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AN EXAMINATION OF THE EFFECTS OF PINEALECTOMY UPON ADRENAL STRUCTURE IN THE WHITE RAT.

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SUMMARY OF THESIS Derek J. Mechan. M.Sc.

The effects of pineal extirpation at three days and thirty days upon adrenal structure in male and female rats were examined. Animals were either reared in a diurnally lighted (12 : 12) environment or in total darkness; those pinealectomized at three days were sacrificed at either twenty or forty days, while those animals which were pinealectomized at thirty days were sacrificed at forty, sixty, eighty or one hundred days. Body weights were monitored at ten daily intervals.

Pinealectomy did not initiate a consistently significant alteration in adrenal weight, adrenal diameter or in the depths of the adrenal cortical zones at any time when compared to unoperated and sham-operated control animals. In addition, no changes in the ultrastructural organisation of the adrenal cortex could be detected.

Animals pinealectomized at thirty days and reared in total darkness possessed adrenals which were slightly lighter than those of light/dark reared animals. This difference became significant when adrenal diameters were compared.

Pinealectomy did not induce a consistently significant alteration in rates of growth, although pinealectomized rats were often slightly heavier than unoperated and sham-operated controls.

It was concluded that adrenal size is influenced by light, but that this influence is not directly mediated by the pineal gland. If, as has been suggested by several workers, the adrenal can be influenced by the pineal, this is probably not via a simple ACTH mechanism. In addition, the pineal does seem to exert a very slight retarding effect upon body weight increase.

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I INTRODUCTION

1.1. A Survey of the Relevant Literature

1.1.1. Introduction

The volume of literature describing studies on the pineal organ is very large. In addition to original research papers and review articles, a number of monographs have appeared; they include the volumes by Gladstone and Wakeley (1940), Kitay and Alshule (1954), Kappers and Schade (eds.) (1965), Wurtman, Axelrod and Kelly (1968), Wolstenholme and Knight (eds.) (1971), Eakin (1973), and Quay (1974).

The application of electron microscopy to the investigation of pineal structure has been partially responsible for the upsurge in interest observed in recent years. The fine structure of the pineal in both lower and higher vertebrates has proved to be extraordinarily interesting and in the first case, highly indicative of function.

Improved biochemical and physiological techniques have encouraged investigations into pineal function. The results of these studies, when linked with structure, have greatly enhanced our understanding of pineal activity.

In surveying the literature concerning the pineal, it is convenient to examine it from two standpoints; firstly, that of histological structure and ultrastructure, and secondly that of function.

1.1.2. Structure of the Pineal Organ in Vertebrates

The majority of vertebrates examined so far exhibit pineal organs, although they may differ in shape and size.

The pineal, together with the pituitary, choroid plexuses (and others) represents one of the smaller derivatives of the brain wall: one of the 'circumventricular organs'. It is a simple or complex evagination of the diencephalic roof and may retain a lumen which maintains a direct communication with the third ventricle. It is often referred to as the

epiphyseal system, since, in many species it consists of a pineal proper (epiphysis cerebri) together with a parapineal (frontal organ). The latter is also referred to as the 'parietal organ' and should not be confused with the paraphysis, another diencephalic outgrowth which lies anterior to the pineal system.

Kelly (1962) has suggested that two primordia lying side by side constitute the beginning of the pineal mass; these then fuse to create a single, midline, presumptive area. A secondary doubling might then occur to create the tandem arrangement found in many adults. This does not necessarily exclude the possibility that the pineal and parapineal could have developed directly from the two primordia. Indeed, the skulls of certain primitive fishes exhibit bilaterally placed indentations, suggesting that the pineal and parapineal were located side by side (Edinger, 1956).

A detailed study of the epiphyseal system reveals that within the various vertebrate classes, at least two basic types of organisation are apparent. First is the primitive saccular structure, characteristic of the lower vertebrates and second is the compact, parenchymal type found in more advanced forms (Okshe etal., 1970).

1.1.2.1. General Structure of Pineal Systems

A large number of Anamniote pineals have been studied, with investigations being carried out on representatives of all of the major groups.

The pineal and parapineal (when present) always exhibit a superficial position with respect to the cranium (figs. 1-5). In the Cyclostome, Lampetra planeri Collin (1969a) and Meiniel (1969) showed that a well developed epiphysis was connected via a well-defined nerve tract to the posterior commissure, while a separate parapineal linked to the habenular commissure (fig. 1).

A more compact vesicular structure has been observed in many Teleost

species including Thynnus thynnus (Holmgren, 1958), Mugil auratus, Uranoscopus scaber (Rudeberg, 1966), Sardina pilchardus (Rudeberg, 1968a), Gobius, Dermogenys, Arius, Macrones, Plotosus (Friedrich-Freksa, 1932), and Esox lucius (Owman and Rudeberg, 1970). Holmgren (1965) examined the early development of the pineal region in Salvinellus fontinalis and Salmo salar and found that the more posterior of two dorsal diencephalic outgrowths developed into the pineal. The anterior outgrowth comes to lie posterior to the mature pineal. This corresponds to the parapineal of the Cyclostomes, but appears to be degenerate in the adult fish (fig. 2 shows the origin of this structure).

The Chondrichthyan Squalus was examined by Holmgren (1918). He described a vesicular structure joined to the third ventricle by a long stalk. The pineal region of the dogfish, Scyliorhinus canicula has been studied by Balfour (1878), Cattie (1882), Gallioti (1897), Studniĉka (1905), Rudeberg (1968, 1969) and Mechan (1971). Structurally it is very similar to that of Squalus and possesses a rather diffuse pineal tract leading to the posterior commissure. A distinct parapineal is not observed in the cartilaginous fish (fig. 3).

In the Amphibia, the epiphysis is supplemented by an outgrowth which penetrates the roof of the skull so that the end vesicle lies immediately below the skin on the top of the head (Kelly and Smith, 1964). This outgrowth is known as the 'frontal organ' and is usually held to be the homologue of the parapineal of Cyclostomes. However, an examination of the nerve tracts shows that this 'parapineal' sends fibres to the posterior commissure while fibres from the anterior region of the epiphysis terminate in the habenular commissure; this is an apparent reversal of the expected pattern. Kuwano (1964) has suggested that the anterior epiphyseal region is more probably homologous with the parapineal (fig. 4).

Members of the Class Reptilia often exhibit an elaborate pineal system with a well developed (true) parapineal (fig. 5). Indeed, in

Sphenodon, the extracranial parietal organ has undergone such differentiation as to confer an eye-like morphology. The cells in the roof of the structure have become modified to form a thickened, well developed transparent lens while the layers immediately above form a transparent cornea (Stebbins and Eakin, 1958).

According to Studnicka (1905), the Avian pineal can exhibit any of three basic structural patterns: saccular with thick walls, follicular and/or tubular and solid. Many intermediate types may also exist (fig. 6). Stammer (1961) for example observed a fairly constant structure in several species, consisting of follicular or glandular tissue, being well innervated and with a rich blood supply. Renzoni (1970) showed that the pineal is merely one of several diencephalic outgrowths and that a second one, which persists as a parenchymal accessory structure, may be homologous with the parapineal.

Similarities to the pineals of lower vertebrates are often discussed; Wetzig (1961) suggested that the gull Larus canus exhibits a direct connection between the lumen of the pineal and the third ventricle. This connection is also observed in Gallus, but only during the first three months of life (Spiroff, 1958).

In spite of earlier reports, the pineal appears to be a richly innervated structure (Ralph, 1970) containing nerve cells (Uek, 1970) and receiving fibres from the cranial portion of the sympathetic system. There may still be fibres which terminate in the posterior or habenular commissures (Quay and Renzoni, 1963).

A true parenchymal pineal is characteristic of the Class Mammalia. The familiar (multiple) vesicular structure is replaced by a single compact parenchymatous organ (fig. 7). One exception to this is the Hamster which possesses both a superficial and a deep pineal (Sheriden and Reiter, 1970a, b).

Ariens Kappers (1960) showed that in the rat, sympathetic fibres (some of which may be cholinergic (Machado and Lemos 1971)) from the superior

cervical ganglion enter the pineal through two 'nervi conarii'. Other fibres from the habenular and posterior commissures appear to loop into and out of the organ without making synaptic contact, although recent findings by David and Herbert (1973) on the ferret suggest that habenular fibres may terminate within the organ.

Romijn (1973a, b) suggested that in the rabbit some parasympathetic fibres are present, but could detect no afferent or efferent connections with the central nervous system.

1.1.2.2. Microscopic Structure of Pineal Systems

The majority of morphological investigations of the pineal carried out in recent years have involved the use of the electron microscope, and there now exists a considerable volume of literature devoted to the ultrastructure of the pineals of a large number of animal species.

Many authors support the view that, ignoring connective elements, blood vessels etc., the pineal organ of the lower vertebrates contains three primary cell types (fig. 8), sensory cells, supporting cells and ganglion cells.

In the higher vertebrates, the pineal is no longer a saccular structure but compact or glandular. The cell types also do not resemble those found in the pineals of lower vertebrates. Is it possible that there is a direct evolutionary relationship between the two pineal types and is there cellular continuity?

Collin (1969b, 1971) has proposed in his 'cell-line theory' that the photoreceptive and conductive elements of the Anamniota persists in the Amniota but undergo important modifications. Therefore, there is not cellular replacement but cellular transformation.

The pineal photoreceptor consists of an outer segment, which protrudes into the lumen of the organ, together with an inner segment which forms a part of the cell mass. The outer segment contains a variable number of flattened discs or sacs formed by infolding of the

plasma membrane. The lumenii of the discs are in contact with the external environment and are therefore held to be cone-like (Cohen, 1968). The outer segment may, in some species, e.g. Lampetra (Collin, 1969a) exhibit a cap-like appearance, in others e.g. Rana (Kelly and Smith, 1964) it may be long and narrow.

The outer segment is joined to the inner by a narrow connecting piece with a 9+0 fibrillar structure, the latter deriving from the axial centriole of the inner segment. Striated rootlet fibres are sometimes observed to derive from the centriolar base, e.g. in Scyliorhinus (Mechan, 1971).

The proximal and distal portions of the inner segment are connected by a narrow neck region bounded by a junctional apparatus. In addition to the centriole, the distal portion also contains a large number of mitochondria and sometimes a distinct 'ellipsoid' is described (Okshe and Vaupel-von Harnack, 1965).

The proximal portion of the inner segment contains a large nucleus, Golgi apparatus with associated vesicles, lysosomes and rough endoplasmic reticulum. In addition, scattered glycogen granules may also be present.

The base of the inner segment narrows to form the synaptic pedicle which usually appears branched. The pedicles appear to synapse with branches of the pineal ganglion cells. They contain dense (80-120nm) or clear (30-50nm) vesicles and in many species synaptic ribbons have been observed (Okshe, 1971). In his review, Okshe (1971) described large stellate and small bipolar neurons in the pineal of Rana esculenta, although he could furnish no evidence for typical (retinal) horizontal cells. He suggested that the large neurons gave rise to a myelinated portion of the pineal tract, while the smaller non-myelinated fibres derive from the bipolar cells.

Vigh-Teichman etal (1973) have also described non-myelinated axons running within the pineal lumen in Pleurodeles, they have been

compared to known C.S.F. contacting axons.

In spite of the fact that the supporting cells of the pineal 'retina' constitute between 60% and 90% of the total cell mass lining the pineal lumen, they have received comparatively little attention. The supporting cells are specialised ependymal elements bearing numerous microvilli and sometimes cilia on their free borders. In Scyliorhinus at least two distinct types are seen, 'light' cells and 'dark' cells (Mechan, 1971), the relative translucency or opacity of the cytoplasm being determined by the number of microtubules present. Cells normally contain smooth endoplasmic reticulum and sometimes stacks of membranes or myeloid bodies (Kelly and Smith, 1964). The bases of the cells are intimately connected with the bases of the photoreceptors and neurones, making determination of the exact structural arrangements extremely difficult. The function of these cells is not known although it is suggested that it might be nutritive.

Photoreceptors which possess sufficiently well developed outer segments as to confer a direct photosensitive function have been observed in a number of species. These include the Cyclostomes, Lampetra lamottei (Julyan, 1964), Loplaneri (Julyan, 1964; Colin, 1969a; Meiniel, 1969) and the Teleosts, Onchorynchus (Hafeez and Ford, 1967), Esox (Owman and Rudeberg, 1970), Mugil (Rudeberg 1966, 1968a), Uranoscopus (Rudeberg, 1966), Thynnus (Murphy, 1971), Phoxinus (Okshe and Kirschstein, 1971), Sardina (Rudeberg, 1968a), Salmo (Breuker and Horstman, 1965), Pterophyllum (Bergmann, 1971). The Chondrichthyan genera, Scyliorhinus (Rudeberg, 1968b, Mechan, 1971), Squalus and Galeus (Altner, 1965) also contain photoreceptors, as do the Amphibia, Rana (Kelly and Smith, 1964), and Taricha (Henderson and Kelly, 1969) and the Reptiles, Cordylus (Steyn, 1959, 1960; Steyn and Steyn, 1965), Sceloporus (Eakin and Westfall, 1959, 1960), Lacerta (Collin, 1969b) and Pseudemys (Vivien-Roels, 1970).

The outer segments of photoreceptors in the pineals/frontal organs of many of the animals mentioned exhibit some signs of degeneration. Very few published micrographs show structures which are comparable to those found in the lateral eyes. There are several possible causes for these observations. The outer segments never possess the close packed arrangement typical of the lateral eye retina and therefore they may be subjected to physical damage since they merely protrude (apparently) indiscriminately into the pineal lumen. Secondly, there is evidence that the outer segments are very sensitive to osmium tetroxide fixation so that some degeneration may be a fixation artefact (Rudeberg, 1968a). Thirdly, there is evidence in some species that the outer segments undergo a degeneration/regeneration cycle which may be associated with the removal of degenerative material by lumenal macrophages or supporting cells (Kelly and Smith, 1964; Kelly, 1971). Alternatively, the majority of pineal photoreceptors may just be naturally degenerative.

There is strong evidence that there is progressive atrophy of the true (?) outer segment to form a variably shaped body containing a number of components, including vesicles, vacuoles and various dense bodies. These are termed 'rudimentary photoreceptors (Collin, 1969b). It is possible that these photoreceptors with seriously degenerated outer segments which still, however, contain discs, may represent an intermediate stage of development between the photoreceptor proper and the rudimentary photoreceptor.

Rudimentary photoreceptors may first appear in the fishes (Collin, 1971) and are certainly present in reptiles (Collin and Ariens Kappers, 1968; Petit, 1969) and especially turtles where the majority of the receptor cells are of the rudimentary type (Vivien-Roels, 1969, 1970; Collin and Meiniel, 1971). They also form the bulk of the sensory cells in birds and, for example, Passer possesses typical inner segments associated with irregularly defined outer segments (Okshe and Vaupel-von

Harnack, 1965; Okshe and Kirschstein, 1969).

The mammalian pinealocyte appears quite different from the photoreceptor and rudimentary photoreceptor described above, although occasionally unusual structures which suggest a close link to these two cell types are seen (see below). It contains an endoplasmic reticulum of varying complexity together with a large Golgi apparatus, lysosomes, mitochondria and possibly lipid droplets contained within the pale, abundant cytoplasm. The nucleus may be so deeply indented as to suggest a polymorphous organisation. The pinealocyte may have one or more branches, but it is not segmented. The pinealocyte's processes may contact other pinealocytes or terminate in a dilation near to the pericapillary space (fig. 9).

In addition to this 'typical' structure some investigators have observed 'vesicle-crowned lamellae', which appear very similar to the synoptic ribbons of photoreceptors, (Wolfe, 1965; Arstila, 1967). Anderson (1965) also found a bulbous modified cilium in the bovine pineal, while Clabough (1973) observed a similar structure in the developing rat pineal.

Small glial cells and numerous non-myelinated fibres may also be present, the latter ending in bulbs containing both granular and agranular vesicles and terminating close to the pinealocytes.

The optical and electron microscopical structure of the mammalian pineal has been studied in a number of animals including rat (Arstila and Hopsu, 1964; Arstila, 1967; Gusek *etal*, 1965), hamster (Clabough, 1973), rhesus monkey (Wislocki and Dempsey, 1948; Wartenberg, 1968), rabbit (Wartenberg and Gusek, 1965; Romijn, 1973a,b), ferret (David and Herbert, 1973; David *etal*, 1973), cow and sheep (Anderson, 1965), hyrax (Quay and Miller, 1971) and seal (Cuello, 1973).

The true, mammalian pinealocyte is also found in large numbers in the Ophidia and appears in Reptilia and Aves.

A single pineal may contain up to three cell types from the 'sensory cell line' (Collin, 1971), for example reptiles and birds possess true photoreceptors, rudimentary photoreceptors and pinealocytes; snakes and mammals contain (primarily) pinealocytes and possibly rudimentary photoreceptors.

If, indeed, the true photoreceptor cell has evolved into the pinealocyte, it is not surprising that (present day) individual pineals should contain more than one cell type.

1.1.3. Function of the Pineal Organ in Vertebrates

The structural considerations already dealt with provide strong pointers to pineal function. It would seem logical that an organ which occupies a superficial position within or without the brain case and possesses very distinctive cone-like cells should possess a photosensory function. In the lower vertebrates the investigation of this function has been of paramount interest.

In the higher vertebrates a glandular structure with a rich blood supply and containing large numbers of neurones has been identified; this has suggested possible neuroendocrine function. Research upon compact pineals has been influenced by considerations of photosensitivity, although it has quickly become apparent that it is not directly responsive to light.

1.1.3.1. Biochemistry of the Pineal

One of the most significant advances in pineal physiology was the discovery of a number of indoles which appeared to be specific to that organ.

It had long been known that bovine pineal extracts cause skin lightening reactions when fed to amphibians (McCord and Allen, 1917). This reverses the darkening induced by melanocyte stimulating hormone (MSH) from the pars intermedia of the pituitary. Lerner etal (1958) isolated the skin lightening agent 5-methoxy-N-acetyltryptamine or melatonin (Lerner etal, 1959a).

Melatonin is a pale yellow crystalline material with a melting point of between 116°C and 118°C (Szmuskovicz and Heinzelman, 1960) and exhibits characteristic fluorescence in a number of solvents.

Although melatonin is often regarded as the pineal hormone, small amounts have been detected in peripheral nerves in, for example, monkey and cow (Lerner etal, 1959b).

Only very small quantities of melatonin are present within the pineal, e.g. the bovine epiphysis contains approximately 0.2 g/g (Lerner etal, 1960) and biological methods for estimating its presence have been necessary. Quantitative biological assays using frog skin were described by Lerner and Wright (1960) and Mori and Lerner (1960). However, experiments by Hadley and Bagnara (1969) cast doubts upon the accuracy of these techniques. Cattabeni etal (1972) have developed a gas chromatographic-mass spectrometric method which is reported to be extremely sensitive.

Melatonin is synthesised by a step-wise conversion from tryptophan (fig. 10).

Tryptophan is converted into 5-hydroxytryptophan under the action of tryptophan hydroxylase. 5-hydroxytryptophan is then converted to 5-hydroxytryptamine or SEROTONIN by L-aromatic amino acid decarboxylase (Shein etal, 1967). N-acetyltransferase then converts serotonin into N-acetyl serotonin (McIsaac and Page, 1959) in the presence of acetyl coA (Weissbach etal, 1960). Finally, 5-methoxy-N-acetyltryptamine is formed by the action of hydroxyindole-O-methyl transferase (HIOMT) (Axelrod and Weissbach, 1961).

The metabolism of melatonin is less well understood than its synthesis. Since most investigations have necessitated the introduction of large quantities of melatonin into the experimental animals, the pathways identified may not be those which are typically used (Wurtman etal, 1968a). Melatonin appears to be converted into 6-hydroxymelatonin

which is then conjugated. The primary metabolites are 6-hydroxymelatonin sulphate, 6-hydroxymelatonin glucuronide and free 6-hydroxymelatonin. The latter represents about 12% of the excreted material (Taborsky etal, 1965). Approximately 80% of these metabolites are excreted in the urine and 20% in the faeces (Kopin etal, 1961; Kveder and McIsaac, 1961).

The pineal complexes of many species have been assayed with respect to melatonin, serotonin and HIOMT.

Melatonin has been found in rats (Prop and Kappers, 1961), cow (Lerner etal, 1960), Kangaroo (Quay and Baker, 1965), amphibia (Van der Veerdonk, 1965), fish (Fenwick, 1970a) and birds (Backstrom etal, 1972; Axelrod and Wurtman, 1964).

Serotonin has been identified in a number of animals including rats (Quay, 1963), cow and monkey (Quay, 1966), sheep and guinea pig (Owman, 1965), turtle, lizard and snake (Quay and Wilhoft, 1964) and birds (Hedlund etal, 1971).

HIOMT has also been identified in several species, including monkey, cat and cow (Axelrod and Weissbach), rat (Wurtman etal, 1963), hen (Axelrod and Wurtman, 1964), toads, reptiles and fish (Quay, 1965b). A recent study by Cardinali and Wurtman (1972) also indicated the presence of HIOMT in the rat retina and Harderian gland.

Several quantitative studies of pineal biochemistry have indicated that light influenced rhythms are present.

Serotonin is present in larger quantities during the hours of light than during the night. This rhythm persists in continuous darkness, but is abolished by continuous light (Illnerova, 1971). Serotonin is converted by N-acetyltransferase (see above) whose activity is much higher during the hours of darkness, estimates varying from a factor of 15 (Klein and Weller, 1970), to 30 (Ellison etal, 1972) up to 50 (Deguchi and Axelrod, 1972) and 70 (Binkley etal, 1973).

Several studies (Deguchi and Axelrod, 1972; Klein and Weller, 1970; Shein, 1971; Ellison et al, 1972; Brownstein etal, 1973) have indicated that N-acetyl transferase activity is controlled by the release of nor-adrenaline from sympathetic nerves and probably acting via a -adrenergic receptor. Since sympathetic activity increases in the dark there is good correlation with enzyme activity. However, there is also strong evidence that an endogenous clock exists (Deguchi and Axelrod, 1972; Binkley etal, 1973) which controls this neuronally indicated activity. Volkman etal (1971) showed that a diminution of enzyme activity could be observed even when sympathetic stimulation continues. Moore and Klein (1974) have suggested that a rhythm generator might be situated in the suprachiasmatic nuclei.

It is believed that the diurnal rhythms exhibited by both serotonin and melatonin are regulated by n-acetyltransferase activity (Klein and Weller, 1970). Serotonin accumulates during the daylight hours and is then converted into n-acetylserotonin during the night; the latter is then converted into melatonin by HIOMT, also in the dark. The latter also exhibits a diurnal rhythm with a peak in the dark period (Axelrod etal, 1965).

It is interesting that the avian pineal exhibits a different pattern of activity. Axelrod etal, (1964) showed that in Passer a decrease in HIOMT activity occurred during the dark phase while Backstrom etal (1972) found no significant alterations in HIOMT levels between the light and dark phases in the Japanese quail. In contrast to this result Oishi and Lauber (1973) found an increased HIOMT activity in the light phase. Recently Gallardo and Piezzi (1973) examined the pineal serotonin activity in the Antarctic penguin and found a diurnal variation completely in reverse to that observed in

the rat.

Other substances of physiological interest found in the pineal include nor-adrenaline (Pellegrino de Iraldi, 1965, 1966, 1971), lipids (Prop, 1965; Zweens, 1965) and lipolytic substances (Rudman 1970, 1972). Nir (1973a, b) has examined the water soluble protein and free amino acid components of the pineal. He found a similar protein content to the brain, and also that diurnal variations exist in both fractions (Nir etal, 1971a).

1.1.3.2. Function of Saccular Pineals

The functional activities of saccular pineals have primarily been studied from two standpoints: (i) The electrophysiological response to variations in environmental illumination. (ii) The role of the pineal in the mediation of colour change in response to variations in environmental lighting.

The presence of photoreceptors within the pineal structure has led many workers to investigate direct photic responses. However, the normal reaction to increased illumination appears to be a reduction in the number of impulses carried by the pineal nerve.

Dodt and Heerd (1962) and Dodt (1964) examined afferent impulses from the cut stalk of the frontal organ in Rana. They found that two possible types of response could be elicited. Some pineals were inhibited by light of all wavelengths, that is they exhibited an 'achromatic response', while others showed varying responses dependent upon the wavelength used. These 'chromatic responses' exhibited maximum inhibition at 355 nm. and excitation at 515 nm. Hamasaki (1970) believes, that the achromatic response is probably due to the inhibitory effect of light stimulated photoreceptors upon spontaneously discharging second order neurones. In the chromatic response two types of sensory cells may be present, one absorbing in the ultraviolet or

blue, the other in the green. These two components may then synapse with a common ganglion cell. Since the inhibitory (355 nm.) response has a significantly shorter latent period than the excitatory component, the inhibitory photoreceptor is thought to synapse directly with the soma of the ganglion cell. The photoreceptor producing ganglionic excitation would therefore synapse with a dendrite.

Dodt (1971) has postulated that the achromatic response may be obtained from myelinated fibres, while the chromatic response derives from non-myelinated fibres. This view is not strongly supported by structural studies which, in most species, show a preponderence of non-myelinated fibres.

Hamasaki and Streck (1971) and Morita and Bergmann (1973) using cartilaginous and teleost fish respectively have demonstrated that the pineal is extremely sensitive to light. Hamasaki and Streck found that the pineal of Scanicula is sensitive to $4 \times 10^{-4} lm/m^2$ (full moonlight would give a luminescence of $4 \times 10^{-2} lm/m^2$ at the surface of the water).

Many workers have observed photopigmentary responses in the lower vertebrates and have attempted to relate these responses to pineal function. McCord and Allen (1917) observed a temporary blanching in frogs and tadpoles fed on minced mammalian pineal. Young (1935) found that the diurnal variations in colour exhibited by Lampetra were interrupted by pinealectomy so that the melanophores remained expanded.

This line of research was extended to the teleost fish. In general it was found that those fish which exhibited particularly exposed pineals also exhibited rapid photopigmentary responses (Breder and Rasquin, 1950). It is perhaps a little surprising that the degree

of exposure should be of any great significance when one considers the extreme sensitivity of the pineal photoreceptors as evidenced by electrophysiological studies.

Bagnara (1965) suggested that, in the absence of light, the pineal releases a melanophore contracting agent (melatonin?) which initiates the blanching response. When light is reapplied, the melatonin is gradually destroyed and the skin darkens, alternatively darkening is brought about by the release of MSH.

Joss (1973a) found that the paling response of the ammocoete is brought about by a melatonin-like substance, since synthetic melatonin only had a slight effect. Wilson and Dodd (1973) suggested that in S. canicula the pineal merely interacts with a more important lateral eye mediated inhibition.

There is a trend towards the view that the Anamniote pineal is not merely a photoreceptive structure but is, in the words of Collin (1971) a 'photo-neuro (CSL)-endocrine organ'.

This is borne out by evidence which suggests a link between the pineal and the reproductive organs, as is often seen in the higher vertebrates. Stebbins and Wilhoft (1966) showed that in the lizard impairment (shielding) of the parietal eye accelerated the reproductive cycle. In the goldfish, Carasius, Fenwick (1970b) discovered that gonad size was related to environmental lighting via the pineal, since pinealectomy induces accelerated gonadal enlargement. Conversely Joss (1973b) pinealectomized Lampetra at the start of their spawning

run and found that there was a delay in gonadal maturation in both male and female together with a delay in the development of the secondary sexual characteristics in the female.

Some other, apparently unconnected aspects of Anamniote pineal function have been studied.

Wakahara (1972) investigated mitosis in the tail fin epidermis of Xenopus, and found a circadian rhythm, apparently mediated by the pineal and subcommissural organ. Clausen and Mofshin (1939) showed that in the lizard Anolis carolineus the pineal plays a part in the regulation of gas metabolism but is less important than either lateral eyes or dermal receptors. Chugunov and Kispoev (1969), however, were not able to demonstrate any such relationship in Rana.

1.1.3.3. Function of Compact Pineals

The majority of investigations into the activity of the mammalian pineal carried out in recent years have examined the possibility of endocrine function. A large number of these have employed the classic techniques of extirpation, implantation and the introduction of extracts, although a significant number of in vitro studies have also been carried out.

1.1.3.3.1. Effects of light upon the compact pineals

One especially significant aspect of pineal function is light sensitivity. The pineal is indirectly light sensitive via the lateral eyes and this has two practical consequences. Firstly all investigators must take careful note of lighting conditions when designing experiments.

Secondly, it suggests that a study of possible light/dark influenced physiological phenomena might prove to be profitable.

Fiske et al (1960) showed that subjecting rats to continuous light for a period of nine or ten weeks resulted in up to a 25% reduction in the weight of the pineal compared to animals kept in constant darkness or light/dark cycles. This study was further developed by Axelrod etal (1965) who found that the pineal exhibits a significant weight variation over the twenty-four hour cycle, being lowest at the end of the daily light period. This is mirrored by the metabolic rate which also follows a cyclical pattern, being greatest during the hours of darkness (Roth, 1965).

Lues (1971) examined the effects of illumination upon the ultrastructure of the guinea pig pineal. He described two types of pinealocyte, 'light' and 'dark'. Under conditions of constant illumination there is a decrease in the organelle content of the light cells associated with an increase in the number of 'vesicle crowned rodlets'. In darkness there is an increase in the number of dark cells together with an apparent 'activation' of the light cells. An apparent reversal of this is seen in the hamster where light deprivation coincides with the appearance of myeloid whorls which are taken to represent signs of degeneration (Clabough, 1971). Continuous illumination was found to induce alteration in the rat pineal mitochondrial structure, causing them to enlarge and lose their cristae.

1.1.3.3.2. Pineal-Gonadal Relationships

Perhaps the most popular line of research into mammalian pineal function developed in recent years has examined its relationship with the reproductive system. These studies have probably generated the largest volume of (recent) literature devoted to the pineal.

In rodents, removal of the pineal frequently accelerates gonadal development although this effect may be masked when animals are subjected to long day lengths. Kitay (1954) found that pinealectomy in prepubertal rats increased gonadal weight in fifty day old rats but only if the operation was performed between twenty-six and thirty days. Several authors have confirmed this inhibitory role of the pineal upon gonadal development (see review of Reiter, 1972d) although Wragg (1967) found that pinealectomy of three day old rats was without effects. In young male rats, pinealectomy results in accelerated reproductive organ growth, with heavier seminal vesicles and prostates but not, in most cases heavier testicles (Motta etal, 1967), although testicular hypertrophy was reported by Thieblot and Blaise (1963) and by Relkin (1972) when compared to dark adapted sham pinealectomized rats.

In the adult female rat, pinealectomy brings about an increased frequency of oestrous smears (Reiter, 1972), although a more spectacular effect is observed when normal animals are subjected to continuous illumination. Ovarian weight (which probably results from accelerated development) is increased in both cases, but in the latter, after prolonged illumination there is a significant weight reduction (Fiske, 1941). This would suggest that not all of the light induced responses

of the reproductive system are mediated by the pineal.

It is interesting that in avian species the effects of light and pinealectomy are quite different from those exhibited by mammals. In the Japanese quail, Arrington etal (1969) found that pinealectomy was completely ineffective in counteracting gonadal regression when maintained in short days. Menaker etal (1970) showed that pinealectomy did not affect testis growth in the sparrow.

Light is not the only factor which influences the gonad inhibiting properties of the pineal; a further three have been described in recent years.

Karden and Hoffman (1967) found that injections of testosterone proprionate into very young male and female rats rendered their reproductive systems extremely sensitive to the effects of darkness, inducing ovarian and uterine involution in the female and tubular and interstitial cell atrophy in the male ('Androgen sterilization'). Reiter etal (1968a) showed that removal of the pineal partially counteracted these effects, reversing the effect of darkness (in this case blinding at 21 days) but not that due to androgen treatment.

The olfactory system also seems to affect the development and activity of the reproductive system, especially when considered in relation to the lateral eyes. Removal of the olfactory bulbs and the eyes together, at weaning severly restricts growth of the gonads and related structures in both male and female rats (Reiter, 1969b).

The effects of combined anosmia and blinding are much greater than either, by itself, but they are counteracted by pinealectomy (Reiter, 1972c). It is thought unlikely that anosmia affects the pineal directly, but more probably influences the brain areas upon which the pineal factors act.

A third factor which influences the pineal-gonadal axis is underfeeding (Reiter, 1972d). Little information exists relating to this phenomenon, although it seems to act in a similar manner to the other two influences already described.

The action of the pineal upon the reproductive tract may possibly be direct but is more likely to be exerted via the hypothalamo-adenohypophyseal system. Several studies have attempted to define a relationship between the pineal, the adenohypophysial gonadotrophins and their releasing factors.

Attempts to establish a link between pineal action and luteinising hormone (LH) release have produced somewhat confusing results.

Fraschini and Martini (1970) observed a rise in pituitary LH in young adult male rats within twelve days of pinealectomy. Adams *etal*, (1965) on the other hand described an increase in pituitary LH following melatonin injection in young female rats. Fraschini *etal* (1968, 1970) introduced various pineal derivatives into several areas of the CNS in adult male castrated rats. Melatonin and 5-hydroxytryptophol retarded the accumulation of pituitary LH while 5-methoxytryptophol and serotonin had no effect. In the midbrain and reticular formation only serotonin

failed to produce a response. The authors suggested that indole-sensitive receptors are present in the areas of the median eminance and reticular formation. Reiter and Sorrentino (1971) showed that in rats treated with pregnant mares serum, melatonin prevents the rise in LH which normally causes shedding of the ova.

Talbot and Reiter (1973/74) found that castration induced rises in pituitary and plasma LH were not influenced by melatonin or 5-methoxytryptophol or pinealectomy. However, Orts and Benson (1973) demonstrated that a melatonin-free aqueous pineal extract significantly inhibited castration induced LH rises in plasma and pituitary. They believed that a small polypeptide might be responsible.

Many experiments designed to examine the pineals relationship to follicle stimularing hormone (FSH) have involved removal of one ovary from the experimental animal and then observing compensatory hypertrophy (COH) of the other, since this is believed to be FSH dependent. Vaughan *etal* (1972 a) examined the ability of a number of pineal indoles to inhibit COH. N-acetylserotonin, 5-hydroxytryptophol, 5-methoxytryptophol and melatonin all proved to be effective inhibitors while 5-hydroxytryptophan serotonin, 6-hydroxymelatonin, 5-hydroxy indoleacetic acid exerted little or no effect. Interestingly (when compared to the work of Orts and Benson (1973)) they also found that aqueous pineal extracts were also effective inhibitors of COH. Moszkowska *etal* (1971) further showed the anti FSH properties of a partially purified pineal polypeptide using *in vitro* techniques.

Fraschini and Martini (1969, 1970) believed that 5-methoxytryptophol and serotonin are the 'pineal envoys' responsible for the control of FSH secretion, while melatonin and 5-hydroxytryptophol are normally concerned with the regulation of LH. This view has gained little support from more recent studies (see above).

Kamberi etal (1971) speculated that melatonin influenced discharge of the hypothalamic releasing factors normally responsible for the regulation of FSH.

Several recent studies, particularly by Relkin (1972a, d, 1973) and Relkin etal (1972) have examined the relationship between the pineal and prolactin secretion. Relkin etal investigated the effects of light and pinealectomy upon plasma and pituitary prolactin in 8 week old male and female rats. They found that constant darkness resulted in a decrease in pituitary prolactin associated with a rise in plasma levels. These effects were reversed by pinealectomy or constant light. It is suggested that the pineal inhibits prolactin inhibiting factor thereby causing pituitary stored prolactin to be released into the plasma.

It is now believed that the pineal functions to regulate the seasonal reproductive rhythms of photosensitive animals.

If hamsters are maintained for long periods in complete darkness, after about twenty-four weeks there is spontaneous regeneration of the gonads in the presence of an active pineal (Reiter, 1969). No real explanation can be offered for this phenomenon, although it has been suggested that the neuroendocrine axis becomes refractory to the pineal's

influence (Reiter and Fraschini, 1969).

The significance of the phenomenon, however, has become apparent when seen in the context of later experiments by Reiter (1969, 1972b, 1973b, 1973/4, 1974), Reiter and Sorrentino (1970) and Herbert (1971). It is now believed that the (burrowing) hibernating hamster is sexually dormant because of pineal action. After about twenty weeks, the gonads begin to regenerate so that when the animal emerges in the Spring, it is ready to breed. At the end of the breeding season, the pineal is 'activated' by the shortening days so that once again the gonads regress. Thus the animals will not attempt to breed at an inopportune time.

Herbert (1972) demonstrated that in the ferret in the first year following pinealectomy, pinealectomized, sham and central animals all came into oestrous at the same time. In the next year animals in the pinealectomized group were twenty to thirty weeks late. Pinealectomy did not alter the length of the reproductive period, only its timing. This experiment underlines the importance of the pineal, at least in the ferret, since it indicates that the activities of the pituitary (?)-gonadal axis are not just influenced by the pineal, they are dependent upon it.

It is interesting that in neither hamster nor ferret is the reproductive system significantly influenced by melatonin.

1.1.3.3.3. Pineal-Pituitary and Thyroid Relationships.

In addition to the pineal-gonadal relationship described above, there is also strong evidence that the pineal influences (and is

probably influenced by) other endocrine organs.

Shiino etal (1974) have recently examined the effect of blinding, anosmia and pinealectomy upon growth hormone (GH). They found that blinding and anomia decreased the levels of serum GH, the effect being prevented by pinealectomy. The possibility of the epiphyseal control of GH is interesting because several workers have reported weight changes in pinealectomized rats. Malm etal (1959) found that pinealectomy induced accelerated growth, Karpannen etal (1970) obtained a weight decrease while Wragg (1967) found no change at all. These three examples indicate some of the confusion which exists.

Reiter etal (1965) found that the subcutaneous introduction of melatonin resulted in a decrease in thyroid activity, while Thieblot etal (1966) showed that melatonin administration in prepubertal rats produced the reverse effect. Panda and Turner (1968) suggested that melatonin exerts a direct effect on TSH which subsequently influences thyroid activity. As a result of his experiments (Relkin, 1972c) postulated that the pineal is capable of exerting a significant, but short-lived inhibitory influence over the pituitary-thyroid axis of prepubertal male rats, probably affecting TSH-RF recretion. This inhibitory role is supported by the work of Csaba and Barath (1974).

1.1.3.3.4. Pineal-Adrenal Relationships

Evidence has been put forward by many authors that the pineal exerts an influence over the adrenal cortex (Palkovits, 1965). Farrell

(1959a, b; 1960) postulated that the pineal produced a substance which he called 'glomerulotrophin', capable of stimulating aldosterone release from the adrenal cortex. In addition, in 1960 he proposed that a second substance might exist, capable of inhibiting the synthesis and release of steroids. It was unfortunate that in his experiments pinealectomy only appeared to exert a marginal effect upon aldosterone synthesis (Farrell, 1964). Later workers supported the idea that an aldosterone stimulating factor might be present, although it need not be located exclusively in the pineal but could be otherwise distributed within the C.N.S. (Jouan and Semperez, 1965).

Several workers have examined the effects of pinealectomy upon adrenal size both in adult and growing animals. Wurtman etal (1959) observed that pinealectomy (in rats) resulted in adrenal hypertrophy. This was also observed by Relkin (1972b), and Vaughan etal (1972b) but not by Wragg (1967), Kinson etal (1968) Seibel and Schweisthal (1973) or Shiino etal (1974).

Reiter and Hester (1966) demonstrated that bilateral enucleation in the hamster resulted in a retardation in growth of the adrenal which was counteracted by pinealectomy. However, their results only appear to be significant for males.

Very little quantitative data has appeared which relates adrenal structure to pinealectomy or replacement experiments. Bugnon etal (1964) found that neither pinealectomy nor massive doses of a ketonic (ox) pineal extract produced any histological modification of the zona

glomerulosa. Relkin (1972b) found that the adrenals of pinealectomized rats of both sexes reveal hypertrophy of the zona fasciculata and reticularis but not the glomerulosa when compared to sham-operated and control animals. He did not, however present any quantitative data.

Giordano and Balestreri (1964) showed that in rats treated with an aqueous pineal extract there is an increased biosynthesis of aldosterone. Gromova etal (1967) on the other hand, found that melatonin inhibited aldosterone production in vivo, while corticosterone levels increased. They also noted that melatonin treatment promoted an increase in adrenal weight.

Kinson etal (1967; 1968) found that both aldosterone and corticosterone are elevated in pinealectomized rats, although the pineal does not appear to be involved in the adrenal response to dietary sodium deficiency.

Jacobs (1974) has studied the diurnal rhythm of plasma corticosterone in the rat. He found that blinding obliterates this rhythm while pinealectomy restores it. He has hypothesised that the pineal may play a part in the light-mediated circadian periodicity of pituitary-adrenal function. Vaughan etal (1972b) suggested that it is probably melatonin (or some other related indole) which is responsible for pineal restriction of adrenal function.

Nir etal (1971b) studied the influence of pinealectomy upon plasma corticosterone. The observed elevated levels in pinealectomized animals maintained for ten days in alternating light and constant darkness.

However, only in constant darkness did the increase become significant. This disparity had vanished by thirty days after the operation. Light was also found to stimulate corticosterone secretion whether the pineal was present or not.

It has been suggested in several of the above-mentioned reports that the pineal may affect the anterior pituitary, exerting an inhibitory effect upon ACTH. This is in agreement with current views upon the functioning of the pituitary-gonadal axis as described above. However, it does not really explain why aldosterone levels should vary so spectacularly in pinealectomized animals. Karppanen etal (1970-1974) in a series of papers have examined pinealectomy-induced hypertension in rats and found that it is partly dependent upon aldosterone, but mainly due to increased tone within the nervous system. They suggest that the renin-angiotensin system might be important in the promotion of aldosterone release in pinealectomized rats, although this is difficult to test out directly.

The relationship, between the pineal and the adrenal is much less well-defined than than which exists between the pineal and the gonads. The extreme sensitivity of the adrenal cortex to stresses set up within the body present great problems when surgical procedures are to be employed so that 'basal levels' of adrenal hormones are difficult to determine. A possible alternative is to study the effects of pineal removal upon adrenal structure, which is, itself, well known to be influenced by hormone (ACTH) levels within the blood.

1.2. Introduction to the Present Study

The present study sets out to examine the structural dependence of the adrenal cortex upon the pineal. Pinealectomy is currently, most commonly held to promote an increase in hormone release (see above). This increased adrenal activity may or may not be associated with hypertrophy, either as an increase in weight or an increase in volume. If the increased adrenal activity (?) associated with pinealectomy is due to ACTH release (or even to some other reason) structural or ultrastructural changes within the cortical zones should appear.

The present study is an attempt to clarify the following:

(i) Does pinealectomy alter body weight when the operation is carried out immediately after birth or post weaning?

(ii) Is the outcome of (i) influenced by the light regime employed?

(iii) Does pinealectomy influence adrenal weight at various times after the operation?

(iv) Does pinealectomy induce a histologically or ultrastructurally detectable alteration in adrenal structure at various times after the operation?

Explanation of text figures.

1. Pineal region of the Lamprey after Wurtman, Axelrod and Kelly (1968a). H.C. - habenular commissure, P.C. - posterior commissure.
2. Pineal region of the teleost, after Holmgren (1965).
3. Pineal region of the Elasmobranch, Scyliorhinus canicula, after Rudeberg, (1969).
4. Pineal region of the Amphibian-Anuran, after Kelly (1962).
5. Pineal region of the Reptile, after Nowikoff (1910).
6. Pineal region of the Bird - Gallus domesticus after Studnika (1905).
7. Pineal region of the Mammal - Rattus, after Wurtman and Axelrod (1965a).
8. Saccular pineal:cell types, after Okshe and Vaupel von-Harnack, (1965). S.C. - supporting cell, N.L. - nerve fibre layer, I.S. - inner segment, ellipsoid-granule within inner segment.
9. Mammalian pineal: cell types, modified from Anderson (1965). P.C. - pericapillary space, nerve-myelinated nerve fibre.
10. Biosynthesis and metabolism of melatonin from Wurtman, Axelrod and Kelly (1968a).

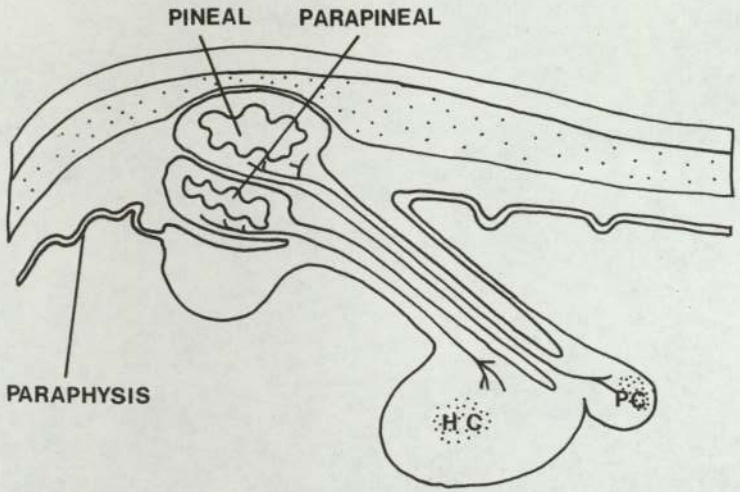


fig. 1.

PINEAL REGION OF THE LAMPREY (WURTMAN et al.)

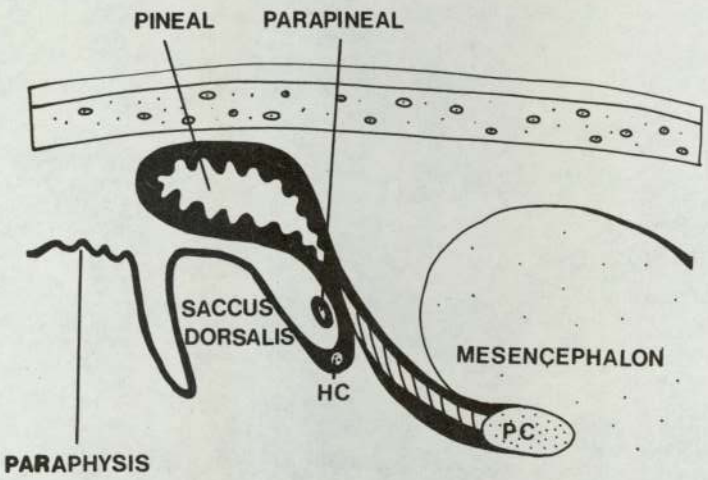


fig. 2

PINEAL REGION OF THE TELEOST (HOLMGREN.)

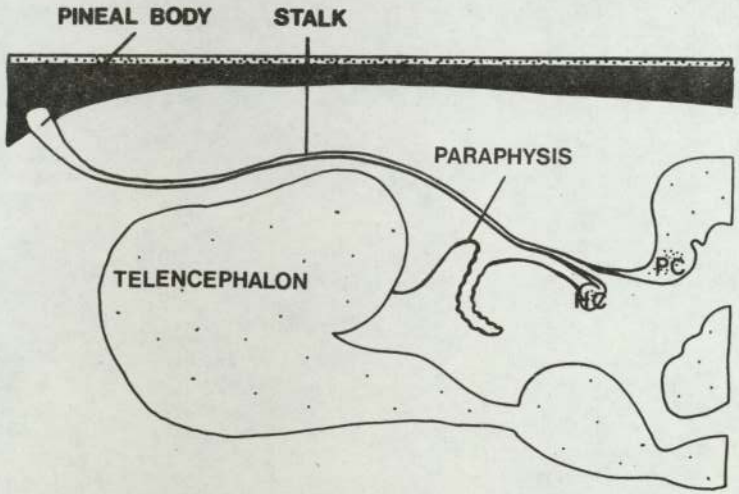


fig. 3.

PINEAL REGION OF THE ELASMOBRANCH (RUDEBERG.)

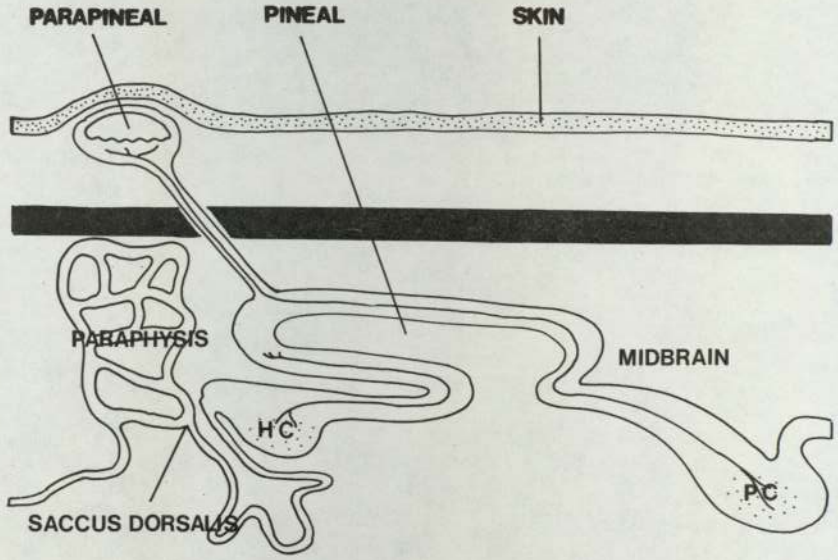
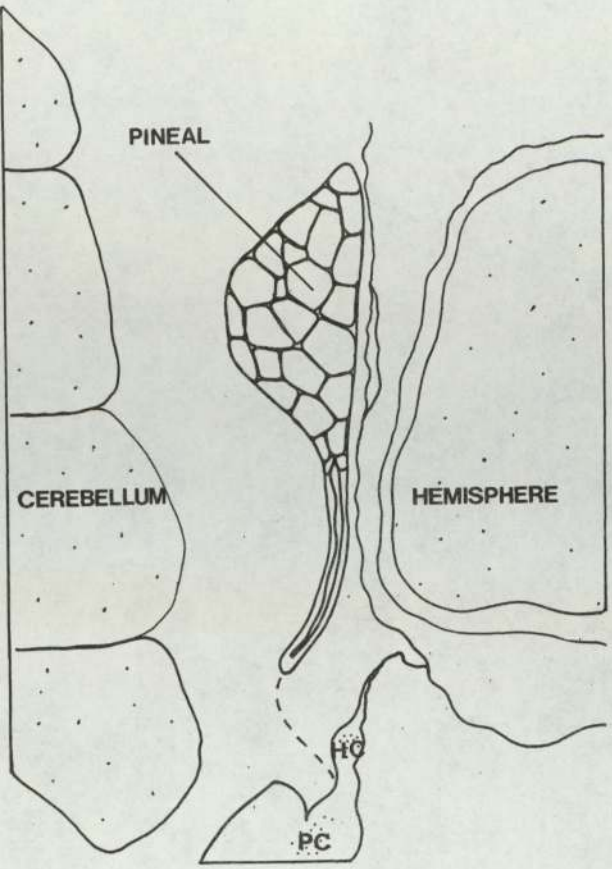
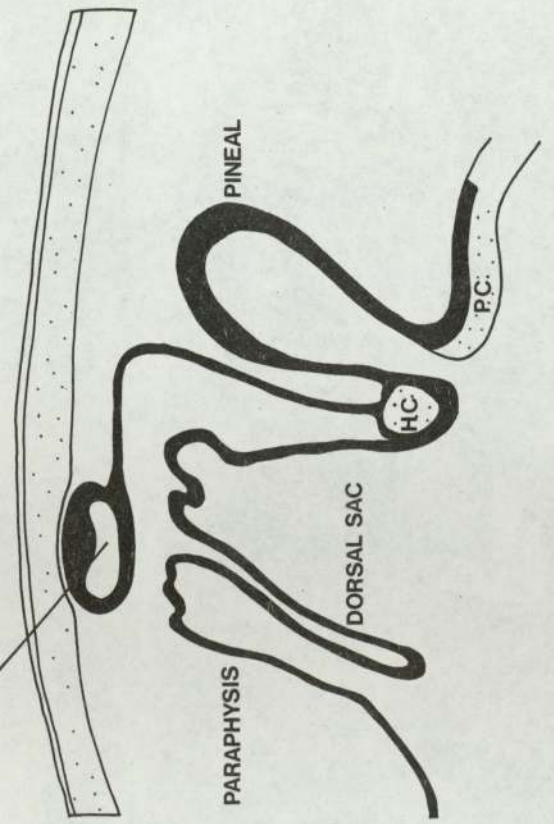


fig. 4.

PINEAL REGION OF THE AMPHIBIAN (KELLY.)



PINEAL REGION OF THE BIRD (STUDNIKA.) fig.6.



PINEAL REGION OF THE REPTILE (NOWIKOFF.)

fig.5.

SACCULAR PINEAL: CELL TYPES

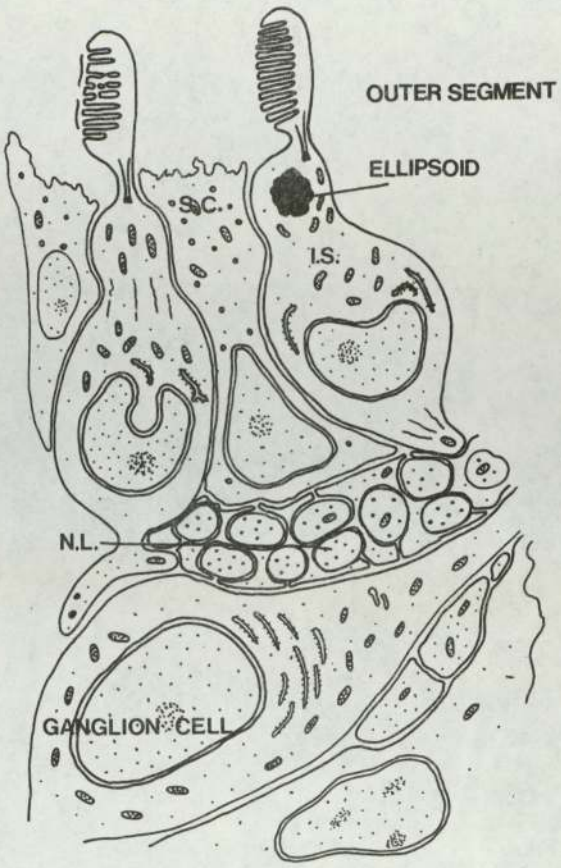
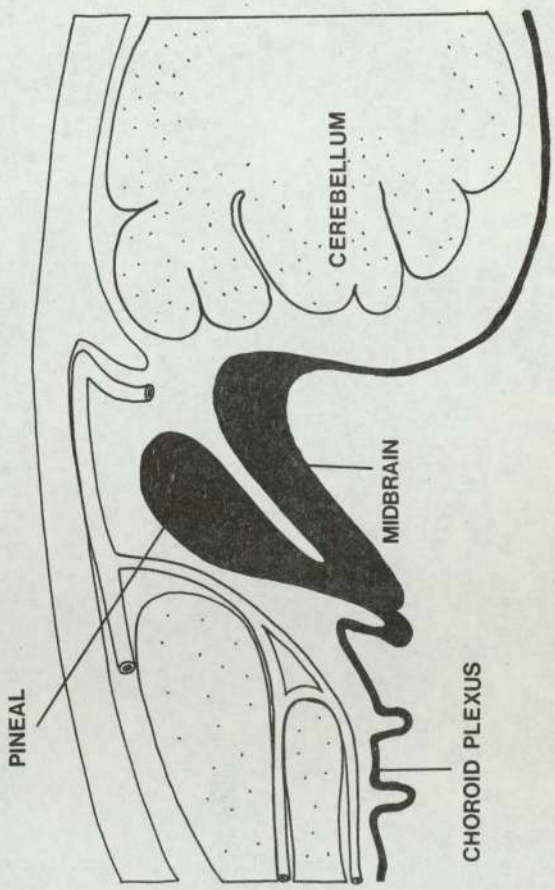
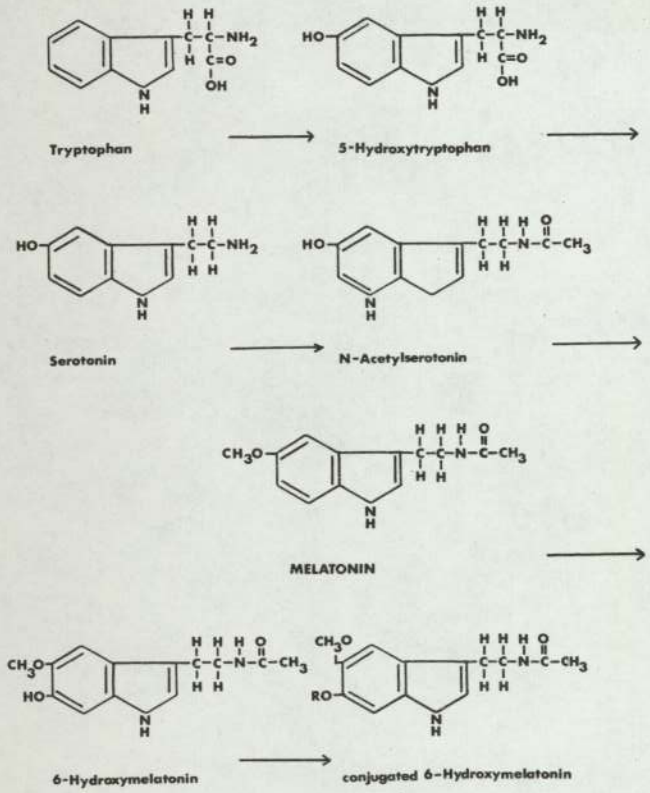


fig.8.



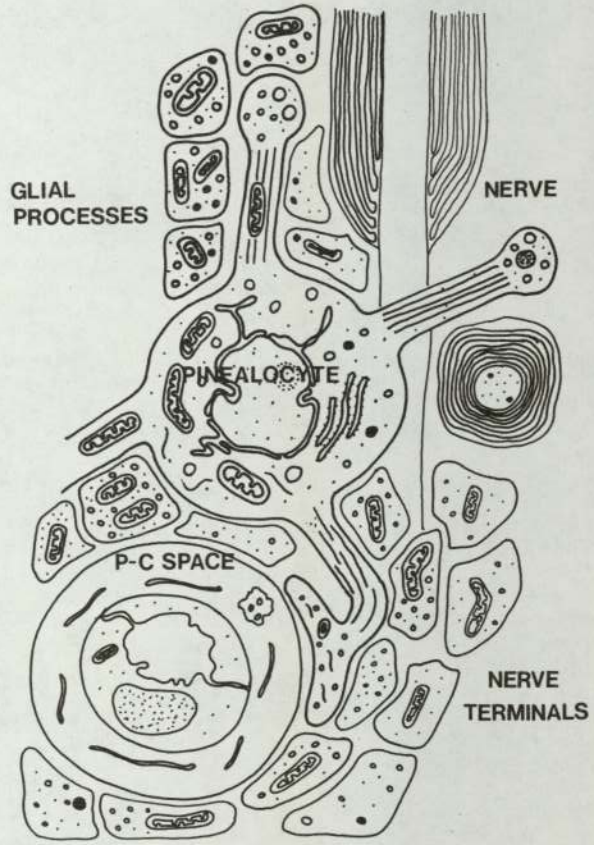
PINEAL REGION OF THE MAMMAL (WURTMAN, AXELROD.)

fig.7.



Biosynthesis and Metabolism of Melatonin

fig.10.



MAMMALIAN PINEAL: CELL TYPES.

fig.9.

II MATERIALS AND METHODS

2.1 Introduction

Groups of male and female rats were pinealectomized at thirty days or three days. Body weights and adrenal weights were then monitored in animals maintained in a light : dark (12 : 12) environment or in total darkness. Adrenal structure was examined by light and electron microscopy. In all experiments litter-mate and non-littermate sham-operated and normal (unoperated) controls were taken.

Laboratory bred Wistar rats from the colony in the animal house of City of Birmingham Polytechnic were used. They were maintained under constant temperature conditions (22°C - 23°C) with free access to Oxoid rat and mouse diet and tap water. Litters were housed in translucent plastic cages (50 cms x 39 cms x 19 cms) for the duration of each experiment.

The experimental period extended throughout the entire year.

2.2 Methods of pinealectomy

2.2.1 Pinealectomy of 30 day old rats

Litters were separated from their mothers at thirty days. Male and female rats were separated into two cages and then each sex-group was randomly sorted into three sub-groups. The animals in sub-groups one and two were pinealectomized and sham-pinealectomized (respectively) by the method of Hoffman and Reiter (1965). The animals in the third group were used as normal controls. Following the operations, operated and non-operated groups (single sex) were mixed together again.

A simple head holder was constructed with a lucite base into which were fitted two brass uprights (2" x 0.375"). Ear bars (3" x 0.375") tapered) and a mouth bar (6" x 4" x 0.375") were fitted to the uprights.

During the course of an operation an anaesthetised rat was placed on a pad of cotton wool, its head immobilised by mouth and ear bars (see plate).

A disc drill was made from stainless steel stock, turned down and drilled so as to form a thin walled tube. Coarse teeth were filed at one end, the other being attached to the shaft of a dental drill (see diagram).

Following some initial experimentation with Nembutal which was found to produce a high mortality rate, all rats were anaesthetised with Avertin (Winthrop Laboratories). 0.25 $cm.^3$ of Avertin was diluted in 10 $cm.^3$ of sterile saline at 37°C. The diluted anaesthetic was mixed well and stored in a tightly capped vessel in a water bath at 37°C during each experiment. (Avertin is not miscible with water below 37°C).

Rats were anaesthetised by an intraperitoneal injection of 0.8 $cm.^3$ of warm dilute Avertin per 100 g. body weight. Since the great majority of rats weighed 70 g. to 110 g., the normal dose was between 0.6 $cm.^3$ and 0.9 $cm.^3$. No anaesthesia deaths were obtained in any of the experiments.

Immediately the animal lost consciousness (normally this took less than two minutes) the top of its head was shaved with an electric clipper. It was then immobilised as described above. The shaved area was then painted with a strong solution of Betadine or Pevidine in an attempt to cleanse the area of the operation.

A midline incision was made antero-posteriarly from a point between the eyes back beyond the base of the skull.

The skin flaps were reflected and anchored. The underlying fascia were then scraped off and the skull surface wiped with an alcohol soaked cotton ball.

The drill was centred upon the confluence of the superior

Fig. 11 Disc drill

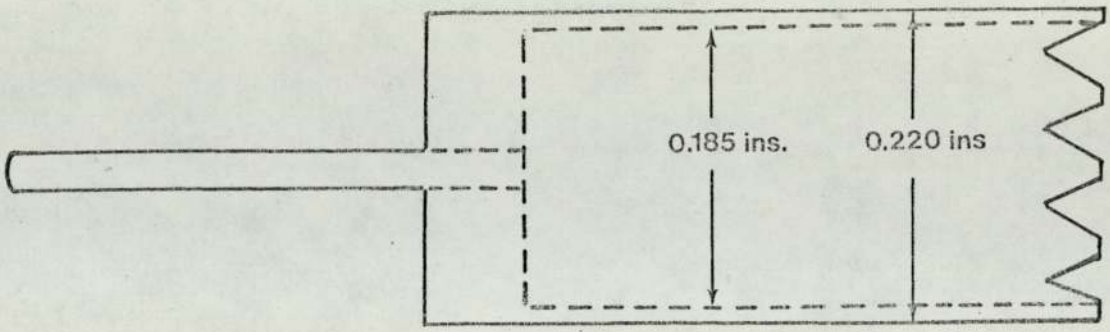
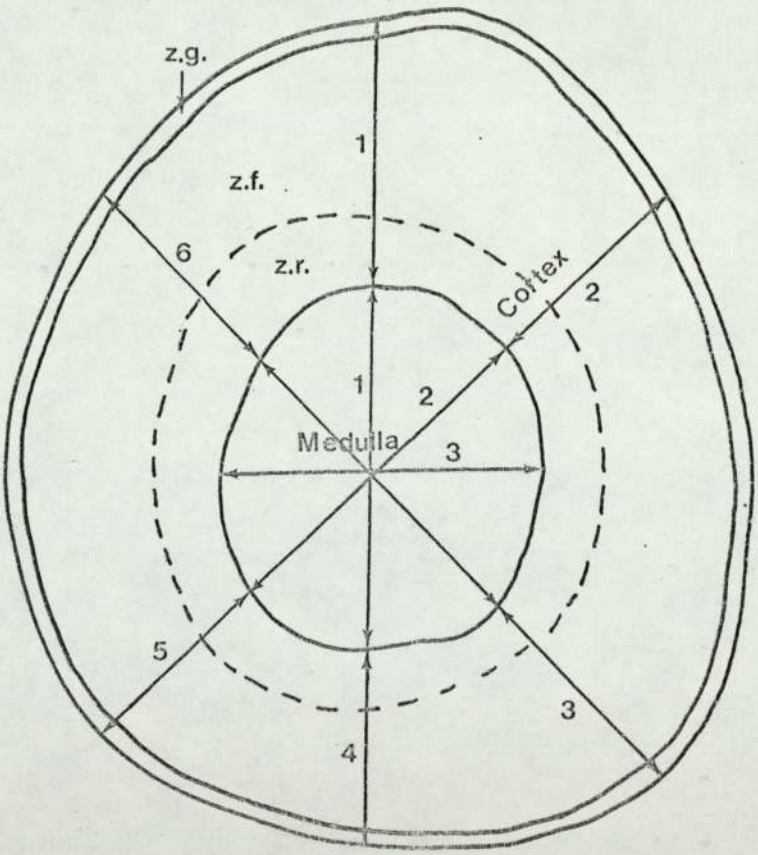
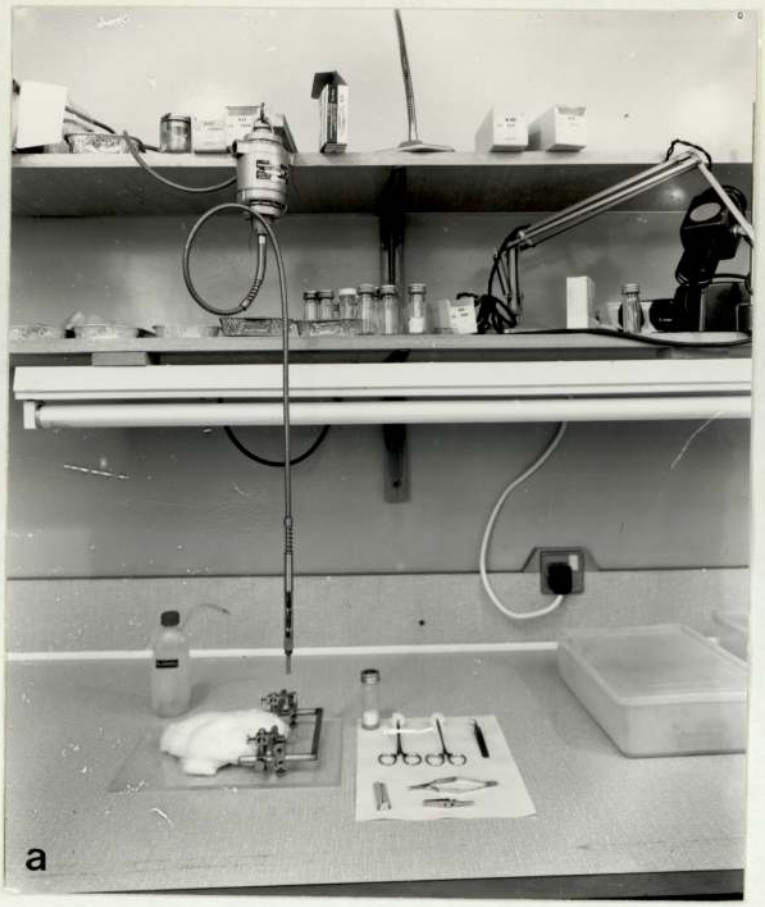


Fig. 12 Grid for adrenal measurements

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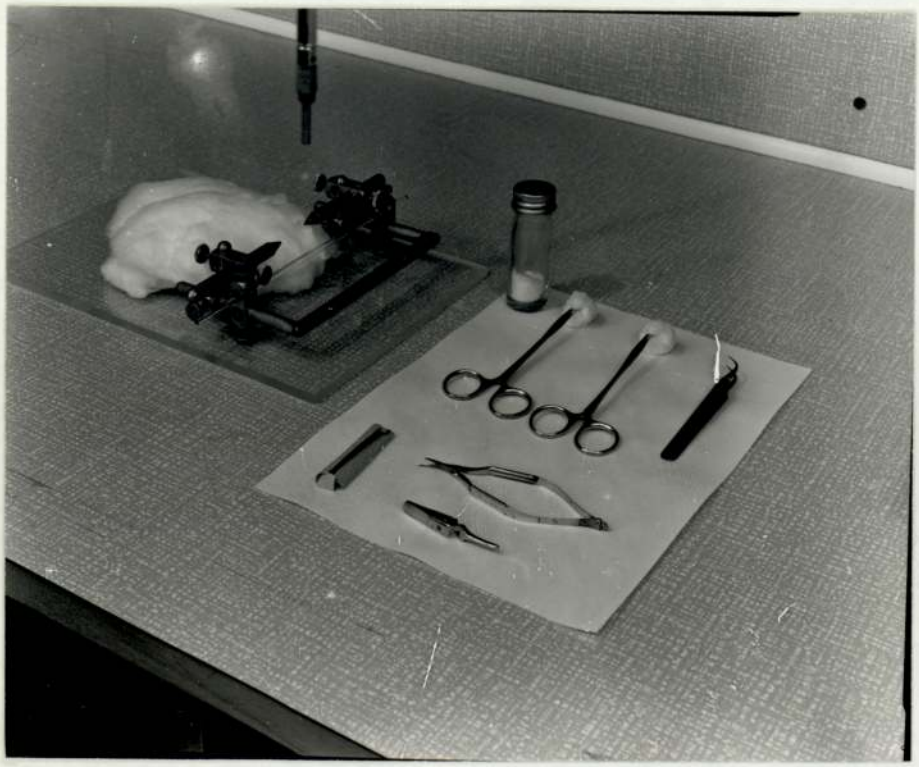

Fig. 13a.b. Apparatus used for pinealectomy of thirty day old rats.

a. Entire bench assembled to perform operation. Strip light and intense light sources are evident.

b. Rat head holder with ancilliary mouth bar for small animals.

sagittal and transverse sinuses and the bone was drilled to a depth not quite equal to the thickness of the skull. The drill was removed and any bone particles cleaned off.

The bone disc was freed by gentle application of a flat seeker, any bleeding being contained by pressure from a cotton wool ball.

A pair of fine curved watchmakers forceps were inserted into the junction of the superior sagittal and transverse sinuses; the pineal stalk was grasped and the pineal body removed in one motion.

The bone disc was replaced rapidly and pressure applied to reduce bleeding. A small piece of Sterispon foam was placed over the disc and the skin flaps closed over the top. The flaps were anchored by two or three gut sutures.

The operation was carried out using alcohol-stored instruments and a clean (but not sterile technique). At no time did an infection develop in an animal post-operatively. Normally animals recovered from the anaesthetic within fifteen minutes of its administration. The operation took six to seven minutes to perform.

Sham-pinealectomies were carried out as described above except that when the sinuses had been ruptured, the pineal was not removed.

2.2.2 Pinealectomy of 3 day old rats

Male and female rats were pinealectomized or sham-pinealectomized at three days by the method of Wragg (1965).

The mother was removed from her litter and placed in a separate cage for the duration of the operation. The young rats were then separated by sex and members of each sex-group were randomly selected for pinealectomy, sham-pinealectomy or to be used as control animals.

In preparation for an operation, an animal was placed in the freezing compartment of a refrigerator until motionless. The inert animal was placed on a pad of cotton wool on the stage of a Vickers

Zoomax stereomicroscope. The operative site was cleaned with alcohol and alcohol-stored instruments were used throughout the operation.

A midline incision was made from a point midway between the eyes (not yet open) and back beyond the base of the skull. The skin flaps were then pulled down on either side, with the fingers, to expose the skull. A transverse incision through the suture between the occiptal and interparietal sutures was made using a sharp scalpel blade. From this, two short incisions were directed forward to a point beyond the transverse sinus. The skull flap was then lifted to expose the pineal organ partially visible below the intact confluence of the sinuses. The pineal stalk was grasped with a pair of curved watchmakers forceps and the organ removed through the dura. The bone flap was replaced immediately and the skin closed by a single gut suture.

Sham-pinealectomies were carried out using an identical technique with the exception that the pineal was left intact.

The young rats were warmed under an infra-red lamp following the operation, which normally took two to five minutes. Once the animals had revived they were returned to the nest for about thirty minutes, when the mother was added.

No deaths resulted directly from the anaesthesia although several litters were destroyed by their mothers.

Pinealectomized, sham-pinealectomized and control aminals were ear-marked for ease of identification.

2.3 Experimental regimes

2.3.1 Pinealectomy at 30 days - light/dark

Litters of mixed male and female rats (each litter contained

between eight and fourteen animals) were taken at birth and placed in translucent plastic cages on the floor of the light-tight experimental room. Two 400 watt Phillips white daylight lamps were attached to a frame at a level of two metres above the floor and orientated so as to allow even illumination of the floor area. The lamps were on a time switch to allow a twelve hour 'day' and a twelve hour 'night' : 'day' - 0600 - 1800 hrs., 'night' - 1800 - 0600 hrs.

Litters were maintained under these conditions until the age of thirty days when they were removed to the operating room. The mother rat was removed from the litter and the young were then pinealectomized, sham-pinealectomized (as described above) or left as normal controls. Animals were selected randomly for the operations, although regard was made to sex so that approximately equal numbers of operated, sham-operated and control males and females were taken within each litter. All animals were weighed and ear-marked; their litter number, individual number, class (whether operated), date of birth and sex were noted on a printed form.

Males and females from each litter were then separated from each other. Thus, from each litter two cages were obtained.

Cages were returned to the experimental room and there maintained for the duration of the experiment.

All animals were weighed at ten-day intervals, i.e. 40, 50, 60, 70, 80, 90 and 100 days. At twenty-day intervals, i.e. 40, 60, 80 and 100 days, animals were selected for sacrifice. In order to ensure that a random sample of animals was taken at each stage, operated, sham-operated and control male and female rats were taken from a number of litters.

Animals were sacrificed by passing coal gas into a large, sealed jar.

Following sacrifice, adrenal glands were removed, weighed and treated as described below.

2.3.2 Pinealectomy at 30 days - Dark

Litters were taken as described in 2.3.1 above and placed in complete darkness in the light-tight experimental room. Animals were removed to the operating room at thirty days, and pinealectomized, sham-pinealectomized or left as normal controls. All animals were weighed and ear-marked and the particulars noted as previously described.

Males and females were separated from their mothers and each other. Cages were then returned to the experimental room and there maintained for the duration of the experiment.

All animals were weighed at ten-day intervals as previously described. All cleaning, routine maintenance and weighings were carried out using a 15 watt bulb fitted to an Anglepoise lamp for illumination.

Animals were sacrificed at twenty day intervals as previously described.

2.3.3 Pinealectomy at 3 days - light/dark

Litters of mixed male and remale rats were taken at birth (each litter contained at least eight animals) and placed in translucent plastic cages on a rack. The two white daylight lamps employed in 2.3.1 above were set up facing the rack. The lamps were controlled by a time switch to give a twelve hour 'day' (0600 - 1800 hrs.) and a twelve hour night (1800 - 0600 hrs.).

At three days, the mother was removed from the litter and males and females were separated. Within each sex group animals were selected for pinealectomy or sham-pinealectomy or to be left as normal controls (see 2.2.2 above).

Following the operations, all animals were ear-marked, weighed and their particulars noted; the sexes were recombined and after a short recovery period (about thirty minutes) were returned to the mother.

All animals were weighed at twenty days when some were selected for sacrifice. The remaining animals were weighed and sacrificed at

forty days. On sacrifice the adrenals were removed, weighed and treated as described below.

2.3.4 Pinealectomy at 3 days - dark

Animals were taken as described in 2.3.3 above and maintained in total darkness in a light-tight room. They were removed at three days for operation and left in a normal experimental room for the succeeding twenty four hour period. This was found to be necessary, since on several occasions entire litters were eaten by their mothers when the cages were returned to the dark room immediately after an operation. The brief period of normal daylight allowed mothers to become reaccustomed to their litters.

Litters were then returned to the dark room. All animals were weighed at twenty days and some were selected for sacrifice. The remaining rats were weighed and sacrificed at forty days. On sacrifice the adrenals were removed, weighed and treated as described below.

All cleaning, routine maintenance and weighings were carried out using a 15 watt bulb fitted to an Anglepoise lamp for illumination.

2.4 Methods of weighing

All animal weighings were carried out between 1000 and 1200 hrs. Animals were active at the time of weighing and were confined in a small plastic bucket. All measurements were obtained using a Sartorius top-pan balance.

Immediately after an animals weight had been recorded it was returned to its cage or sacrificed. All weighings were carried out in the experimental room.

2.5 Microscopy

Animals were sacrificed by coal-gas poisoning. Coal-gas was passed through a rubber tube into a wide-neck glass jar; the top was

then replaced and left until the animals respiratory movements ceased.

The animal was removed to a cork board and a midline incision through skin and muscle made from the genital area to the base of the sternum. Transverse incisions were then continued down on either side below the lower ribs. Skin and muscle flaps were reflected back and the intestines displaced to the left side of the animal to expose the right adrenal. The right adrenal was removed swiftly in order to prepare it for examination by the electron microscope.

The intestines were then moved back and deflected to the right side of the animal in order that the left adrenal could be removed. The gland was removed to a white tile, washed in saline and the attached fat was carefully dissected away. It was then subjected to a further saline wash, blotted dry, placed on a cover slip and weighed by difference on a Sartorius Analytical balance. Immediately after weighing the gland was placed in fixative as a first stage in its preparation for examination by the optical microscope.

Pinealectomized and sham-operated animals were examined to determine the efficiency of these operations. The skin was removed from the surface of the skull and a large disc of bone cut out to expose the pineal region. The absence of the pineal was confirmed visually in pinealectomized animals. The presence of the pineal was similarly confirmed in sham-operated animals.

2.5.1 Electron microscopy

The right adrenal gland was placed on a precooled white tile. It was cut into two with a sharp scalpel blade and ice cold 3% (M/15 phosphate) buffered gluteraldehyde was dripped onto the cut surfaces. Thin slices were cut from the half adrenals and then small wedges removed from these slices. One or two wedges were minced to produce blocks of approximately 0.5 mm side. Pieces from the outer and middle areas of each wedge (containing cortex) were placed into an excess of ice cold gluteraldehyde in a stoppered Bijou bottle. The fragments were fixed for three to four

hours at 4°C.

Following gluteraldehyde fixation the specimens were washed in isotonic sucrose buffer overnight and then post-fixed in osmium tetroxide (Caulfield, 1957) for two hours. Specimens were further washed in sucrose buffer and then dehydrated through a series of cold acetone solutions and embedded in Epon 812 (Taab laboratories) according to Luft (1961). All specimens were embedded in gelatine capsules. Following the hardening process (for forty-eight hours in a 56°C. oven) the gelatin was cut away and the block hand trimmed.

Sections were cut on an LKB Ultratome III, using triangular glass knives with a knife angle of 45°, at 60-150 mμ according to the Peachey (1958) scale.

Sections were floated out on 1% ethanol in water and stretched by allowing trichlorethylene vapour to settle upon them. The sections were then collected on uncoated copper grids.

Following drying on a filter paper in a Petri dish the grids were stained with a freshly prepared saturated solution of (Analar) uranyl acetate (Taab laboratories) in absolute ethanol. The staining solution was centrifuged immediately prior to use and the working solution removed from the surface of the fluid in the centrifuge tube. Grids were immersed in stain for ten to fifteen minutes at room temperature and washed by repeated immersion in a series of alcohols. They were then allowed to dry on clean filter paper in a Petri dish.

Dried grids were then stained by Reynolds (1963) lead citrate. 1.33 g. of lead nitrate and 1.76 g. of sodium citrate were shaken vigourously for one minute in 30 $cm.^3$ of distilled water in a 50 $cm.^3$ volumetric flask. The solution was allowed to stand for thirty minutes with intermittent shaking. 8 $cm.^3$ of Normal sodium hydroxide solution was added to the flask which was then shaken. Distilled water was added to give a final

volume of 50 $cm.^3$. The solution was centrifuged prior to use.

A glass Petri dish containing a thin layer of wax was used as a staining vessel. Drops of stain were placed on the wax, the grids being floated face down upon these drops. A few pellets of sodium hydroxide were placed in the centre of the dish. Grids were stained for seven minutes at $4^{o}C$. The low temperature was found to be useful in the prevention of lead salt precipitation. They were then thoroughly washed in 0.02 Normal sodium hydroxide solution and distilled water.

All sections were examined on an A.E.I. EM6B electron microscope at 60 Kilovolts and photographs taken by the standard plate camera.

2.5.2 Optical microscopy

The left adrenal gland was fixed in 10% formol saline at room temperature for a minimum of seventy two hours. They were then washed, dehydrated through a series of alcohols and cleaned with chloroform, prior to embedding in paraffin wax.

After blocking out the adrenal was trimmed and roughly sectioned until the central medulla could be observed in the block face; a 5 µ.m. section was cut. Several 10 µ.m. sections were removed from the block and then another 5 µ.m. section. A total of three 5 µ.m. sections were collected from each block and mounted on a single slide.

Sections were dewaxed in xylene, hydrated and stained by Harris haematoxylin and eosin. After some initial experimentation it was found to be advantageous to overstain with eosin, since this allowed easy differentiation of the layers of the adrenal cortex.

The depths of the various zones of the adrenal were then measured using a micrometer eyepiece fitted to a Vickers stereomicroscope with a quartz iodine base and Microplan (flat field) objectives. Only the section with the largest medulla was measured since this was taken to be a section from the centre of the block. If the medulla was small or absent in every section, the block was recut. Six measurements of

the zona glomerulosa, zona faciculata and zona reticularis were taken, together with four measurements of the medulla. A standard grid was employed for all measurements (see diagram).

The mean values from each of the four groups of measurements were taken. It was hoped that by making multiple measurements that errors due to irregularities in the shape of the adrenals could be eliminated.

III RESULTS

3.1 Introduction

Within each experiment all results were combined into groups as follows: control (c), pinealectomized (p) or sham-operated (s) for each sampling time, i.e. three day, twenty day, forty day, etc. Means and standard deviations were obtained for each group and were then analysed by means of a t-test; controls were compared with both pinealectomized and sham-operated animals which were also compared with respect to each other. All calculations were performed on an ICL 1902 computer.

Means, standard deviations and levels of significance are presented in tables 1 to 28. Mean body weights are presented in graphs 1 to 8 and % adrenal weights in graphs 9 to 16. Adrenal measurements are not presented graphically.

3.2 Body weights

Male rats reared in a 12 : 12 (L : D) environment maintained very similar patterns of growth whether they had been pinealectomized or not. Although at any particular age, there are wide variations in body weight, even when comparatively large samples were taken there was no statistically significant variation between the three groups of animals. It may be noted, however, that the pinealectomized animals were consistently (except at forty days) heavier than either controls or sham-operated animals. This could be explained by the fact that the initial weights were also slightly higher than those of both the sham-operated and control groups (Table 1).

Female rats also exhibited comparable body weight increases irrespective of the operative treatment. The control animals appeared to differ from both operated groups at the commencement of the experiment, although this difference vanished at forty days when the weights of thirteen animals which had inadvertently been left out of the records at thirty days

were included. A significant difference in weight between sham-operated and control animals also appeared at seventy days (Table 2).

Male and female rats pinealectomized at thirty days and reared in the absence of light also failed to demonstrate a consistently significant alteration in growth rate when compared to normal or sham-operated controls. Males exhibited a significantly elevated body weight when compared to sham-operated but not normal controls only at ninety and one hundred days. Females showed a much more complicated picture, particularly at sixty and seventy days when control, sham-operated and pinealectomized animals all appeared to differ significantly (Tables 3 and 4).

It is interesting that in dark reared animals, pinealectomized groups were consistently, if not significantly heavier than the other two groups.

A comparison of light/dark reared and dark reared rats (graphs 1 and 3, 2 and 4) reveal no obvious differences in growth rates.

Rats operated at three days whether reared in a diurnally lighted environment or constant darkness also revealed no consistently significant variation between the three groups. However, in three of the four experiments (graphs 5, 7 and 8) pinealectomized animals appeared to be slightly lighter than the other two groups. In graph 6 (12 : 12 females) there is a very slight retardation at twenty days only. Several animals pinealectomized at three days exhibited severe retardation of growth together with symptoms of hydrocephalus. On examination of the brain in these animals it was obvious that brain damage had occurred during the operation. Although none of these animals were included in the final results it does indicate that the operation is liable to produce trauma and may, therefore, induce a retardation in growth. This view is strengthened by the observation that in two of the experiments (graphs 7 and 8) body weights converged again at forty days.

3.3 Adrenal weights

Adrenal weights for all experiments are presented both as actual organ weight and as a percentage of body weight. In male animals there is a steep increase in adrenal weight from forty to one hundred days, but this does not quite keep pace with the very steep rise in body weight. Therefore, a slight decrease in the % adrenal weight is observed. In female animals adrenal weight increases keep pace with body weight increases so that % adrenal weight remains fairly constant.

Variations in absolute adrenal weight tend to reduce markedly when this figure is expressed as a % of body weight, e.g. at forty days the adrenal weights of light/dark reared males are: control 21.47 mg., pinealectomized 17.16 and sham 14.78. Both pinealectomized and sham values differ from that of the control group at the 5% level of significance. When expressed as a % of body weight the values become 12.54 (C), 11.49 (Px) and 12.38 (S) respectively which do not differ from one another at any level of significance.

In light/dark (12 : 12) reared males, pinealectomized animals exhibited a slightly increased adrenal weight when compared to those of the sham-operated or control groups, except at forty days. This difference was only significant at one hundred days when both sham-operated and pinealectomized values were significantly heavier than that for the control group (Table 9).

Female adrenal weights exhibited slightly higher values than those observed in the male groups; the difference became much more apparent (Table 10) when related to body weight.

Both male and female light/dark (12 : 12) reared animals failed to show consistently significant alterations in adrenal weight when the pineal was removed at thirty days.

Dark reared males exhibited adrenal weights which demonstrated a similar pattern to that already described. Only at forty days were any

significant differences between the three groups observed; the sham-operated animals had significantly heavier adrenals than the control group. Female adrenal weights failed to exhibit significant variations at all times. (Tables 11 and 12).

It is interesting that, generally speaking, both male and females raised in a diurnally lighted environment possessed adrenals that were slightly heavier than those of dark reared animals. These differences were particularly consistent in the male group although statistical significance was not observed.

Animals operated at three days and their controls exhibited rapid adrenal growth between twenty and forty days. In the light/dark reared male groups, the adrenal weights of the pinealectomized animals were lower than those of either control or sham-operated animals. This difference became significant at forty days, although it vanished when body weight was considered (Table 13). The adrenals of light/dark reared females showed an opposite picture at forty days when the adrenals of pinealectomized females were slightly heavier than those of either of the other two groups.

Dark reared males and females failed to demonstrate consistent weight differences between the various groups. (Tables 15 and 16). In addition, dark reared male and female rats did not present adrenal weights that were consistently and/or significantly different from those of light/dark reared animals.

3.4 Optical microscopy

Male rats reared in as 12/12 light : dark environment demonstrated a steady increase in adrenal diameter between forty and one hundred days (Table 17a). During this period, the medulla and zona glomerulosa and zona reticularis of the cortex especially were seen to increase in diameter, while the extent of the zona fasciculata remained fairly constant. The adrenal diameter of the pinealectomized animals was significantly elevated at sixty and one hundred, but not at forty and eighty days. This appeared

to be primarily due to growth of the medulla and the zona reticularis.

It is interesting to examine table 17b which expresses the depths of the various zones as a percentage of the total diameter. A (fairly) consistent decrease in the zona fasciculata associated with an increase in the zona reticularis is observed. The other regions appear to maintain a constant level of development. Pinealectomy at thirty days does not appear to produce any consistently significant effects upon development of the various regions.

Female adrenals exhibited a more complicated pattern (Table 18a). The zona reticularis was deepest in animals which had been pinealectomized, at all times, although the total adrenal diameter was only greater at forty and sixty days. Other than this, no consistent differences were apparent in the three experimental groups.

Males and females reared in total darkness exhibited no consistent variations between the adrenal zones in the three experimental groups, at any time (Tables 19a, 19b, 20a and 20b).

A comparison of the total adrenal diameters of both male and female light/dark and dark reared animals indicates that the former are (nearly) consistently larger than the latter. In males three of the twelve pairs of figures are significant at the 0.1% level and two at the 1% level (Table 25). In females eight pairs are significant at the 2% (or less) level (Table 26).

Animals pinealectomized at three days whether reared in an alternating light/dark cycle or in total darkness failed to demonstrate consistently significant differences between the various adrenal zones in the three groups (Tables 21a - 24b). A comparison of light/dark and dark reared animals showed a reverse to the result described above, i.e. the dark reared animals demonstrated larger adrenals than those of light/dark reared animals (Tables 27 and 28). However, only two results demonstrated significance at a level of 5% or less.

Examples of optical sections of adrenals are presented in figs. 14, 15, 16 and 17. These examples were randomly chosen and demonstrate the variations in depth of the adrenal zones at any one age and are not intended to show differences between experimental groups. The measuring techniques employed attempted to eliminate the effects of these eccentricities in the adrenal zones.

3.5 Electron microscopy

All three zones of the adrenal cortex could be differentiated ultrastructurally. Differences occurred particularly in the morphology of the mitochondria, which, in the zona glomerulosa, varied in shape from round to oblong and contained tubular or lamellar cristae within a dense matrix. Cells of the zona fasciculata contained round or oval mitochondria which most often contained vesicular cristae, although sometimes tubular structures were present. The mitochondria observed in cells of the zona reticularis were variable in shape and contained tubular or vesicular cristae.

In this work the zona fasciculata was studied with particular interest since it is known to be sensitive to influence by externally applied hormones.

The zona fasciculata was observed to be permeated by numerous capillaries lined by a layer of extremely thin and apparently fenestrated endothelial cells (fig. 21). Below this layer in the sub-endothelial spaces, microvilli may often be observed. The latter may also be seen between adjacent cells (fig. 22).

The parenchymal cells contained large central nuclei which appeared to be round or oval in shape, and in the normally pale cytoplasm a rich smooth endoplasmic reticulum with a tubular or vesicular organisation was seen. The cytoplasm is also packed by very large numbers of mitochondria (described previously) and liposomes. The latter often appeared as completely clear vesicles (presumably an artefact) (fig. 20), denser cored vesicles (fig. 26a), vesicles containing a pale flocculent material

(fig. 26b) or completely dense vesicles (fig. 18a). Occasionally, aggregations of mitochondria, liposomes and/or endoplasmic reticulum were observed (fig. 21); these have been termed 'double' or 'triple' complexes by other authors.

No structures were observed which have not previously been described in the literature.

Rearing animals in total darkness and/or removal of the pineal did not appear to induce any long term alterations in the fine structure of the adrenal cortex. Numbers and appearance of mitochondria or liposomes which are known to be useful monitors of function did not appear to be altered significantly in any of the experimental groups.

KEY TO FIGURES

GRAPHS:

Control ○– – – – – ○
Pinealectomized ▽————▽
Sham-operated □—·—·— □

HISTOGRAMS:

Control **C**
Pinealectomized **P**
Sham-operated **S**

MICROGRAPHS:

z.f. - zona fasciculata
z.g. - zona glomerulosa
z.r. - zona reticularis

Bv. - blood vessel
D - dark cell
e - erythrocyte
er - endoplasmic reticulum
j - junction
L - light cell
l - liposome
ly - lysosome
M - medulla
m - mitochondrion
mv - microvilli
X - cytoplasmic inclusion
* - double/triple complex

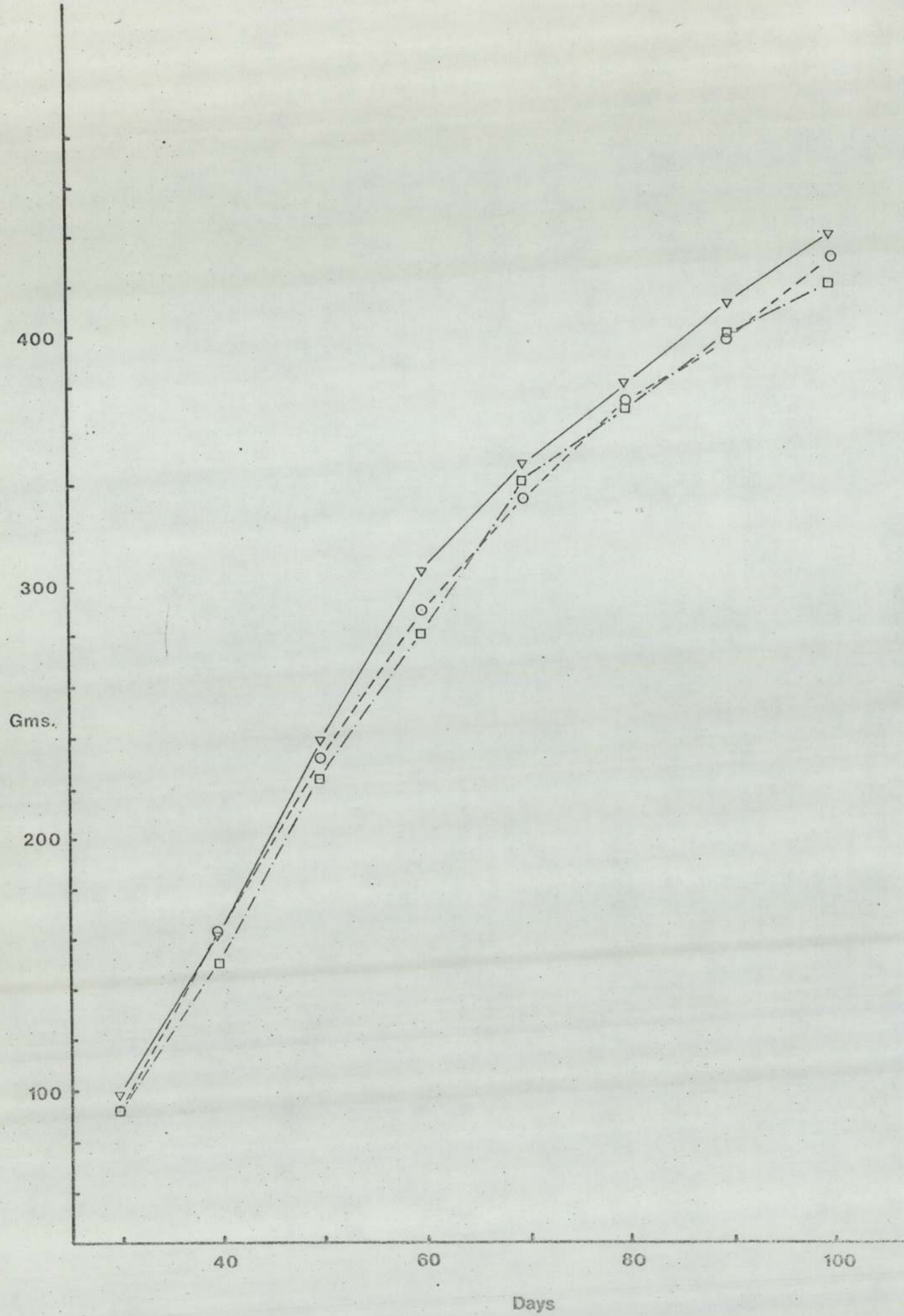
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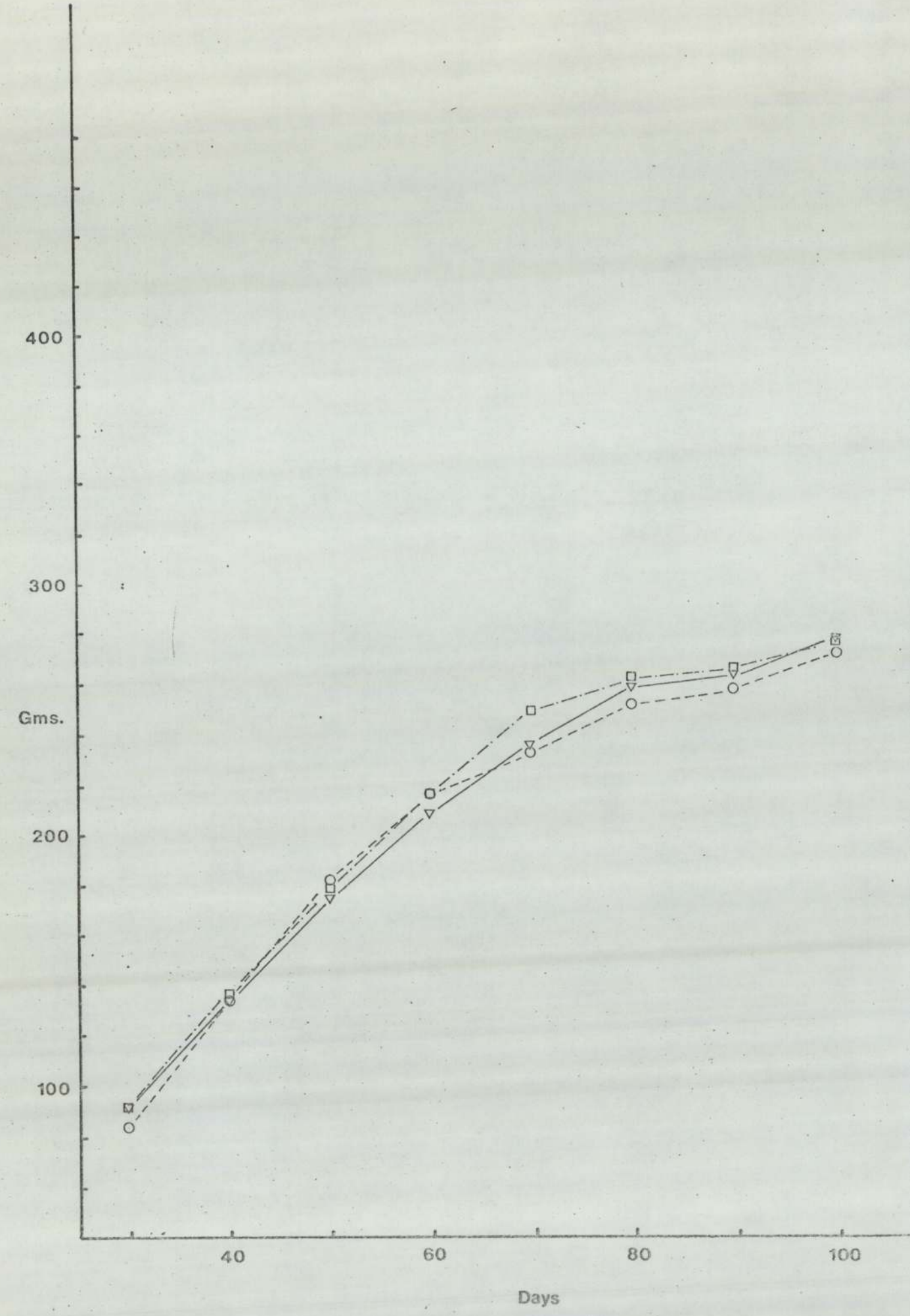
Table 1. Body weights : Males (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
30	C	31	92.35 ± 14.883	
	P	18	99.11 ± 23.967	
	S	22	93.18 ± 29.238	
40	C	52	162.84 ± 22.842	
	P	18	161.44 ± 28.247	
	S	22	150.09 ± 43.034	
50	C	46	232.19 ± 23.086	
	P	13	239.62 ± 28.621	
	S	18	223.78 ± 50.674	
60	C	46	290.51 ± 22.355	
	P	13	306.08 ± 35.359	
	S	18	282.50 ± 50.773	
70	C	40	335.95 ± 19.433	
	P	9	349.56 ± 42.802	
	S	13	342.77 ± 45.231	
80	C	40	374.01 ± 20.196	
	P	9	381.22 ± 39.004	
	S	13	371.46 ± 42.058	
90	C	22	398.84 ± 24.082	
	P	6	413.83 ± 40.193	
	S	8	401.12 ± 45.531	
100	C	22	431.73 ± 24.627	
	P	6	441.83 ± 43.445	
	S	8	422.62 ± 47.650	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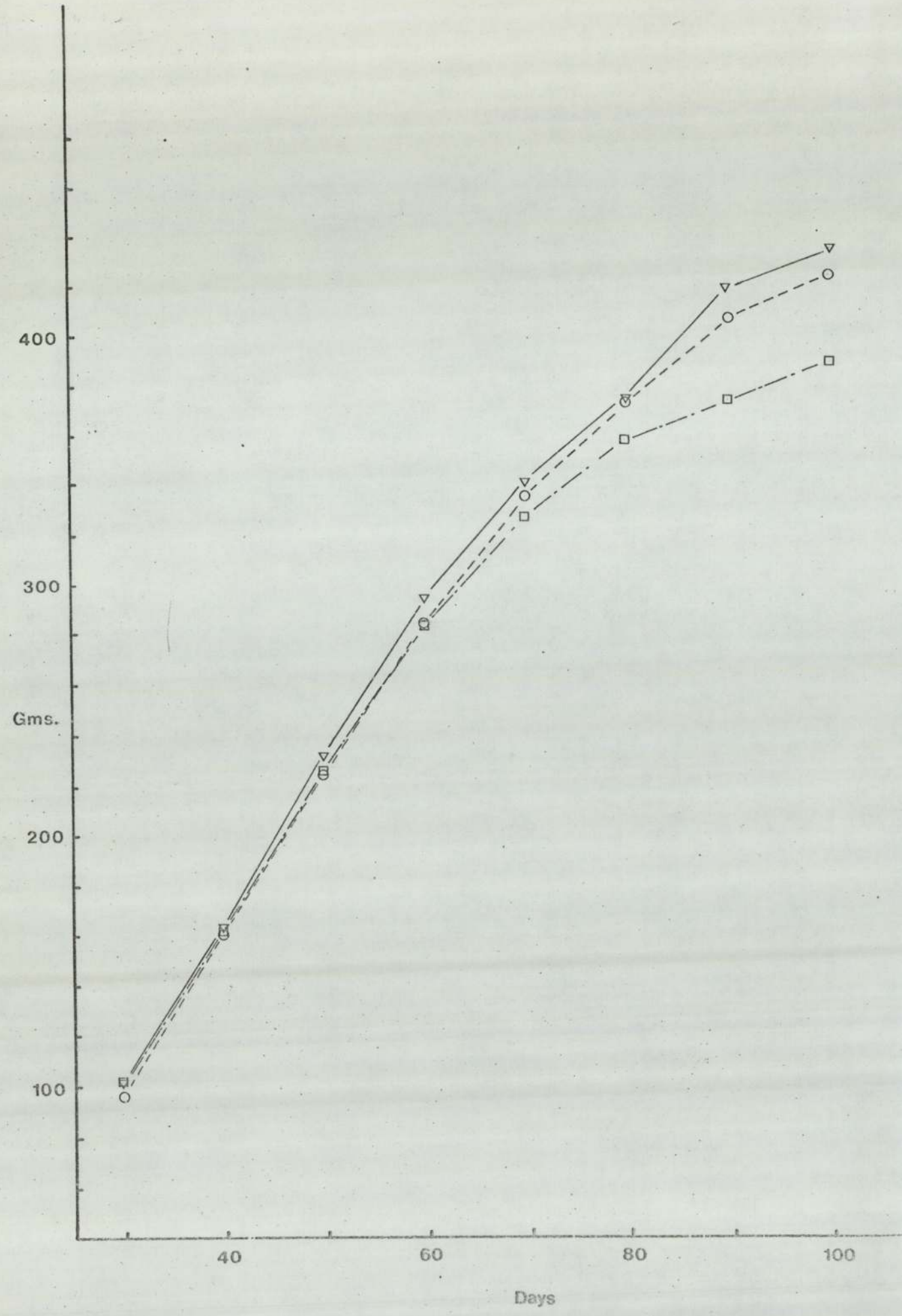
Days

Table 2. Body weights : Females (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
30	C	29	83.53 ± 10.812	
	P	21	92.14 ± 14.194	*P < 2.5%
	S	18	91.44 ± 19.181	
40	C	42	134.36 ± 15.248	
	P	21	134.95 ± 22.436	
	S	18	137.39 ± 22.581	
50	C	35	181.67 ± 15.857	
	P	17	175.12 ± 27.027	
	S	14	179.31 ± 23.407	
60	C	35	216.30 ± 15.554	
	P	17	209.47 ± 24.210	
	S	14	215.57 ± 26.701	
70	C	24	233.71 ± 18.222	
	P	12	236.83 ± 25.751	*P < 2.5%
	S	10	250.50 ± 14.431	
80	C	24	252.65 ± 15.263	
	P	12	259.83 ± 32.088	
	S	10	262.90 ± 20.825	
90	C	11	258.00 ± 15.021	
	P	6	264.00 ± 24.014	
	S	6	267.00 ± 18.430	
100	C	11	272.55 ± 13.780	
	P	6	279.17 ± 20.578	
	S	6	277.33 ± 19.880	

* w.r.t. control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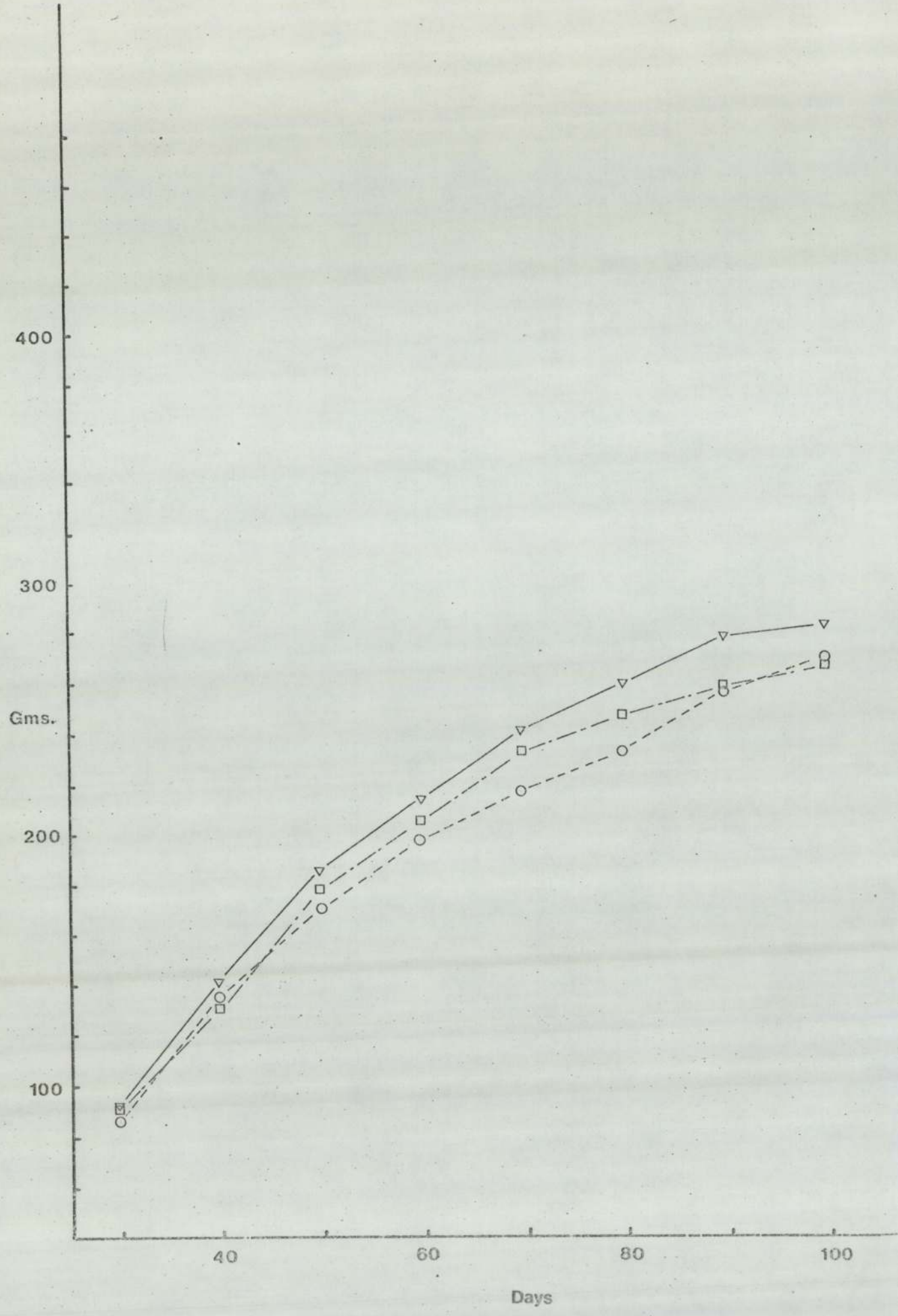
Days

Table 3. Body weights : Males (0 : 24 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
30	C	32	95.78 ± 20.795	
	P	27	100.74 ± 21.813	
	S	25	102.36 ± 16.441	
40	C	40	160.70 ± 22.856	
	P	27	162.22 ± 27.941	
	S	25	161.00 ± 18.139	
50	C	30	224.43 ± 26.950	
	P	21	232.00 ± 34.425	
	S	20	226.60 ± 23.857	
60	C	30	284.90 ± 25.833	
	P	21	295.38 ± 30.483	
	S	20	283.75 ± 24.085	
70	C	21	335.43 ± 34.697	
	P	15	341.73 ± 37.309	
	S	13	327.08 ± 22.714	
80	C	21	372.71 ± 44.320	
	P	15	374.73 ± 43.378	
	S	13	357.77 ± 23.324	
90	C	10	406.20 ± 32.735	
	P	8	418.63 ± 30.377	**P < 5%
	S	6	373.50 ± 33.520	
100	C	10	423.80 ± 33.129	
	P	8	434.25 ± 29.596	**P < 2.5%
	S	6	388.83 ± 30.411	

** w.r.t. sham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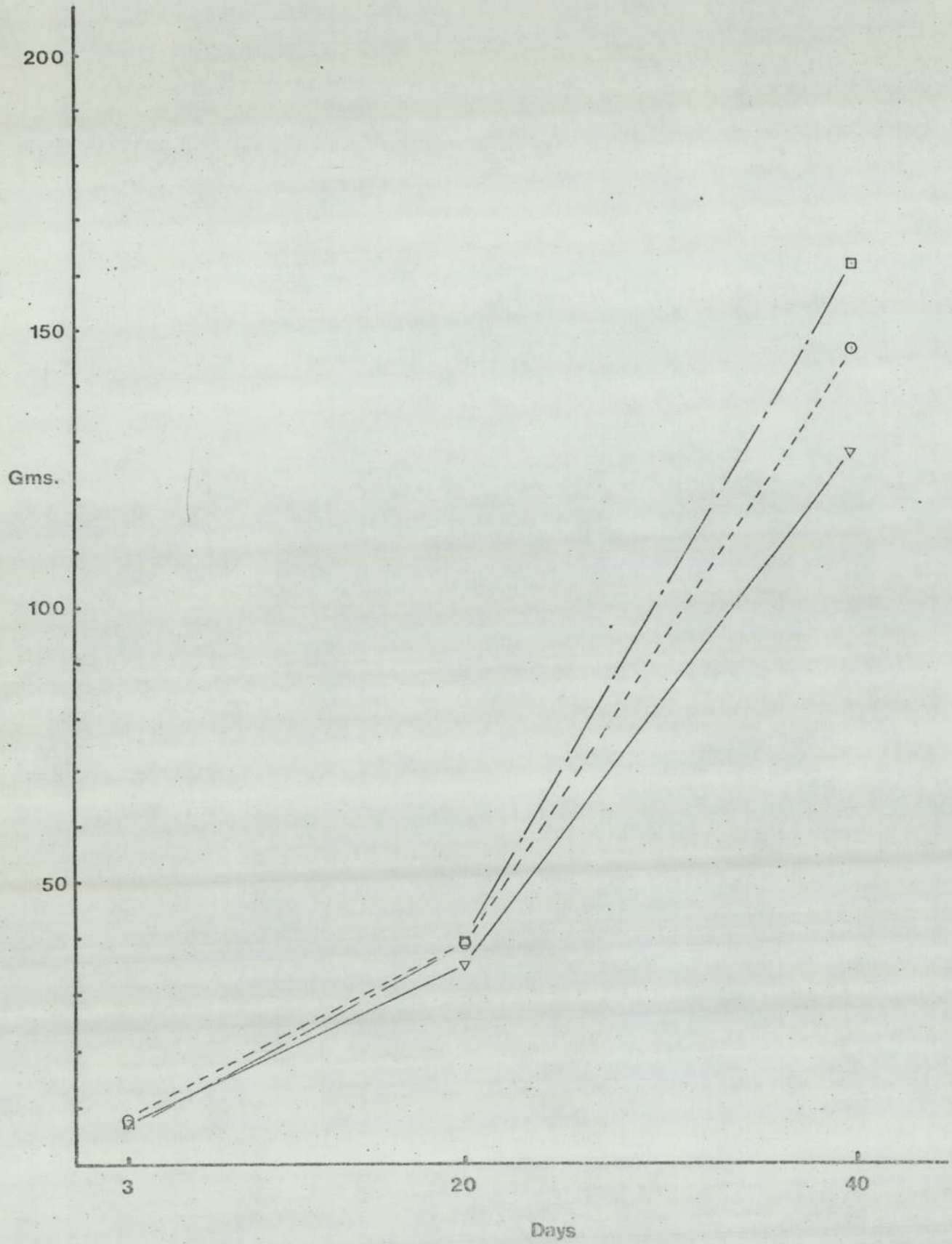
Days

Table 4. Body weights : Females (0 : 24 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
30	C	28	87.57 ± 11.700	
	P	26	92.81 ± 12.487	
	S	27	91.96 ± 12.548	
40	C	34	136.62 ± 12.445	
	P	26	142.23 ± 13.993	**P < 2.5%
	S	27	132.15 ± 14.349	
50	C	23	171.78 ± 14.056	
	P	19	185.26 ± 17.862	*P < 2.5%
	S	21	179.76 ± 13.012	
60	C	23	197.87 ± 14.480	
	P	19	215.26 ± 18.806	*P < 0.5%
	S	21	206.67 ± 10.111	*P < 5%
70	C	15	217.13 ± 18.355	
	P	12	242.75 ± 15.385	*P < 0.5%
	S	13	233.31 ± 17.380	*P < 5%
80	C	15	233.47 ± 21.416	
	P	12	260.17 ± 14.519	*P < 0.5%
	S	13	247.00 ± 20.331	
90	C	7	257.14 ± 25.765	
	P	5	279.00 ± 20.890	
	S	6	259.00 ± 15.155	
100	C	7	270.29 ± 22.821	
	P	5	283.40 ± 15.318	
	S	6	267.33 ± 16.519	

* w.r.t. control
** w.r.t. sham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Days

Table 5. Body weights : Males (12 : 12 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
3	C	13	7.72 ± 1.659	
	P	11	7.37 ± 0.921	
	S	8	7.01 ± 1.545	
20	C	13	39.91 ± 8.657	
	P	11	35.88 ± 8.384	
	S	8	40.40 ± 9.004	
40	C	8	147.25 ± 14.686	
	P	4	128.25 ± 16.679	**P < 2.5%
	S	5	162.40 ± 14.691	

** w.r.t. sham

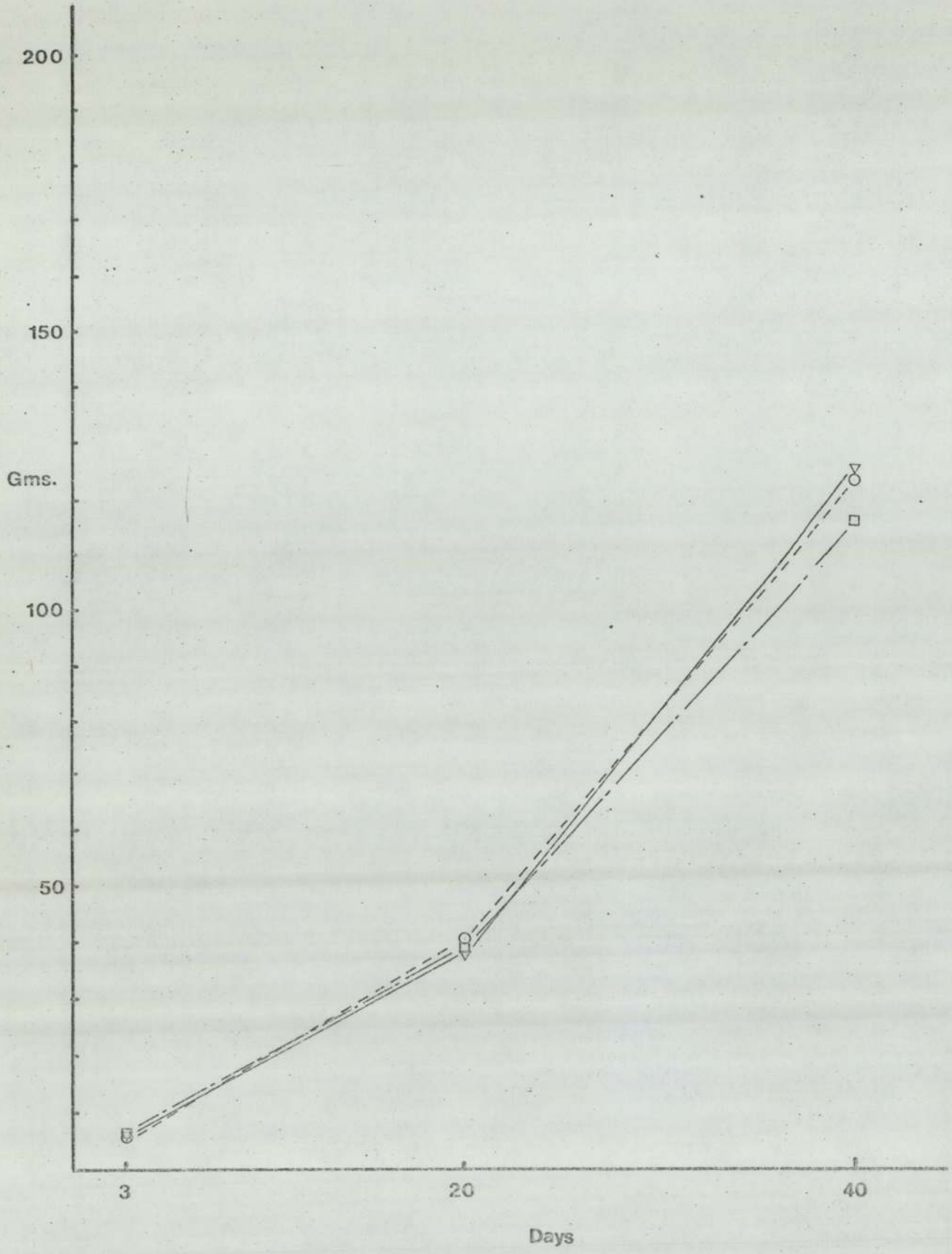
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Table 6. Body weights : Females (12 : 12 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
3	C	14	6.10 ± 1.241	
	P	9	6.76 ± 0.596	
	S	12	6.83 ± 1.050	
20	C	14	41.15 ± 7.694	
	P	9	38.86 ± 8.942	
	S	12	40.05 ± 8.088	
40	C	8	124.12 ± 15.767	
	P	6	126.33 ± 15.217	
	S	6	116.17 ± 16.211	

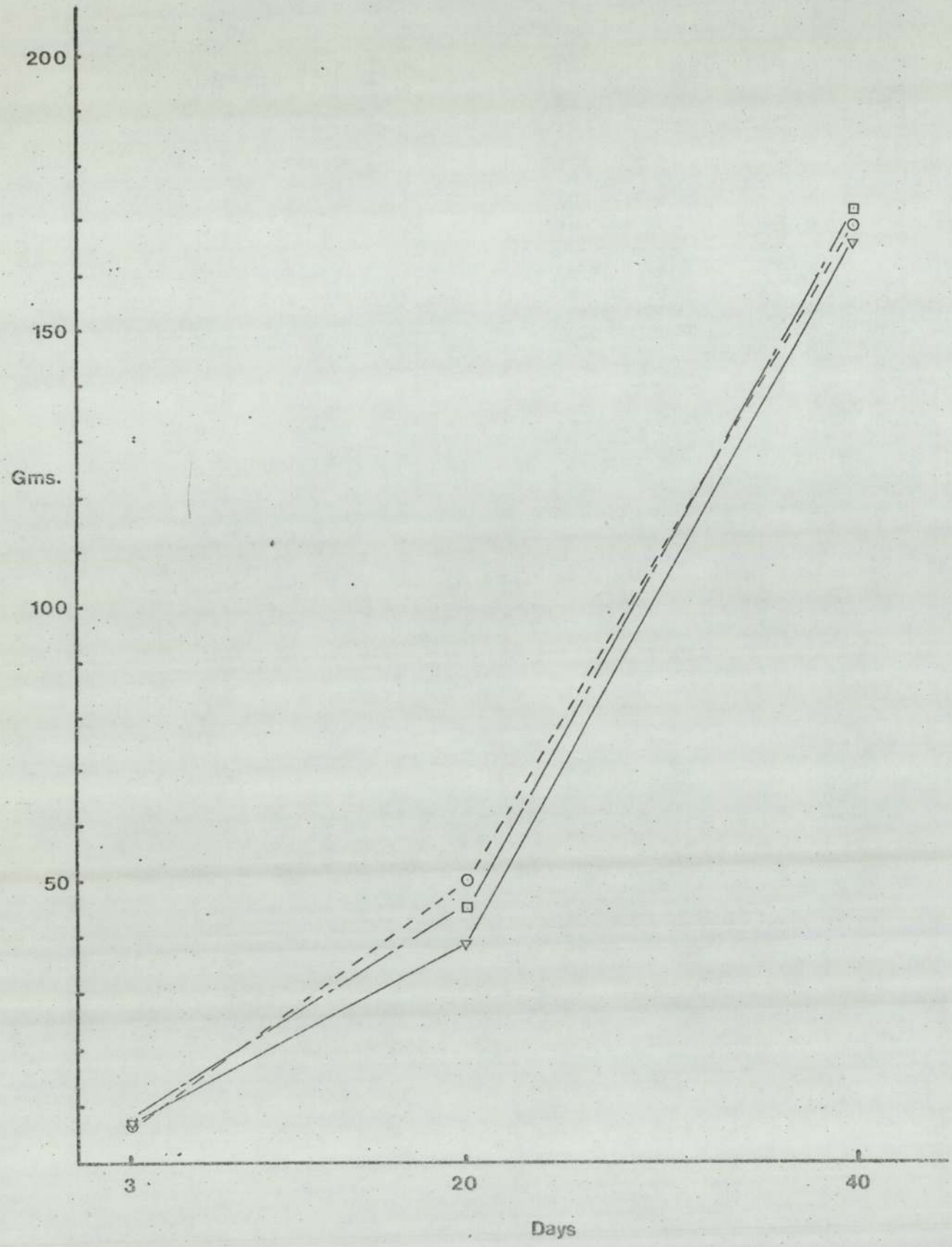

200
150
Gms.
100
50
3
20
40
Days

Table 7. Body weights : Males (0 : 24 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
3	C	25	7.48 ± 1.259	
	P	12	7.66 ± 1.454	
	S	14	7.89 ± 1.176	
20	C	25	51.10 ± 14.965	
	P	12	39.71 ± 7.584	*P < 2.5%
	S	14	46.21 ± 9.608	
40	C	15	169.80 ± 14.154	
	P	7	165.57 ± 11.159	
	S	9	172.00 ± 13.548	

* w.r.t. control

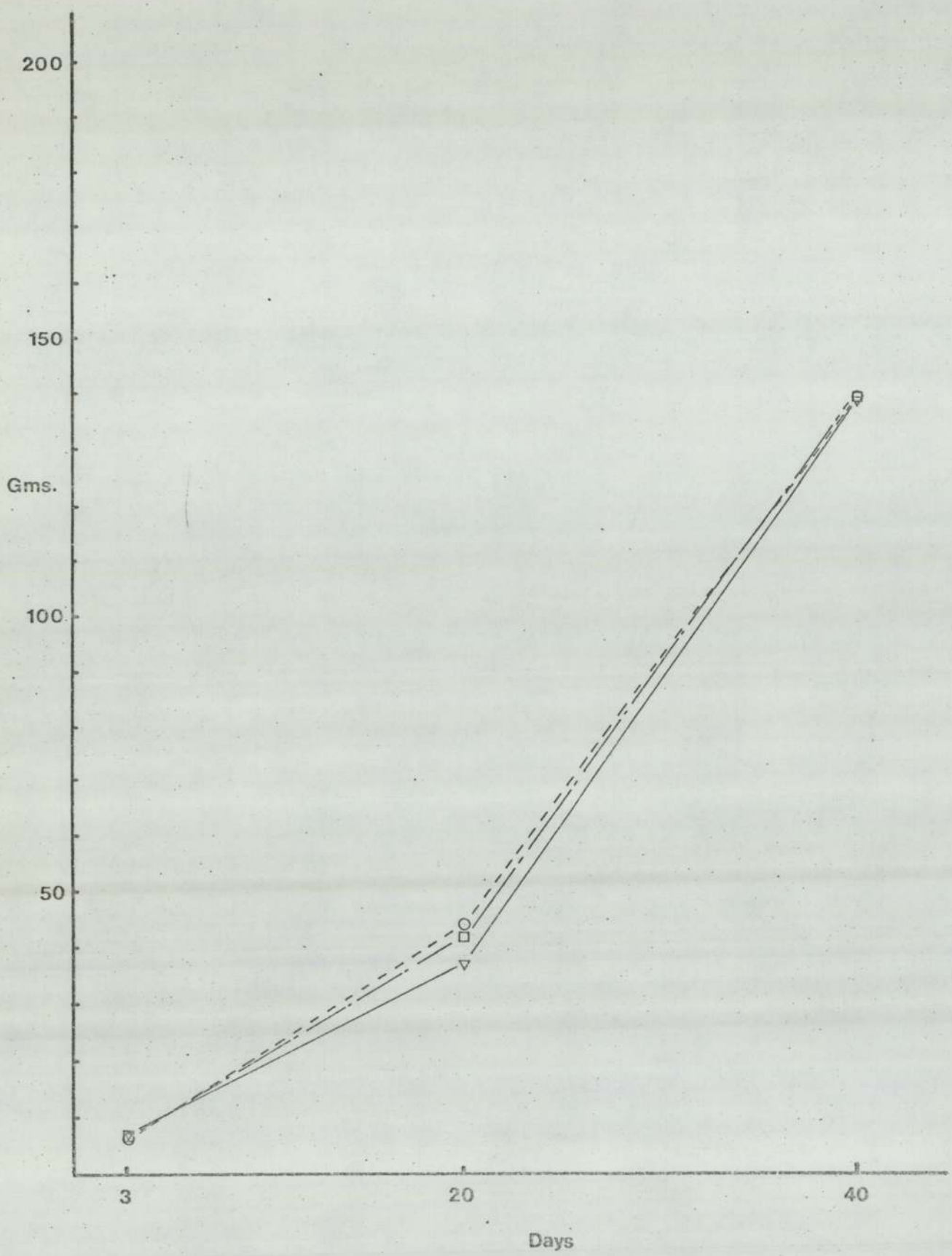

200
150
Gms.
100
50
3
20
40
Days

Table 8. Body weights : Females (0 : 24 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Body Wt. (± S.D.)gms.	Significance
3	C	29	6.91 ± 1.279	
	P	16	7.39 ± 0.971	
	S	18	7.23 ± 1.332	
20	C	29	45.54 ± 10.669	
	P	16	37.92 ± 9.991	
	S	18	44.82 ± 11.905	
40	C	17	141.06 ± 10.446	
	P	11	139.23 ± 8.500	
	S	10	141.20 ± 10.381	

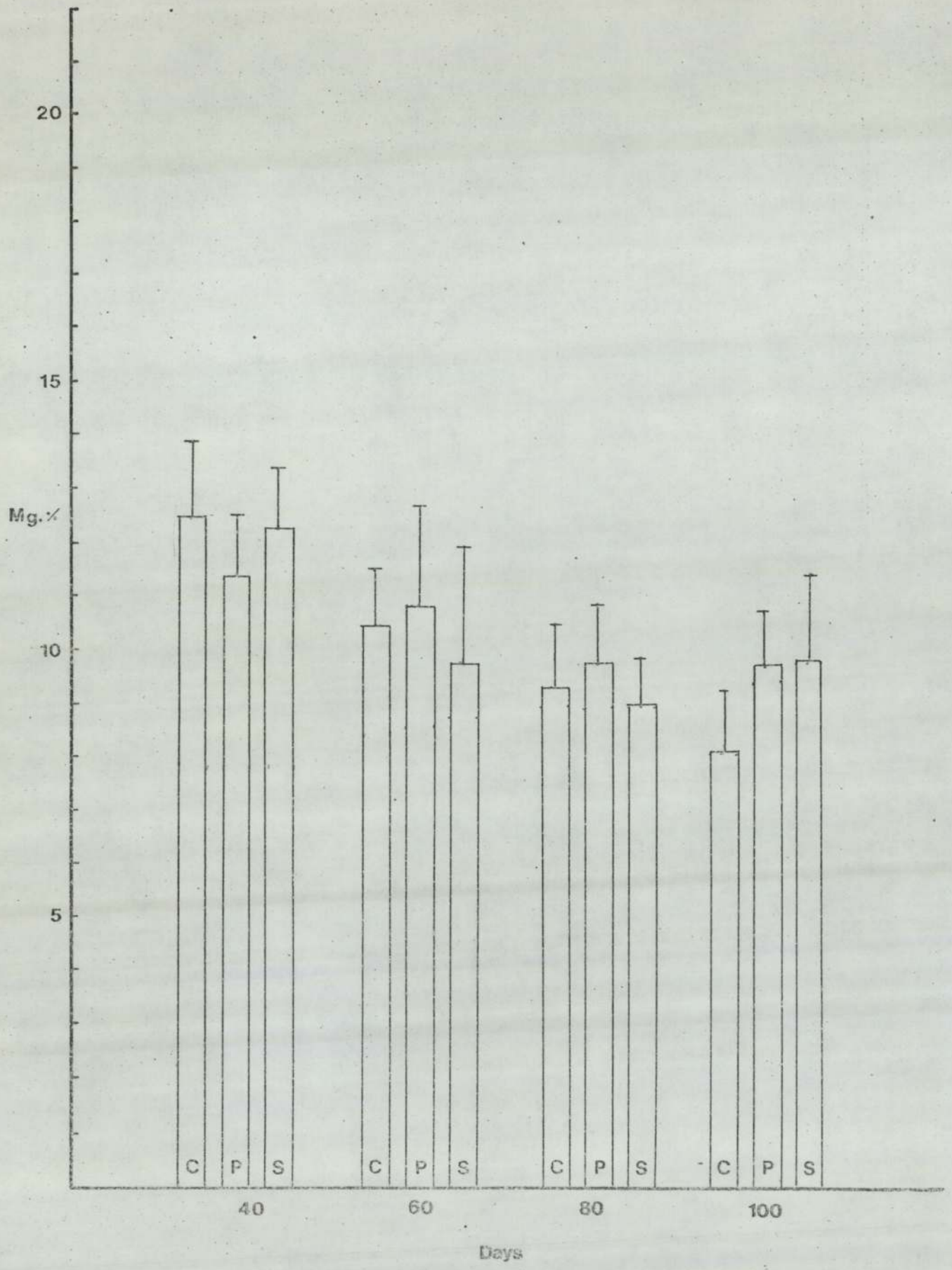

20
15
Mg.%
10
5
C
P
S
C
P
S
C
P
S
C
P
S
40
60
80
100
Days

Table 9. Adrenal weights : Males (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance	
40	C	7	21.47 ± 2.876	12.54 ± 1.410		
	P	5	17.16 ± 3.088	11.49 ± 1.152	*P<5%	
	S	4	14.78 ± 4.966	12.38 ± 1.113	*P<5%	
60	C	6	29.35 ± 5.433	10.53 ± 0.953		
	P	4	32.18 ± 6.087	10.92 ± 1.840		
	S	6	24.63 ± 7.032	9.86 ± 2.109		
80	C	18	34.61 ± 4.442	9.34 ± 1.206		
	P	3	36.63 ± 5.951	9.87 ± 1.076		
	S	5	32.90 ± 2.613	9.01 ± 0.855		
100	C	22	35.10 ± 5.224	8.13 ± 1.169		
	P	6	43.37 ± 7.343	9.78 ± 1.076	*P<1%	*P<1%
	S	6	41.95 ± 8.713	9.85 ± 1.673	*P<5%	*P<1%

* w.r.t. control

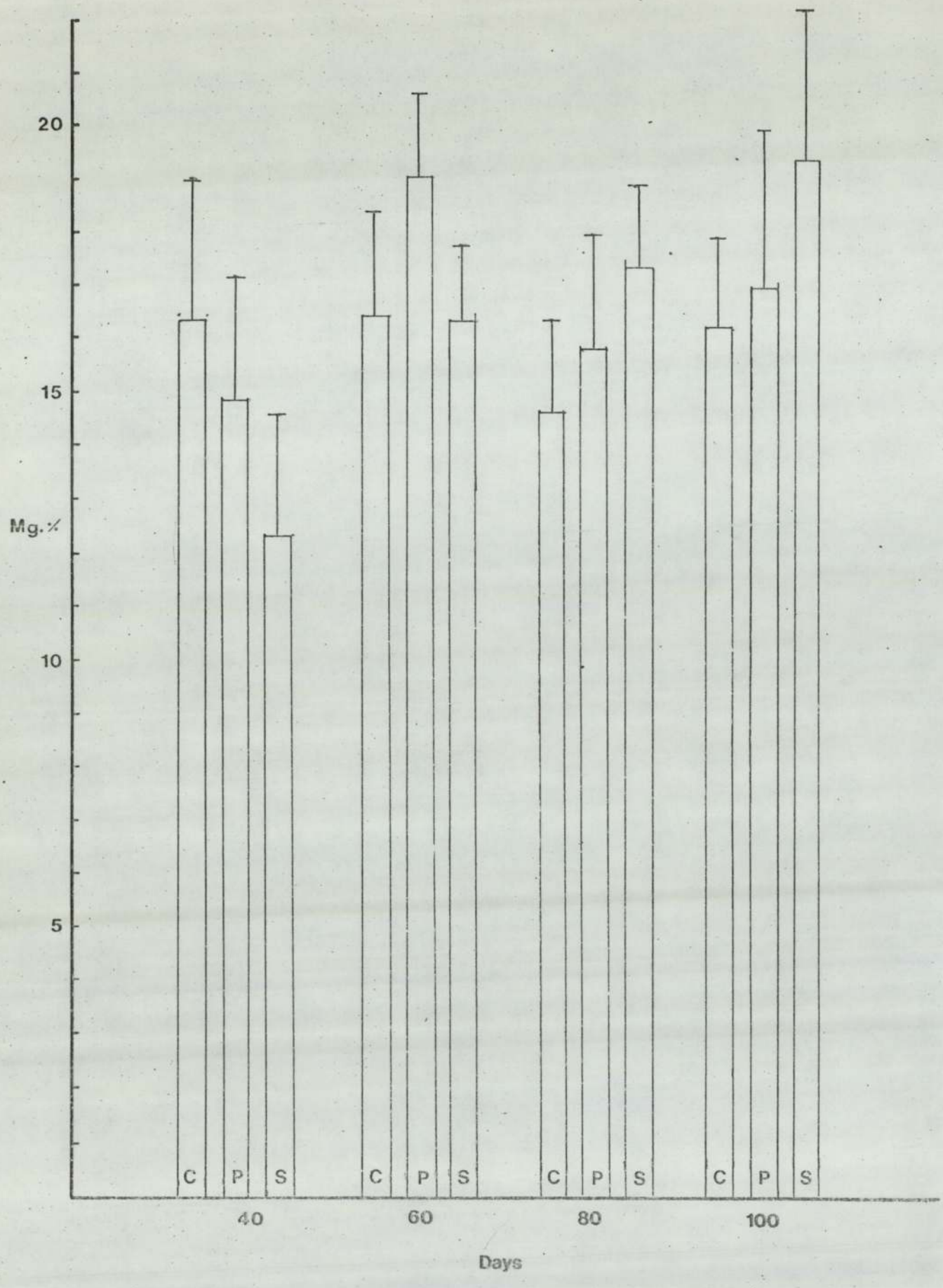

20
15
10
5
Mg.%
C
P
S
C
P
S
C
P
S
C
P
S
40
60
80
100
Days

Table 10. Adrenal weights : Females (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance	
40	C	7	23.31 ± 4.129	16.34 ± 2.687		
	P	4	21.45 ± 3.714	14.90 ± 2.310		
	S	4	16.82 ± 5.035	12.36 ± 2.238		*P 5%
60	C	11	35.70 ± 4.424	16.45 ± 1.956		
	P	5	38.02 ± 6.564	19.05 ± 2.622		
	S	5	30.46 ± 2.944	16.32 ± 1.487	*P 5%	
80	C	13	36.82 ± 3.879	14.67 ± 1.687		
	P	6	40.95 ± 4.170	15.80 ± 2.239		
	S	4	47.08 ± 2.927	17.37 ± 1.527	*P 0.1%	*P 2.5%
100	C	11	44.11 ± 5.535	16.18 ± 1.763		
	P	6	47.72 ± 10.049	17.00 ± 2.954		
	S	6	53.55 ± 8.260	19.32 ± 2.907	*P 2.5%	*P 2.5%

* w.r.t. control

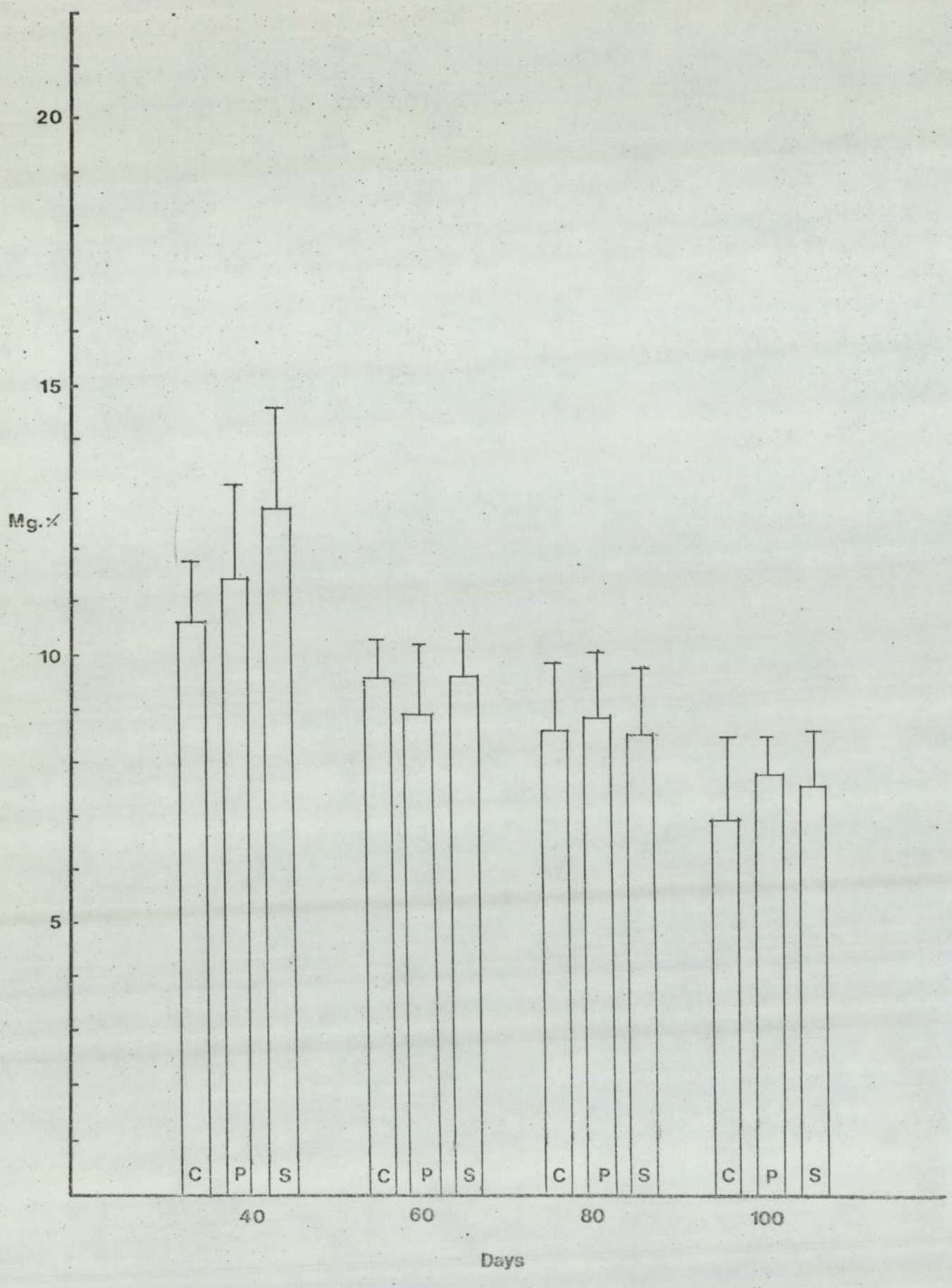

20
15
Mg.%
10
5
C
P
S
C
P
S
C
P
S
C
P
S
40
60
80
100
Days

Table 11. Adrenal weights : Males (O : 24 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
40	C	10	16.36 ± 2.167	10.65 ± 1.126	
	P	5	18.16 ± 2.849	11.44 ± 1.755	
	S	5	20.00 ± 2.902	12.77 ± 1.886	*P 2.5% *P 5%
60	C	9	26.63 ± 2.164	9.48 ± 0.808	
	P	6	25.02 ± 3.206	8.89 ± 1.290	
	S	7	27.36 ± 2.669	9.56 ± 0.820	
80	C	11	30.24 ± 5.445	8.53 ± 1.228	
	P	7	32.66 ± 5.074	8.75 ± 1.246	
	S	7	30.56 ± 3.965	8.44 ± 1.276	
100	C	10	29.23 ± 6.454	6.93 ± 1.492	
	P	8	33.10 ± 3.220	7.65 ± 0.765	
	S	6	28.80 ± 3.678	7.44 ± 0.953	

* w.r.t. control

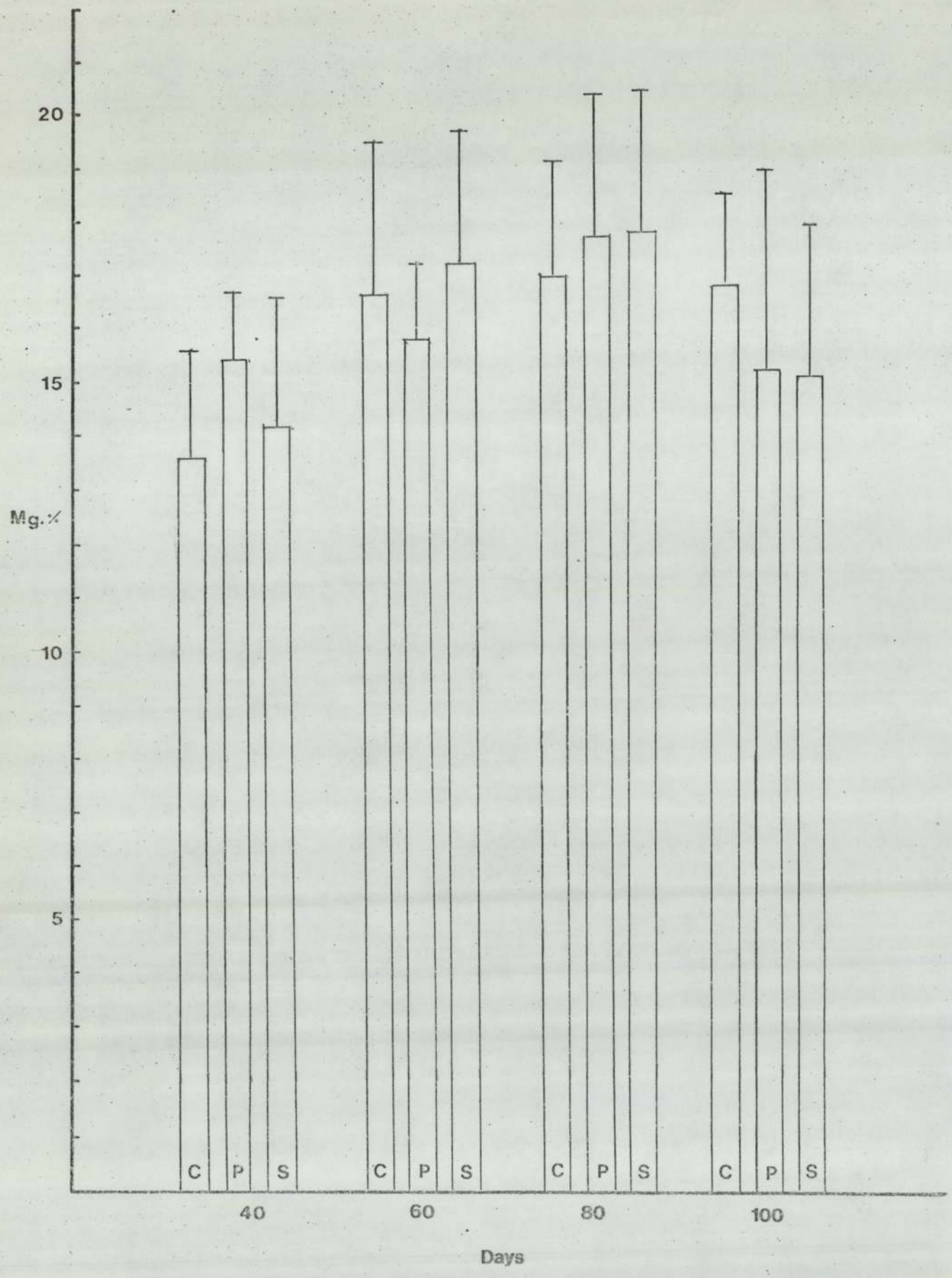

20
15
10
5
Mg.%
C
P
S
C
P
S
C
P
S
C
P
S
40
60
80
100
Days

Table 12. Adrenal weights : Females (0 : 24 Light/Dark)
Animals pinealectomized at thirty days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
40	C	11	18.96 ± 2.115	13.56 ± 2.065	
	P	6	20.85 ± 2.824	15.42 ± 1.270	
	S	5	17.24 ± 2.635	14.15 ± 2.428	
60	C	9	32.80 ± 6.043	16.58 ± 2.928	
	P	7	32.50 ± 3.619	15.69 ± 1.513	
	S	8	34.30 ± 5.478	17.18 ± 2.545	
80	C	7	38.57 ± 5.665	16.97 ± 2.090	
	P	8	43.94 ± 5.678	17.66 ± 2.673	
	S	6	42.67 ± 6.109	17.82 ± 2.646	
100	C	7	45.23 ± 6.008	16.74 ± 1.721	
	P	5	43.20 ± 11.940	15.17 ± 3.792	
	S	7	40.79 ± 7.637	14.98 ± 2.880	

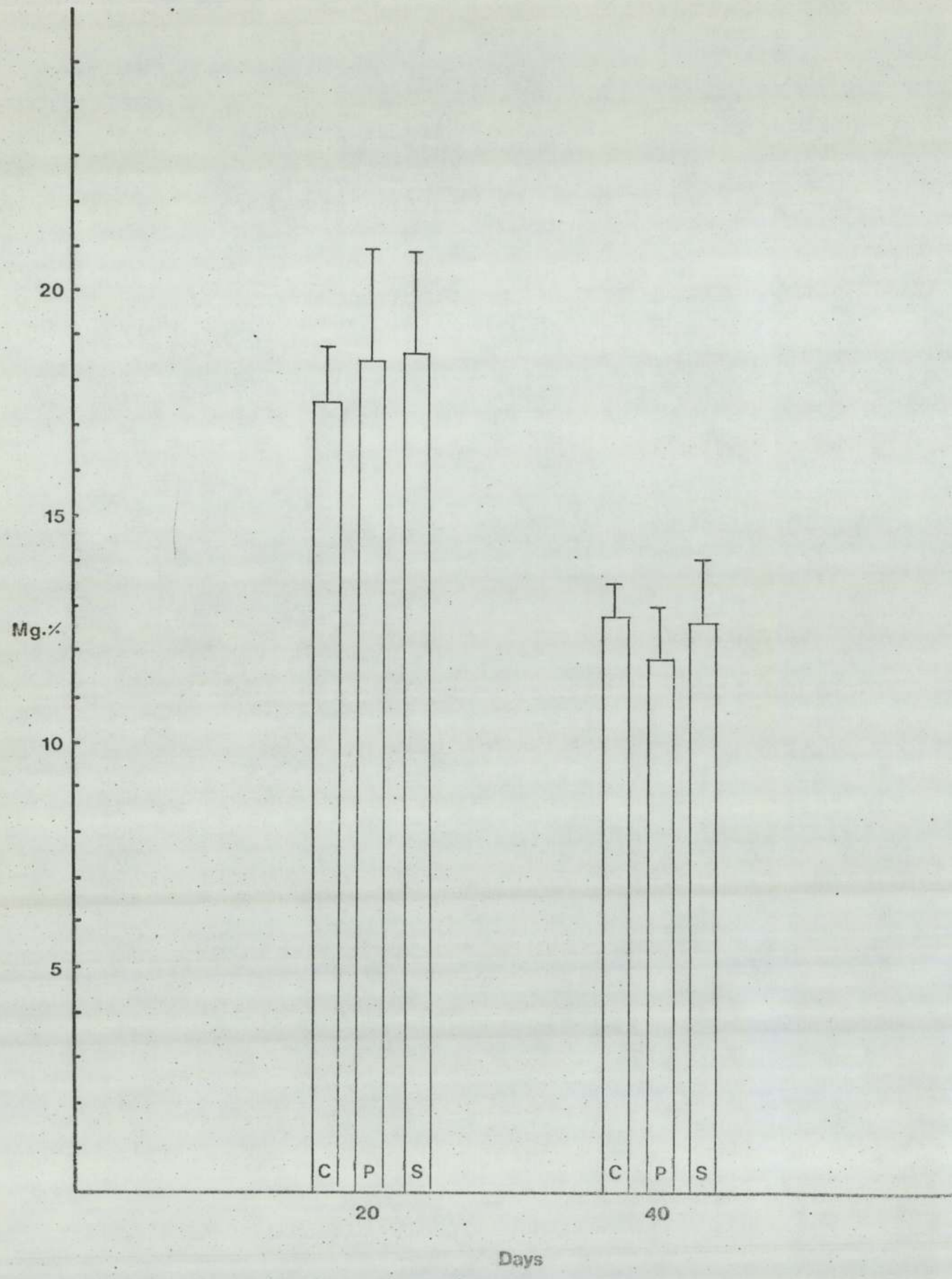

20
15
Mg.%
10
5
C
P
S
C
P
S
20
40
Days

Table 13. Adrenal weights : Males (12 : 12 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
20	C	6	7.30 ± 1.528	17.39 ± 1.403	
	P	6	6.48 ± 1.527	18.50 ± 2.566	
	S	7	7.04 ± 1.036	18.70 ± 1.970	
40	C	8	18.68 ± 1.911	12.69 ± 0.675	
	P	4	14.95 ± 1.203	11.77 ± 1.122	*P<1%
	S	5	20.56 ± 3.111	12.62 ± 1.223	***P<2.5%

* w.r.t. control
*** w.r.t. pinealectomized

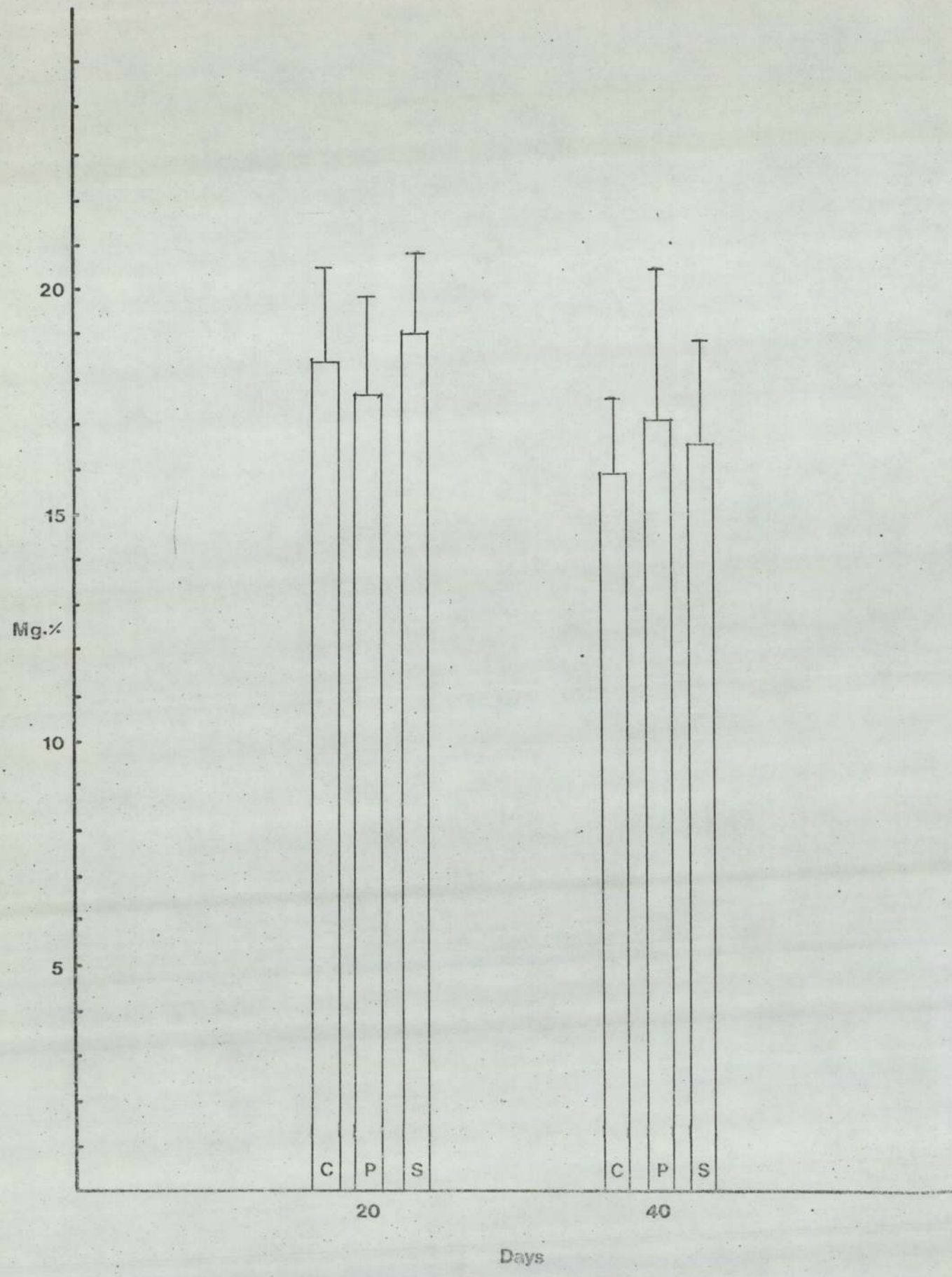

20
15
Mg.%
10
5
C
P
S
C
P
S
20
40
Days

Table 14. Adrenal weights : Females (12 : 12 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
20	C	7	8.11 ± 1.255	18.64 ± 1.822	
	P	7	7.07 ± 1.641	17.68 ± 2.145	
	S	9	7.71 ± 1.405	19.02 ± 1.754	
40	C	8	19.71 ± 2.889	15.92 ± 1.759	
	P	6	21.55 ± 3.029	17.27 ± 3.232	
	S	6	19.20 ± 2.897	16.64 ± 2.352	

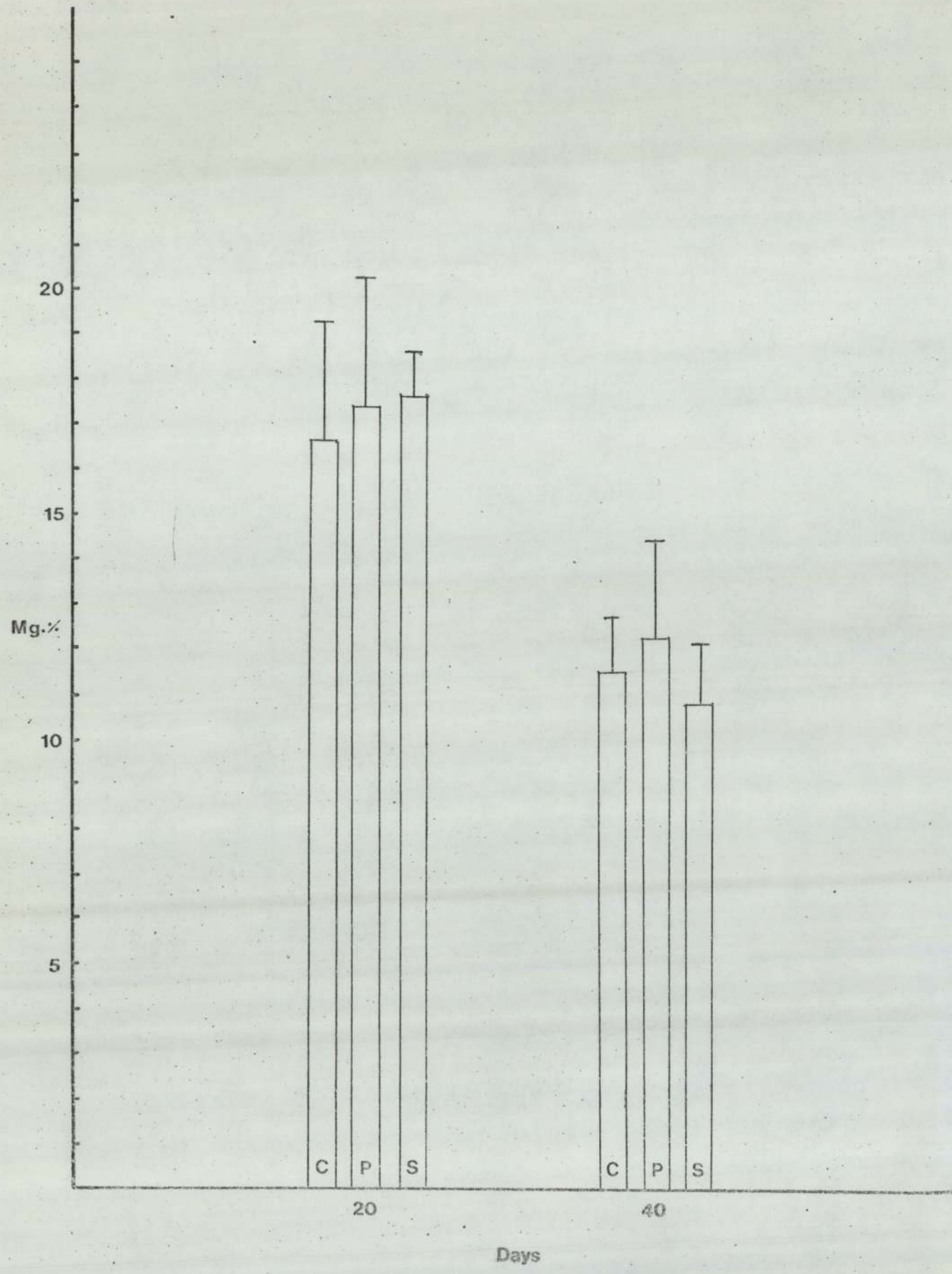

20
15
Mg.%
10
5
C
P
S
C
P
S
20
40
Days

Table 15. Adrenal weights : Males (0 : 24 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
20	C	14	8.57 ± 2.747	16.68 ± 2.564	
	P	7	6.34 ± 1.473	17.31 ± 2.873	
	S	7	7.83 ± 1.913	17.72 ± 0.942	
40	C	15	19.37 ± 2.408	11.43 ± 1.354	
	P	7	20.10 ± 3.462	12.16 ± 2.136	
	S	8	18.74 ± 2.025	10.79 ± 1.130	

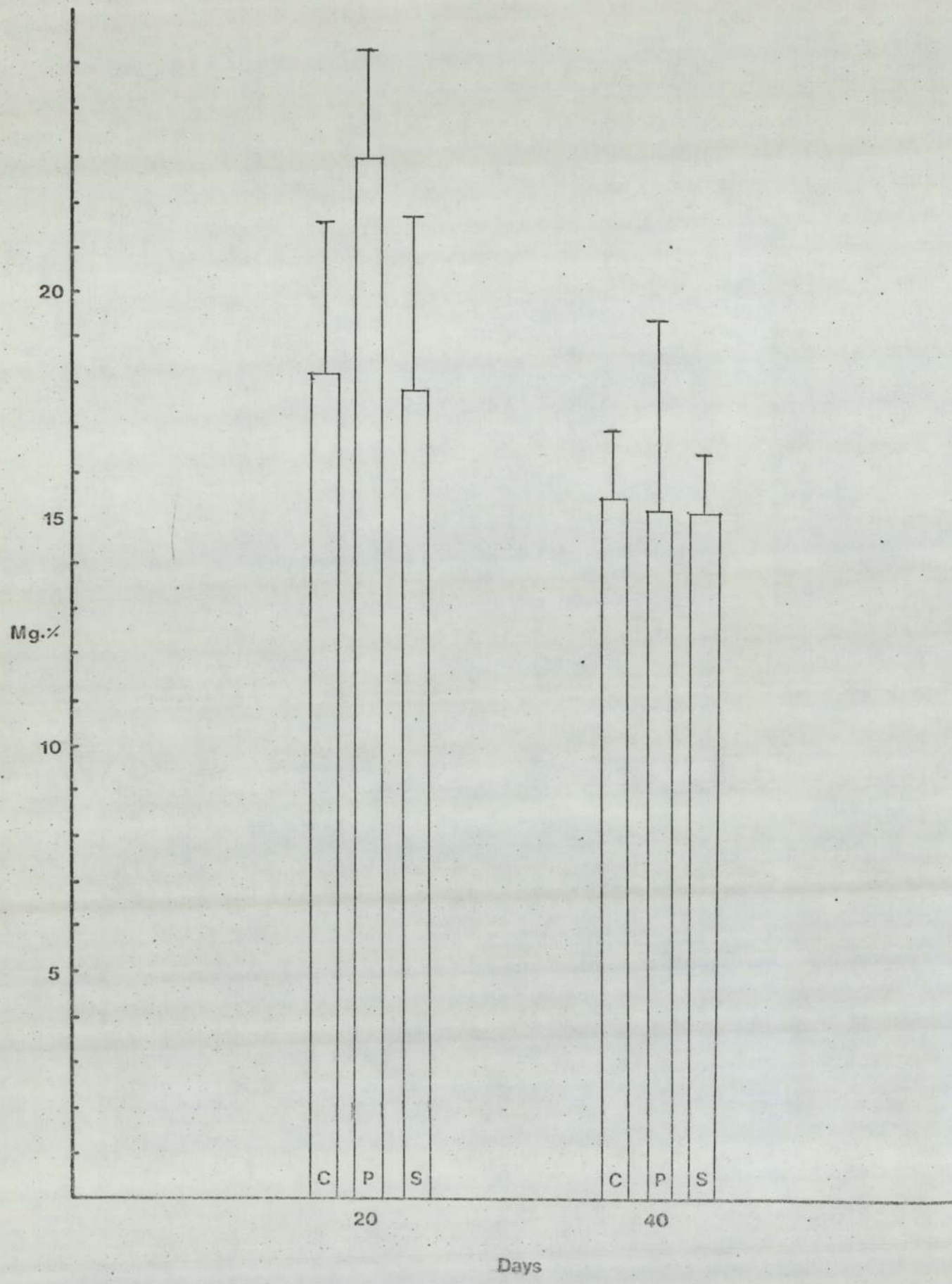

20
15
Mg.%
10
5
C
P
S
C
P
S
20
40
Days

Table 16. Adrenal weights : Females (0 : 24 Light/Dark)
Animals pinealectomized at three days.

Day	Class	No.	Mean Adrenal Wt. (± S.D.)mgs. Actual	%	Significance
20	C	12	7.95 ± 1.789	18.25 ± 3.318	
	P	4	7.55 ± 0.783	22.93 ± 2.318	*P < 5%
	S	8	8.26 ± 2.558	17.86 ± 3.889	
40	C	17	21.58 ± 2.322	15.35 ± 1.540	
	P	11	21.08 ± 5.775	15.18 ± 4.053	
	S	10	21.31 ± 1.640	15.15 ± 1.434	

* w.r.t. control

Table 17a. Adrenal Measurements : Males (12 : 12 Light/Dark)
Animals pinealectomized at thirty days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	7	2.55 ± 0.401	0.797 ± 0.158	0.959 ± 0.091	0.041 ± 0.009	0.571 ± 0.122	0.184 ± 0.044
	P	5	2.69 ± 0.291	0.876 ± 0.118	0.942 ± 0.064	0.042 ± 0.013	0.580 ± 0.054	0.256 ± 0.085
	S	4	2.40 ± 0.413	0.785 ± 0.171	0.830 ± 0.186	0.030 ± 0.009	0.573 ± 0.136	0.185 ± 0.030
60	C	5	2.50 ± 0.235	e0.778 ± 0.072	0.940 ± 0.117	0.035 ± 0.008	0.436 ± 0.134	0.306 ± 0.128
	P	4	a b3.24 ± 0.338	f1.025 ± 0.181	h1.185 ± 0.080	0.039 ± 0.011	0.590 ± 0.133	j0.395 ± 0.055
	S	4	2.22 ± 0.139	g0.643 ± 0.036	i0.930 ± 0.083	0.032 ± 0.002	0.435 ± 0.056	0.175 ± 0.030
80	C	16	c3.32 ± 0.348	1.032 ± 0.132	1.252 ± 0.191	0.041 ± 0.010	0.594 ± 0.083	0.397 ± 0.099
	P	3	3.29 ± 0.575	1.070 ± 0.227	1.150 ± 0.121	0.045 ± 0.012	0.623 ± 0.102	0.403 ± 0.143
	S	5	2.94 ± 0.272	0.948 ± 0.127	1.048 ± 0.065	0.040 ± 0.014	0.570 ± 0.075	0.336 ± 0.067
100	C	22	3.62 ± 0.330	1.127 ± 0.114	1.383 ± 0.225	0.054 ± 0.012	0.600 ± 0.089	0.473 ± 0.088
	P	7	d4.05 ± 0.344	k1.239 ± 0.090	1.573 ± 0.270	0.058 ± 0.006	0.630 ± 0.112	10.556 ± 0.052
	S	6	3.79 ± 0.374	1.215 ± 0.062	1.528 ± 0.133	0.051 ± 0.009	0.567 ± 0.072	m0.623 ± 0.114

a w.r.t. control P<2.5%
b w.r.t. sham P<0.5%
c w.r.t. sham P<5%
d w.r.t. control P<1%

e w.r.t. sham P<2.5%
f w.r.t. control<P 5%
g w.r.t. pinealectomized P<2.5%
h w.r.t. control P<2.5%

i w.r.t. pinealectomized P<1%
j w.r.t. sham P<0.1%
k w.r.t. control P<1%
l w.r.t. control P<5%
m w.r.t. pinealectomized P<0.5%

Table 17b. Adrenal Measurements : Males (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
	C	7	31.25	37.61	1.61	22.39	7.22
40	P	5	32.57	35.02	1.56	21.56	9.52
	S	4	32.71	34.58	1.25	23.88	7.71
	C	5	31.12	37.60	1.40	17.44	12.24
60	P	4	31.63	36.57	1.20	18.21	12.19
	S	4	28.96	41.89	1.44	19.59	7.88
	C	16	31.08	37.70	1.23	17.89	11.96
80	P	3	32.52	34.95	1.37	18.94	12.25
	S	5	32.24	35.64	1.36	19.39	11.43
	C	22	31.13	38.20	1.49	16.57	13.07
100	P	7	30.59	38.83	1.43	15.56	13.73
	S	6	32.05	40.31	1.35	14.96	16.44

Table 18a. Adrenal Measurements : Females (12 :12 Light/Dark)
Animals pinealectomized at thirty days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	6	2.92 ± 0.337	0.943 ± 0.146	1.032 ± 0.101	0.039 ± 0.005	0.607 ± 0.092	0.298 ± 0.111
	P	4	3.08 ± 0.167	1.055 ± 0.068	0.970 ± 0.076	0.048 ± 0.010	0.685 ± 0.063	0.320 ± 0.081
	S	5	2.91 ± 0.537	0.964 ± 0.214	0.984 ± 0.185	0.041 ± 0.017	0.632 ± 0.0119	0.290 ± 0.169
60	C	10	3.08 ± 0.358	d0.995 ± 0.112	1.091 ± 0.178	f0.034 ± 0.004	g0.639 ± 0.063	i0.322 ± 0.082
	P	5	3.36 ± 0.675	e1.124 ± 0.261	1.144 ± 0.160	0.034 ± 0.008	h0.710 ± 0.102	0.378 ± 0.154
	S	5	a 2.65 ± 0.155	0.814 ± 0.058	1.026 ± 0.062	0.027 ± 0.004	0.558 ± 0.048	0.228 ± 0.026
80	C	12	3.72 ± 0.310	j1.144 ± 0.118	1.344 ± 0.195	0.038 ± 0.007	k0.604 ± 0.062	0.501 ± 0.110
	P	6	3.79 ± 0.452	1.247 ± 0.192	1.298 ± 0.085	0.043 ± 0.014	l0.695 ± 0.127	0.508 ± 0.125
	S	4	3.81 ± 0.169	1.300 ± 0.058	1.213 ± 0.094	0.041 ± 0.008	0.888 ± 0.056	0.373 ± 0.058
100	C	11	3.71 ± 0.290	1.211 ± 0.109	n1.287 ± 0.219	0.047 ± 0.004	0.723 ± 0.089	q0.440 ± 0.090
	P	5	b4.17 ± 0.223	m1.456 ± 0.089	o1.258 ± 0.316	p0.042 ± 0.004	0.812 ± 0.122	r0.602 ± 0.177
	S	6	c4.25 ± 0.358	1.322 ± 0.150	1.605 ± 0.081	0.047 ± 0.005	0.702 ± 0.132	0.572 ± 0.043

a w.r.t. control P<5%
b w.r.t. control P<1%
c w.r.t. pinealectomized P<1%
d w.r.t. sham P<1%
e w.r.t. sham P<5%
f w.r.t. sham P<2.5%
g w.r.t. sham P<5%
h w.r.t. sham P<5%
i w.r.t. sham P<5%
j w.r.t. sham P<5%
k w.r.t. sham P<0.1%
l w.r.t. sham P<5%
m w.r.t. control P<0.5%
n w.r.t. sham P<1%
o w.r.t. sham P<5%
p w.r.t. control P<5%
q w.r.t. sham P<1%
r w.r.t. control P<5%

Table 18b. Adrenal Measurements : Females (12 : 12 Light/Dark)
Animals pinealectomized at thirty days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	6	32.29	35.34	1.34	20.79	10.21
	P	4	34.25	31.49	1.56	22.24	10.39
	S	5	33.13	33.81	1.41	21.72	9.97
60	C	10	32.31	35.42	1.10	20.75	10.45
	P	5	33.45	34.04	1.01	21.13	11.25
	S	5	30.72	38.71	1.02	21.06	8.60
80	C	12	30.75	36.12	1.02	16.24	13.47
	P	6	32.90	34.24	1.13	18.34	13.40
	S	4	34.12	31.83	1.08	23.31	9.79
100	C	11	32.64	34.69	1.27	19.49	11.86
	P	5	34.91	30.16	1.01	19.47	14.44
	S	6	31.10	37.76	1.11	16.52	13.46

Table 19a. Adrenal Measurements : Male (0 : 24 Light/Dark)
Animals pinealectomized at thirty days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	9	2.36 ± 0.253	0.758 ± 0.095	0.844 ± 0.126	0.032 ± 0.006	0.549 ± 0.074	0.178 ± 0.045
	P	6	2.35 ± 0.157	0.733 ± 0.035	0.883 ± 0.124	0.031 ± 0.005	0.517 ± 0.055	0.185 ± 0.057
	S	5	2.30 ± 0.075	0.752 ± 0.012	0.796 ± 0.078	0.030 ± 0.006	0.520 ± 0.018	0.202 ± 0.013
60	C	8	2.42 ± 0.195	0.779 ± 0.050	0.859 ± 0.194	0.030 ± 0.006	0.515 ± 0.064	0.234 ± 0.045
	P	6	2.42 ± 0.231	0.822 ± 0.053	0.777 ± 0.147	0.030 ± 0.007	0.493 ± 0.057	a0.298 ± 0.050
	S	7	2.43 ± 0.222	0.747 ± 0.079	0.939 ± 0.105	0.028 ± 0.006	0.466 ± 0.086	0.253 ± 0.059
80	C	11	2.69 ± 0.360	0.839 ± 0.138	1.016 ± 0.128	0.032 ± 0.007	0.482 ± 0.070	0.324 ± 0.097
	P	7	2.79 ± 0.188	0.876 ± 0.092	1.043 ± 0.193	0.029 ± 0.004	0.519 ± 0.096	0.327 ± 0.049
	S	7	2.72 ± 0.176	0.879 ± 0.067	0.959 ± 0.142	0.030 ± 0.006	0.481 ± 0.088	0.367 ± 0.050
100	C	10	2.77 ± 0.282	0.859 ± 0.093	1.050 ± 0.177	0.040 ± 0.008	0.416 ± 0.042	0.402 ± 0.077
	P	8	3.05 ± 0.271	b0.971 ± 0.085	1.107 ± 0.199	0.035 ± 0.005	0.455 ± 0.043	0.480 ± 0.094
	S	6	2.82 ± 0.244	0.907 ± 0.069	1.002 ± 0.142	0.035 ± 0.008	0.457 ± 0.094	0.417 ± 0.083

a w.r.t. control P<5%
b w.r.t. control P<2.5%

Table 19b. Adrenal Measurements : Male (0 : 24 Light/Dark)
Animals pinealectomized at thirty days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	9	32.12	35.76	1.36	23.26	7.54
	P	6	31.19	37.57	1.32	22.00	7.87
	S	5	32.70	34.61	1.30	22.61	8.78
60	C	8	32.19	35.50	1.24	21.28	9.67
	P	6	33.97	32.11	1.24	20.37	12.31
	S	7	30.74	38.64	1.15	19.18	10.41
80	C	11	31.19	37.76	1.19	17.92	12.04
	P	7	31.40	37.38	1.04	18.60	11.72
	S	7	32.32	35.26	1.10	17.68	13.49
100	C	10	31.10	37.90	1.44	15.02	14.51
	P	8	31.84	36.29	1.15	14.92	15.74
	S	6	32.16	35.53	1.24	16.21	14.79

Table 20a. Adrenal Measurements : Females (0 : 24 Light/Dark)
Animals pinealectomized at thirty days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	9	2.40 ± 0.121	0.771 ± 0.056	0.858 ± 0.057	0.027 ± 0.005	0.532 ± 0.044	0.209 ± 0.065
	P	6	2.46 ± 0.281	0.820 ± 0.097	0.822 ± 0.129	0.026 ± 0.004	0.540 ± 0.055	0.253 ± 0.082
	S	4	2.31 ± 0.115	0.757 ± 0.029	0.790 ± 0.086	0.031 ± 0.004	0.530 ± 0.007	0.195 ± 0.035
60	C	9	2.59 ± 0.329	0.883 ± 0.100	0.823 ± 0.224	0.028 ± 0.006	0.573 ± 0.083	0.281 ± 0.125
	P	7	2.68 ± 0.356	0.901 ± 0.054	0.880 ± 0.260	0.027 ± 0.006	0.581 ± 0.077	0.291 ± 0.108
	S	8	2.71 ± 0.226	0.921 ± 0.068	0.869 ± 0.162	0.028 ± 0.010	0.622 ± 0.065	0.269 ± 0.068
80	C	7	2.97 ± 0.324	1.024 ± 0.087	0.924 ± 0.208	0.030 ± 0.006	0.664 ± 0.064	0.330 ± 0.073
	P	7	3.07 ± 0.318	1.033 ± 0.114	1.007 ± 0.117	0.025 ± 0.003	0.641 ± 0.064	0.366 ± 0.140
	S	6	3.11 ± 0.299	1.023 ± 0.118	1.062 ± 0.099	0.031 ± 0.007	0.658 ± 0.122	0.335 ± 0.063
100	C	7	3.31 ± 0.263	1.081 ± 0.112	1.151 ± 0.127	0.030 ± 0.005	0.734 ± 0.122	0.317 ± 0.029
	P	5	3.16 ± 0.406	1.006 ± 0.121	1.144 ± 0.189	0.032 ± 0.003	0.628 ± 0.066	0.346 ± 0.088
	S	5	3.09 ± 0.218	1.052 ± 0.121	0.982 ± 0.186	0.031 ± 0.006	a 0.570 ± 0.094	b 0.450 ± 0.077

a w.r.t. control P< 5%
b w.r.t. control P<0.5%

Table 20b. Adrenal Measurements : Females (0 : 24 Light/Dark)
Animals pinealectomized at thirty days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
40	C	9	32.13	35.75	1.13	22.17	8.71
	P	6	33.33	33.41	1.06	21.95	10.28
	S	4	32.77	34.20	1.34	22.94	8.44
60	C	9	34.09	31.78	1.08	22.12	10.85
	P	7	33.62	32.84	1.01	21.68	10.86
	S	8	33.99	32.07	1.03	22.95	9.93
80	C	7	34.47	31.11	1.01	22.36	11.11
	P	7	33.64	32.80	0.81	20.88	11.92
	S	6	32.89	34.14	1.00	21.16	10.77
100	C	7	32.65	34.77	0.91	22.18	9.58
	P	5	31.83	36.20	1.01	19.87	10.95
	S	5	34.04	31.78	1.00	18.45	14.56

Table 21a. Adrenal Measurements : Male (12 : 12 Light/Dark)
Animals pinealectomized at three days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	5	1.87 ± 0.377	0.564 ± 0.133	0.744 ± 0.169	0.025 ± 0.005	0.494 ± 0.115	0.044 ± 0.045
	P	6	1.77 ± 0.271	0.495 ± 0.094	0.777 ± 0.092	0.027 ± 0.007	0.432 ± 0.088	0.033 ± 0.028
	S	6	1.74 ± 0.129	0.495 ± 0.066	0.757 ± 0.089	0.026 ± 0.006	0.423 ± 0.084	0.045 ± 0.055
40	C	8	2.68 ± 0.195	0.837 ± 0.048	1.004 ± 0.139	0.033 ± 0.004	0.571 ± 0.060	0.216 ± 0.070
	P	3	2.51 ± 0.143	0.840 ± 0.110	a0.833 ± 0.152	0.030 ± 0.004	0.537 ± 0.059	0.270 ± 0.071
	S	5	2.75 ± 0.116	0.836 ± 0.066	1.082 ± 0.043	0.034 ± 0.006	0.630 ± 0.084	0.172 ± 0.029

a w.r.t. sham P<2.5%

Table 21b. Adrenal Measurements : Males (12 : 12 Light/Dark)
Animals pinealectomized at three days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	5	30.16	39.79	1.34	26.42	2.35
	P	6	27.97	43.90	1.53	24.41	2.54
	S	6	28.45	43.51	1.49	24.31	2.59
40	C	8	31.23	37.46	1.23	21.31	8.05
	P	3	33.47	33.19	1.20	21.39	10.76
	S	5	30.40	39.34	1.24	22.91	6.25

Table 22a. Adrenal Measurements : Female (12 : 12 Light/Dark)
Animals pinealectomized at three days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	2	1.78 ± 0.005	0.510 ± 0.030	0.775 ± 0.045	0.031 ± 0.002	0.480 ± 0.030	0.000 ± 0.000
	P	5	1.58 ± 0.131	0.452 ± 0.057	0.676 ± 0.054	0.026 ± 0.005	a0.384 ± 0.030	0.044 ± 0.038
	S	7	1.79 ± 0.240	0.567 ± 0.110	0.656 ± 0.086	0.027 ± 0.007	0.503 ± 0.118	0.039 ± 0.055
40	C	8	2.78 ± 0.254	0.876 ± 0.074	1.054 ± 0.157	0.029 ± 0.003	0.600 ± 0.056	0.248 ± 0.056
	P	6	2.78 ± 0.244	0.860 ± 0.069	1.057 ± 0.111	0.032 ± 0.004	b0.622 ± 0.040	0.207 ± 0.071
	S	6	2.78 ± 0.196	0.872 ± 0.090	1.037 ± 0.076	0.028 ± 0.007	0.558 ± 0.045	0.283 ± 0.092

a w.r.t. control $P<2.5\%$
b w.r.t. sham $P<5\%$

Table 22b. Adrenal Measurements : Females (12 : 12 Light/Dark)
Animals pinealectomized at three days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	2	28.65	43.54	1.74	26.97	0.00
	P	5	28.61	42.78	1.65	24.30	2.78
	S	7	31.68	36.65	1.51	28.10	2.18
40	C	8	31.51	37.91	1.04	21.58	8.92
	P	6	30.94	38.02	1.15	22.37	7.45
	S	6	31.37	37.30	1.01	20.07	10.18

Table 23a. Adrenal Measurements : Male (0 : 24 Light/Dark)
Animals pinealectomized at three days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	12	2.19 ± 0.306	0.648 ± 0.089	0.878 ± 0.159	0.033 ± 0.006	0.559 ± 0.039	0.055 ± 0.085
	P	8	2.03 ± 0.281	0.639 ± 0.103	0.754 ± 0.161	0.034 ± 0.007	0.565 ± 0.072	0.038 ± 0.053
	S	8	2.00 ± 0.400	0.588 ± 0.132	0.826 ± 0.152	0.030 ± 0.005	0.530 ± 0.099	0.026 ± 0.038
40	C	13	2.83 ± 0.363	0.862 ± 0.068	1.065 ± 0.154	0.035 ± 0.004	0.602 ± 0.095	0.226 ± 0.070
	P	5	2.85 ± 0.256	0.900 ± 0.072	1.050 ± 0.120	a0.041 ± 0.007	0.686 ± 0.094	0.174 ± 0.055
	S	8	2.84 ± 0.180	0.899 ± 0.090	1.045 ± 0.138	0.037 ± 0.006	0.654 ± 0.095	0.208 ± 0.057

a w.r.t. control P<5%

Table 23b. Adrenal Measurements : Males (O : 24 Light/Dark)
Animals pinealectomized at three days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
	C	12	29.59	40.09	1.51	25.53	2.51
20	P	8	31.48	37.14	1.67	27.83	1.87
	S	8	29.40	41.30	1.50	26.50	1.30
	C	13	30.46	37.63	1.24	21.27	7.99
40	P	5	31.58	36.84	1.44	24.07	6.11
	S	8	31.65	36.79	1.30	23.03	7.32

Table 24a. Adrenal Measurements : Female (0 : 24 Light/Dark)
Animals pinealectomized at three days. Means (± S.D.)mm.

Day	Class	No.	Adrenal dia.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	11	2.20 ± 0.354	0.671 ± 0.119	0.858 ± 0.159	0.031 ± 0.008	0.572 ± 0.063	0.066 ± 0.082
	P	4	2.30 ± 0.522	0.698 ± 0.180	0.908 ± 0.168	0.030 ± 0.004	0.557 ± 0.093	0.108 ± 0.111
	S	8	2.33 ± 0.339	0.719 ± 0.100	0.895 ± 0.194	0.034 ± 0.007	0.588 ± 0.052	0.097 ± 0.081
40	C	14	3.00 ± 0.188	0.942 ± 0.044	1.120 ± 0.150	0.031 ± 0.005	0.655 ± 0.072	0.255 ± 0.080
	P	10	2.93 ± 0.218	0.905 ± 0.075	1.120 ± 0.121	0.034 ± 0.005	0.624 ± 0.078	0.246 ± 0.079
	S	8	2.87 ± 0.321	0.886 ± 0.122	1.096 ± 0.102	0.032 ± 0.005	0.639 ± 0.070	0.215 ± 0.067

Table 24b. Adrenal Measurements : Females (0 : 24 Light/Dark)
Animals pinealectomized at three days.
Zones as % adrenal diameter.

Day	Class	No.	Cortex	Medulla	Z.glomerulosa	Z.fasciculata	Z.reticularis
20	C	11	30.50	39.00	1.41	26.00	3.00
	P	4	30.35	39.48	1.30	24.22	4.70
	S	8	30.86	38.41	1.46	25.24	4.16
40	C	14	31.40	37.33	1.03	21.83	8.50
	P	10	30.89	38.22	1.16	21.30	8.40
	S	8	30.87	38.18	1.11	22.26	7.49

Table 25. Comparison of adrenal diameters with respect to light regime.

Animals pinealectomized at thirty days : Males

Day	Class	Light/Dark	Dark	Significance
40	C	2.55 ± 0.401	2.36 ± 0.253	
	P	2.69 ± 0.291	2.35 ± 0.157	
	S	2.40 ± 0.413	2.30 ± 0.075	
60	C	2.50 ± 0.235	2.42 ± 0.195	
	P	3.24 ± 0.338	2.42 ± 0.231	P<1%
	S	2.22 ± 0.139	2.43 ± 0.222	
80	C	3.32 ± 0.348	2.69 ± 0.360	P<1%
	P	3.29 ± 0.575	2.79 ± 0.188	
	S	2.94 ± 0.272	2.72 ± 0.176	
100	C	3.62 ± 0.330	2.77 ± 0.282	P<0.1%
	P	4.05 ± 0.344	3.05 ± 0.271	P<0.1%
	S	3.79 ± 0.374	2.82 ± 0.244	P<0.1%

Table 26. Comparison of adrenal diameters with respect to light regime.

Animals pinealectomized at thirty days : Females

Day	Class	Light/Dark	Dark	Significance
40	C	2.92 ± 0.337	2.40 ± 0.121	P<1%
	P	3.08 ± 0.167	2.46 ± 0.281	P<1%
	S	2.91 ± 0.537	2.31 ± 0.115	
60	C	3.08 ± 0.358	2.59 ± 0.329	P<5%
	P	3.36 ± 0.675	2.68 ± 0.356	
	S	2.65 ± 0.155	2.71 ± 0.226	
80	C	3.72 ± 0.310	2.97 ± 0.324	P<0.1%
	P	3.79 ± 0.452	3.07 ± 0.318	P<2%
	S	3.81 ± 0.169	3.11 ± 0.299	P<1%
100	C	3.71 ± 0.290	3.31 ± 0.263	P<2%
	P	4.17 ± 0.223	3.16 ± 0.406	P<1%
	S	4.25 ± 0.358	3.09 ± 0.218	P<0.1%

Table 27. Comparison of adrenal diameters with respect to light regime.

Animals pinealectomized at three days : Males

Day	Class	Light/Dark	Dark	Significance
20	C	1.87 ± 0.377	2.19 ± 0.306	
	P	1.77 ± 0.271	2.03 ± 0.281	
	S	1.74 ± 0.129	2.00 ± 0.400	
40	C	2.68 ± 0.195	2.83 ± 0.363	
	P	2.51 ± 0.143	2.85 ± 0.256	
	S	2.75 ± 0.116	2.84 ± 0.180	

Table 28. Comparison of adrenal diameters with respect to light regime.

Animals pinealectomized at three days : Females

Day	Class	Light/Dark	Dark	Significance
20	C	1.78 ± 0.005	2.20 ± 0.354	
	P	1.58 ± 0.131	2.30 ± 0.522	
	S	1.79 ± 0.240	2.33 ± 0.339	P<1%
40	C	2.78 ± 0.254	3.00 ± 0.188	P<5%
	P	2.78 ± 0.244	2.93 ± 0.218	
	S	2.78 ± 0.196	2.87 ± 0.321	

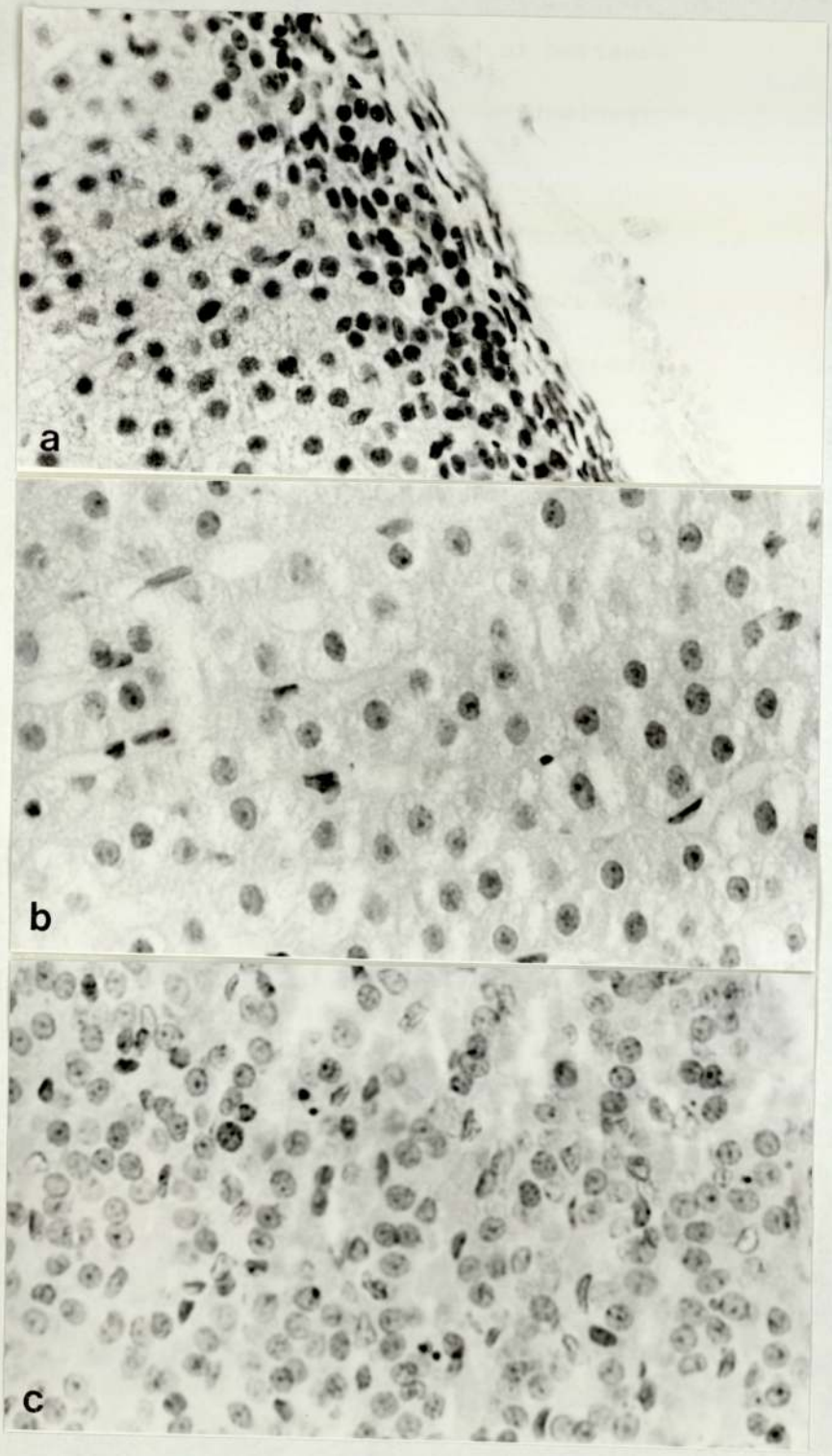
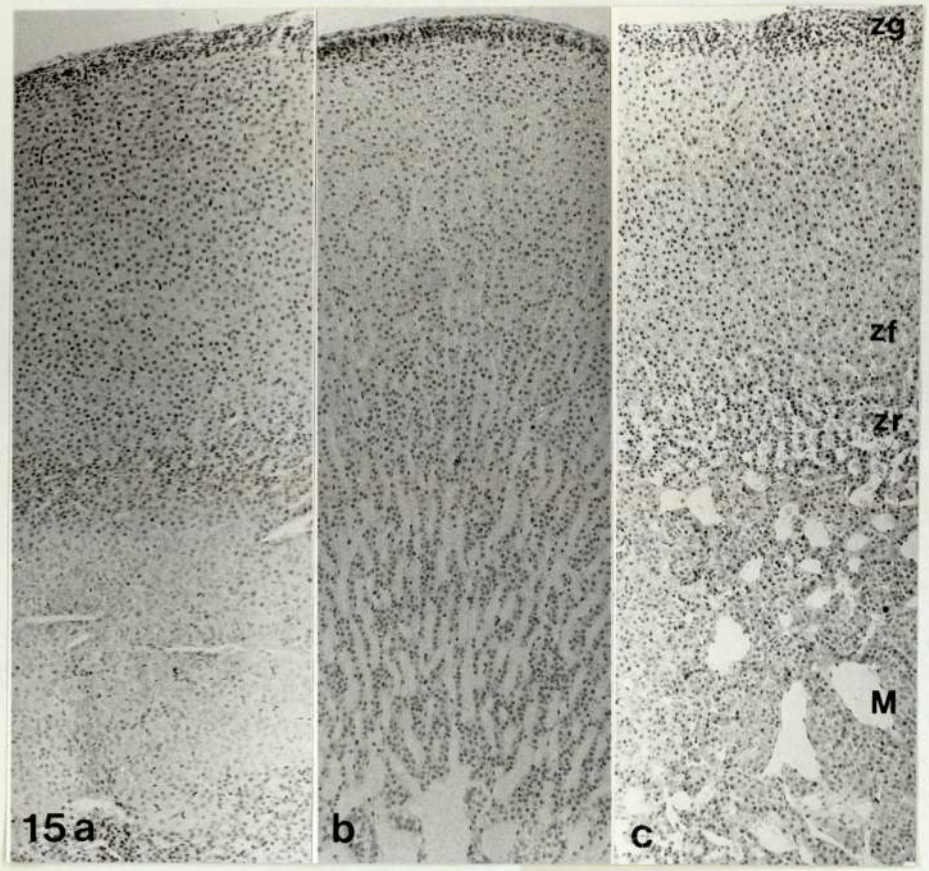

a
b
c

Fig. 14 The three zones of the adrenal cortex:

a. Zona glomerulosa. Close packed nuclei below the fibrous capsule of the adrenal demonstrate the extent of the z. glomerulosa. A thin layer observed to be 0.025 - 0.060 mm. deep in these experiments. x 600.

b. Zona fasciculata. A layer of comparatively consistent nuclear density lying below the zona glomerulosa. It does not have a uniform depth within each adrenal. x 600.

c. Zona reticularis. The innermost layer of the cortex. This layer contains a large number of blood vessels which impart a reticular appearance when observed optically. Like the zona fasciculata, this layer does not exhibit uniform thickness within each adrenal. x 600.


zg
zf
zr
M
15a
b
c

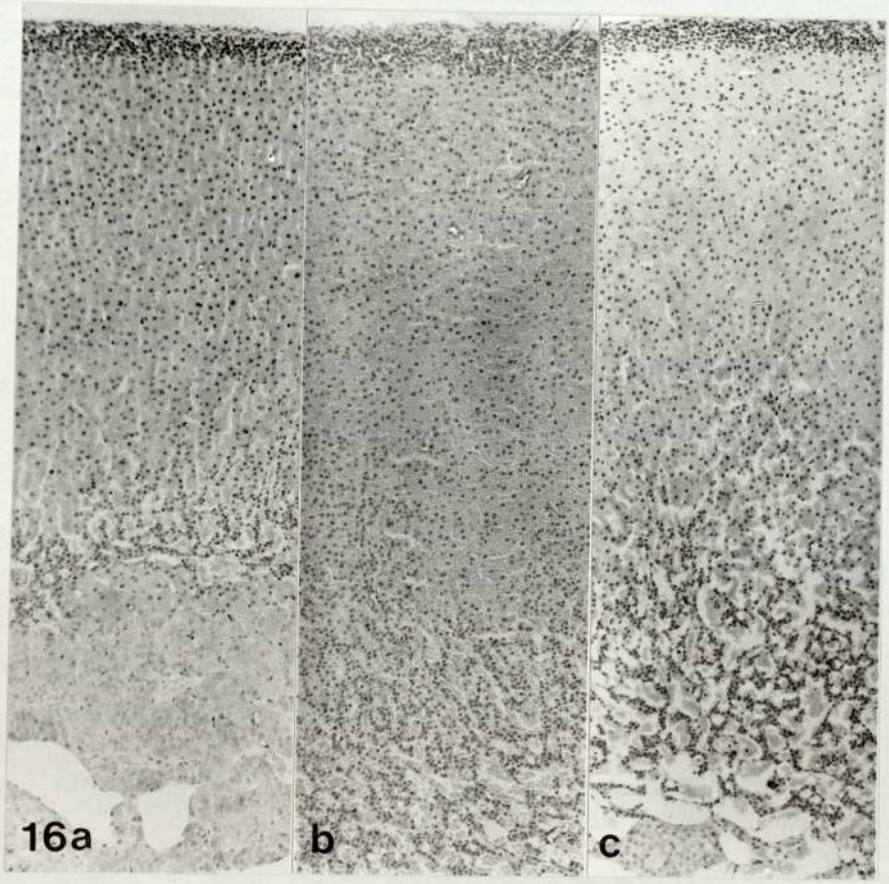

16a
b
c

Fig. 15 Sections of adrenals from 40 day old dark reared females (see Table 12). The sections are randomly chosen and are not strictly comparable. x 100.

a. Control

b. Pinealectomized.

c. Sham-operated.

Fig. 16 Sections from 100 day old dark reared females (see Table 12). The sections are randomly chosen and are not strictly comparable. x 100.

a. Control

b. Pinealectomized.

c. Sham-operated.

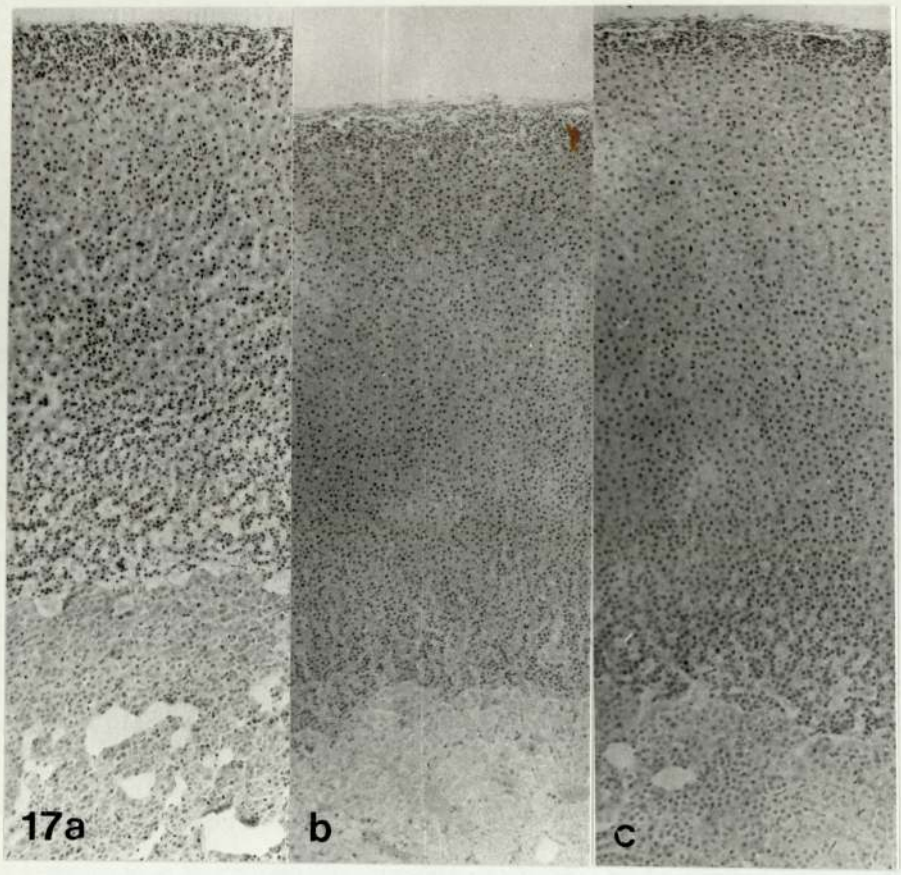

17a
b
c

Fig. 17 Sections of adrenals from:

a. 100 day old dark reared control male (Table 11).

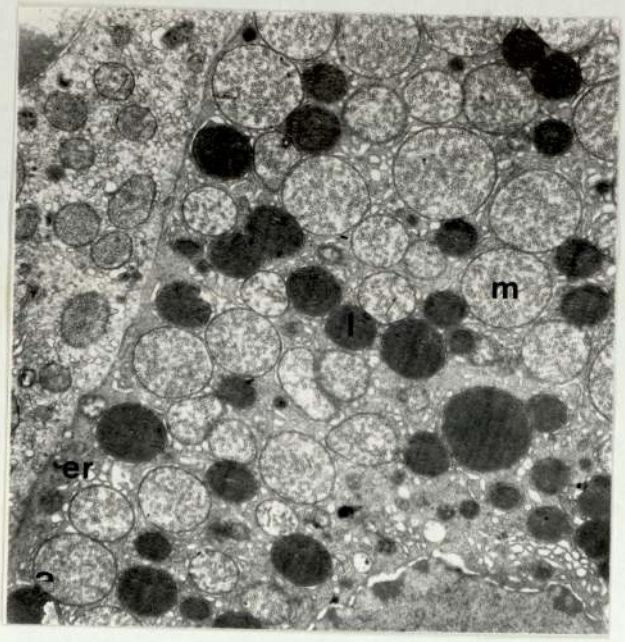
x 100.

b. 40 day old light/dark reared control make (Table 9).

x 100.

c. 20 day old light/dark reared control female (Table 14).

x 100.


m
l
er

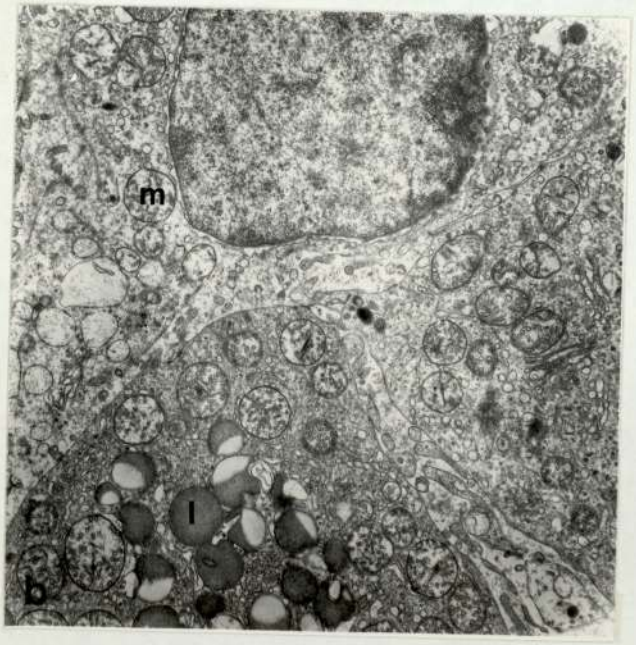
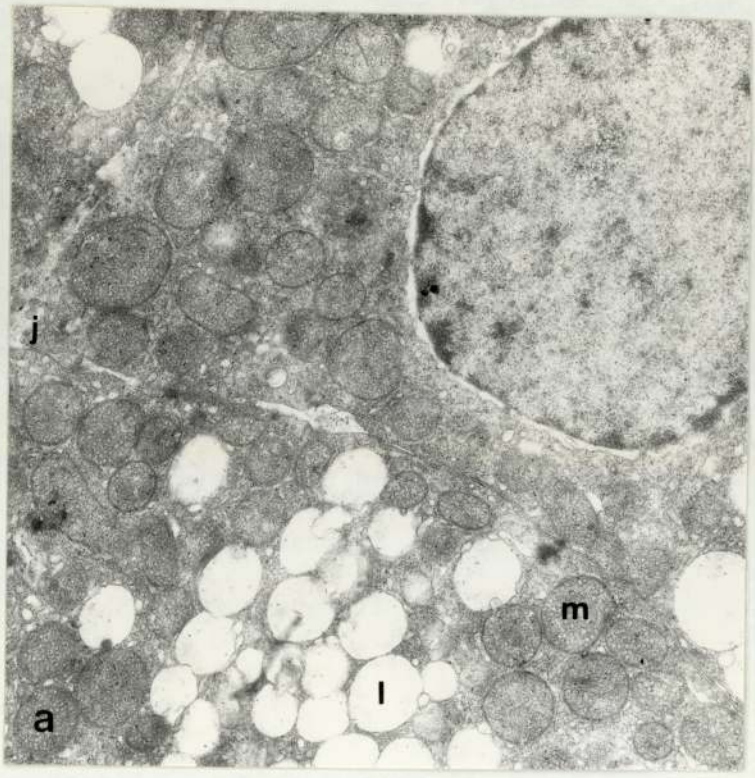

m
l
b

Fig. 18 a.b. Adrenal sections from twenty-day old dark-reared rats. (Operated at three days).

a. Pinealectomized female: zona fasciculata. Darkly stained liposomes (l) and typical mitochondria (m) are evident, together with agranular endoplasmic reticulum (er). x 7,500.

b. Control male: zona fasciculata. Liposomes (l) and some atypical mitochondria (m) are present. x 7,500.


j
m
l
a

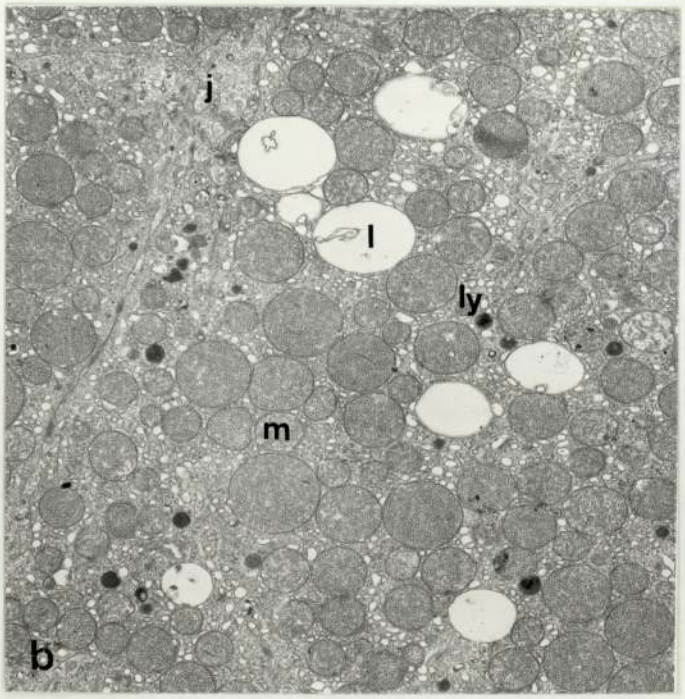
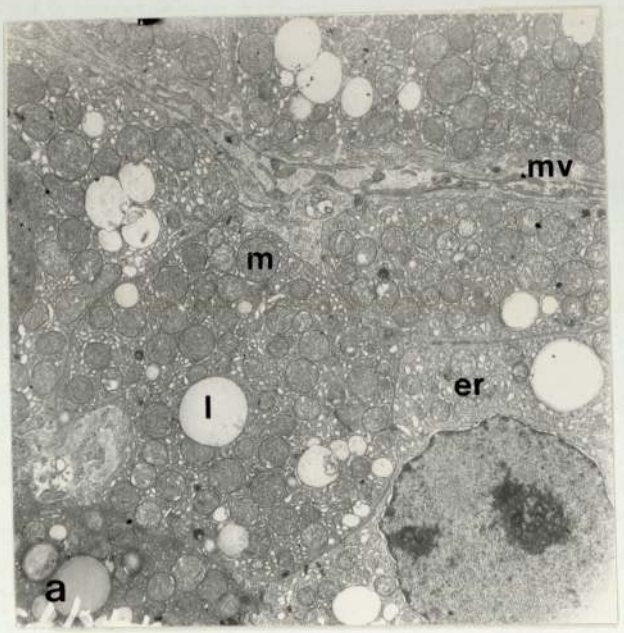

j
l
ly
m
b

Fig. 19 a.b. Adrenal sections from forty day old male rats. (Operated at thirty days).

a. Dark reared control animal: zona fasciculata. Numerous mitochondria (m), liposomes (l) and a large nucleus are present. At the left hand edge the junction (j) of three cells is apparent. x 10,000.

b. Light/dark reared pinealectomized male: zona fasciculata. Numerous mitochondria (m), lysosomes (ly) and fewer liposomes. At the upper edge, the junction (j) of three cells is apparent. x 10,000.


mv
m
er
l
a

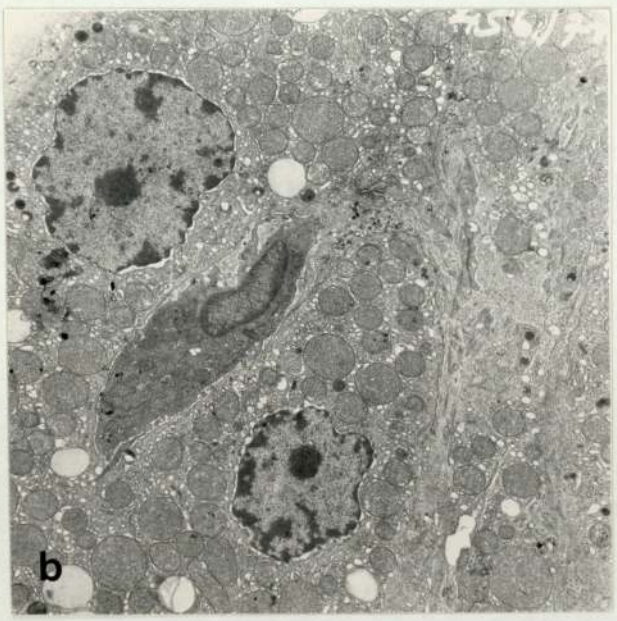
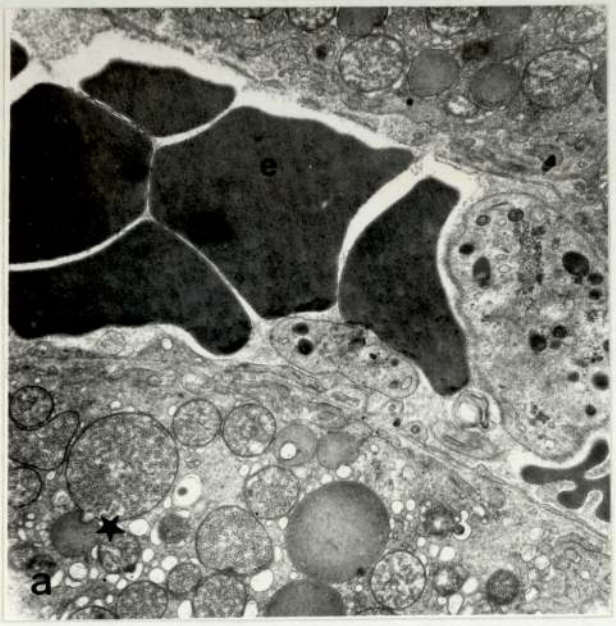

b

Fig. 20 a.b. Adrenal sections from forty day old light/dark reared pinealectomized male rats. (Operated at thirty days).

a. Zona fasciculata. Typical mitochondria (m), liposomes (l), smooth endoplasmic reticulum (er) and intercellular spaces containing microvilli (mv). x 7,500.

b. Zona fasciculata from a different adrenal x 7,500.


e
a

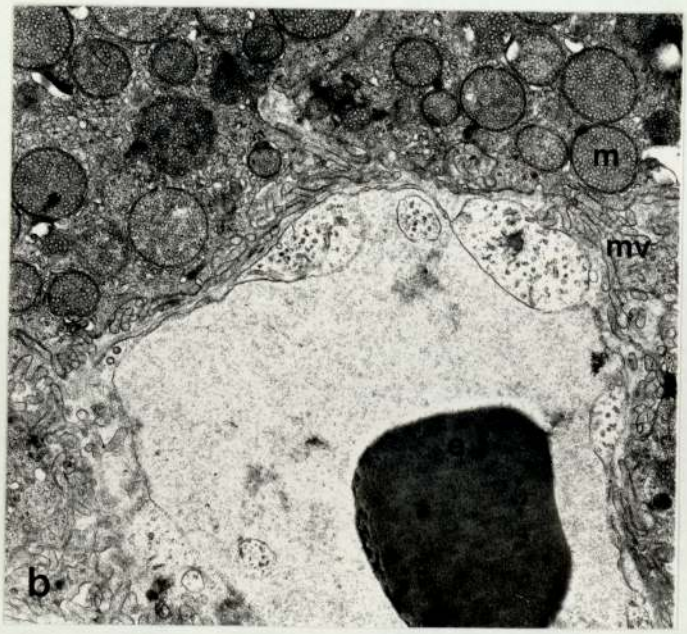
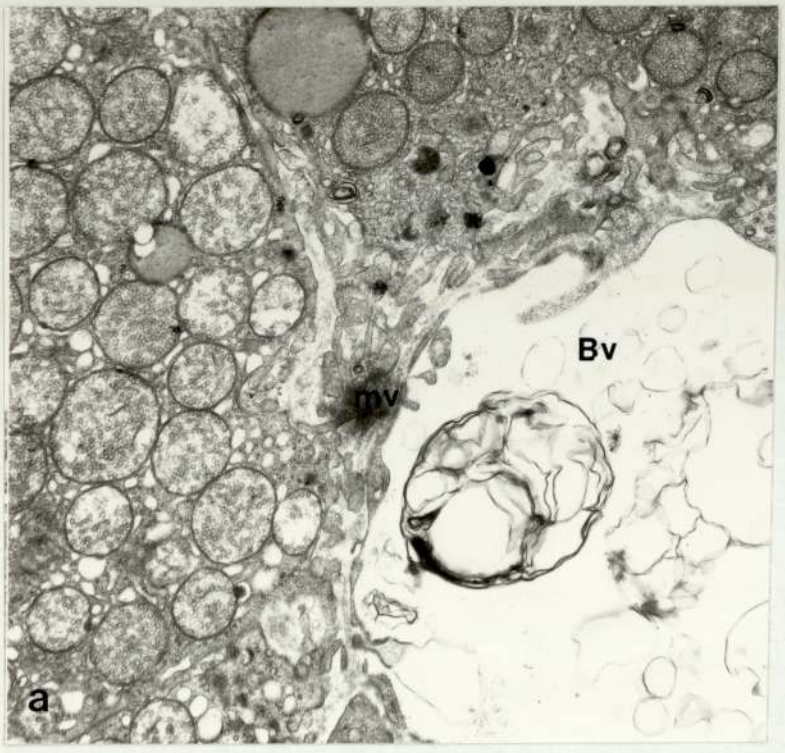

m
mv
e
b

Fig. 21 a.b. Adrenal sections from dark reared rats.

a. Forty day old pinealectomized female (operated at three days): zona fasciculata. Blood vessel containing a number of erythrocytes (e). A well defined double complex (*) is present in one of the parenchymal cells. x 10,000.

b. Sixty day old pinealectomized male (operated at thirty days): zona fasciculata. Blood vessel containing one erythrocyte. Numerous mitochondria (m) but no liposomes are present. x 10,000.


Bv
mv
a

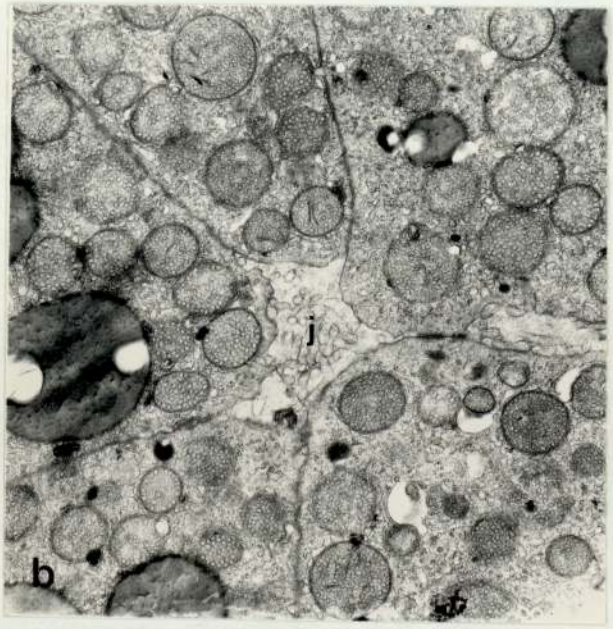
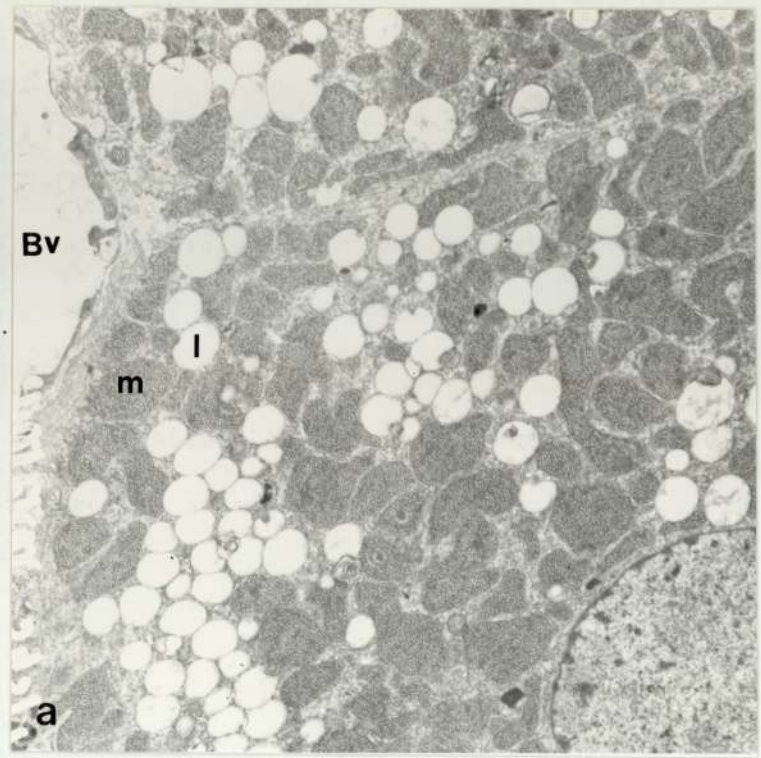

j
b

Fig. 22 a.b. Adrenal sections from sixty day old dark reared pinealectomized male rats. (Operated at thirty days).

a. Zona fasciculata. Blood vessel (Bv) with microvilli (mv) in the sub endothelial space. x 10,000.

b. Zona fasciculata. Junction (j) between five cells. x 12,000.


Bv
l
m
a

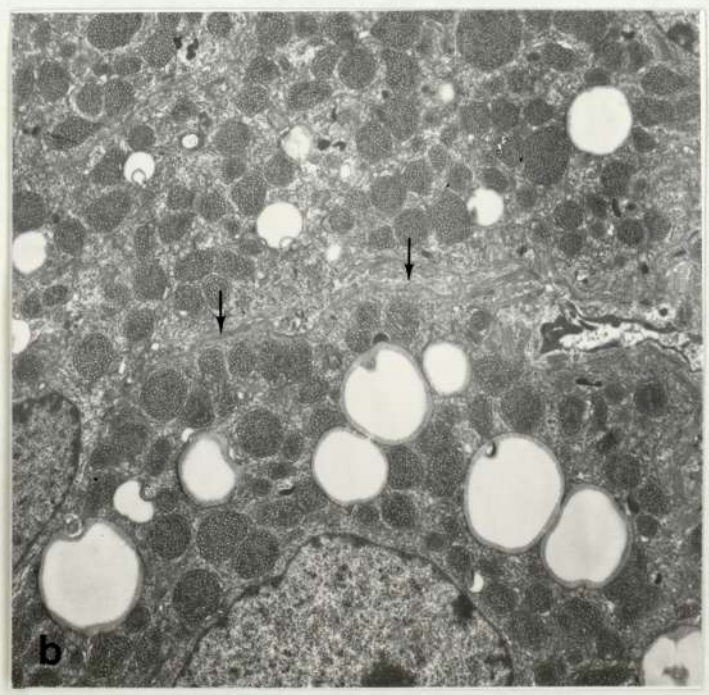
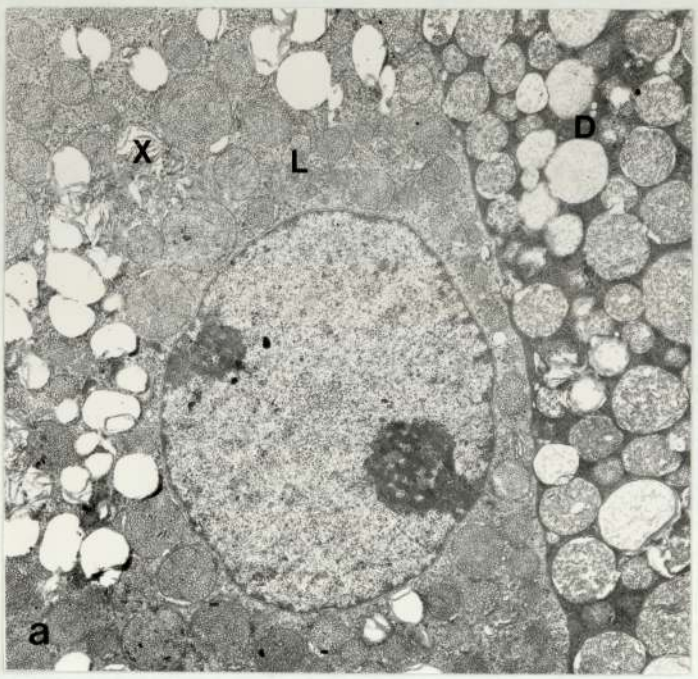

b

Fig. 23 a.b. Adrenal sections from one hundred day old light/dark reared sham-operated male rats. (Operated at thirty days).

a. Zona fasciculata. Two cells rich in liposomes (l) and mitochondria (m) are evident. The lumen of a blood vessel (Bv) is evident on the left hand side of the micrograph. x 9,000.

b. Zona fasciculata. The boundary (↑) between two cells can be seen containing microvilli. x 8,000.


X
L
D
a

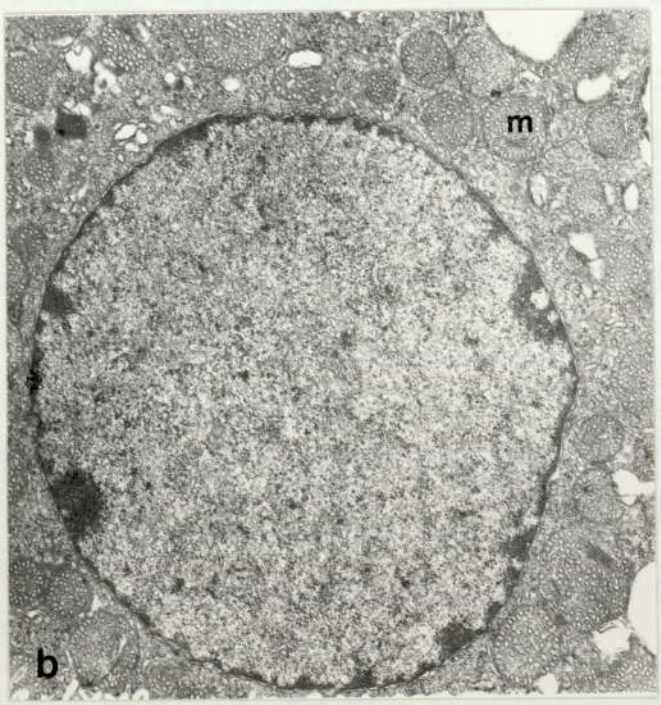
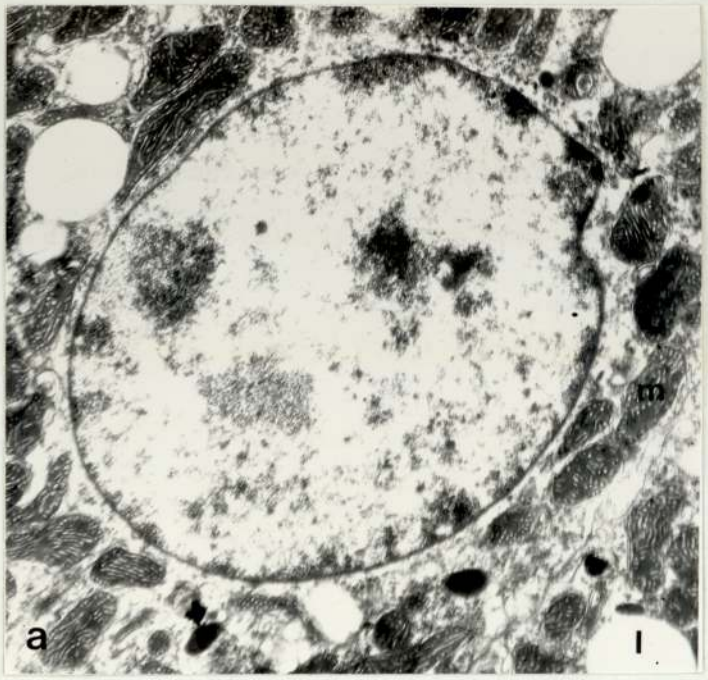

m
b

Fig. 24 a.b. Adrenal sections from one hundred day old light/dark reared pinealectomized male rats. (Operated at thirty days).

a. Deep zona fasciculata. Light (L) and dark (D) cells can be seen. Some disintegration of cytoplasmic inclusions (X) which may be a fixation artefact. x 8,000.

b. Zona fasciculata. Detail of nucleus and surrounding mitochondria (m). x 10,000.


m
a
l

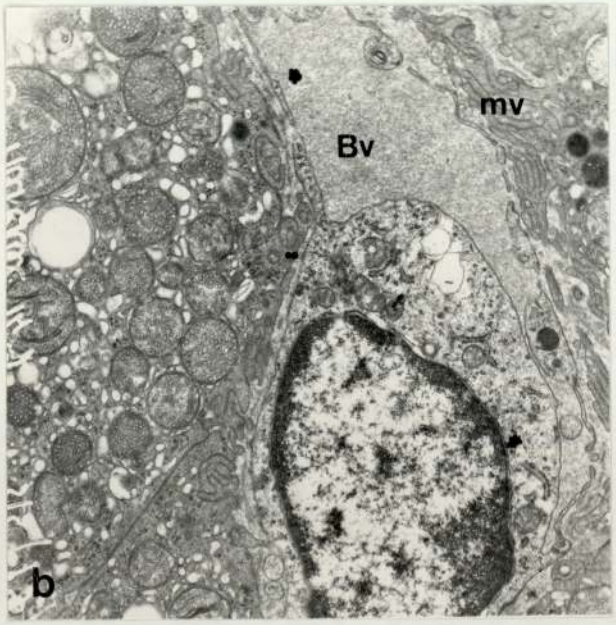
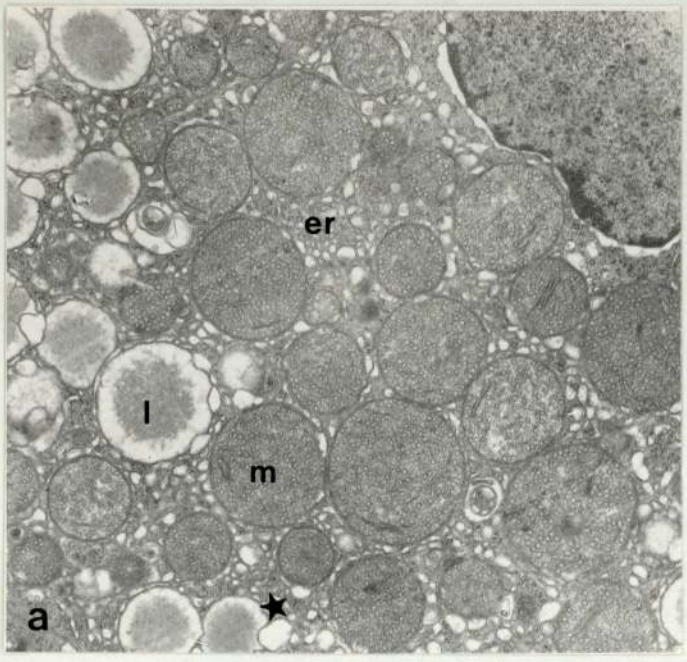

mv
Bv
b

Fig. 25 a.b. Adrenal sections from one hundred day old light/dark reared pinealectomized rats. (Operated at thirty days).

a. Zona glomerulosa from a male rat. Mitochondria (m), liposomes (l). x 11,000.

b. Zona fasciculata from a female rat. Blood vessel (Bv) with microvilli (mv) in the sub-endothelial space. x 10,000.


er
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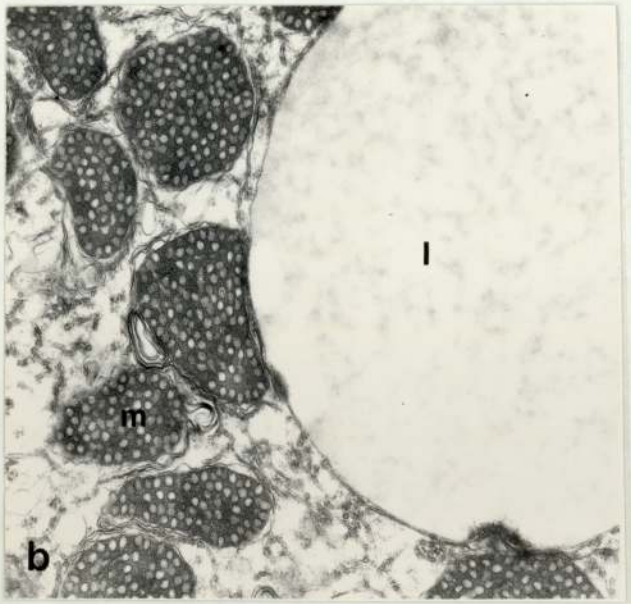
l
m
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Fig. 26 a.b. Adrenal sections from one hundred day old light/dark reared rats. (Operated at thirty days).

a. Zona fasciculata from sham-operated female. Mitochondria (m), liposomes (l), endoplasmic reticulum (er) and a double complex (*) are evident. x 12,000.

b. Zona fasciculata from sham-operated male. Several mitochondria (m) with vesicular cristae and a dense matrix lie close to a single large liposome (l). x 20,000.

IV DISCUSSION

Pinealectomy did not induce a consistent and/or significant alteration in growth rate (as measured by body weight increase) in male or female rats when carried out at three or thirty days. In addition growth rates were not found to be altered significantly when animals were reared in the absence of light.

A large number of published reports have included data on body weight although very few have investigated the influence of pinealectomy upon growth rates as a primary parameter. Several of these reports have referred to the white rat, fewer to the hamster and other species. Many workers only describe a single value, taken at the end of the experiment, while others also include an initial value; surprisingly few have observed weight gain over a period of time. In addition, many of the published accounts do not refer to the animals' age at the commencement of the experiment, only to weight. This could mean that there is considerable variation in initial age which might influence the outcome of the experiment, since animals are normally sacrificed at the same age.

Anderson and Wolfe (1934) examined the effect of surgical removal of the pineal in the rat. They carried out their operation at one, two or three days and employed both sham-operated and normal controls. Animals were then weighed weekly until the age of sixty - seventy days (male) and eighty six - ninety days (female). In all cases growth curves, within a sex-group, were very close and no significant variations were noted.

More recently, Karppanen (1970) employed an electrocoagulation method to pinealectomize male rats weighing 170-180g., observing body weight regularly up until ten weeks after the operation. He found that pinealectomized rats demonstrated a consistent retardation in growth compared to normal and sham-operated control animals.

Vaugham and Reiter (1971) examined the influence of pinealectomy upon body weight in male mice. They carried out their operation at forty two - forty five days, weighing them at sacrifice, twelve, twenty four, thirty six, forty eight and sixty days later. They discovered that pinealectomized animals were always lighter than those which had been sham-operated and that this difference became significant in groups killed at twelve, thirty six and forty eight days. Although body weight increases over a period of time are given, different animals were weighed at each age, and this is perhaps better looked at as a series of initial weight-final weight experiments.

Morin (1973) obtained female hamsters weighing 85-90g. and pinealectomized them six weeks later. He does not, therefore, provide information of age or weight at the commencement of the experiment. He weighed the animals five, nine, thirteen and seventeen days post-operatively and found that the growth curves exhibited by pinealectomized and sham-operated animals were identical, although both were significantly depressed when related to a normal control group. Maximal growth was obtained in a group maintained in constant light. It is assumed that operative stress has resulted in a retardation in growth rate in the operated groups.

Several authors have failed to detect weight changes in pinealectomized rats when single readings are taken at sacrifice. Kitay (1954) failed to obtain any alteration in weight gain in female rats at fifty days whether pinealectomized at twenty one, twenty six or thirty days. In 1964, Bruinvels etal (1964) pinealectomized male rats twenty one - twenty eight days old as part of an experiment to investigate the influence of pinealectomy and hypophysectomy upon the renin content of the kidney. When examined six weeks later, no significant alterations in body weight were detected when compared to sham-operated control groups.

Kinson and Singer (1967) pinealectomized male rats weighing 100-120g., but failed to observe any differences from the controls seventeen

days later. Motta etal (1967) pinealectomized mature male rats, weighing approximately 245g., but could find no alterations in growth twelve days later when compared to a sham-operated control group. They also found that daily doses of melatonin appeared to exert no influence upon growth rates.

More recently, Reiter (1972a) pinealectomized rats at sixty days, and sacrificed them at one hundred and twenty five days. He could find no differences in body weight compared to intact controls irrespective of the light regime (L : D, 14 : 10, 24 : 0) employed. On the other hand, Relkin (1972e) found that male rats pinealectomized at about twenty three days and sacrificed at fifty one - fifty three days gained weight more rapidly when animals were raised in darkness.

Several of the more recent investigations have become more complicated as the role of light in the inhibition of pineal function has been appreciated. Many of the earlier experimenters did not describe the light regimes which were employed while others included normal daylight. It is now more common to use controlled lighting, e.g. L : D hrs., 24 : 0, 14 : 10, 12 : 12, 8 : 16, 1 : 23 or 0 : 23. In addition, since the pineal indoles and the influence of the nervous system upon the pineal were discovered, experiments involving the implantation of chemical substances, bilateral enucleation and removal of the olfactory bulbs have been carried out.

Sorrentino etal (1971) operated on forty four day old male rats, weighing them at eight day intervals until the age of one hundred and eleven days. They found that blinded-pinealectomized animals grew at the same rate as the controls, but animals which were either blinded or pinealectomized both demonstrated a slight (not statistically significant) lag. Bilateral removal of the olfactory bulbs, whether alone or together with blinding or blinding and pinealectomy, induces a significant depression in growth rate. Pinealectomy seems to exert a slight negating

effect, reversing the effect of blinding only.

Shiino etal (1974) also examined the effects of blinding and anosmia on rats. They operated on twenty three - twenty four day old animals, sacrificing them fifty two days later. Like Sorrentino etal, they found that blinding and anosmia depressed body weight increases, but that this was completely reversed by pinealectomy.

Osman etal (1972) examined the effects of pinealectomy under various lighting conditions in twenty two day old female rats. They showed that growth was retarded in animals maintained in continuous darkness, but that this effect was counteracted by pinealectomy.

An examination of the literature relating pinealectomy to growth rates produces a confusing picture. Pinealectomy appears to either facilitate growth, inhibit growth or not affect it at all. It is very difficult to relate the various experimental accounts because the operations appear to have been carried out at different ages and using different techniques. In many of the reports insufficient information has been provided to discover exactly when or how the operation has been carried out.

It has been observed in the present experiments that pinealectomy at three days induces a retardation in growth which is most evident at twenty days. This result is probably an artefact due to operative damage resulting from damage to the dural sinuses as the pineal is removed. Sham-operations tend to result in rather less damage.

Pinealectomy at thirty days seems to induce a slight (but not significant) acceleration in growth, especially in dark reared animals. This result would be expected if the pineal exerted a growth inhibiting effect, since this would be most effective in the absence of light. It is possible that pinealectomy relieves some inhibitory effect upon the hypothalamus so that increased amounts of growth hormone (GH) can be released (Sorrentino etal, 1971; Shiino etal, 1974). The fact that the

growth facilitating action of pinealectomy is so slight might mean that an alternative endocrinological pathway is involved, not involving a general growth regulator like GH.

The role of light in the regulation of growth is an added complication. Some authors have described a retardation in growth in blinded animals when compared to normal controls. The results presented here do not confirm this, since dark reared animals do not exhibit growth curves which are significantly different from those reared under alternating light/dark conditions. It is interesting that this result confirms that of Nir *etal* (1972) who found that extremes of illumination and pinealectomy did not influence body weight although they *did* influence tibial growth. This suggests that perhaps simple body weight is not the most useful monitor of growth.

A number of workers have studied the effects of pinealectomy upon adrenal weight under a variety of conditions.

Anderson and Wolfe (1934) included an examination of adrenal weight in their survey of the effects of pinealectomy. They observed slight differences between experimental groups, but explained them in terms of infection and in the females by relating them to the reproductive cycle.

Wurtman *etal* (1959) reported that pinealectomy resulted in adrenal hypertrophy which could be partially negated by bovine pineal extract. Relkin (1972b) also pinealectomized male rats, later castrating them. Pinealectomy had resulted in adrenal hypertrophy when animals were sacrificed between the sixty seventh and seventy second day. It is unfortunate that his paper only notes absolute adrenal weight with no reference to body weight.

Vaughan and Reiter (1971) also observed adrenal hypertrophy (in terms of % body weight) in pinealectomized mice, twelve, twenty four, thirty six, forty eight and sixty days postoperatively. They believed that this was due to increased release of (pituitary) adrenocorticotrophic

hormone (ACTH). Vaughan etal (1972) again found that pinealectomy produced adrenal hypertrophy in male mice which could be reversed by the application of melatonin. Furthermore, pinealectomy also augmented adrenal enlargement caused by castration, although melatonin would only counteract that portion due to removal of the pineal.

Reiter and Hester (1966) demonstrated in the hamster that blinding initiated a retardation in adrenal growth which was counteracted by pinealectomy or superior cervical ganglionectomy. In non-blinded animals, the two operations were without effect.

Wragg (1967) failed to demonstrate that pinealectomy in three day old rats produced any alterations in adrenal growth rates. Relkin (1972e) also showed that pinealectomy under various lighting conditions did not induce significant alterations in adrenal weight. However, if expressed as a percentage of body weight his (small) dark reared, sham-operated animals do exhibit an elevated value, and pinealectomy has therefore produced a retardation in growth of the gland.

Sorrentino etal (1971) found that pinealectomy alone did not induce hypertrophy of the adrenal, although those of blinded rats were smaller than those of the control animals.

The results obtained by Shiino etal (1974) are somewhat confusing. In blinded anosmic rats they demonstrated a decrease in body weight which is counteracted by pinealectomy. Adrenal weight remains constant while pituitary weights (expressed as half organ and whole organ weights) increase significantly. However, when these results are related to body weight, pituitary weights remain almost constant while adrenal weights of blinded anosmic animals are reduced.

Reiter and Sorrentino (1972) studied the effect of pinealectomy and isolation of the medial-basal hypothalamus (MBH) in the hamster. They did not observe significant differences in adrenal weight between experimental groups and straight forward pinealectomy exerted no effect.

However, isolation of the MBH induced a reduction in adrenal weight, which when related to body weight became very marked. Maintaining animals in short photoperiods was without effect.

It is evident from the literature that removal of the pineal does not produce a consistently demonstrable alteration in adrenal weight. This might be due to a genuine ineffectiveness or perhaps to factors which mask the genuine effects of removal. The present work attempted to take account of environmental and other factors, but still no consistent results were obtained. It must be concluded, then, that pinealectomy does not induce a significant alteration in adrenal weight of the white rat.

Several workers have suggested that dark reared animals exhibit lighter adrenals then animals reared under normal lighting schedules. The results presented here show that seven out of twelve female and eleven out of twelve male groups reared in the absence of light exhibited mean (absolute) adrenal weights which were lighter than those of the corresponding light/dark reared animals. This tends to support the view that adrenal weight may be influenced by environmental lighting.

While adrenal weight is a parameter which has been studied by many workers, adrenal diameter has been used by very few. This is perhaps surprising, since from the results presented here it would seem that changes in adrenal diameter are detected more easily than changes in adrenal weight.

Relkin (1972b) described hypertrophy of the zona fasciculata and the zona reticularis, but not the zona glomerulosa in pinealectomized rats; he did not, however, provide any quantitative evidence to support these statements. Devercerski (1965) described a similar result (Miline, 1971). Seibel and Schweisthal (1973) also performed a histological study of the adrenal following pinealectomy in the hamster, but again they provide no

quantitative results.

Although few structural investigations have been carried out, many biochemical studies have been described in the literature.

Farrell (1959a, b; 1960; 1964) proposed that the pineal exerted a stimulatory effect on the adrenal cortex, particularly in the release of aldosterone. In the later accounts he also considered the existence of an inhibitory factor. Most of the subsequent workers, when they could find any effect at all, ascribe an inhibitory role to the pineal.

Tanner and Hungerford (1962) examined sodium and potassium retention following pinealectomy. They found that both pinealectomized and sham-operated animals exhibited increased sodium retention for the first five days, although the sham-operated group returned to the basal rate faster than the pinealectomized group. Potassium did not appear to possess any pineal dependence. Later Bruinvels etal (1964) found that pinealectomy produced no alteration in the levels of plasma renin, a finding which has recently been contradicted by Karappanen (1970) who discovered raised renin levels.

Perhaps the most complete studies of the influence of pinealectomy upon adrenal function are those described by Kinson and Singer (1967) and Kinson etal (1967, 1968). In a series of experiments they examined the effects of pinealectomy upon plasma aldosterone and corticosterone levels under a variety of circumstances in the rat. They found that seventeen days post-operatively in both sodium replete and deplete animals, aldosterone levels, but not corticosterone levels were raised in the pinealectomized group. In rats with one clipped renal artery they found that pinealectomy raised both aldosterone and corticosterone levels. Straight forward pineal removal, on the other hand, raised aldosterone levels at thirty and ninety, but not one hundred and eighty days, while corticosterone levels were only slightly raised at thirty days.

Nir etal (1971b) pinealectomized rats at twenty one days and found

that corticosterone levels were raised in dark reared animals only, ten days post-operatively. These differences had vanished thirty days post-operatively. They also found elevated corticosterone levels in all light reared groups. These results led them to believe that corticosterone output is light dependent, but not completely pineal dependent.

Jacobs (1974) examined the diurnal variation in plasma corticosterone in pinealectomized animals, but found that the operation was without effect. Blinding appeared to destroy the cyclicity, but when combined with pinealectomy it was restored. Like Nir *etal* he found that pinealectomy influenced adrenal action only in dark adapted (blinded) animals.

Although the experiments of Nir *etal* and Jacobs are both simple and elegant, they *could* be objected to on the grounds of the results produced by Krieger (1973). In an exhaustive study into the effects of various light regimes, she showed that although continuous dark or continuous light or blinding destroyed the normal diurnal rhythms, it did not cause a complete flattening of corticosterone output. Therefore, the results of Nir *etal* and Jacobs could result from either a shift in the curve or a completely new curve arising.

Motta *etal* (1971) demonstrated that melatonin reduced plasma corticosterone in non-stressed rats after only one hour. However, the doses of melatonin employed and the levels of corticosterone reported are extremely high which leads one to doubt the physiological significance of these results. Singer (1971) in a similar experiment found that melatonin was without effect.

Relkin (1972b) examined the effect of pinealectomy at four days on testosterone output, but could find no significant effect. It is interesting that the hormone levels that he presents for his pinealectomized and sham-operated animals are both significantly different from the unoperated controls.

The ultrastructural zonation of the adrenal cortex is well known from studies by such workers as Sabatini and de Robertis (1961), Lever (1955) and Sheridan and Belt (1964). It is also known that the cortical ultrastructure is comparatively labile, changing in response to externally applied substances. Size, appearance and numbers of liposomes and mitochondria and extent of the agranular reticulum are particularly useful monitors in such studies.

Several workers have examined the effect of ACTH upon adrenocortical structure.

Ashworth etal (1958) found that ACTH induced marked changes in the zona fasciculata with dark staining compact cells apparently replacing the normal light staining parenchyma. This work was confirmed by Carr (1961). Most recently Alvarez and Lavender (1974) found that ACTH promoted hypertrophy of the zona fasciculata, but could not find any evidence suggesting abnormal levels of mitosis which led them to believe that cellular hypertrophy was responsible.

Idelman (1970) showed that ACTH treatment affected both zona fasciculata and zona reticularis, causing a decrease in the number of liposomes associated with an increase in the numbers of mitochondria and microvilli (where present). The agranular endoplasmic reticulum also appeared to exhibit increased development. In an extremely careful study of the effect of ACTH upon the rat adrenal cortex, Rhodin (1971) also found a decrease in the number of liposomes in the zona fasciculata and an apparent reduction in the extent of the agranular endoplasmic reticulum. The mitochondria seemed to enlarge but maintained a constant number.

It is interesting that Manuelidis and Mulrow (1973) found that in tissue culture ACTH treatment brought about an alteration of mitochondria from the type normally found in the zona glomerulosa to the type found in the zona fasciculata. The significance of this is not understood.

The results of the various studies on adrenal function are by no means conclusive. In what is often held as being one of the most elegant studies, Kinson and his co-workers found corticosterone increases at thirty days in (presumably) diurnal lighting conditions. Nir etal on the other hand could only demonstrate such a response at ten days and then only in dark-adapted animals. Jacobs similarly only found an effect in dark reared animals.

The results presented here suggest that some light-mediated factor influences adrenal growth (in terms of increased diameter) such that it is retarded in dark reared animals. This effect is most obvious in females and increases with age. It does not, however, appear to be influenced by the pineal.

The development of the adrenal zones is interesting. Relkin (1972b) describes hypertrophy of the zona reticularis and yet can find no increase in testosterone output. On the other hand he can determine no alteration in the zona glomerulosa when a number of studies have shown that the zona glomerulosa becomes hyperactive in pinealectomized animals.

There is no conclusive evidence presented here that pinealectomy produces hypertrophy of any particular zone. In some of the light/dark reared animals pinealectomy appears to induce a non-significant hypertrophy in some age groups. Also, in some groups the zona fasciculata and zona reticularis appear to be developing more quickly, while in others it is the zona glomerulosa. Generally speaking these areas of 'special development' only reflect a general hypertrophy. This becomes evident when each zone is considered as a fraction of the whole. The only exception to this is the zona reticularis, which takes over a larger proportion of the cortex with age, generally at the expense of the zona fasciculata.

Several studies have examined the effects of low sodium and raised potassium concentrations upon the ultrastructure of the adrenal cortex.

Giacomelli etal (1965) found that sodium restriction resulted in an enlargement of the zona glomerulosa together with an apparent increase in the number of mitochondria, quantity of agranular endoplasmic reticulum and a decrease in the number of liposomes. This type of response has been largely confirmed in the mouse by Shelton and Jones (1971) and the opossum by Long and Jones (1967; 1969).

Devercerski (1965) studied the morphology of the adrenal following pinealectomy in adult male rats. He observed hyperplasia of the zona fasciculata and zona reticularis and increased polymorphism in the mitochondria. This work appears to be the sole account of the influence of pinealectomy upon adrenal ultrastructure, but does not appear to have been published widely (Miline, 1971).

It has been suggested by many workers that the pineal influences the adrenal cortex through the hypothalamus and/or adenohypophysis employing ACTH as a mediator. If this were true then some alteration in adrenal structure should be obtained when the pineal is removed, since this would alleviate an inhibition on ACTH and blood levels would rise. It would also mean, of course, that corticosterone output from the adrenal would increase significantly together with a smaller rise in aldosterone. It is unfortunate that aldosterone increases are often observed while corticosterone increases are not. Therefore, if the pineal does genuinely influence adrenal activity it is likely that it is not through ACTH.

Ultrastructural changes of the adrenal cortex are not only dependent upon ACTH, it has already been seen that low levels of plasma sodium induce a morphologically observable effect. Thus, whatever the supposed influence is, it too might produce such an effect. This

hypothesis does not seem to be confirmed by the results presented here. Considerable variations in numbers and appearance of mitochondria, liposomes, etc. appear to exist within all experimental groups, but no obvious differences appear to exist between the groups. It is therefore concluded that pineal removal does not induce a recognizable alteration in the adrenal cortex.

The results presented in this thesis suggest that the pineal exerts a very slight inhibitory effect on normal growth such that its removal allows a slight acceleration of growth to be observed. It is also suggested that the pineal does not demonstrate a consistent retardation of adrenal development (and perhaps function), for when observed at a number of different times post-pinealectomy, weight and structure fail to show consistent deviation from the norm. It is noted that rearing animals in darkness induces a consistently significant retardation in adrenal growth, but again this is not affected by pinealectomy and is therefore presumably not controlled by the pineal.

In support of the results obtained by other workers it is suggested that the pineal may have a transitory influence upon the adrenal under certain experimental conditions, but that this influence is probably not mediated in a simple manner by ACTH, but rather by an indirect route, perhaps with the involvement of other endocrine organs.

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