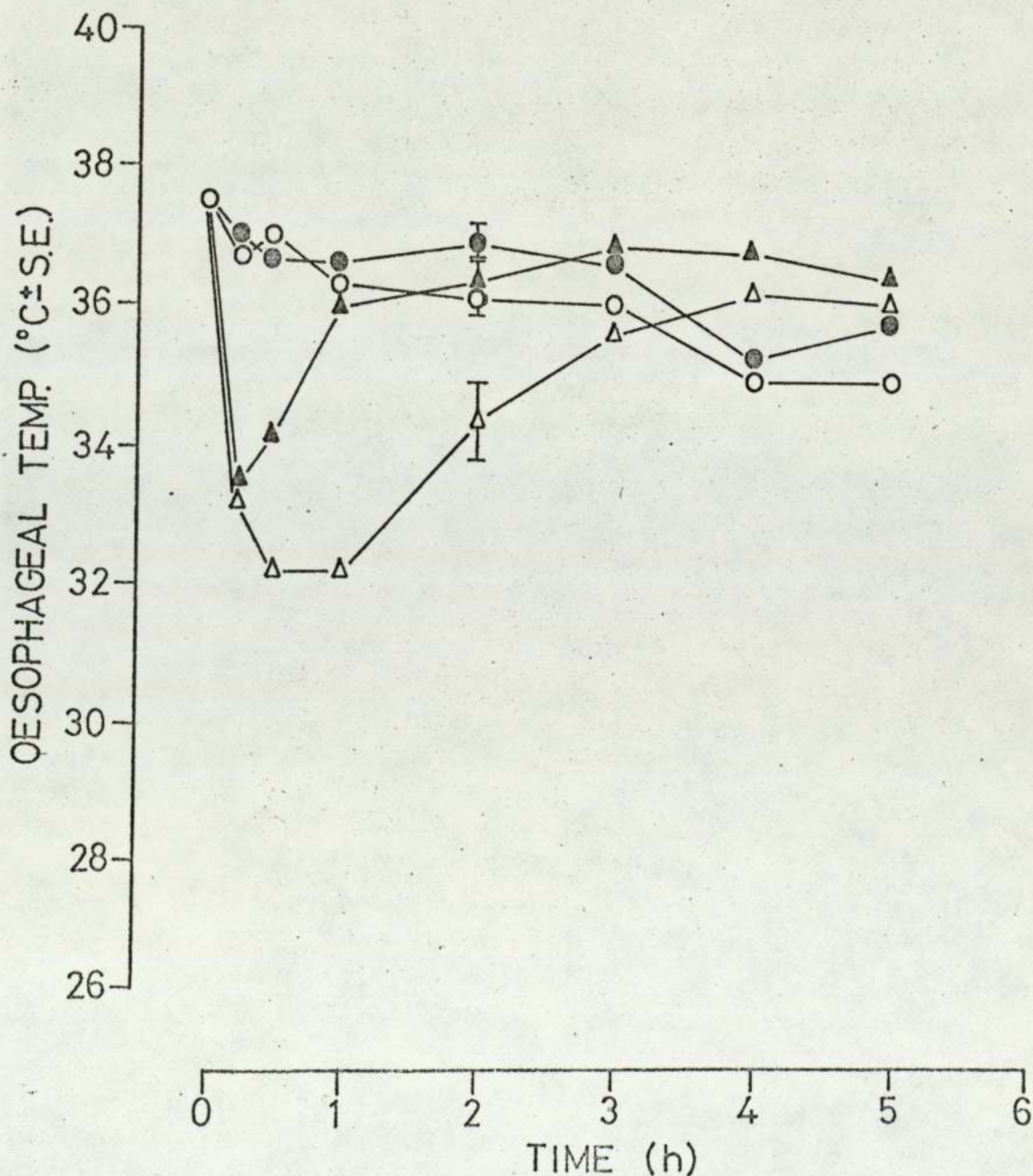

FIG. 54

Effect of desmethylimipramine pretreatment on the hypothermia produced by icv sodium diethyldithiocarbamate (DDC) in the mouse. (o——o) 10 ml/kg saline ip 5 min before 10 μl saline icv at time 0; (Δ——Δ) 10 ml/kg saline ip, (x——x) 5 mg/kg desmethylimipramine ip or (▲——▲) 10 mg/kg desmethylimipramine ip 5 min before 1 mg DDC icv at time 0.

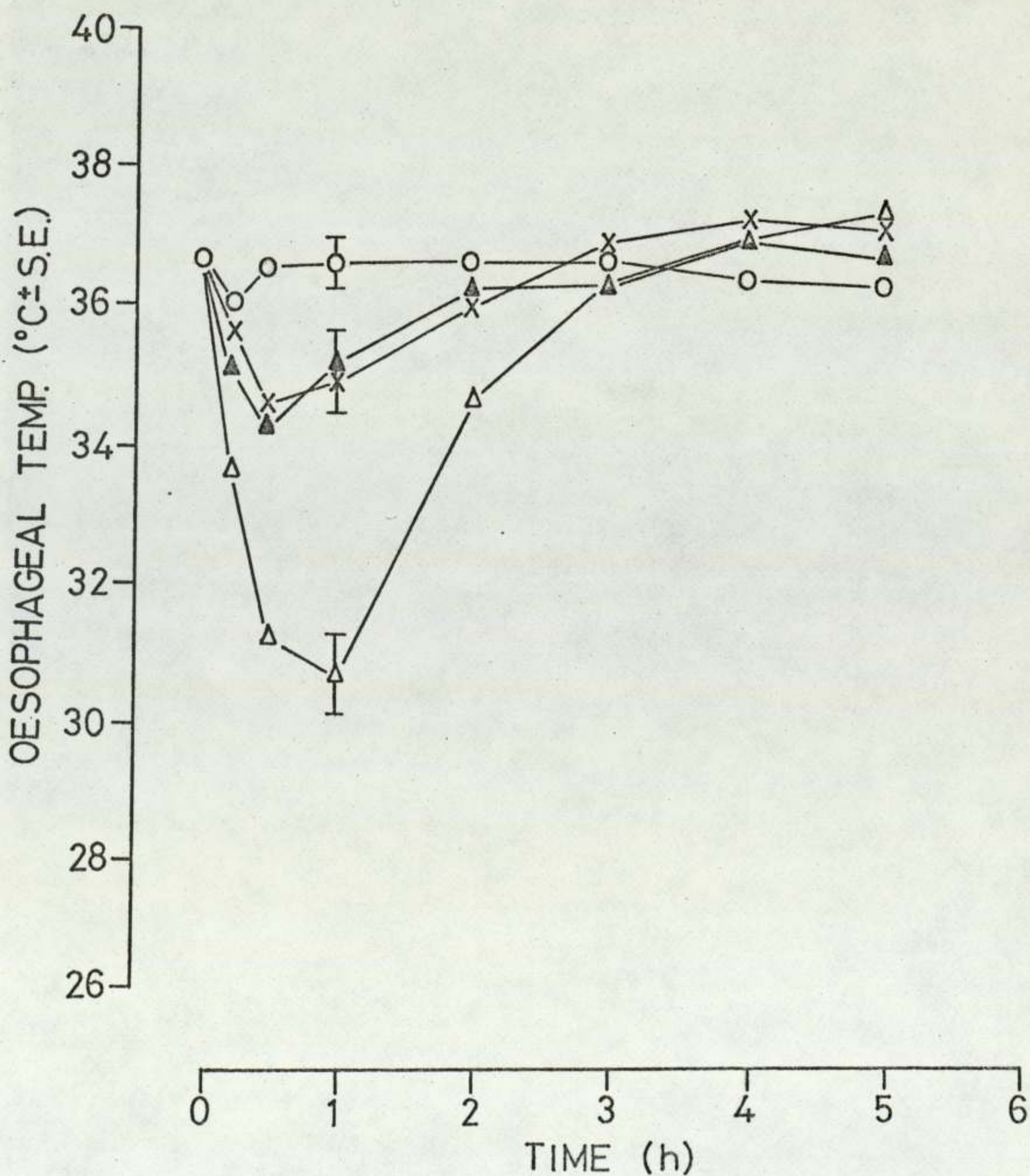

FIG. 55

Effect of nialamide pretreatment on the hypothermia produced by icv sodium diethyldithiocarbamate (DDC) in the mouse. (o——o) 20 mg/kg nialamide ip 2 h before 10 μl saline icv at time 0; (▲——▲) 20 mg/kg nialamide ip or (Δ——Δ) 10 ml/kg saline ip 2 h before 1 mg DDC icv at time 0.

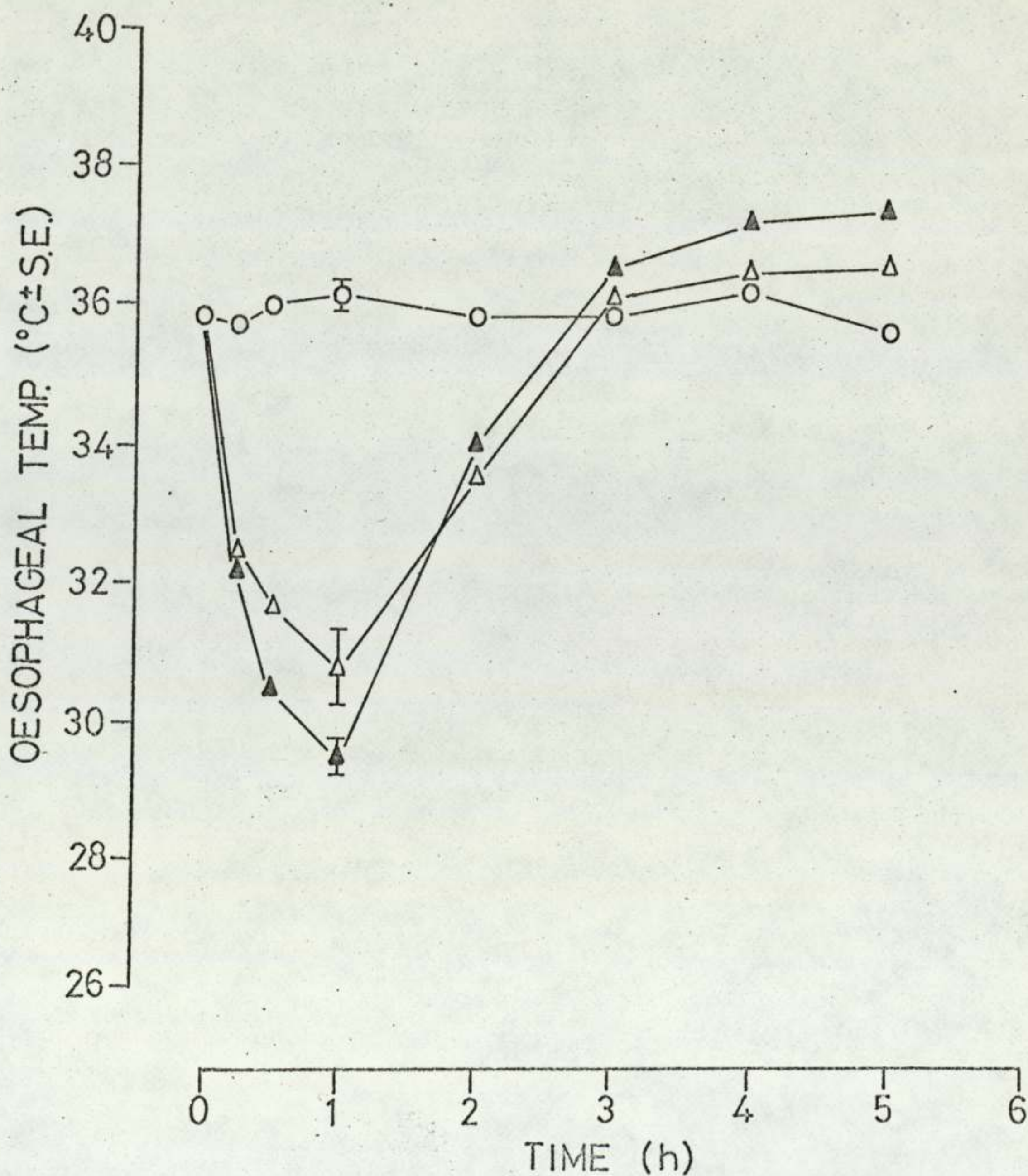

FIG. 56

Effect of icv sodium diethyldithiocarbamate (DDC) on the anti-nociceptive activity of morphine in the tail flick test. Mice received (o—o) 10 ml/kg saline sc at time 0, 15 min before 500 μg DDC icv at the arrow; 2.5 mg/kg morphine sc at time 0, 15 min before (x—x) 10 ml/kg saline icv, (●—●) 20 μg DDC icv, (▲—▲) 100 μg DDC icv, or (Δ—Δ) 500 μg DDC icv at the arrow.

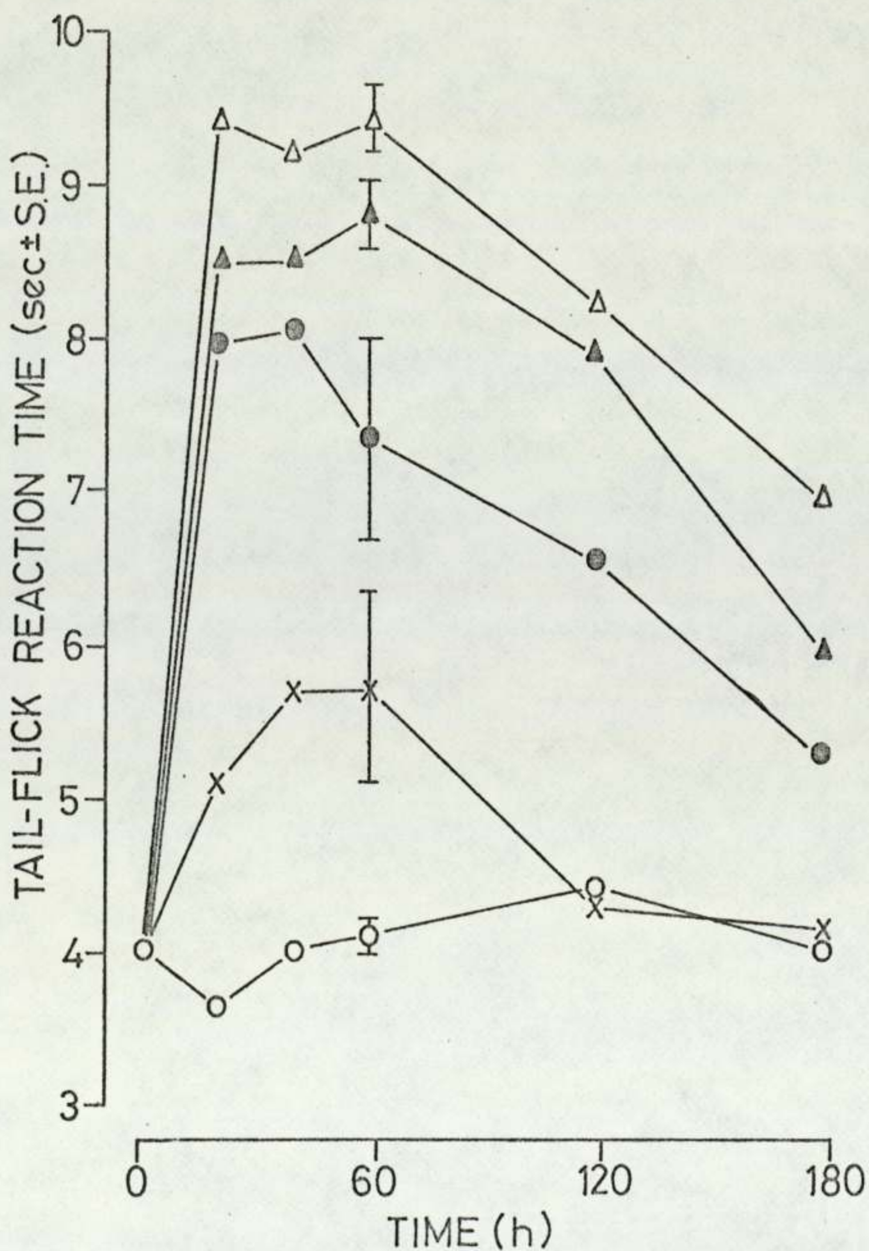

FIG. 57

Effect of icv 6-hydroxydopamine (6-OHDA) on the body temperature of the mouse. (x——x) 10 µl saline; (o——o) 1 µg 6-OHDA; (●——●) 10 µg 6-OHDA; (Δ——Δ) 25 µg 6-OHDA; (▲——▲) 50 µg 6-OHDA.

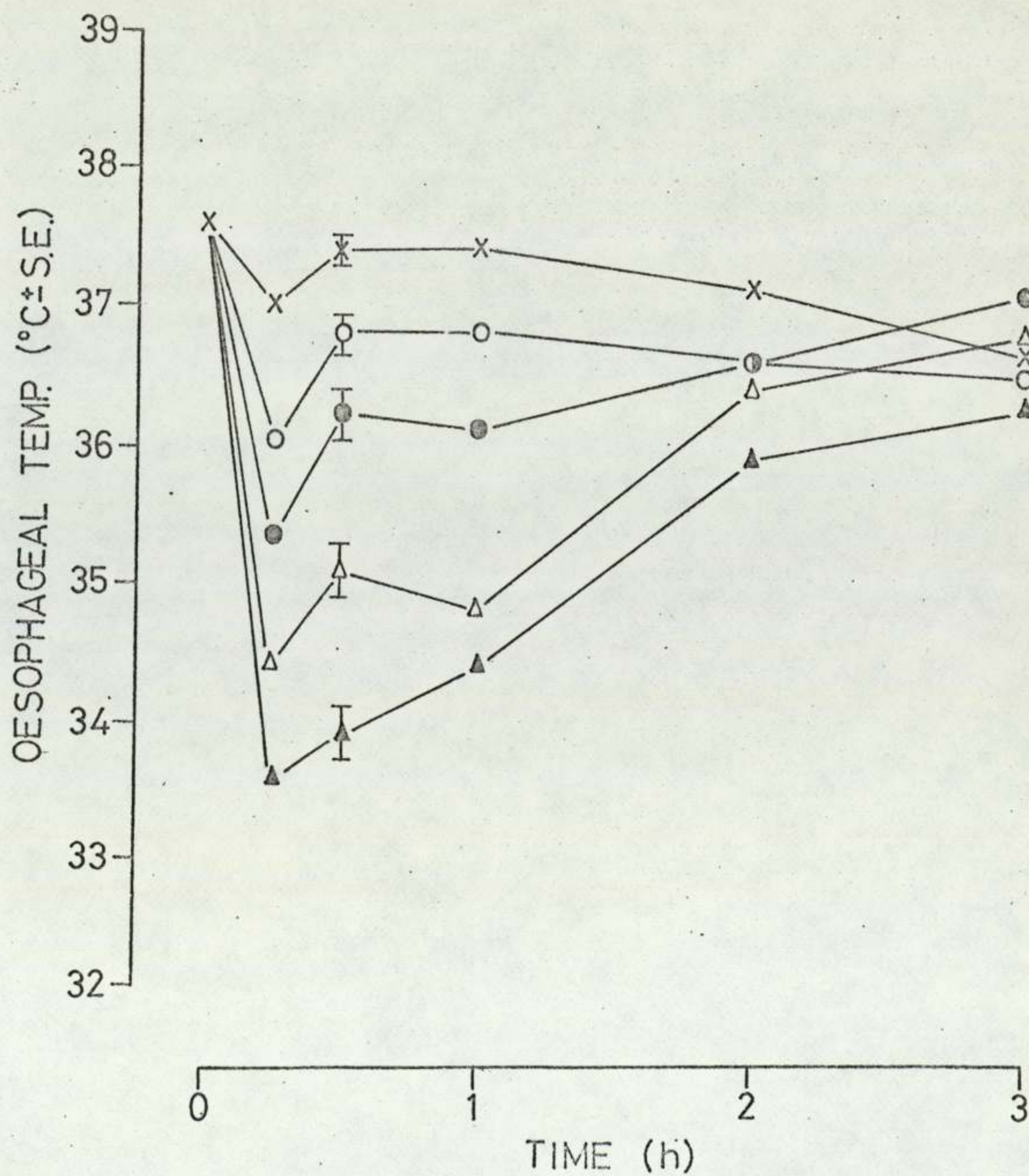

FIG. 58

Effect of icv chlorpromazine (CPZ) on the body temperature of the mouse. (x——x) 10 µl saline; (o——o) 1 µg CPZ; (●——●) 5 µg CPZ; (Δ——Δ) 10 µg CPZ.

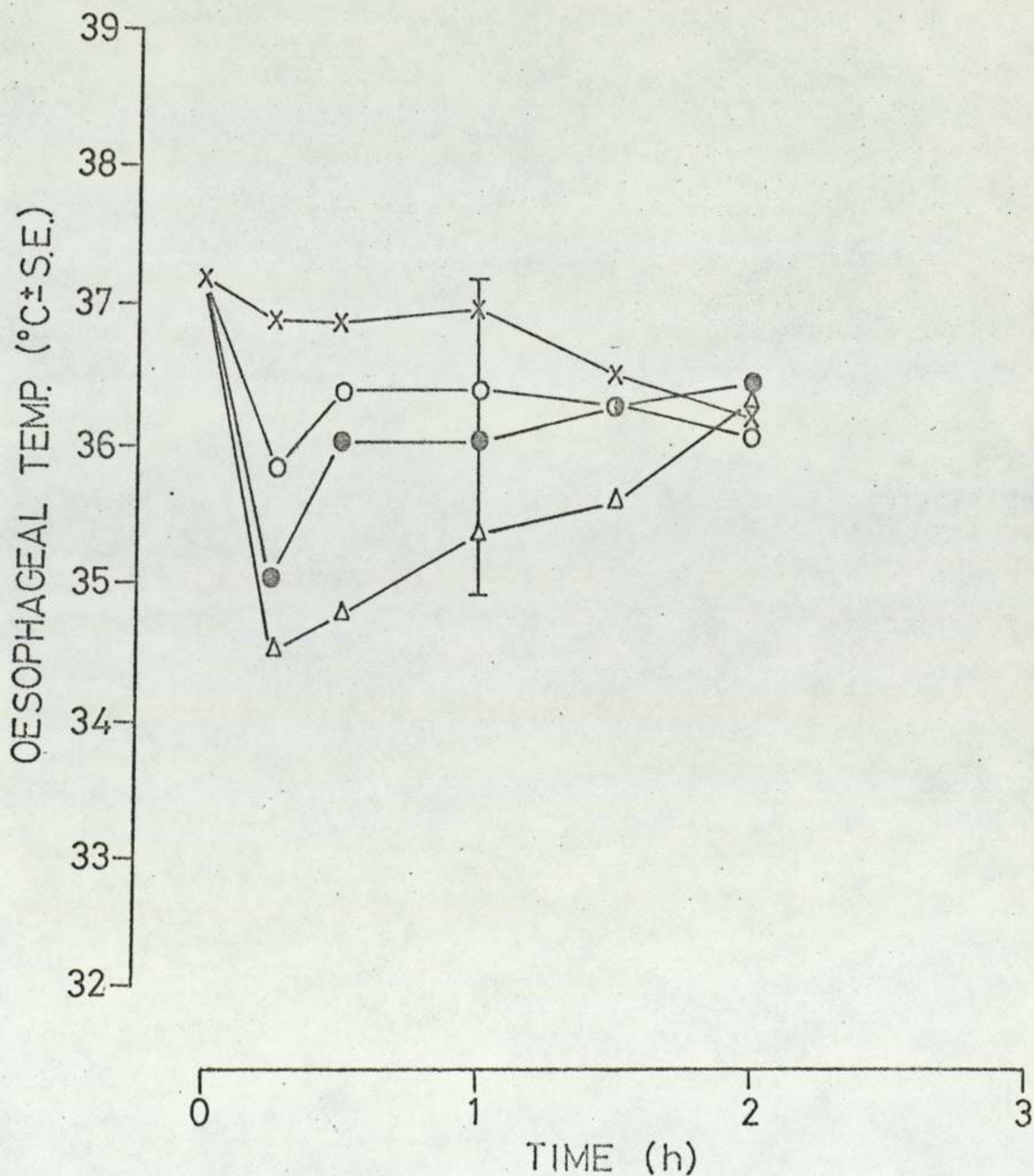

FIG. 59

Effect of icv dibutyryl cyclic 3',5'-adenosine monophosphate (dbcAMP) on reserpine-induced hypothermia in the mouse. Animals received 2 mg/kg reserpine ip 20 h before (o——o) 10 µl saline icv or (●——●) 25 µg dbcAMP icv at time 0.

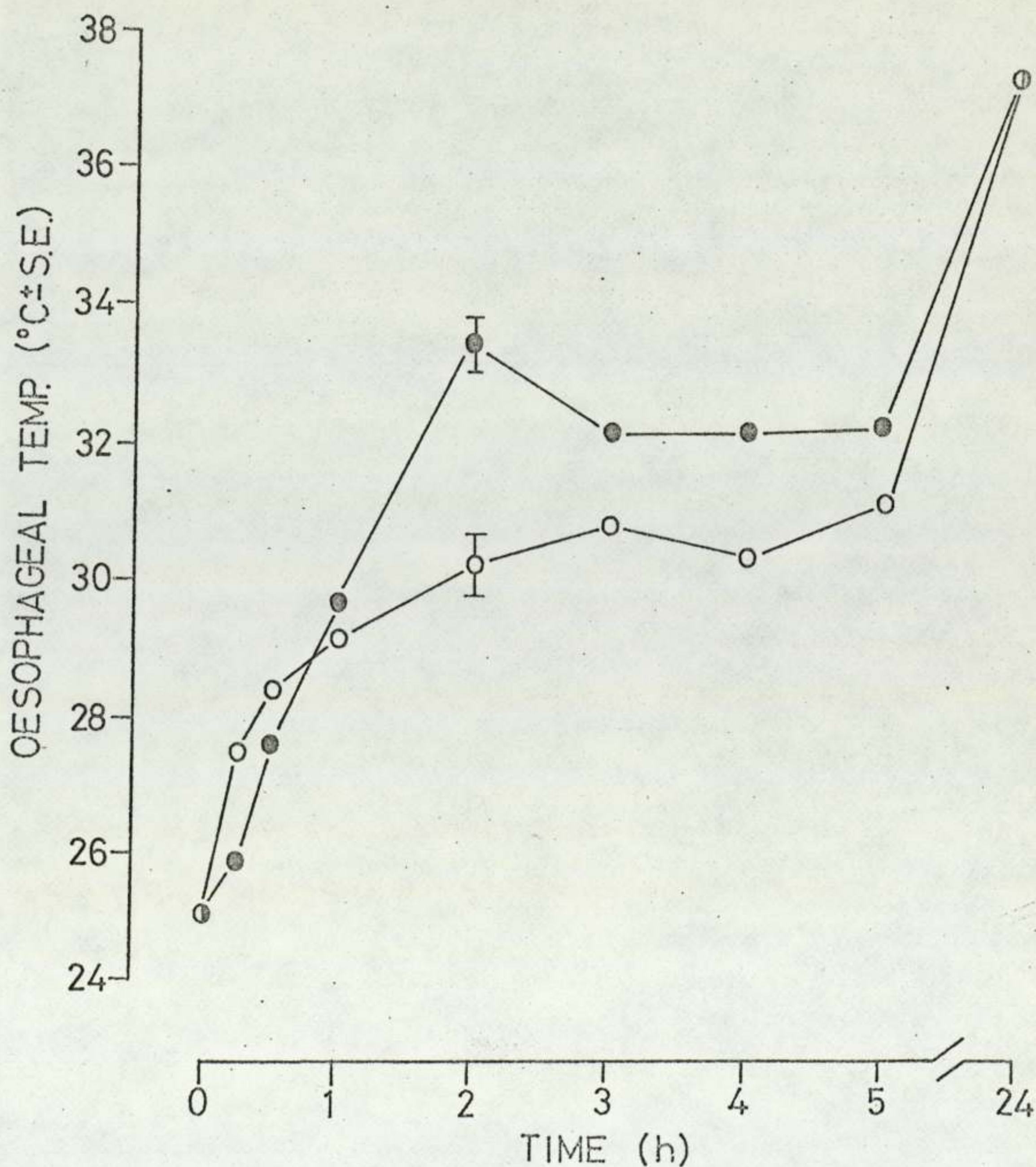

TABLE 18

Effect of chlorpromazine given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Controls received saline (10 µl icv) 10 min before trial.

DOSE (µg)	PARAMETER MEASURED	MEAN RESPONSES (% ± S.E.)		
		control	10 min after inj.	30 min after inj.
50	% avoid	95 ± 3	0	93 ± 3
	escape failure	0	0	0
	latency	404 ± 20	1060 ± 180	387 ± 53

GENERAL DISCUSSION

GENERAL DISCUSSION

In order to maintain normal function, the complex interrelated systems of the central nervous system must be held in delicate balance. One would expect the whole organism to be profoundly affected by any disturbance in the constant internal environment of the central neurones. The administration of substances which interfere directly with energy-yielding or energy-consuming processes in the brain would probably produce gross changes in animals behaviour. Depending on the particular areas to which the drugs gained access or on the extent of interference with energy metabolism, the induced changes would vary both qualitatively and quantitatively. Agents which interfere with central metabolism may induce either depression or excitation, and the behavioural changes produced will reflect both the distribution of the agent and the functions of the areas of the brain affected by it.

It has been demonstrated that compounds such as 2,4-dinitrophenol (DNP) and pentachlorophenol (PCP), which can interfere with energy production, and ouabain, which can interfere with energy utilisation, are all capable of producing central nervous effects.

Following icv injection, the uncouplers of oxidative phosphorylation, after an initial excitatory phase, cause central nervous depression to the extent of producing a complete loss of righting reflex, similar to that seen following the icv administration of pentobarbitone. They are more potent in their hypnotic actions than pentobarbitone, but do not seem to produce the same overall widespread depression of central nervous tissue; for example, they are not anti-convulsant and do not non-selectively block either conditioned or unconditioned avoidance behaviour. This may adequate-

ly be explained by considering the physico-chemical properties of the two classes of compounds. In the unionised form pentobarbitone has an affinity for non-polar solvents which favours its retention within, and its distribution throughout, the brain. On the other hand, the more acidic polar structures of DNP and PCP render them hydrophilic at physiological pH and lead to their rapid removal from the site of injection into the continuous aqueous phase, and thence into the blood. Furthermore, even after direct injection into the cerebral ventricles, only small quantities of the uncouplers would be expected to reach an intracellular site. It is not therefore surprising that DNP and PCP can give rise to hypnosis, possibly by a depressant action on the reticular formation (part of which lies just below the injection site) although they produce no substantial anti-convulsant activity. However, icv pentobarbitone can produce widespread depression with anti-convulsant activity, and this may perhaps result from its diffusion into a larger area of the brain, including the cerebral motor cortex.

The depressant effects seen after the central administration of uncouplers of oxidative phosphorylation differ from those reported after their peripheral administration in both dogs and rodents (Kaiser, 1964). Administration of phenolic uncouplers in these species produced a marked elevation in body temperature, an increase in heart rate and respiration, salivation and weight-loss. Further administration could produce convulsions and death. With the exception of that on body temperature, these effects correlate well with the excitatory stage through which all animals pass after the icv injection of DNP and PCP, and before the secondary depression becomes apparent.

Peripherally-administered uncouplers do differ from centrally-administered uncouplers in their effects on body temperature, since the

former route of administration produces a hyperthermia and the latter a hypothermia. This apparent difference in effect may result from a common mechanism, namely an increase in heat production brought about by uncoupling oxidative phosphorylation. In the case of pheripheral administration, this results in an overt elevation of body temperature due to widespread excessive heat production. After central administration the hyperthermia may affect the hypothalamic centres responsible for temperature control, which in turn results in a compensatory feed-back, and this, together with the associated loss of locomotor activity results in a whole-body hypothermia.

Centrally-administered DNP is capable of producing a marked potentiation of pentobarbitone sleeping time which appears to be at least partially caused by a direct interaction in the central nervous system. A large number of other substances can interfere with oxidative phosphorylation, including antibiotics, barbiturates, benzimidazoles, coumarins, phenothiazines, phenylbutazones and salicylates (see Weinbach & Garbus, 1969, for references). There have also been reports that the pharmacological potencies of several centrally acting drugs, including barbiturates (Prange, Lipton, Shearin & Love, 1966) and morphine (Bhagat, 1964), are increased in hyperthyroid animals. Furthermore, the toxicities of imipramine, nortryptyline, chlorpromazine, perphenazine, chlordiazepoxide (Ashford & Ross, 1968) and amphetamine (Dolfini & Kobayashi, 1967) are also increased in hyperthyroidism. Although this may be partially caused by an effect on the rates of drug metabolism, the possibility exists that the observed enhancement of the action of these drugs may be a result of a thyroxine-induced increase in sensitivity of the central nervous system. This may include an interference with oxidative phosphorylation, since thyroxine has been shown to be an uncoupler of this process in brain mitochondria (Bain, 1954).

These findings serve to reiterate the need for caution in multiple drug administration, since unpredictable drug interactions might occur which would involve effects on the mitochondrial respiratory chain.

The icv administration of the cardiac glycosides ouabain and digitoxin in small doses also produced central nervous depression, but was unable to cause hypnosis. The compounds can block the sodium pump mechanism, and hence interfere with energy utilisation in the brain. In view of the considerable amount of energy expended in the operation of these pumps in neural tissue, one would expect their inhibition to produce a diversity of effects. Previous reports have suggested that the cardiac glycosides produce death by convulsions, particularly in rodents. The results in this thesis demonstrate conclusively that the cardiac glycosides have a biphasic action in the central nervous system, with small doses causing a depression and higher doses an excitation, together with convulsions.

Small depressant doses of icv ouabain cause an increase in dopamine levels but have little effect on noradrenaline levels, whereas larger excitant doses cause both an increase in dopamine and a decrease in noradrenaline (Anagnoste & Goldstein, 1967). It may therefore be that noradrenergic systems have a rôle to play in the development of the excitant actions. In support of this Butterbaugh & Spratt (1970b) found that, after peripheral administration in the rat, brain noradrenaline and 5-hydroxytryptamine levels were lowered after lethal convulsant doses of digitoxigenin, whereas brain dopamine levels were unchanged.

The part played by noradrenaline in the aetiology of both the depressant and excitant effects of icv ouabain is complicated by the fact that

it has both inhibitory and excitatory effects on the population of neurones responsive to this amine but has no effect on others (Salmoiraghi, 1966). A paradoxical action of noradrenaline is seen in the experiments reported in Results V 2. Noradrenaline causes hypothermia when given by icv injection, yet, since it partly reverses the hypothermia produced by icv ouabain, it may be that noradrenaline acts as a partial agonist, competing with ouabain for a receptor site at the pre-synaptic neuronal membrane and thus diminishing the effect of ouabain.

The effects seen after small doses of icv ouabain are comparable to those following peripheral administration of neuroleptics such as reserpine, tetrabenazine and chlorpromazine. These drugs induce a characteristic pattern of central nervous depression - poikilothermia, motor inactivity, ptosis, catalepsy and loss of conditioned avoidance behaviour. Reserpine and tetrabenazine block amine uptake by an effect on the granular uptake mechanism in adrenergic neurones, leading to a depletion of tissue amine stores. Chlorpromazine and ouabain, however, prevent uptake by blocking the membrane pump, chlorpromazine having little effect on tissue monoamine stores, ouabain elevating only dopamine levels. In the case of ouabain and chlorpromazine, evidence for a non-intracellular site of action is furnished by the failure of pretreatment with monoamine oxidase inhibitors to prevent the central nervous depression produced by these agents. Such pretreatment in the case of reserpine and tetrabenazine, on the other hand, can reverse the depression, and thus indicates an intracellular site of action. Compounds blocking amine uptake without associated α-receptor blocking activity are capable of potentiating the effects of sympathomimetics, and, in this respect, ouabain is typical, since it sensitises animals to the excitant and toxic effects of dexamphetamine.

The available evidence suggests that the central nervous depressant effects of icv ouabain involve a dopaminergic component. Other compounds, such as sodium diethyldithiocarbamate, which cause an increase in dopamine levels, albeit, by a different mechanism, produce similar effects to icv ouabain when given by the same route of administration. Moreover, it has been postulated that the hypothermic effects of icv 6-hydroxydopamine, which depletes both noradrenaline and dopamine, are brought about by a release of dopamine in an active form. Compounds, such as desmethylimipramine, which do not affect dopamine uptake do not produce hypothermia and depression but do cause excitation. Experiments with apomorphine indicate that, whereas the production of certain central nervous depressant effects including catalepsy and the abolition of conditioned avoidance behaviour may involve a blockade of central dopamine receptors by ouabain, the hypothermia does not involve such a blockade. The effects of icv ouabain thus appear to be a result of an increased synthesis of catecholamines brought about by an inhibition of amine re-uptake, together with a blockade of dopamine receptors at higher doses. Whereas it is obvious that dopamine has an important rôle in the depressant effects of icv ouabain, any function of noradrenaline in the production of these effects has yet to be defined.

Digitalis does not act specifically on the heart, but at appropriate concentrations it affects every living tissue in the body (Withering, 1785; Somlyo, 1960). Wherever it has been found to act, a digitalis-sensitive ion transport mechanism has been identified (Bonting, 1964). The biochemical evidence suggests that, after absorption, the therapeutic and toxic actions of the cardiac glycosides are inseparable and share a common mechanism, hence there exists the possibility of drug interactions at many sites.

Centrally-administered ouabain is capable of potentiating the effects of peripherally-administered dexamphetamine, pentobarbitone and morphine. Since digitalis-like drugs are given at doses closer to the toxic level than any other medicine in general use, and the toxicity of the cardiac glycosides is manifested by central nervous symptoms, an appreciation of possible interactions with centrally-acting drugs is desirable. A frequent clinical manifestation of digitalis therapy is nausea and vomiting and, in view of the neuroleptic-like depression produced by ouabain through an effect on the central nervous system, concurrent administration of neuroleptics, such as chlorpromazine, either as an anti-emetic or tranquillising agent, may result in a potentiation of their activity.

The central nervous effects of the cardiac glycosides are particularly pronounced following peripheral administration in young animals, which have a poorly developed blood-brain barrier. Since this barrier is poorly developed in embryos and neonates, the use of digitalis in the treatment of cardiac insufficiency in premature infants should be undertaken with extreme caution, particularly since children require about 50 % more digitoxin than adults for the production of a similar effect (Mathes, Gold, Marsh, Greiner, Palumbo & Messeloff, 1952). Furthermore, Levine & Blumenthal (1962) have reported a marked susceptibility to intoxication by digoxin in infants less than 72 h old. Since the permeability of the blood-brain barrier may also break down in various pathological states, the administration of digitalis in heart disease concurrent with other diseases should again be approached with caution, since it may exhibit undesirable side effects, which in such patients are attributable to an action on the central nervous system.

As expected, the inhibition of energy metabolism within the brain, whether by a decrease in production or utilisation, does produce definite pharmacological effects, ranging from behavioural depression and hypnosis to excitation, convulsions and death. The results obtained after icv administration of uncouplers of oxidative phosphorylation show that such compounds can produce central nervous depression *per se*, and direct attention to the possibility of an uncoupling process being responsible for a number of drug interactions. Results obtained after icv administration of cardiac glycosides lead one to postulate the use of icv ouabain as a possible pharmacological tool in the screening of certain anti-depressant drugs, and at the same time focus attention onto possible clinical side effects during digitalis therapy.

Studies with icv ouabain also direct attention to the enzymes of active transport, thus emphasising that it is not only the transformation of substances which plays a part in disturbances of brain function, but also the translocation of these substances which is of considerable importance.

It has long been the hope that experimental pharmacology might elucidate the mechanisms of mood disorders. Over the years the enthusiastic optimism of some workers has been tempered by the more cautious views of the majority. The results presented in this thesis underline the fact that a fundamental interference with cell metabolism in the brain can produce changes in animal behaviour which are not the overall non-specific depression or excitation expected from such a generalised interference with function, but can vary in degrees of subtlety.

Although it may perhaps be premature to impute the mechanism of action of all centrally acting drugs to direct effects on cell metabolism, nevertheless it would only be prudent to consider them a real possibility, since lack of therapeutic specificity may be explained in some instances by effects of this nature, and apparent therapeutic specificity may be due more to a specific site than mode of action.

APPENDIX

Tables for figures presented with

incomplete data in the text.

FIG. 8

(n = 6)

TIME (min)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm S.E.$)		
	10 µl Saline icv @ 35°C	10 µl Saline icv @ 42°C	10 µl Saline icv @ 45°C
0	37.0 ± 0.3	37.0 ± 0.2	36.9 ± 0.1
0	37.0 ± 0.2	36.9 ± 0.2	37.1 ± 0.2
5	36.1 ± 0.3	35.8 ± 0.3	35.6 ± 0.1
10	36.4 ± 0.1	35.3 ± 0.4	35.3 ± 0.4
15	36.3 ± 0.4	35.5 ± 0.4	35.8 ± 0.3
20	36.7 ± 0.4	36.2 ± 0.2	36.1 ± 0.2
25	36.7 ± 0.4	36.4 ± 0.3	36.5 ± 0.1
30	36.7 ± 0.4	36.5 ± 0.3	36.4 ± 0.2

FIG. 9

(n = 8)

TIME (min)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm S.E.$)			
	10 µl Saline 0.9 %; pH 7	10 µl Saline 0.9 %; pH 9.5	10 µl Saline 5 %; pH 7	10 µl Saline 5 %; pH 9.5
0	37.4 ± 0.1	37.3 ± 0.1	37.3 ± 0.1	37.2 ± 0.1
15	36.5 ± 0.4	36.4 ± 0.3	35.5 ± 0.4	35.3 ± 0.4
30	36.8 ± 0.2	36.9 ± 0.2	35.4 ± 0.4	36.2 ± 0.3
60	36.9 ± 0.2	36.8 ± 0.1	36.1 ± 0.3	36.4 ± 0.4
120	36.9 ± 0.2	36.7 ± 0.2	35.8 ± 0.3	36.6 ± 0.1
180	36.9 ± 0.3	36.8 ± 0.2	36.2 ± 0.3	36.7 ± 0.3

FIG. 10

(n = 10)

PENTOBARBITONE		DNP		PCP	
Dose (µg)	Duration of Sleep (sec ± S.E.)	Dose (µg)	Duration of Sleep (sec ± S.E.)	Dose (µg)	Duration of Sleep (sec ± S.E.)
80	150 ± 24	20	127 ± 22	40	184 ± 26
130	208 ± 26	40	233 ± 44	50	203 ± 32
150	286 ± 48	80	426 ± 39	60	301 ± 100
200	354 ± 25	100	550 ± 62	80	448 ± 76
		200	669 ± 82	160	664 ± 109
				400	862 ± 78

FIG. 15

(n = 6)

TIME (min)	MEAN OESOPHAGEAL TEMPERATURE ($^oC \pm$ S.E.)		
	Saline (10 µl icv)	Pentobarbitone (120 µg icv)	DNP (40 µg icv)
0	36.6 ± 0.1	36.9 ± 0.3	37.0 ± 0.3
5	35.8 ± 0.2	34.5 ± 0.5	34.6 ± 0.1
10	35.8 ± 0.5	33.7 ± 0.4	33.6 ± 0.2
15	35.9 ± 0.2	34.4 ± 0.6	33.8 ± 0.3
20	35.9 ± 0.2	34.9 ± 0.6	34.1 ± 0.3
25	36.2 ± 0.3	35.8 ± 0.5	34.5 ± 0.3
30	36.3 ± 0.2	36.1 ± 0.4	34.7 ± 0.3
60	36.3 ± 0.4	36.5 ± 0.3	35.6 ± 0.3

FIG. 17

(n = 10)

DOSE µg	MEAN SLEEPING TIME (sec ± S.E.)	
	ATP	ADP
200	135 ± 46	340 ± 81
400	463 ± 60	845 ± 118
800	1226 ± 134	1601 ± 181

FIG. 18

(n = 10)

DOSE OF PENTOBARBITONE (µg)	PENTOBARBITONE + SALINE (10 ml/kg ip)		PENTOBARBITONE + DEXAMPHETAMINE (5 mg/kg ip)	
	Sleep Duration (sec ± S.E.)	% Mortality	Sleep Duration (sec ± S.E.)	% Mortality
100	181 ± 26	0	61 ± 12	0
130	285 ± 30	0	109 ± 11	0
200	354 ± 25	47	278 ± 23	20
300	-	100	789 ± 75	35

FIG. 19

(n = 5)

AMBIENT TEMP.	TIME AFTER ip INJECTION (min)	MEAN OESOPHAGEAL TEMPERATURE (sec ± S.E.)	
		Pentobarbitone ip Saline icv	Pentobarbitone ip DNP icv
21 ± 1°C	0	37.6 ± 0.2	37.8 ± 0.3
	5	37.5 ± 0.1	36.6 ± 0.4
	10	36.8 ± 0.3	35.4 ± 0.3
	15	36.7 ± 0.4	34.8 ± 0.5
	20	36.5 ± 0.3	35.7 ± 0.6
	30	36.9 ± 0.2	36.6 ± 0.3
32 ± 1°C	0	37.6 ± 0.2	37.5 ± 0.2
	5	37.6 ± 0.1	37.3 ± 0.2
	10	37.3 ± 0.1	37.1 ± 0.2
	15	37.1 ± 0.3	37.4 ± 0.1
	20	37.2 ± 0.2	37.7 ± 0.1
	30	37.5 ± 0.1	37.6 ± 0.2

FIG. 21

(n = 10)

TIME (h).	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Saline	0.1 µg	0.2 µg	0.4 µg
0	37.1 ± 0.2	36.5 ± 0.2	37.0 ± 0.2	36.3 ± 0.2
¼	35.9 ± 0.3	35.3 ± 0.3	33.8 ± 0.7	32.5 ± 0.1
½	36.6 ± 0.5	34.9 ± 0.4	33.3 ± 0.7	30.1 ± 0.1
1	36.9 ± 0.3	36.0 ± 0.3	33.0 ± 1.1	27.2 ± 0.1
1 ½	36.8 ± 0.4	36.0 ± 0.3	34.2 ± 1.0	25.6 ± 0.1
2	36.9 ± 0.4	36.0 ± 0.3	35.2 ± 0.5	26.0 ± 0.1
2 ½	36.7 ± 0.2	36.7 ± 0.2	36.4 ± 0.2	27.0 ± 0.2
3	36.7 ± 0.3	36.5 ± 0.3	36.5 ± 0.3	28.2 ± 0.3
3 ½	36.6 ± 0.2	36.5 ± 0.4	36.6 ± 0.4	29.7 ± 0.4
4	36.7 ± 0.3	36.6 ± 0.4	37.0 ± 0.4	31.6 ± 0.2
4 ½	36.8 ± 0.3	36.8 ± 0.3	37.1 ± 0.4	32.8 ± 0.5
5	36.9 ± 0.3	36.7 ± 0.4	37.1 ± 0.3	35.2 ± 0.4
5 ½	37.1 ± 0.2	36.9 ± 0.4	37.1 ± 0.3	36.1 ± 0.3
6 ½	37.2 ± 0.3	37.3 ± 0.4	37.3 ± 0.3	36.8 ± 0.2
8	37.7 ± 0.2	37.8 ± 0.3	37.5 ± 0.2	37.3 ± 0.1

FIG. 22

(n = 10)

TIME (min)	DURATION OF CATALEPSY (sec ± S.E.)		
	0.2 μg	0.25 μg	0.3 μg
10	9 ± 2	14 ± 2	12 ± 3
30	8 ± 3	8 ± 2	20 ± 3
45	6 ± 1	12 ± 3	15 ± 3
60	4 ± 2	12 ± 2	15 ± 3
90	2 ± 1	7 ± 2	10 ± 2
120	1 ± 1	5 ± 1	5 ± 1
180	0	1 ± 1	0

FIG. 23

(n = 5)

TIME (h)	MEAN SKIN TEMPERATURE (^{o}C ± S.E.)	
	Saline	0.4 μg
0	24.5 ± 0.2	24.4 ± 0.2
¼	-	28.0 ± 0.2
½	-	26.4 ± 0.2
1	24.5 ± 0.2	24.9 ± 0.1
2	-	23.8 ± 0.2
2 ½	25.2 ± 0.3	-
3	-	23.8 ± 0.3
4	25.2 ± 0.5	24.3 ± 0.6
5	25.2 ± 0.4	23.7 ± 0.2
6	25.6 ± 0.8	23.5 ± 0.1

FIG. 24

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMP. ($^{o}C \pm S.E.$)	
	Saline	0.3 μg
0	38.8 ± 0.1	38.8 ± 0.2
¼	37.5 ± 0.2	37.1 ± 0.3
½	38.0 ± 0.1	36.7 ± 0.1
1	38.1 ± 0.2	37.9 ± 0.2
1 ½	37.8 ± 0.2	39.0 ± 0.2
2	37.8 ± 0.2	38.8 ± 0.4
3	38.0 ± 0.2	37.4 ± 0.3
4	38.2 ± 0.2	37.5 ± 0.3
5	38.5 ± 0.1	37.6 ± 0.2
6	38.3 ± 0.1	38.0 ± 0.1

FIG. 26

(n = 6)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm S.E.$)			
	Saline		0.2 μg	
	Day 1	Day 2	Day 1	Day 2
0	37.7 ± 0.2	37.1 ± 0.3	37.8 ± 0.3	37.3 ± 0.3
¼	-	-	34.1 ± 0.3	34.8 ± 0.4
½	37.7 ± 0.3	36.7 ± 0.3	33.0 ± 0.5	33.1 ± 0.5
1	37.3 ± 0.1	36.3 ± 0.3	32.6 ± 0.4	32.8 ± 0.5
1 ½	-	36.4 ± 0.2	-	34.6 ± 0.4
2	36.1 ± 0.3	36.8 ± 0.1	35.3 ± 0.4	35.7 ± 0.3
3	36.2 ± 0.3	36.6 ± 0.2	36.0 ± 0.4	36.2 ± 0.4
4	36.8 ± 0.2	36.6 ± 0.4	36.8 ± 0.3	37.0 ± 0.2
5	36.7 ± 0.2	36.0 ± 0.2	37.1 ± 0.2	37.2 ± 0.1

FIG. 27

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMP. ($^{o}C \pm$ S.E.)	
	Saline	2.5 µg
0	37.7 ± 0.2	38.2 ± 0.2
¼	35.7 ± 0.1	32.5 ± 0.3
½	36.0 ± 0.4	30.4 ± 0.4
1	36.8 ± 0.4	29.1 ± 0.5
1 ½	37.1 ± 0.3	29.3 ± 0.6
2 ½	37.8 ± 0.3	30.9 ± 0.3
3 ½	38.0 ± 0.4	34.1 ± 0.5
4 ½	38.4 ± 0.3	35.9 ± 0.3
5 ½	38.4 ± 0.2	37.0 ± 0.3
6 ½	38.4 ± 0.2	37.8 ± 0.2

FIG. 28

(n = 4)

TIME (h)	MEAN RECTAL TEMP. ($^{o}C \pm$ S.E.)	
	Saline	1.0 µg
0	37.1 ± 0.1	36.9 ± 0.1
¼	37.0 ± 0.1	35.6 ± 0.3
½	37.6 ± 0.1	34.5 ± 0.6
1	37.4 ± 0.1	34.4 ± 0.7
1 ½	37.4 ± 0.1	35.3 ± 0.9
2	37.2 ± 0.2	36.5 ± 0.7
3	37.0 ± 0.1	37.5 ± 0.2

FIG. 30

(n = 5)

TIME (h)	MEAN RECTAL TEMPERATURE ($^{o}C \pm$ S.E.)			
	Saline	0.2 mg/kg	0.3 mg/kg	0.5 mg/kg
0	39.4 ± 0.2	39.6 ± 0.2	39.6 ± 0.3	39.4 ± 0.2
¼	39.7 ± 0.3	39.1 ± 0.2	38.7 ± 0.1	38.7 ± 0.3
½	39.7 ± 0.2	38.9 ± 0.4	37.7 ± 0.1	37.4 ± 0.3
1	39.6 ± 0.2	38.9 ± 0.3	37.2 ± 0.6	36.7 ± 0.7
1 ½	39.7 ± 0.2	39.3 ± 0.2	38.1 ± 0.5	37.5 ± 0.4
2	39.8 ± 0.2	39.5 ± 0.2	38.5 ± 0.5	38.1 ± 0.6
3	39.5 ± 0.2	39.9 ± 0.2	39.5 ± 0.5	38.9 ± 0.5
4	39.5 ± 0.1	39.9 ± 0.1	39.9 ± 0.3	39.8 ± 0.2

FIG. 31

(n = 5)

TIME (h)	MEAN RECTAL TEMPERATURE (°C ± S.E.)									
	Day 2		Day 3		Day 4		Day 5		Day 10	
	Saline	Ouabain	Saline	Ouabain	Saline	Ouabain	Saline	Ouabain	Saline	Ouabain
0	38.1 ± 0.2	38.0 ± 0.3	39.1 ± 0.2	39.2 ± 0.2	39.4 ± 0.2	39.6 ± 0.2	39.4 ± 0.2	39.7 ± 0.1	39.5 ± 0.2	39.9 ± 0.1
¼	37.9 ± 0.2	37.1 ± 0.5	38.7 ± 0.1	37.8 ± 0.3	39.7 ± 0.3	38.4 ± 0.3	39.7 ± 0.3	39.4 ± 0.3	39.5 ± 0.3	39.9 ± 0.1
½	37.8 ± 0.2	35.6 ± 0.3	38.7 ± 0.1	36.7 ± 0.3	39.7 ± 0.2	37.3 ± 0.4	39.7 ± 0.2	38.2 ± 0.3	39.3 ± 0.1	39.2 ± 0.1
1	37.9 ± 0.2	34.4 ± 0.4	38.9 ± 0.1	36.2 ± 0.2	39.6 ± 0.2	38.4 ± 0.7	39.6 ± 0.2	38.7 ± 0.2	39.5 ± 0.2	39.6 ± 0.2
1½	37.9 ± 0.2	32.7 ± 0.2	39.0 ± 0.1	36.1 ± 0.2	39.7 ± 0.2	38.6 ± 0.5	39.7 ± 0.2	39.2 ± 0.2	39.6 ± 0.1	39.7 ± 0.2
2	37.7 ± 0.1	31.9 ± 0.2	38.9 ± 0.1	36.5 ± 0.3	39.8 ± 0.2	39.0 ± 0.5	39.8 ± 0.2	39.7 ± 0.2	39.7 ± 0.1	39.8 ± 0.1
3	38.2 ± 0.2	30.0 ± 0.3	38.7 ± 0.1	37.4 ± 0.9	39.5 ± 0.2	39.9 ± 0.2	39.5 ± 0.2	40.0 ± 0.1	39.8 ± 0.2	40.4 ± 0.2
4	38.0 ± 0.1	28.4 ± 0.7	38.7 ± 0.2	37.7 ± 1.0	39.5 ± 0.1	40.2 ± 0.3	39.5 ± 0.1	40.2 ± 0.1	39.4 ± 0.3	39.7 ± 0.1

FIG. 33

(n = 10)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)				
	Saline icv Saline ip	Saline icv DEX 10 mg/kg ip	Ouabain icv Saline ip	Ouabain icv DEX 10 mg/kg ip	Ouabain icv DEX 0.5 mg/kg ip
0	37.9 ± 0.1	38.0 ± 0.2	37.9 ± 0.4	38.1 ± 0.2	37.9 ± 0.2
¼	37.3 ± 0.2	37.0 ± 0.3	32.5 ± 0.2	32.5 ± 0.2	32.5 ± 0.2
½	37.6 ± 0.1	37.5 ± 0.3	30.3 ± 0.2	30.3 ± 0.2	30.3 ± 0.2
1	37.6 ± 0.2	38.4 ± 0.6	27.3 ± 0.9	37.2 ± 0.6	27.3 ± 0.2
1½	37.2 ± 0.2	38.0 ± 0.2	26.3 ± 0.8	39.2 ± 0.7	27.8 ± 0.2
2	37.2 ± 0.3	37.7 ± 0.3	25.7 ± 0.3	40.2 ± 0.4	28.8 ± 0.3
2½	37.3 ± 0.2	37.2 ± 0.2	26.3 ± 0.5	40.2 ± 0.4	29.2 ± 0.3
3	37.5 ± 0.2	36.8 ± 0.2	27.6 ± 0.5	36.6 ± 0.7	30.5 ± 0.3
3½	37.2 ± 0.3	37.2 ± 0.4	28.9 ± 0.8	36.2 ± 0.5	31.6 ± 0.3
4	37.5 ± 0.3	37.3 ± 0.3	30.1 ± 1.0	35.5 ± 0.6	39.9 ± 0.3
5	37.6 ± 0.2	37.7 ± 0.1	34.5 ± 0.3	35.8 ± 0.7	35.6 ± 0.3
6	37.6 ± 0.2	37.6 ± 0.1	37.3 ± 0.2	36.3 ± 0.7	36.5 ± 0.3

FIG. 34

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)			
	DMI (5 mg/kg ip) Saline icv	DMI (5 mg/kg ip) Ouabain (0.3 µg icv)	DMI (10 mg/kg ip) Ouabain (0.3 µg icv)	Saline ip Ouabain (0.3 µg icv)
0	36.5 ± 0.2	36.4 ± 0.2	36.5 ± 0.2	36.5 ± 0.1
¼	35.9 ± 0.2	34.3 ± 0.2	35.5 ± 0.3	32.5 ± 0.3
½	36.6 ± 0.2	33.7 ± 0.2	35.3 ± 0.2	29.4 ± 0.2
1	36.8 ± 0.2	34.5 ± 0.4	36.0 ± 0.5	27.8 ± 0.4
1 ½	36.6 ± 0.2	35.4 ± 0.5	35.7 ± 0.4	27.6 ± 0.3
2	36.5 ± 0.2	36.8 ± 0.3	35.9 ± 0.6	27.9 ± 0.2
2 ½	36.7 ± 0.1	37.0 ± 0.2	36.8 ± 0.3	28.4 ± 0.5
3	36.5 ± 0.1	37.0 ± 0.2	37.2 ± 0.3	38.9 ± 0.5
4	36.5 ± 0.1	37.1 ± 0.1	37.2 ± 0.2	31.0 ± 0.7
5	36.5 ± 0.1	37.2 ± 0.1	37.2 ± 0.1	32.8 ± 0.5
6	36.4 ± 0.2	37.1 ± 0.1	37.1 ± 0.1	35.8 ± 0.3

FIG. 35

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)			
	Nialamide (10 mg/kg ip) Saline icv	Nialamide (10 mg/kg ip) Ouabain (0.3 µg icv)	Nialamide (20 mg/kg ip) Ouabain (0.3 µg icv)	Saline ip Ouabain (0.3 µg icv)
0	37.1 ± 0.2	37.2 ± 0.3	37.1 ± 0.3	37.1 ± 0.2
¼	36.9 ± 0.4	34.1 ± 0.2	31.6 ± 0.5	34.5 ± 0.2
½	37.2 ± 0.3	32.1 ± 0.5	29.3 ± 0.4	32.9 ± 0.3
1	37.6 ± 0.2	29.7 ± 0.3	27.3 ± 0.6	30.5 ± 0.5
1 ½	37.4 ± 0.4	28.3 ± 0.4	27.5 ± 0.8	28.8 ± 0.3
2	37.6 ± 0.4	30.6 ± 0.5	29.2 ± 0.7	29.1 ± 0.2
3	37.4 ± 0.3	33.0 ± 0.2	33.2 ± 1.0	30.0 ± 0.5
4	38.1 ± 0.1	34.5 ± 0.2	35.0 ± 0.6	32.0 ± 0.7
5	38.3 ± 0.1	36.0 ± 0.2	36.5 ± 0.3	34.0 ± 0.5
6	38.3 ± 0.1	37.5 ± 0.2	37.8 ± 0.2	36.9 ± 0.3

FIG. 36

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE (^{o}C ± S.E.)			
	Saline	Phentolamine	Noradrenaline	Ouabain
0	38.0 ± 0.1	38.0 ± 0.2	37.9 ± 0.1	37.9 ± 0.1
½	37.3 ± 0.3	35.5 ± 0.6	35.9 ± 0.7	34.0 ± 0.7
1	37.5 ± 0.5	36.1 ± 0.3	36.8 ± 0.6	33.0 ± 1.0
1½	37.3 ± 0.3	36.6 ± 0.3	36.7 ± 0.3	34.9 ± 0.6
2	37.2 ± 0.3	36.6 ± 0.3	36.3 ± 0.5	35.8 ± 0.5
3	37.0 ± 0.2	37.1 ± 0.2	36.9 ± 0.5	36.6 ± 0.4
4	36.5 ± 0.1	37.3 ± 0.2	36.9 ± 0.6	36.8 ± 0.2
5	36.8 ± 0.3	37.3 ± 0.1	37.1 ± 0.6	37.0 ± 0.3
6	36.6 ± 0.1	37.3 ± 0.2	37.2 ± 0.6	37.2 ± 0.4
7	36.5 ± 0.2	37.1 ± 0.1	37.2 ± 0.4	36.9 ± 0.4

FIG. 37

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE (^{o}C ± S.E.)				
	Phentolamine	Ouabain	Noradrenaline	Phentolamine + Ouabain	Phentolamine + Noradren.
0	38.0 ± 0.2	37.9 ± 0.1	37.9 ± 0.1	38.0 ± 0.2	38.0 ± 0.1
½	35.5 ± 0.6	34.0 ± 0.7	35.9 ± 0.7	33.4 ± 0.8	35.5 ± 0.1
1	36.1 ± 0.3	33.0 ± 1.0	36.8 ± 0.6	30.2 ± 0.6	35.2 ± 0.4
1½	36.6 ± 0.3	34.9 ± 0.6	36.7 ± 0.3	30.0 ± 1.2	35.5 ± 0.3
2	36.6 ± 0.3	35.8 ± 0.5	36.3 ± 0.5	31.1 ± 0.8	35.5 ± 0.3
3	37.1 ± 0.2	36.6 ± 0.4	36.9 ± 0.5	32.1 ± 0.7	36.6 ± 0.2
4	37.3 ± 0.2	36.8 ± 0.2	36.9 ± 0.6	34.0 ± 0.3	36.9 ± 0.2
5	37.3 ± 0.1	37.0 ± 0.3	37.1 ± 0.6	35.9 ± 0.9	36.9 ± 0.1
6	37.3 ± 0.2	37.2 ± 0.4	37.2 ± 0.6	36.4 ± 0.5	37.2 ± 0.2
7	37.1 ± 0.1	36.9 ± 0.4	37.2 ± 0.4	36.9 ± 0.4	37.1 ± 0.2

FIG. 38

(n = 6)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE (^{o}C ± S.E.)			
	Saline	Noradrenaline	Ouabain	Noradrenaline + Ouabain
0	37.7 ± 0.2	37.9 ± 0.1	37.8 ± 0.3	37.9 ± 0.1
¼	37.0 ± 0.2	36.1 ± 0.4	34.1 ± 0.3	35.7 ± 0.4
½	37.7 ± 0.3	35.6 ± 0.5	33.0 ± 0.5	34.9 ± 0.6
1	37.3 ± 0.1	36.5 ± 0.4	32.6 ± 0.4	34.9 ± 0.6
2	36.1 ± 0.3	36.5 ± 0.5	35.3 ± 0.4	36.0 ± 0.5
3	36.2 ± 0.3	36.6 ± 0.5	36.0 ± 0.4	36.9 ± 0.3
4	36.8 ± 0.2	36.7 ± 0.4	36.8 ± 0.3	37.0 ± 0.4
5	36.7 ± 0.2	36.7 ± 0.4	37.1 ± 0.2	36.9 ± 0.3

FIG. 39

(n = 6)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)		
	Saline ip Ouabain icv	α - MMT ip Ouabain icv	α - MMT ip Saline icv
0	38.0 ± 0.2	38.0 ± 0.2	38.2 ± 0.1
¼	35.0 ± 0.1	35.5 ± 0.3	38.9 ± 0.2
½	32.8 ± 0.3	32.5 ± 0.4	38.9 ± 0.3
1	32.7 ± 0.6	33.2 ± 0.8	38.3 ± 0.2
1 ½	33.6 ± 0.5	34.4 ± 1.0	38.1 ± 0.3
2 ½	35.4 ± 0.3	35.9 ± 0.6	37.8 ± 0.3
3 ½	36.7 ± 0.4	37.2 ± 0.4	37.6 ± 0.4
4 ½	37.4 ± 0.3	37.8 ± 0.2	37.4 ± 0.2

FIG. 40

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)			
	Saline	dbcAMP	Ouabain	dbcAMP + Ouabain
0	37.0 ± 0.3	37.1 ± 0.4	36.8 ± 0.4	37.5 ± 0.1
¼	36.4 ± 0.3	35.5 ± 0.5	32.7 ± 0.3	33.8 ± 0.3
½	36.7 ± 0.4	34.5 ± 0.9	29.6 ± 0.2	-
1	-	36.3 ± 0.5	27.9 ± 0.3	29.6 ± 0.6
1 ½	37.0 ± 0.3	36.1 ± 0.3	27.8 ± 0.3	-
1 ¾	-	-	-	29.8 ± 0.9
2	37.1 ± 0.3	36.5 ± 0.1	28.1 ± 0.2	-
2 ½	-	-	-	32.6 ± 0.5
3	36.9 ± 0.2	36.7 ± 0.4	29.0 ± 0.5	-
3 ½	-	-	-	33.8 ± 0.6
4	-	36.4 ± 0.2	31.0 ± 0.7	35.1 ± 0.4
5	36.5 ± 0.1	36.2 ± 0.4	33.0 ± 0.5	35.2 ± 0.6
6	36.6 ± 0.2	35.9 ± 0.4	35.8 ± 0.3	35.6 ± 0.4

FIG. 41

(n = 8)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Caffeine (40 mg/kg ip) Saline icv	Caffeine (20 mg/kg ip) Ouabain icv	Caffeine (40 mg/kg ip) Ouabain icv	Saline ip Ouabain icv
0	37.3 ± 0.3	38.5 ± 0.1	37.5 ± 0.3	37.5 ± 0.3
½	39.1 ± 0.2	34.4 ± 0.4	34.7 ± 0.7	32.2 ± 0.5
1	38.5 ± 0.3	34.4 ± 0.5	35.2 ± 0.5	29.8 ± 0.8
1 ½	38.9 ± 0.2	36.0 ± 0.4	35.6 ± 0.6	29.3 ± 0.8
2	38.5 ± 0.1	35.6 ± 0.4	35.9 ± 1.2	29.4 ± 1.2
3	37.8 ± 0.2	36.3 ± 0.3	37.1 ± 0.6	31.0 ± 1.3
4	37.8 ± 0.1	39.9 ± 0.2	37.6 ± 0.2	32.4 ± 1.2
5	37.5 ± 0.2	37.2 ± 0.2	37.8 ± 0.1	34.1 ± 0.9
6	37.5 ± 0.2	37.6 ± 0.3	38.3 ± 0.3	36.5 ± 1.0

FIG. 42

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Saline icv Saline ip	Saline icv Pentobarbitone ip	Ouabain (0.1 µg icv) Pentobarbitone ip	Ouabain (0.2 µg icv) Pentobarbitone ip
0	37.0 ± 0.2	37.1 ± 0.2	37.1 ± 0.1	37.0 ± 0.2
½	36.5 ± 0.3	36.4 ± 0.2	34.8 ± 0.2	33.2 ± 0.7
1	36.9 ± 0.3	36.8 ± 0.3	35.8 ± 0.2	32.8 ± 1.0
2	36.9 ± 0.4	36.8 ± 0.4	35.9 ± 0.3	35.0 ± 0.5
3	36.7 ± 0.3	36.8 ± 0.4	36.5 ± 0.3	36.4 ± 0.2
4	36.7 ± 0.3	36.7 ± 0.3	36.6 ± 0.4	37.0 ± 0.2
5	36.9 ± 0.3	36.8 ± 0.3	36.6 ± 0.4	37.1 ± 0.3
6	37.0 ± 0.2	36.8 ± 0.3	36.8 ± 0.2	37.2 ± 0.1

FIG. 43

(n = 10)

TIME (min)	DURATION OF CATALEPSY (sec ± S.E.)	
	Ouabain (0.3 µg icv) Saline sc	Ouabain (0.3 µg icv) Apomorphine (20 mg/kg sc)
10	12 ± 3	8 ± 4
30	19 ± 3	0
60	17 ± 3	0
90	11 ± 2	0
120	6 ± 3	0
180	0	0

FIG. 44

(n = 8)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)		
	Saline icv Saline sc	Ouabain icv Saline sc	Ouabain icv Apomorphine sc
0	36.7 ± 0.1	37.1 ± 0.1	36.9 ± 0.1
¼	36.4 ± 0.1	34.6 ± 0.4	33.9 ± 0.2
½	37.0 ± 0.1	33.0 ± 0.5	33.4 ± 0.4
1	37.1 ± 0.1	32.3 ± 0.6	33.0 ± 0.6
2	36.3 ± 0.2	33.4 ± 0.8	33.3 ± 0.3
3	36.0 ± 0.2	35.4 ± 0.5	34.9 ± 0.5
4	36.3 ± 0.2	36.2 ± 0.4	35.9 ± 0.3
5	36.3 ± 0.2	36.4 ± 0.3	36.5 ± 0.3

FIG. 45

(n = 10)

TIME (min)	REACTION TIME (sec ± S.E.)		
	Saline icv	Morphine (5 mg/kg sc)	Morphine (1.5 μg icv)
0	4.0 ± 0.2	4.0 ± 0.2	4.0 ± 0.2
20	3.8 ± 0.1	7.3 ± 0.5	7.8 ± 0.4
40	4.2 ± 0.2	8.0 ± 0.6	8.0 ± 0.5
60	4.4 ± 0.2	8.0 ± 0.6	7.8 ± 0.8
120	4.1 ± 0.2	5.7 ± 0.4	6.6 ± 0.3
180	4.2 ± 0.1	3.8 ± 0.2	4.0 ± 0.2

FIG. 46

(n = 10)

TIME (min)	REACTION TIME (sec ± S.E.)		
	0.1 μg	0.2 μg	0.3 μg
0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
10	3.5 ± 0.1	4.4 ± 0.2	4.4 ± 0.3
20	3.5 ± 0.1	4.2 ± 0.1	5.1 ± 0.4
40	3.9 ± 0.2	5.0 ± 0.3	5.5 ± 0.4
60	4.0 ± 0.4	5.8 ± 0.6	6.1 ± 0.7
120	3.6 ± 0.2	5.4 ± 0.5	7.3 ± 0.8
180	3.4 ± 0.1	4.4 ± 0.1	4.6 ± 0.2

FIG. 48 & 49

(n = 10)

TIME AFTER sc INJ. (min)	REACTION TIME (sec ± S.E.)			
	icv Inj. 15 min before Morphine		Morphine 15 min before icv Inj.	
	Saline icv Morphine sc	Ouabain icv Morphine sc	Morphine sc Saline icv	Morphine sc Ouabain icv
0	3.4 ± 0.1	3.4 ± 0.2	4.4 ± 0.1	4.5 ± 0.2
10	3.3 ± 0.1	4.8 ± 0.4	-	-
20	4.0 ± 0.1	4.9 ± 0.2	5.8 ± 0.2	8.5 ± 0.5
40	4.5 ± 0.4	5.9 ± 0.7	6.9 ± 0.3	8.7 ± 0.4
60	5.2 ± 0.8	5.0 ± 0.4	8.3 ± 0.4	8.6 ± 0.5
120	4.2 ± 0.3	4.6 ± 0.2	6.2 ± 0.4	6.0 ± 0.3
180	4.0 ± 0.2	4.0 ± 0.1	5.0 ± 0.2	5.7 ± 0.4

FIG. 50

(n = 10)

TIME AFTER sc INJ. (min)	REACTION TIME (sec ± S.E.)		
	Saline sc Dopamine icv	Morphine sc Saline icv	Morphine sc Dopamine icv
0	3.9 ± 0.1	3.9 ± 0.2	3.9 ± 0.2
20	4.0 ± 0.1	4.9 ± 0.6	5.8 ± 0.7
40	4.2 ± 0.2	4.7 ± 0.3	6.5 ± 0.8
60	3.9 ± 0.1	5.4 ± 0.5	7.4 ± 0.7
120	4.0 ± 0.2	4.7 ± 0.3	5.9 ± 0.6
180	3.9 ± 0.1	4.2 ± 0.7	4.2 ± 0.3

FIG. 51

(n = 10)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Saline icv Morphine sc	Dopamine icv Morphine sc	Ouabain icv Saline sc	Ouabain icv Morphine sc
0	36.5 ± 0.1	36.4 ± 0.2	36.5 ± 0.2	36.5 ± 0.1
¼	36.3 ± 0.2	36.1 ± 0.2	35.3 ± 0.2	35.3 ± 0.4
½	35.8 ± 0.4	35.7 ± 0.3	34.9 ± 0.4	34.1 ± 0.4
1	35.9 ± 0.3	35.9 ± 0.3	36.0 ± 0.3	34.3 ± 0.5
1½	36.8 ± 0.2	36.5 ± 0.2	36.0 ± 0.2	34.4 ± 0.5
2	37.0 ± 0.1	36.8 ± 0.1	36.0 ± 0.2	35.1 ± 0.5
3	36.6 ± 0.1	36.8 ± 0.1	36.4 ± 0.1	35.4 ± 0.3
4	37.0 ± 0.1	37.1 ± 0.2	36.6 ± 0.2	35.8 ± 0.4
5	37.2 ± 0.1	37.3 ± 0.1	36.7 ± 0.1	35.9 ± 0.2
6	37.2 ± 0.1	37.3 ± 0.1	37.0 ± 0.1	36.2 ± 0.2

FIG. 52

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)				
	Saline	0.2 mg	0.5 mg	1 mg	2 mg
0	36.7 ± 0.2	36.5 ± 0.2	36.8 ± 0.2	36.8 ± 0.2	36.9 ± 0.2
¼	36.0 ± 0.2	34.4 ± 0.3	32.9 ± 0.2	32.1 ± 0.3	32.6 ± 0.1
½	36.8 ± 0.1	34.6 ± 0.7	32.7 ± 0.4	30.5 ± 0.4	30.9 ± 0.3
1	36.7 ± 0.2	36.2 ± 0.5	34.9 ± 0.4	30.7 ± 0.8	30.4 ± 0.6
2	36.5 ± 0.2	36.7 ± 0.2	36.4 ± 0.2	32.5 ± 0.2	33.4 ± 0.7
3	36.8 ± 0.2	36.9 ± 0.1	37.0 ± 0.1	35.5 ± 0.2	36.0 ± 0.3
4	36.3 ± 0.1	37.5 ± 0.1	37.3 ± 0.2	37.0 ± 0.3	37.2 ± 0.1
5	36.5 ± 0.1	37.7 ± 0.1	37.5 ± 0.2	37.0 ± 0.3	37.4 ± 0.1

FIG. 53

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Saline icv Saline ip	Saline icv DEX ip	DDC icv Saline ip	DDC icv DEX ip
0	37.4 ± 0.1	37.7 ± 0.2	37.3 ± 0.2	37.8 ± 0.2
¼	36.9 ± 0.1	37.1 ± 0.2	33.4 ± 0.5	33.6 ± 0.2
½	37.1 ± 0.2	36.8 ± 0.3	32.3 ± 0.4	34.3 ± 0.6
1	36.4 ± 0.2	36.8 ± 0.4	32.3 ± 0.4	36.3 ± 0.5
2	36.3 ± 0.2	37.0 ± 0.2	34.5 ± 0.6	36.4 ± 0.3
3	36.1 ± 0.3	36.8 ± 0.2	35.7 ± 0.2	36.8 ± 0.2
4	35.0 ± 0.4	35.3 ± 0.4	36.3 ± 0.3	36.9 ± 0.2
5	35.0 ± 0.4	35.9 ± 0.2	36.0 ± 0.2	36.5 ± 0.3

FIG. 54

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE (°C ± S.E.)			
	Saline ip Saline icv	Saline ip DDC icv	DMI 5 mg/kg ip DDC icv	DMI 10 mg/kg ip DDC icv
0	36.7 ± 0.1	36.7 ± 0.2	36.5 ± 0.3	36.5 ± 0.2
¼	36.1 ± 0.2	33.7 ± 0.2	35.7 ± 0.3	35.2 ± 0.3
½	36.6 ± 0.3	31.3 ± 0.3	34.6 ± 0.4	34.4 ± 0.3
1	36.7 ± 0.4	30.7 ± 0.6	34.9 ± 0.5	35.3 ± 0.5
2	36.7 ± 0.4	34.7 ± 1.0	36.1 ± 0.5	36.2 ± 0.3
3	36.7 ± 0.3	36.3 ± 0.3	36.6 ± 0.4	36.7 ± 0.4
4	36.4 ± 0.3	37.0 ± 0.2	37.1 ± 0.3	37.0 ± 0.3
5	36.3 ± 0.3	37.3 ± 0.1	37.1 ± 0.2	36.7 ± 0.2

FIG. 55

(n = 5)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)		
	Nialamide ip Saline icv	Saline ip DDC icv	Nialamide ip DDC icv
0	35.8 ± 0.1	35.9 ± 0.1	35.9 ± 0.1
¼	35.7 ± 0.1	32.4 ± 0.1	32.1 ± 0.1
½	36.0 ± 0.1	31.6 ± 0.3	30.4 ± 0.2
1	36.1 ± 0.2	30.7 ± 0.6	29.4 ± 0.3
2	35.8 ± 0.2	33.5 ± 0.4	34.0 ± 0.2
3	35.9 ± 0.2	36.0 ± 0.2	36.6 ± 0.2
4	36.2 ± 0.1	36.5 ± 0.2	37.3 ± 0.3
5	35.6 ± 0.2	36.6 ± 0.1	37.4 ± 0.3

FIG. 56

(n = 10)

TIME AFTER sc INJ. (min)	REACTION TIME (sec ± S.E.)				
	Morphine sc Saline icv	Saline sc DDC 500 μg icv	Morphine sc DDC 20 μg icv	Morphine sc DDC 100 μg icv	Morphine sc DDC 500 μg icv
0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
20	5.1 ± 0.2	3.6 ± 0.2	8.0 ± 0.6	8.6 ± 1.0	9.5 ± 0.5
40	5.7 ± 0.6	4.0 ± 0.1	8.1 ± 0.5	8.6 ± 0.6	9.3 ± 0.6
60	5.7 ± 0.7	4.1 ± 0.2	7.4 ± 0.7	8.9 ± 0.5	9.5 ± 0.5
120	4.3 ± 0.3	4.4 ± 0.2	6.6 ± 0.9	8.0 ± 0.7	8.3 ± 0.7
180	4.1 ± 0.1	4.1 ± 0.1	5.3 ± 0.7	6.0 ± 0.8	7.0 ± 0.6

FIG. 57

(n = 5)

TIME	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)				
(h)	Saline	1 μg	10 μg	25 μg	50 μg
0	37.6 ± 0.2	37.7 ± 0.1	37.6 ± 0.1	37.8 ± 0.2	37.6 ± 0.3
¼	37.0 ± 0.2	36.0 ± 0.3	35.3 ± 0.4	34.4 ± 0.4	33.6 ± 0.3
½	37.4 ± 0.2	36.8 ± 0.3	36.2 ± 0.4	35.1 ± 0.4	33.9 ± 0.4
1	37.4 ± 0.1	36.8 ± 0.2	36.1 ± 0.3	34.8 ± 0.2	34.4 ± 0.5
2	37.1 ± 0.3	36.6 ± 0.1	36.5 ± 0.2	36.5 ± 0.4	35.9 ± 0.5
3	36.6 ± 0.3	36.5 ± 0.3	37.1 ± 0.1	36.8 ± 0.2	36.3 ± 0.3

FIG. 58

(n = 5)

TIME (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)			
	Saline	1 μg	5 μg	10 μg
0	37.2 ± 0.4	37.2 ± 0.4	37.2 ± 0.3	37.1 ± 0.3
¼	36.9 ± 0.3	35.8 ± 0.5	35.0 ± 0.6	34.5 ± 0.6
½	36.9 ± 0.2	36.4 ± 0.3	36.0 ± 0.7	34.8 ± 0.6
1	37.0 ± 0.2	36.4 ± 0.6	36.0 ± 0.3	35.4 ± 0.5
1 ½	36.5 ± 0.2	36.3 ± 0.3	36.3 ± 0.4	35.6 ± 0.6
2	36.2 ± 0.1	36.2 ± 0.1	36.4 ± 0.4	36.4 ± 0.6

FIG. 59

(n = 8)

TIME AFTER icv INJ. (h)	MEAN OESOPHAGEAL TEMPERATURE ($^{o}C \pm$ S.E.)	
	Reserpine ip Saline icv	Reserpine ip dbcAMP icv
0	25.0 ± 0.3	24.9 ± 0.6
¼	27.4 ± 0.6	25.8 ± 0.2
½	28.3 ± 0.7	27.5 ± 0.5
1	29.1 ± 0.6	29.6 ± 0.7
2	30.2 ± 0.5	33.4 ± 0.4
3	30.8 ± 0.4	32.1 ± 0.5
4	30.3 ± 0.6	32.1 ± 0.5
5	31.1 ± 0.7	32.2 ± 0.2
24	37.4 ± 0.7	37.4 ± 0.3

R E F E R E N C E S

Abbreviations used follow World Medical Periodicals, 3rd edn, 1961 and Supplement, 1968.

REFERENCES

ABDULLA, Y.H. & HAMADAH, K. (1970). 3',5'-cyclic adenosine monophosphate in depression and mania. *Lancet*, 1, *378 - 381.*

ADAM, H.M., McKAIL, R.A., OBRADOR, S. & WILSON, W.C. (1938). Acetylcholine in the cerebrospinal fluid. *J. Physiol. (Lond.)*, 93, *45P.*

ALDRIDGE, W.N. & PARKER, V.H. (1960). Barbiturates and oxidative phosphorylation. *Biochem. J.*, 76, *47 - 56.*

ANAGNOSTE, B. & GOLDSTEIN, M. (1967). The effects of ouabain on catecholamine biosynthesis in different areas of rats' brains. *Pharmacologist*, 9, *210.*

ANDÉN, N.-E. (1964). On the mechanism of noradrenaline depletion by α-methyl metatyrosine and metaraminol. *Acta pharmacol. (Kbh.)*, 21, *260-271.*

ANDÉN, N.-E., RUBENSON, A., FUXE, K. & HÖKFELT, T. (1967). Evidence for dopamine receptor stimulation by apomorphine. *J. Pharm. Pharmacol.*, 19, *627 - 629.*

ANDERSON, E.G. & BONNYCASTLE, D.D. (1960). A study of the central depressant action of pentobarbital, phenobarbital and diethyl ether in relationship to increases in brain 5-hydroxytryptamine. *J. Pharmacol. exp. Ther.*, 130, *138 - 143.*

ANDERSSON, B., JOBIN, M. & OLSSON, K. (1966). Serotonine and temperature control. *Acta physiol. scand.*, 67, *50 - 56.*

ANGELUCCI, L., LORENZ, G. & BALDIERI, J. (1966). The relation between noradrenaline content of rabbit heart muscle and the amount of K-strophanthin needed to produce arrhythmias. *J. Pharm. Pharmacol.*, 18, *775 - 782.*

ASGHAR, K. & WAY, E.Leong. (1970). Active removal of morphine from the cerebral ventricles. *J. Pharmacol. exp. Ther.*, 175, *75 - 83.*

ASHCROFT, G.W. & SHARMAN, D.F. (1960). 5-hydroxyindoles in human cerebrospinal fluids. *Nature (Lond.)*, 186, *1050 - 1051.*

ASHFORD, A. & ROSS, J.W. (1968). Toxicity of depressant and antidepressant drugs in hyperthyroid mice. *Brit. med. J.*, 2, *217 - 218.*

BAIN, J.A. (1954). The effect of magnesium upon thyroxine inhibition of phosphorylation. *J. Pharmacol. exp. Ther.*, 110, *2 - 3.*

BANERGEE, U., BURKS, T.F., FELDBERG, W. & GOODRICH, C.A. (1968). Temperature effects of reserpine injected into the cerebral ventricles of rabbits and cats. *J. Physiol. (Lond.)*, 197, *221 - 231.*

BARBOUR, H.G. (1912). Die Wirkung unmittelbarer Erwärmung und Abkühlung der Wärmezentra auf die Körpertemperatur. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 70, *1 - 26.*

BARNETT, A. & TABER, R.I. (1968). The effects of diethyldithiocarbamate and L-dopa on body temperature in mice. *J. Pharm. Pharmacol.*, 20, 600 - 604.

BATTERMAN, R.C. & GUTNER, L.B. (1948). Hitherto undescribed neurological manifestations of digitalis toxicity. *Amer. Heart J.*, 36, 582 - 586.

BERKOWITZ, B.A., TARVER, J.H. & SPECTOR, S. (1969). Norepinephrine release by theophylline and caffeine. *Fed. Proc.*, 28, 415.

BERNARD, C. (1875). Septième leçon: Anésthésiques. In: *Leçons sur les anésthésiques et sur l'asphyxie*, p. 213. Paris: Librairie J.B. Baillière et fils.

BERTI, F. & SHORE, P.A. (1967a). A kinetic analysis of drugs that inhibit the adrenergic neuronal membrane amine pump. *Biochem. Pharmacol.*, 16, 2091 - 2094.

BERTI, F. & SHORE, P.A. (1967b). Interaction of reserpine and ouabain on amine concentrating mechanisms in the adrenergic neurone. *Biochem. Pharmacol.*, 16, 2271 - 2274.

BERTLER, A., CARLSSON, A. & ROSENGREN, E. (1958). A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, 44, 273 - 292.

BERTLER, A., FALCK, B., OWMAN, C.R. & ROSENGREN, E. (1966). Properties of adrenergic tissues. B. The localization of monoaminergic blood-brain barrier mechanisms. *Pharmacol. Rev.*, 18, 369 - 385.

BHAGAT, B. (1964). The effects of a deficiency in thiamine and an excess of thyroid hormone on the analgesic action of morphine. *Arch. int. Pharmacodyn.*, 148, 536 - 544.

BIANCHETTI, A., PUGLIATTI, C. & JORI, A. (1967). On the hyperthermia induced by 2,4-dinitrophenol. *Med. Pharmacol. exp.*, 17, 401 - 408.

BIANCHI, C. & FRANCESCHINI, J. (1954). Experimental observations on Hoffner's method for testing analgesic drugs. *Brit. J. Pharmacol.*, 9, 280 - 284.

BIDDER, T.G. (1966). Hemi-infusion of the brain of rats: blood to brain penetration. *J. appl. Physiol.*, 21, 1073 - 1077.

BLACKBURN, K.J., FRENCH, P.C. & MERRILLS, R.J. (1967). 5-hydroxytryptamine uptake by rat brain in vitro. *Life Sci.*, 6, 1653 - 1663.

BLIGH, J. (1966). Effects on temperature of monoamines injected into the lateral ventricles of sheep. *J. Physiol. (Lond.)*, 185, 46P - 47P.

BOGDANSKI, D.F., TISSARI, A. & BRODIE, B.B. (1968). Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. *Life Sci.*, 7, 419 - 428.

BONTING, S.L. (1964). Na-K activated ATPase and active cation transport. In: *Water and Electrolyte Metabolism II*, ed. de Graeff, J. & Leijnse, B., *pp. 36 - 58*. Amsterdam: Elsevier.

BORISON, H.L. (1959). Effect of ablation of medullary chemoreceptor trigger zone on vomiting response to cerebral intraventricular adrenaline, apomorphine and pilocarpine in the cat. *J. Physiol. (Lond.)*, *147*, *172 - 177*.

BRITTAIN, R.T. & HANDLEY, S.L. (1967). Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. *J. Physiol. (Lond.)*, *192*, *805 - 813*.

BRITTAIN, R.T. & SPENCER, P.S.J. (1964). Measurement of body temperature in conscious small laboratory animals by means of an oesophageal thermocouple. *J. Pharm. Pharmacol.*, *16*, *497 - 499*.

BRODIE, B.B. & HOGBEN, C.A.M. (1957). Some physico-chemical factors in drug action. *J. Pharm. Pharmacol.*, *9*, *345 - 380*.

BRODY, T.M. (1955). The uncoupling of oxidative phosphorylation as a mechanism of drug action. *Pharmacol. Rev.*, *7*, *335 - 363*.

BRODY, T.M. & BAIN, J.A. (1951). Effect of barbiturates on oxidative phosphorylation. *Proc. Soc. exp. Biol. (N.Y.)*, *77*, *50 - 53*.

BRODY, T.M. & BAIN, J.A. (1954). Barbiturates and oxidative phosphorylation. *J. Pharmacol. exp. Ther.*, *110*, *148 - 156*.

BRODY,T.M. & KILLAM, K.F. (1952). Potentiation of barbiturate anaesthesia by 2,4-dinitrophenol. *J. Pharmacol. exp. Ther.*, *106*, *375*.

BROOKSBANK, B.W.L. & COPPEN, A. (1967). Plasma II-hydroxycorticosteroids in affective disorders. *Brit. J. Psychiat.*, *113*, *395 - 404*.

BUNNEY, W.E. jr., JANOWSKY, D.S., GOODWIN, F.K., DAVIS, J.M., BRODIE, H.K.H., MURPHY, D.L. & CHASE, T.N. (1969). Effect of L-dopa on depression. *Lancet*, *1*, *885 - 886*.

BUTCHER, R.W. & SUTHERLAND, E.W. (1962). Adenosine 3',5'-phosphate in biological materials. *J. biol. Chem.*, *237*, *1244 - 1250*.

BUTCHER, R.W. & SUTHERLAND, E.W. (1967). The effects of the catecholamines, adrenergic blocking agents, prostaglandin E, and insulin on cyclic AMP levels in the rat epididimal fat pad. *Ann. N.Y. Acad. Sci.*, *139*, *849 - 859*.

BUTLER, T.C. (1942). The delay in onset of intravenously injected anaesthetics. *J. Pharmacol. exp. Ther.*, *74*, *118 - 128*.

BUTTERBAUGH, G.G. & SPRATT, J.L. (1970a). The possible role of brain monoamines in the acute toxicity of digitoxigenin. *J. Pharmacol. exp. Ther.*, *175*, *121 - 130*.

BUTTERBAUGH, G.G. & SPRATT, J.L. (1970b). Effect of digitoxigenin in acute sublethal and lethal doses on biogenic amine content of whole rat brain. *Arch. int. Pharmacodyn.*, *186*, *345 - 351.*

BUTTERBAUGH, G.G. & SPRATT, J.L. (1970c). Observations on the possible role of central mechanisms in acute digitoxigenin toxicity. *Toxicol. appl. Pharmacol.*, *17*, *387 - 399.*

CALCUTT, C.R. & SPENCER, P.S.J. (1971). Activities of narcotic and narcotic-antagonist analgesics following intraventricular injection of various substances. *Brit. J. Pharmacol.*, *41*, *401P - 402P.*

CARLSSON, A. (1966). Modification of sympathetic function. Pharmacological depletion of catecholamine stores. *Pharmacol. Rev.*, *18*, *541 - 549.*

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966). Biochemical and histochemical studies on the effects of imipramine-like drugs and (+)-amphetamine on central and peripheral catecholamine neurons. *Acta physiol. scand.*, *67*, *481 - 497.*

CARLSSON, A., FUXE, K. & HÖKFELT, T. (1967). Failure of dopamine to accumulate in central noradrenaline neurons after depletion with diethyldithiocarbamate. *J. Pharm. Pharmacol.*, *19*, *481 - 483.*

CARLSSON, A. & LINDQVIST, M. (1962). In vivo decarboxylation of α-methyl dopa and α-methyl-meta-tyrosine. *Acta physiol. scand.*, *54*, *87 - 94.*

CARLSSON, A., LINDQVIST, M., FUXE, K. & HÖKFELT, T. (1966). Histochemical and biochemical effects of diethyldithiocarbamate on tissue catecholamines. *J. Pharm. Pharmacol.*, *18*, *60 - 62.*

CARLSSON, A., LINDQVIST, M. & MAGNUSSON, T. (1957). 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature (Lond.)*, *180*, *1200.*

CARLSSON, A. & WALDECK, B. (1958). A fluorimetric method for the determination of dopamine (3-hydroxytyramine). *Acta physiol. scand.*, *44*, *293 - 298.*

CARLSSON, A. & WALDECK, B. (1965). Inhibition of 3H-metaraminol uptake by antidepressive and related agents. *J. Pharm. Pharmacol.*, *17*, *243 - 244.*

CARPI, A. & OLIVERIO, A. (1965). Effects of reserpine on the heart-lung preparation of guinea-pig. *Arch. int. Pharmacodyn.*, *157*, *470 - 486.*

CARR, L.A. & MOORE, K.E. (1969). Norepinephrine: release from brain by d-amphetamine *in vivo*. *Science*, *164*, *322 - 323.*

CASHIN, C.H. & JACKSON, H. (1962). An apparatus for testing anti-convulsant drugs by electroshock seizures in mice. *J. Pharm. Pharmacol.*, *14*, *44T - 47T.*

CHARALAMPOUS, K.D. & BROWN, S. (1967). A clinical trial of α-methyl-para-tyrosine in mentally ill patients. *Psychopharmacologia (Berl.)*, *11*, *422 - 429.*

CHEUNG, W.Y. (1967). Properties of cyclic 3',5'-nucleotide phosphodiesterase from rat brain. *Biochemistry*, 6, *1079 - 1087*.

CONNEY, A.H. & GARREN, L. (1961). Contrasting effects of thyroxin on zoxazolamine and hexobarbital metabolism. *Biochem. Pharmacol.*, 6, *257 - 262*.

COPPEN, A. (1967). The biochemistry of affective disorders. *Brit. J. Psychiat.*, 113, *1237 - 1264*.

COPPEN, A. (1968). Depressed states and indolealkylamines. *Advanc. Pharmacol.*, 6B, *283 - 291*.

COPPEN, A., SHAW, D.M., MALLESON, A., ECCLESTON, D. & GRUNDY, G. (1965). Tryptamine metabolism in depression. *Brit. J. Psychiat.*, 111, *993 - 998*.

COX, B. & POTKONJAK, D (1967). The effect of ambient temperature on the actions of tremorine on body temperature and on the concentration of noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine in rat brain. *Brit. J. Pharmacol.*, 31, *356 - 366*.

CREMER, J.E. & BLIGH, J. (1969). Body-temperature and responses to drugs. *Brit. med. Bull.*, 25, *299 - 306*.

CREVELING, C.R., DALY, J., TOKUYAMA, T. & WITKOP, B. (1968). The combined use of α-methyltyrosine and threo-dihydroxyphenylserine - selective reduction of dopamine levels in the central nervous system. *Biochem. Pharmacol.*, 17, *65 - 70*.

CRONE, C. (1965). Facilitated transfer of glucose from blood into brain tissues. *J. Physiol. (Lond.)*, 181, *103 - 113*.

CURTIS, D.R. & ECCLES, R.M. (1958). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol. (Lond.)*, 141, *446 - 463*.

D'AMOUR, F.E. & SMITH, D.L. (1941). A method for detecting loss of pain sensation. *J. Pharmacol. exp. Ther.*, 72, *74 - 79*.

DA PRADA, M. & PLETSCHER, A. (1966). Acceleration of the cerebral dopamine turnover by chlorpromazine. *Experientia (Basel)*, 22, *465 - 466*.

DAVSON, H., KLEEMAN, C.R. & LEVIN, E. (1962). Quantitative studies of the passage of different substances out of the cerebrospinal fluid. *J. Physiol. (Lond.)*, 161, *126 - 142*.

DAWSON, W. (1968). The continuous recording of arterial blood pressure in the conscious un-restrained rat. *J. Pharm. Pharmacol.*, 20, *959 - 960*.

DEARING, W.H., BARNES, A.R., KERNOHAN, J.W. & ESSEX, H.E. (1943). Experiments with calculated therapeutic and toxic doses of digitalis. IV. Effects of cellular structure of the central nervous system. *Amer. Heart J.*, 25, *734 - 745*.

DEGKWITZ, R. FROWEIN, R., KULENKAMPFF, C. & MOHS, U. (1960). Über die Wirkungen des L-Dopa beim Menschen und deren Beeinflussung durch Reserpin, Chlorpromazin, Iproniazid und Vitamin B_6. *Klin. Wschr.*, *38*, *120 - 123.*

DEICHMAN, W., MACHLE, W., KITZMILLER, K.V. & THOMAS, G. (1942). Acute and chronic effects of pentachlorophenol and sodium pentachlorophenate upon experimental animals. *J. Pharmacol. exp. Ther.*, *76*, *104 - 117.*

DEWHURST, W.G. (1968). Cerebral amine functions in health and disease. In: *Studies in Psychiatry*, ed. Shepherd, M., Davies, D.L., *pp. 289 - 317.* London: Oxford University Press.

DIKSHIT, B.B. (1934). Action of acetylcholine in the brain and its occurrence therein. *J. Physiol. (Lond.)*, *80*, *409 - 421.*

DIKSHIT, B.B. (1935). Action of acetylcholine on the "sleep center". *J. Physiol. (Lond.)*, *83*, *42P.*

DIXON, W.E. & HALLIBURTON, W.D. (1916). The cerebrospinal fluid. IV. Circulation. *J. Physiol. (Lond.)*, *50*, *198 - 216.*

DOBBING, J. (1961). The blood-brain barrier. *Physiol. Rev.*, *41*, *130 - 188.*

DOLFINI, E. & KOBAYASHI, M. (1967). Studies with amphetamine in hyper- and hypothyroid rats. *Europ. J. Pharmacol.*, *2*, *65 - 66.*

DORAN, W.J. (1959). Barbituric acid hypnotics. In: *Medicinal Chemistry, Vol. IV.*, ed. Blicke, F.F. & Cox, R.H. New York: Wiley.

EBADI, M.S. & CARVER, M.J. (1970). The inhibition of transport ATPase by chlorprothixene in brain. *Europ. J. Pharmacol.*, *9*, *190 - 194.*

EDSTROM, R. (1964). Recent developments of the blood-brain barrier concept. *Int. Rev. Neurobiol.*, *7*, *153 - 190.*

EHRINGER, H., HORNYKIEWICZ, O. & LECHNER, K. (1960). Die Wirkung des Chlorpromazins auf den Katecholamin- und 5-Hydroxytryptaminstoffwechsel im Gehirn der Ratte. *Naunyn Schmiedeberg's Arch. exp. Path. Pharmak.*, *239*, *507 - 519.*

EHRLICH, P. (1885). Experimenteller Theil. I. Das Alizarinblau. D. Ueber die Vertheilungsgesetze des Alizarinblau. In: *Das Sauerstoff-Bedürfnis des Organismus. Eine farbenanalytische Studie*, *pp. 54 - 72.* Berlin: Verlag von August Hirschwald.

ELLIOTT, H.C. (1969). Accessory cells of the central nervous system. In: *Textbook of Neuroanatomy*, *pp. 21 - 35.* Oxford: Blackwell Scientific Publications.

ELSHOVE, A. & ROSSUM, G.D.V. van (1963). Net movements of sodium and potassium, and their relation to respiration, in slices of rat liver incubated in vitro. *J. Physiol. (Lond.)*, *168*, *531 - 553.*

ELSMORE, T.A. & WITHROW, C.D. (1968). Intracellular pH regulation and the Na^+ pump. *Fed. Proc.*, 27, *603.*

ERNST, A.M. (1967). Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia (Berl.)*, 10, *316 - 323.*

ERNST, A.M. & SMELIK, P.G. (1966). Site of action of dopamine and apomorphine on compulsive gnawing behaviour in rats. *Experientia (Basel)*, 22, *837 - 838.*

EULER, C. von (1964). The gain of the hypothalamic temperature regulating mechanisms. *Progr. Brain Res.*, 5, *127 - 131.*

EULER, U.S. von & FLODING, I. (1955). A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. *Acta physiol. scand.*, 33, *suppl. 18, 45 - 56.*

FAWCETT, J.A. & BUNNEY, W.E. jr. (1967). Pituitary adrenal function and depression. An outline for research. *Arch. gen. Psychiat.*, 16, *517 - 535.*

FELDBERG, W. (1963). *A Pharmacological Approach to the Brain from its Inner and Outer Surface.* London: Arnold.

FELDBERG, W. (1966). Adrenergic mechanisms in the nervous system. Introductory remarks. *Pharmacol. Rev.*, 18, *713 - 716.*

FELDBERG, W. & FLEISCHHAUER, K. (1965). A new experimental approach to the physiology and pharmacology of the brain. *Brit. med. Bull.*, 21, *36 - 43.*

FELDBERG, W., HELLION, R.F. & LOTTI, V.J. (1967). The temperature effects produced in dogs and monkeys by injection of monoamines and related substances into the third ventricle. *J. Physiol. (Lond.)*, 191, *501 - 515.*

FELDBERG, W. & MYERS, R.D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol. (Lond.)*, 173, *226 - 237.*

FELDBERG, W. & SHERWOOD, S.L. (1953). A permanent cannula for intraventricular injections in cats. *J. Physiol. (Lond.)*, 120, *3P.*

FELDBERG, W. & SHERWOOD, S.L. (1954). Injections of drugs into the lateral ventricle of the cat. *J. Physiol. (Lond.)*, 123, *148 - 167.*

FIELD, M., PLOTKIN, G.R. & SILEN, W. (1968). Effects of vasopressin, theophylline and cyclic adenosine monophosphate on short circuit current across isolated rabbit ileal mucosa. *Nature (Lond.)*, 217, *469 - 471.*

FINDLAY, J.D. & ROBERTSHAW, D. (1967). The mechanism of body temperature changes induced by intraventricular injections of adrenaline, noradrenaline and 5-hydroxytryptamine in the ox. *J. Physiol. (Lond.)*, 189, *329 - 336.*

FRIEDEMANN, U. (1942). Blood-brain barrier. *Physiol. Rev.*, 22, *125 - 145*.

FUHRMAN, F.A. (1947). The effect of body temperature on the duration of barbiturate anesthesia in mice. *Science*, 105, *387 - 388*.

FUJITA, T. & TEDESCHI, D.H. (1968). Technic to record arterial blood pressure of unanaesthetized rats. *Life Sci.*, 7, *673 - 680*.

FUXE, K. & HÖKFELT, T. (1966). The effects of barbiturates on the activity of the catecholamine neurones in the rat brain. *J. Pharm. Pharmacol.*, 18, *556 - 558*.

FUXE, K. & UNGERSTEDT, U. (1966). Localisation of catecholamine uptake in the rat brain after intraventricular injection. *Life Sci.*, 5, *1817 - 1824*.

FUXE, K. & UNGERSTEDT, U. (1968). Histochemical studies on the effect of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurons after intraventricular injection of catecholamines and 5-hydroxytryptamine. *Europ. J. Pharmacol.*, 4, *135 - 144*.

GERST, E.C., STEINSLAND, O.S. & WALCOTT, W.W. (1966). Use of constant temperature and sodium borohydride in the trihydroxyindole method of catecholamines. *Clin. Chem.*, 12, *659 - 669*.

GEY, K.F. & PLETSCHER, A. (1961). Influence of chlorpromazine and chlorprothixene on the cerebral metabolism of 5-hydroxytryptamine, norepinephrine and dopamine. *J. Pharmacol. exp. Ther.*, 133, *18 - 24*.

GIACHETTI, A. & SHORE, P.S. (1966). Studies in vitro of amine uptake mechanisms in heart. *Biochem. Pharmacol.*, 15, *607 - 614*.

GIBBONS, J.L. (1965). Metabolic function in affective disorders. Endocrine changes in depressive illness. *Proc. roy. Soc. Med.*, 58, *519 - 521*.

GLOWINSKI, J. & IVERSEN, L.L. (1966). Regional studies of catecholamines in the rat brain - I. The disposition of [^{3}H]norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J. Neurochem.*, 13, *655 - 669*.

GLOWINSKI, J., KOPIN, I.J. & AXELROD, J. (1965). Metabolism of noradrenaline-^{3}H in the rat brain. *J. Neurochem.*, 12, *25 - 30*.

GODFRAIND, J.M., KRNJEVIĆ, K. & PUMAIN, R. (1970). Unexpected features of the action of dinitrophenol on cortical neurones. *Nature (Lond.)*, 228, *562 - 564*.

GOLDSTEIN, L. & MUNOZ, C. (1961). Influence of adrenergic stimulant and blocking drugs on cerebral electrical activity in curarized animals. *J. Pharmacol. exp. Ther.*, 132, *345 - 353*.

GOLDSTEIN, M., OHI, Y. & BACKSTROM, T. (1970). The effect of ouabain on catecholamine biosynthesis in rat brain cortex slices. *J. Pharmacol. exp. Ther.*, 174, *77 - 82*.

GOMAHR, H. (1957). Beiträge zur Kenntnis der Wirkung von Adenosin. 2. Mitteilung: Der Einfluss von Adenosin auf die Wirkung von Barbituraten. *Arzneimittel-Forsch.*, *7*, *621 - 623*.

GOODMAN, L.S., TOMAN, J.E.P. & SWINYARD, E.A. (1949). Anticonvulsant drugs: Mechanisms of action and methods of assay. *Arch. int. Pharmacodyn.*, *78*, *144 - 162*.

GREEF, K. & KASPERAT, H. (1961a). Konvulsive und paralytische Wirkungen von Digitalis-Glycosiden und Geninen bei intracerebraler und intravenöser Injektion an Mäusen. *Arzneimittel-Forsch.*, *11*, *908 - 909*.

GREEF, K. & KASPERAT, H. (1961b). Vergleich der neurotoxischen Wirkung von Digitalisglykosiden und Geninen bei intracerebraler und intravenöser Injektion an Mäusen, Ratten und Meerschweinchen. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, *242*, *76 - 89*.

GREEN, H.N. & STONER, H.B. (1950). *Biological Actions of the Adenine Nucleotides*. London: H.K. Lewis.

GRUNDEN, L.R. (1969). Action of intracerebroventricular epinephrine on gross behaviour, locomotor activity and hexobarbital sleeping times in rats. *Int. J. Neuropharmacol.*, *8*, *573 - 586*.

GRUNDEN, L.R. & MARLEY, E. (1970). Effects of sympathomimetic amines injected into the third ventricle in adult chickens. *Int. J. Neuropharmacol.*, *9*, *119 - 128*.

HÄGGENDAL, J. (1968). The depletion and recovery of noradrenaline in the brain and some sympathetically innervated mammalian tissues after tetrabenazine. *J. Pharm. Pharmacol.*, *20*, *364 - 367*.

HALEY, T.J. & McCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Brit. J. Pharmacol.*, *12*, *12 - 15*.

HALEY, T.J. & WEINBERG, S.J. (1955). Comparison of strophanthin-K and tryptamine-strophanthidin after intraventricular injection in unanaesthetised dogs. *Proc. Soc. exp. Biol. (N.Y.)*, *89*, *345 - 349*.

HARRIS, L.S. (1970). Central neurohumoral systems involved with narcotic agonists and antagonists. *Fed. Proc.*, *29*, *28 - 32*.

HAVLIČEK, V. & SKLENOVSKÝ, A. (1967). The deactivating effect of catecholamines upon the electrocorticogram of the rat. *Brain Res.*, *4*, *345 - 357*.

HAYDEN, J.F., JOHNSON, L.R. & MAICKEL, R.P. (1966). Construction and implantation of a permanent cannula for making injections into the lateral ventricle of the rat brain. *Life Sci.*, *5*, *1509 - 1515*.

HEINBECKER, P. & BARTLEY, S.H. (1940). Action of ether and nembutal on the nervous system. *J. Neurophysiol.*, *3*, *219 - 236*.

HERMANSEN, K. (1970). Evidence for adrenergic mediation of ouabain induced arrhythmias in the guinea-pig. *Acta pharmacol. (Kbh.)*, *28*, *57 - 65*.

HERZ, A. (1960). Drugs and the conditioned avoidance response. *Int. Rev. Neurobiol.*, 2, *229 - 277*.

HOCH, F.L. & LIPMAN, F. (1953). Inhibition of mitochondrial phosphorylation by thyroxin. *Fed. Proc.*, 12, *218 - 219*.

HUEPER, W.C. & ICHNIOWSKI, C.T. (1941). Experimental studies in cardiovascular pathology. II. Pathological lesions in organs of cats, guinea pigs, and frogs produced by digitalis poisoning. *J. Lab. clin. Med.*, 26, *1565 - 1574*.

HULME, N.A. & KRANTZ, J.C. (1955). Anaesthesia XLVIII. Anaesthetic potency and the uncoupling of oxidative phosphorylation. *Anaesthesiology*, 17, *43 - 46*.

INNES, I.R. & NICKERSON, M. (1965). Drugs acting on postganglionic adrenergic nerve endings and structures innervated by them. (Sympathomimetic drugs). In: *The Pharmacological Basis of Therapeutics*, 3rd Edn., ed. Goodman, C.S. & Gilman, A., *pp. 477 - 520*. New York: Macmillan.

JACOBSEN, E. (1964). The theoretical basis of the chemotherapy of depression. In: *Depression*, ed. Davies, E.B., *pp. 208 - 213*. Cambridge: Cambridge University Press.

JACOBSEN, E. & SONNE, E. (1955). The effect of benzilic acid diethylaminoethylester, HCl (Benactyzine) on stress-induced behaviour in the rat. *Acta pharmacol. (Kbh.)*, 11, *135 - 147*.

JACOBSEN, E. & SONNE, E. (1956). The effect of benactyzine on the conditioned responses in the rat. *Acta pharmacol. (Kbh.)*, 12, *310 - 320*.

KAISER, J.A. (1964). Studies on the toxicity of disophenol (2,6-diiodo-4-nitrophenol) to dogs and rodents plus some comparisons with 2,4-dinitrophenol. *Toxicol. appl. Pharmacol.*, 6, *232 - 244*.

KAUL, C.L. & LEWIS, J.J. (1963a). The effects of reserpine and some related compounds upon the levels of adenine nucleotides, creotine phosphate and inorganic phosphate in the rat brain *in vivo*. *J. Pharmacol. exp. Ther.*, 140, *111 - 116*.

KAUL, C.L. & LEWIS, J.J. (1963b). Effects of minor tranquillizers on brain phosphate levels *in vivo*. *Biochem. Pharmacol.*, 12, *1279 - 1282*.

KLEINROK, Z., ZEBROWSKA, I. & WIELOSZ, M. (1970). Some central effects of diethyldithio-carbamate administered intraventricularly in rats. *Int. J. Neuropharmacol.*, 9, *451 - 455*.

KRANTZ, K.D. & SEIDEN, L.S. (1968). Effects of diethyldithiocarbamate on the conditioned avoidance response of the rat. *J. Pharm. Pharmacol.*, 20, *166 - 167*.

LABORIT, H. (1963). Stoffwechselstrukturen. Funktionelle und pharmakologische Tätigkeiten. *Ärztl. Forsch.*, 17, *169 - 181*.

LABORIT, H. & WEBER, B. (1965). Essai d'interpretation du mode d'action métabolique de certains agents neurotropes. II. Le sulfate de strychnine. III. Le gamma hydroxybutyrate. IV. Le penthiobarbital. V. L'éther éthylique et le chloroform. VI. Le pentylenetetrazol. *Agressologie*, 6, *157 - 203.*

LACKEY, R.W. (1947). Effect of the injection of glucose into the cerebrospinal fluid. *Science*, 106, *618.*

LAPIN, I.P. & OXENKRUG, G.F. (1969). Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. *Lancet*, 1, *132 - 136.*

LARDY, H.A. & FELDOTT, G. (1951). Metabolic effects of thyroxine in vitro. *Ann. N.Y. Acad. Sci.*, 54, *636 - 648.*

LARSEN, E.R., DYKE, R.A. van & CHENOWETH, M.B. (1968). Mechanisms of narcosis. In: *Drugs Affecting the C.N.S.*, Medicinal Research Series Vol. 2, ed. Burger, A., *pp. 1 - 24.* London: Arnold.

LAVERTY, R. & TAYLOR, K.M. (1970). Effects of intraventricular 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behaviour and brain catecholamine metabolism. *Brit. J. Pharmacol.*, 40, *836 - 846.*

LESSIN, A.W. & PARKES, M.W. (1957). The relation between sedation and body temperature in the mouse. *Brit. J. Pharmacol.*, 12, *245 - 250.*

LEVIN, E. & SCICLI, G. (1969). Brain barrier phenomena. *Brain Res.*, 13, *1 - 12.*

LEVINE, O.R. & BLUMENTHAL, S. (1962). Digoxin dosage in premature infants. *Pediatrics*, 29, *18 - 25.*

LEWIS, J.J. & PATTEN, G.R. van (1962). The effect of amphetamine and related compounds on the concentration of adenine nucleotides, inorganic phosphate and creatine phosphate in the rat brain. *J. Pharmacol. exp. Ther.*, 136, *372 - 377.*

LEWIS, J.J. & PATTEN, G.R. van (1963). The effect of antidepressive drugs and some related compounds on the levels of adenine nucleotides, inorganic phosphate and phosphocreatine in the rat brain. *Brit. J. Pharmacol.*, 20, *462 - 470.*

LITCHFIELD, J.T. jr. & WILCOXON, F. (1949). A simplified method for evaluating dose-effect experiments. *J. Pharmacol. exp. Ther.*, 96, *99 - 113.*

LOMAX, P. (1966). Measurement of 'core' temperature in the rat. *Nature (Lond.)*, 210, *854.*

McILWAIN, H. (1953). The effect of depressants on the metabolism of stimulated cerebral tissues. *Biochem. J.*, 53, *403 - 412.*

McILWAIN, H. (1966). *Biochemistry and the Central Nervous System*, 3rd edn. London: Churchill.

MAJ, J. & PRZEGALIŃSKI, E. (1967). Disulfiram and some effects of amphetamine in mice and rats. *J. Pharm. Pharmacol.*, 19, *341 - 343*.

MAJ, J., SOWIŃSKA, H., BARAN, L. & DUREK, B. (1971). The influence of agents depressing the catecholamine levels on the action of narcotic analgesics. *Diss. pharm. pharmacol.*, 23, *9 - 23*.

MAJ, J. & ZEBROWSKA, I. (1966). The effect of catecholamines on the cataleptic action of chlorpromazine and haloperidol. *Diss. pharm. pharmacol.*, 18, *439 - 448*.

MANDELL, A.J. & SPOONER, C.E. (1968). Psychochemical research studies in man. *Science*, 162, *1442 - 1453*.

MARTIUS, C. & HESS, B. (1951). The mode of action of thyroxin. *Arch. Biochem.*, 33, *486 - 487*.

MATHES, S., GOLD, H., MARSH, R., GREINER, T., PALUMBO, F. & MESSELOFF, C. (1952). Comparison of the tolerance of adults and children to digitoxin. *J. Amer. med. Ass.*, 150, *191 - 194*.

MATHIEU, N. (1960). Potentialisation de la narcose expérimental par l'acide adénosine triphosphorique chez le rat et la souris. *Med. exp. (Basel)*, 2, *278 - 284*.

MATHIEU-LEVY, N. (1967). Métabolisme de quelques hypnotiques (hydrate de chloral, trichloréthanol, mébubarbital, thiopental) chez le rat, au cours du sommeil expérimental potentialisé par l'acide adénosine triphosphorique. *Thérapie*, 22, *945 - 956*.

MATHIEU-LEVY, N. (1968). Mécanisme de la potentialisation du sommeil expérimental par l'acide adénosinetriphosphorique (ATP). Influence des propriétés vasodilatrices et hypothermisante d'ATP. *Thérapie*, 23, *105 - 114*.

MAYER, S., MAICKEL, R.P. & BRODIE, B.B. (1959). Kinetics of penetration of drugs and other foreign compounds into cerebrospinal fluid and brain. *J. Pharmacol. exp. Ther.*, 127, *205 - 211*.

MAYNARD, E.A., SCHULTZ, R.L. & PEASE, D.C. (1957). Electron microscopy of the vascular bed of the rat cerebral cortex. *Amer. J. Anat.*, 100, *409 - 434*.

MEGGES, R. & REPKE, K. (1964). Extracardiac toxicities of various digitalis derivatives. *Med. exp. (Basel)*, 10, *267 - 271*.

MILLER, J.W. & ELLIOTT, H.W. (1955). Rat tissue levels of carbon-14-labelled analgetics as related to pharmacological activity. *J. Pharmacol. exp. Ther.*, 113, *283 - 291*.

MILNE, M.D., SCRIBNER, B.H. & CRAWFORD, M.A. (1958). Non-ionic diffusion and the excretion of weak acids and bases. *Amer. J. Med.*, 24, *709 - 729*.

MINARD, F.N. & DAVIS, R.V. (1962). Effect of chlorpromazine, ether, and phenobarbital on the active-phosphate level of rat brain: an improved extraction technique for acid-soluble phosphates. *Nature (Lond.)*, 193, *277 - 278*.

MONAKOW, C. von (1921). Der Kreislauf des Liquor Cerebrospinalis. *Schweiz. Arch. Neurol. Psychiat.*, 8, *233 - 234*.

MOORE, P.F., IORIO, L.C. & McMANUS, J.M. (1968). Relaxation of the guinea-pig tracheal chain preparation by N^6, 2'-O-dibutyryl 3',5'-cyclic adenosine monophosphate. *J. Pharm. Pharmacol.*, 20, *368 - 372*.

MOSHER, L.R., KLERMAN, G.L. & GREANEY, J.F. (1966). A clinical trial of alpha-methyldopa in elated states. *Amer. J. Psychiat.*, 122, *1185 - 1188*.

NYBACK, H. & SEDVALL, G. (1968). Influence of drugs on catecholamine metabolism in brain as studied by ^{14}C-tyrosine. *Brit. J. Pharmacol.*, 34, *220P - 221P*.

OWMAN, C. & ROSENGREN, E. (1967). Dopamine formation in blood capillaries - an enzymic blood-brain barrier. *J. Neurochem.*, 14, *547 - 550*.

PARE, C.M. & SANDLER, M. (1959). A clinical and biochemical study of a trial of iproniazid in the treatment of depression. *J. Neurol. Neurosurg. Psychiat.*, 22, *247 - 251*.

PERSSON, T. & ROOS, B.-E. (1967). 5-hydroxytryptophan for depression. *Lancet*, 2, *987 - 988*.

PERSSON, T. & WALDECK, B. (1968). Effects of protryptyline on the formation of H^3-noradrenaline from H^3-dopa. *J. Pharm. Pharmacol.*, 20, *966 - 969*.

PFEIFER, A.K., GALAMBOS, E. & GYÖRGY, L. (1966). Some central nervous properties of diethyldithiocarbamate. *J. Pharm. Pharmacol.*, 18, *254*.

PLETSCHER, A. & DA PRADA, M. (1967). Mechanism of action of neuroleptics. In: *Neuropsychopharmacology*. Proceedings of the fifth international congress of the collegium internationale neuro-psychopharmacologicum, Int. Congress Series, No. 129, pp. *304 - 311*. Amsterdam: Excerpta Medica Foundation.

POLLAY, M. & CURL, F. (1967). Secretion of cerebrospinal fluid by the ventricular ependyma of the rabbit. *Amer. J. Physiol.*, 213, *1031 - 1038*.

POPOVIC, V. & POPOVIC, P. (1960). Permanent cannulation of aorta and vena cava in rats and ground squirrels. *J. appl. Physiol.*, 15, *727 - 728*.

PRANGE, A.J., LIPTON, M.A., SHEARIN, R.B. & LOVE, G.N. (1966). The influence of thyroid status on the effects and metabolism of pentobarbital and thiopental. *Biochem. Pharmacol.*, 15, *237 - 248*.

PRINCE, A.L. & HAHN, L.J. (1918). The effect on body temperature induced by thermal stimulation of the heat center in the brain of the cat. *Amer. J. Physiol.*, 46, *412 - 415*.

RALL, D.P., STABENAU, J.R. & ZUBROD, C.G. (1959). Distribution of drugs between blood and cerebrospinal fluid: general methodology and effect of pH gradients. *J. Pharmacol. exp. Ther.*, 125, *185 - 193*.

RALL, D.P. & ZUBROD, C.G. (1962). Mechanisms of drug absorption and excretion: passage of drugs in and out of the central nervous system. *Ann. Rev. Pharmacol.*, 2, *109 - 128.*

RANDRUP, A. & SCHEEL-KRÜGER, J. (1966). Diethyldithiocarbamate and amphetamine stereotype behaviour. *J. Pharm. Pharmacol.*, 18, *752.*

RAVENTÓS, J. (1938). The influence of room temperature on the action of barbiturates. *J. Pharmacol. exp. Ther.*, 64, *355 - 363.*

READING, H.W. & WALLWORK, J. (1969). Oxidation of succinate and pyruvate in rat brain and its effects on barbiturate anaesthesia. *Biochem. Pharmacol.*, 18, *2211 - 2214.*

REID, W.D., VOLICER, L., SMOOKLER, H., BEAVEN, M.A. & BRODIE, B.B. (1968). Brain amines and temperature regulation. *Pharmacology (Basel)*, 1, *329 - 344.*

RI, H.D. & SCHMIDT, G. (1960). Zentrale und periphere Beeinflussung der Atmung durch toxische Herzglykosiddosen an der Ratte. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, 239, *158 - 169.*

RIZZOLO, A. (1929). Clonus and generalised epileptiform convulsions caused by local applications of strophanthin, theine and nicotine on the cephalic end of the central nervous system. *Arch. Farmacol. sper.*, 50, *16 - 30.*

ROSENBLATT, S., CHANLEY, J.D., SOBOTKA, H. & KAUFMAN, M.R. (1960). Interrelationships between electroshock, the blood-brain barrier and catecholamines. *J. Neurochem.*, 5, *172 - 176.*

ROSS, S.B. & RENYI, A.L. (1966). In vitro inhibition of noradrenaline-^{3}H uptake by reserpine and tetrabenazine in mouse cerebral cortex tissues. *Acta pharmacol. (Kbh.)*, 24, *73 - 88.*

ROSS, S.B. & RENYI, A.L. (1967). Inhibition of the uptake of tritiated catecholamines by anti-depressant and related agents. *Europ. J. Pharmacol.*, 2, *181 - 186.*

ROSSUM, J.M. van (1966). The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. *Arch. int. Pharmacodyn.*, 160, *492 - 493.*

RUBIN, R.T. & MANDELL, A.J. (1966). Adrenal cortical activity in pathological emotional states: a review. *Amer. J. Psychiat.*, 123, *387 - 400.*

SAARNIVAARA, L. (1969). Analgesic activity of some sympathetic drugs and their effect on morphine analgesia in rabbits. *Ann. Med. exp. Fenn.*, 47, *180 - 190.*

SACHAR, E.J. (1967). Corticosteroids in depressive illness. I. A re-evaluation of control issues and the literature. II. A longitudinal psychoendocrine study. *Arch. gen. Psychiat.*, 17, *544 - 567.*

SALMOIRAGHI, G.C. (1966). Adrenergic mechanisms in the nervous system. B. Central adrenergic synapses. *Pharmacol. Rev.*, 18, 717 - 726.

SANTESSON, C.G. & STRINDBERG, L. (1917). Digitalisstudien. *Skand. Arch. Physiol.*, 35, 51 - 100.

SCHEEL-KRÜGER, J. (1970). Central effects of anticholinergic drugs measured by the apomorphine gnawing test in mice. *Acta pharmacol. (Kbh.)*, 28, 1 - 6.

SCHEEL-KRÜGER, J. & RANDRUP, A. (1967). Stereotype hyperactivity behaviour produced by dopamine in the absence of noradrenaline. *Life Sci.*, 6, 1389 - 1398.

SCHILDKRAUT, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Amer. J. Psychiat.*, 122, 509 - 522.

SCHOEPFLE, G.M. (1957). Pentothal block of single nerve fibres and subsequent revival by means of anodal polarization. *Fed. Proc.*, 16, 114.

SEAGER, L.D., MURPHEE, O.D. & MONROE, B. (1965). Behaviour changes in the rat induced by subacute and convulsive doses of digitoxin. *Int. J. Neuropsychiat.*, 1, 383 - 390.

SHANKER, L.S. (1961). Mechanisms of drug absorption and distribution. *Ann. Rev. Pharmacol.*, 1, 29 - 44.

SHANKER, L.S. (1962). Passage of drugs across body membranes. *Pharmacol. Rev.*, 14, 501 - 530.

SHEPHERD, M., LADER, M. & RODNIGHT, R. (1968a). Biochemical effects of psychotropic drugs. In: *Clinical Psychopharmacology*, pp. 192 - 241. London: English University Press.

SHEPHERD, M., LADER, M. & RODNIGHT, R. (1968b). The "tranquillizers". The phenothiazines, the rauwolfia derivatives, the butyrophenones and others. In: *Clinical Psychopharmacology*, pp. 85 - 125. English University Press.

SHERWOOD, S.L. (1955). The response of psychotic patients to intraventricular injections. *Proc. roy. Soc. Med.*, 48, 855 - 863.

SIMMONDS, M.A. & URETSKY, N.J. (1970). Acute hypothermic response of the rat to the intraventricular injection of 6-hydroxydopamine. *Brit. J. Pharmacol.*, 40, 564P - 565P.

SJOERDSMA, A., ENGELMAN, K., SPECTOR, S. & UDENFRIEND, S. (1965). Inhibition of catecholamine synthesis in man with alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *Lancet*, 2, 1092 - 1094.

SMITH, H.J. & WILLIAMS, H. (1965a). Aspects of drug action: a comparison with intramolecular processes occurring in pharmaceutical and biochemical systems. *J. Pharm. Pharmacol.*, 17, 529 - 557.

SMITH, H.J. & WILLIAMS, H. (1965b). Some aspects of drug action: a comparison with intramolecular processes occurring in pharmaceutical and biochemical systems. *J. Pharm. Pharmacol.*, *17*, *601 - 618*.

SOMLYO, A.P. (1960). The toxicology of digitalis. *Amer. J. Cardiol.*, *5*, *523 - 533*.

SPARKES, C.G. & SPENCER, P.S.J. (1969). Modification of morphine analgesia in the rat by biogenic amines administered intraventricularly. *Brit. J. Pharmacol.*, *35*, *362P - 363P*.

SPARKES, C.G. & SPENCER, P.S.J. (1971). Antinociceptive activity of morphine after injection of biogenic amines in the cerebral ventricles of the conscious rat. *Brit. J. Pharmacol.*, *42*, *230 - 241*.

SPENCER, P.S.J. & WAITE, R. (1968). The effects of chlorpromazine and reserpine in hyperthyroid mice. *Brit. J. Pharmacol.*, *32*, *419P - 420P*.

SPENCER, P.S.J. & WAITE, R. (1970). Barbiturate-induced sleep in hyperthyroid mice. *Europ. J. Pharmacol.*, *11*, *392 - 394*.

STILL, J.W., PRADHAN, S.N. & WHITCOMB, E.R. (1956). Direct measurements of aortic blood pressure in unanaesthetized rats. *J. appl. Physiol.*, *8*, *575 - 576*.

STILLE, G. & SAYERS, A. (1964). The effect of antidepressant drugs on the convulsive excitability of brain structures. *Int. J. Neuropharmacol.*, *3*, *605 - 609*.

TANZ, R.D. (1967). Possible relationship between ouabain-induced augmentation and endogenous cardiac catecholamine release. In: *Factors Influencing Myocardial Contractility*, ed. Tanz, R.D., Cavaler, F. & Roberts, J.A.Y., *pp. 563 - 577*. New York & London: Academic Press.

TARVE, U. & BRECHTLOVÁ, M. (1967). Effects of psychopharmacological agents on brain metabolism. III. Effects of imipramine and ouabain on the $(Na^+ + K^+)$-activated ATPase from brain microsomes and co-operative interactions with the enzyme. *J. Neurochem.*, *14*, *283 - 290*.

THURANSZKY, K. (1966). Continuous blood pressure measurements in non-anaesthetized animals. *Acta physiol. Acad. Sci. hung.*, *29*, *33 - 40*.

UNGERSTEDT, U. (1968). 6-hydroxydopamine induced degeneration of central monoamine neurones. *Europ. J. Pharmacol.*, *5*, *107 - 110*.

UNGERSTEDT, U., BUTCHER, L.L., BUTCHER, S.G., ANDÉN, N.-E. & FUXE, K. (1969). Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. *Brain Res.*, *14*, *461 - 471*.

URETSKY, N.J. & IVERSEN, L.L. (1969). Effect of 6-hydroxydopamine on noradrenaline-containing neurones in the rat brain. *Nature (Lond.)*, *221*, *557 - 559*.

URETSKY, N.J. & IVERSEN, L.L. (1970). Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. *J. Neurochem.*, *17*, *269 - 278*.

VEDERNIKOV, Y.P. & AFRIKANOV, I.I. (1969). On the role of a central adrenergic mechanism in morphine analgesic action. *J. Pharm. Pharmacol.*, *21*, *845 - 847*.

WAELSCH, H. (1955). Blood-brain barrier and gas exchange. In: *Biochemistry of the Developing Nervous System*, ed. Waelsch, H., *pp. 187 - 207*. New York: Academic Press.

WATANABE, K., MATSUI, Y. & IWATA, H. (1969). Enhancement of the analgesic effect of morphine by sodium diethyldithiocarbamate in rats. *Experientia (Basel)*, *25*, *950 - 951*.

WEEKS, J.R. & DAVIS, J.D. (1964). Chronic intravenous cannulas for rats. *J. appl. Physiol.*, *19*, *540 - 541*.

WEEKS, J.R. & JONES, J.A. (1960). Routine direct measurement of arterial pressure in unanaesthetized rats. *Proc. Soc. exp. Biol. (N.Y.)*, *104*, *646 - 648*.

WEIL-MALHERBE, H., WHITBY, L.G. & AXELROD, J. (1961). The uptake of circulating (^{3}H) norepinephrine by the pituitary gland and various areas of the brain. *J. Neurochem.*, *8*, *55 - 64*.

WEINBACH, E.C. & GARBUS, J. (1969). Mechanism of action of reagents that uncouple oxidative phosphorylation. *Nature (Lond.)*, *221*, *1016 - 1018*.

WEINBERG, S.J. & HALEY, T.J. (1955). Centrally mediated effects of cardiac drugs: Strophanthin-K, Quinidine and Procaine amide. *Circulat. Res.*, *3*, *103 - 109*.

WEINER, N. & HULS, H.N. (1961). Effect of chlorpromazine on levels of adenine nucleotides and creatine phosphate of brain. *J. Neurochem.*, *7*, *180 - 185*.

WEISS, S. (1932). The effects of the digitalis bodies on the nervous system: an analysis of the mechanism of cardiac slowing, nausea, and vomiting, psychosis, and visual disturbance following digitalis therapy. *Med. Clin. N. Amer.*, *15*, *963 - 982*.

WHITE, R.P., BOYAJY, C.D. (1959). Comparison of physostigmine and amphetamine in antagonising EEG effects of C.N.S. depressants. *Proc. Soc. exp. Biol. (N.Y.)*, *102*, *479 - 483*.

WILLIAMS, J.A., WITHROW, C.D. & WOODBURY, D.M. (1969). Effects of ouabain, diphenylhydantoin (DPH), and K^+ on transmembrane potentials and electrolytes of rat liver and muscle. *Pharmacologist*, *11*, *268*.

WILSON, D.F. & MERZ, R. (1969). Inhibition of mitochondrial respiration by uncouplers of oxidative phosphorylation. II. The site of inhibition of succinate oxidation by the uncouplers. *Arch. Biochem.*, *129*, *79 - 85*.

WINTERSTEIN, H. (1961). The actions of substances introduced into the cerebrospinal fluid and the problems of intracranial chemoreceptors. *Pharmacol. Rev.*, *13*, *71 - 107*.

WITHERING, W. (1785). *An Account of the Foxglove and some of its Medicinal Uses: With Practical Remarks on Dropsy and Other Diseases.* Birmingham: M. Swinney.

WOLPERT, A., TRUITT, E.B.jr., BELL, F.K. & KRANTZ, J.C. jr. (1956). Anesthesia. L. The effect of certain narcotics on oxidative phosphorylation. *J. Pharmacol. exp. Ther., 117, 358 - 361.*

WOODWARD, D.L., REED, D.J. & WOODBURY, D.M. (1967). Extracellular space of rat cerebral cortex. *Amer. J. Physiol., 212, 367 - 370.*

ZEBROWSKA-LUPINA, I., KLEINROK, Z. & SMOLARZ, J. (1971). The effect of sodium diethyldithiocarbamate on the action of analgesics in albino mice. *Diss. pharm. pharmacol., 23, 33 - 39.*

ZETLER, G. (1968). Cataleptic state and hypothermia in mice, caused by central cholinergic stimulation and antagonized by anticholinergic and antidepressant drugs. *Int. J. Neuropharmacol., 7, 325 - 335.*