BIOCHEMICAL, SOCIAL AND PSYCHIATRIC ASPECTS OF DRUG OVERDOSES

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Famous last words...

"I think I am about done now."

(Mrs. Beeton 1836–1865)

Summary

In Part A investigations have been made into the problem of deliberate drug overdoses, with the intention of setting up a permanent data base for collection of clinical and social information about patients admitted to the West Midlands Regional Poisoning Treatment Centre after drug overdose incidents. This involved the development and use of a computer-coded questionnaire sheet.

An analysis of the information collected over 40 months was carried out and confirmed many of the findings of similar reports from other geographical locations.

The study probed deeper into the social aspects of the drug overdose problem than is usual and the clinical data was collected in a more purposeful fashion and involved more detailed description of symptoms and their associated drug levels in body fluids than previous studies.

The study also reinforced the intentional nature of adult drug overdoses and suggested that the behaviour seen might itself be interpreted as a "coping strategy" to enable patients to deal with stressful situations rather than as a "cry for help" as is the view held at present by most workers in the field.

It was evident that a high proportion of drug overdose patients took salicylate analgesics and that this proportion tended to be younger and to have bought the drug in large quantity containers.

In Part B the metabolism and kinetics of salicylate were investigated in normal human volunteers and in overdosed patients. This involved the development of an improved colourimetric method for estimating salicylic acid in body fluids. The study yielded further evidence of the rate limitation of some of the pathways of salicylate metabolism.

Lowering of brain glucose associated with salicylate overdose was observed for the first time in humans, confirming the interference of salicylate with carbohydrate metabolism in the central nervous system previously reported in animals.

The study revealed that the ratio of salicylate in the cerebrospinal fluid to that in plasma did not reach the high levels previously reported in infants and this confirmed the difference in metabolic disturbances reported in different age ranges of salicylate overdose patients.

Key Words

Deliberate drug overdoses
Salicylate overdoses
Salicylate metabolism
Salicylate kinetics

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PART A

Drug Overdoses - A Demographic Study with Special Reference to Salicylates

Section 1 Introduction

1. The Scope of the Problem - Statistics

Jones (1969) reported a "remarkable increase in the proportion suicidal deaths due to self poisoning". He also remarked that estimates at that time put the ratio of attempted to successful suicides at between 7:1 and 15:1 and he considered that the problem of deliberate self poisoning was "a tremendous challenge to preventive medicine.". He also states that "in England and Wales, during 1963, 42% of women and 22% of men who killed themselves did so with drugs". These proportions stand out as inordinately high, however the fact remains that the incidence of self poisoning with drugs, whether fatal or otherwise, had been increasing during the twelve year period studied.

Murray, Campbell, Reid and Telfer (1974) studied the yearly admissions to Glasgow Royal Infirmary for self poisoning over the ten years from 1962 to 1972 and showed that these had approximately doubled in number but that their percentage of total admissions had remained little changed.

More recently, Jones (1977) confidently estimated that in 1977 there would have been a total of 100,000 admissions to U.K. hospitals as a result of self poisonings. He quoted a 24 fold increase in self poisoning admissions to Sheffield casualty deparments over the twenty years from 1955 to 1975. In 1975 these represented 11.3% of medical admissions and 29.7% of emergency admissions, but there was no indication of whether this figure represented an increase or whether the proportion had remained stable over this period.

This phenomenon is not limited to the U.K.. Reports from Boston, Massachusetts (U.S.A.) and Oslo (Norway) also indicate an upward trend. O'Brien in Boston (1977) estimated an increase of 11.8% in drug overdose suicidal attempts over the period 1964–72 and Petersen and Brosstad in Oslo (1977) reported an increase of 40% between 1958–1960 and 1970–1973. Again the proportion of hospital admissions as acute drug poisoning showed little change over the period studied by Petersen and Brosstad, remaining stable at between 5.5% and 6.7%. A leading article in the Lancet (1974) put the estimated frequency of hospital admissions in England and Wales for self poisoning and self injury at around 2.5% of all admissions, although this figure may not be directly comparable as it is not clear whether "admissions" refers to general medical admissions or total admissions.

Hamid Ghodse (1977) suggested that the peak of what he called the "epidemic" of self poisoning had not yet been reached, and Smith (1972) estimated that in 1972 this type of behaviour cost the Health Service in the region of two million pounds.

2. General Information

(i) Terminology

When discussing the problem of people who knowingly take an overdose of drugs it is very easy to slip into terminology which, consciously or otherwise, while describing the type of behaviour, also includes spurious references to either the state of mind of the individual or to the intended outcome of the act. This is immediately evident in the foregoing section of the introduction where the present author deliberately, when referring to the reports of this type of behaviour, reproduced other authors' own terminologies to describe it. In a few lines we have a variety of terms including:

- "suicidal deaths due to self poisoning"
- "attempted/successful suicides"
- "deliberate self poisoning"
- "self poisoning"
- "drug overdose suicidal attempts"
- "acute drug poisoning"

Taking those terms which include the words "suicide" or "suicidal", this tends to imply that the person involved did in fact intend that death should be the final outcome and assumes that he or she knew that the amount of drug taken would be likely to produce this outcome. It is questionable whether any observer is qualified to make any such assumptions about another person. The term "acute drug poisoning" is even more dubious as it is more generalised and a reader would be left in doubt on two points: was the poisoning accidental or deliberate also was the poisoning self inflicted or inflicted by another party? The term "self poisoning" also does not

define whether the action was deliberate or otherwise. Of all terms used "deliberate self poisoning" is probably the most descriptive of this type of behaviour and is also open to less misinterpretation, at the same time being less cumbersome than "deliberate self-administered drug overdose" which describes the type of behaviour referred to in the following discussions.

(ii) Legal aspects

Until 1961 "Unlawfully attempting to commit suicide against the peace of our sovereign lady the Queen, her crown and dignity" was a felony in English law and persons who deliberately took an overdose of drugs and survived, if they came to the notice of the forces of law and order, were dealt with as criminals. However, anyone who died from a deliberate self-administered overdose of drugs was deemed to have committed suicide "...while the balance of the mind was disturbed...". This anomalous position was pointed out by Middleton, Ashby and Clark (1961) writing shortly after the repeal of the law concerning suicide. They also stated the important opinion that this type of behaviour was "...merely the individual's last despairing effort to draw attention to his difficulties...", an opinion that largely holds good almost twenty years later.

(iii) Official statistics

When one attempts to discover the incidence of deliberate self poisoning episodes, several problems become immediately apparent. The most comprehensive medical statistics are provided by the Hospital In-Patient Enquiry compiled by the Office of Population and Census Surveys (O.P.C.S.). Unfortunately these only record discharges and deaths caused by adverse effects of

drugs. This type of information would therefore include deliberate self poisoning but also accidental poisonings of all kinds and adverse reactions to therapeutic levels of drugs. It would also only include patients admitted to hospital and not those who died at home or elsewhere or those successfully treated by their general practitioners.

Further problems arise if one attempts to investigate these excluded categories further. If a person dies from a self-administered drug overdose, as with any other death, a certificate has to be completed stating the cause of death. Statistics concerning death by poisoning are compiled by O.P.C.S., being classified according to the information given on the death certificates. The Pharmaceutical Journal publishes these statistics on a yearly basis in their "Public Health" feature, distinguishing between suicidal poisoning, accidental poisoning and poisoning undetermined as to intention. It has been suggested (Ovenstone, 1973, Barraclough, 1974) that because open inquiries are held in this country to ascertain causes of death, a large amount of positive evidence is required by English coroners before a verdict of suicidal poisoning is brought in. As a result of this, it is suggested, many actual suicides may be eventually classified as either open verdicts or accidents, and suicidal poisoning as a result may be under represented. Comparative studies, including countries where open enquiries are not held, have taken place and suggest that the situation is not as simple as this and many other factors have to be considered. It would be difficult to elaborate upon these suggestions without including much detail that would be inappropriate here. Suffice it to say that absolute reliance should not be placed on coroners' verdicts when the choice is

between accidental and suicidal poisoning and that the published figures for suicidal poisoning deaths are likely to be underestimates. Kreitman (1977) suggests that the underestimate might be as much as 40%.

Statistics concerning deliberate self poisoning treated successfully by G.P.s are not collected officially. Studies of "attempted suicide" in this category have been carried out by Kennedy and Kreitman (1973) who, as a result of a survey of a representative sample of G.P.s, found that hospital studies in Edinburgh underestimated the frequency of this type of case by 30%.

(iv) Localised studies

Having stressed the difficulty in obtaining statistics on deliberate self poisoning incidents, a difficulty recognised in a leading article in the Lancet (1974), it seems that, for further information, we must turn to the many and more detailed local studies of these patients. Differences in methods used to obtain information (immediate or retrospective), characteristics of hospital catchment areas and again the semantic confusion about terminology make direct comparisons difficult. However some general points emerge which throw some light on this form of behaviour.

Sex ratio

Perhaps the most obvious fact to emerge from studies of deliberate self poisoning is that many more women than men seem to be involved. Middleton et al. (1961) studied "attempted suicide" (although they only seem to mention the taking of drugs or the use of gas) in Gateshead in 1961 and

discovered that the ratio of female to male patients was 2:1. A similar ratio was seen by Burston (1969) in Sunderland (7:3), Mitchell and Lawson (1974) in Dunfermline (63.5% females, 34.7% males), Murray et al. (1974) in Glasgow (2:1) and Ghodse (1977) in London (2.2:1). O'Brien (1977) however reported from Boston that although this ratio held true for 1964 it had decreased to 1:1 in 1972, a fact that was accounted for by an increase in drug overdoses by males between 20 and 39 years of age. Petersen and Brosstad (1977) also reported no female predominance overall, although in the under 30 age group females tended to outnumber males.

Types of drug used

An interesting feature of deliberate self poisoning is the pattern of drugs used and whether a single drug is taken or a combination. Jones (1969) reported that the incidence of single drugs being involved in deliberate self poisoning episodes was only one in ten. Although Murray et al. (1974) reported an increase in the taking of combinations of drugs, this is not borne out by other studies. Glauser and Smith (1975) reported 63.5% and Holland, Massie, Grant and Plumb (1975) 71% incidence of single drug involvement and two further studies by Jones (1977) and Ghodse (1977) reported 87% and 55% respectively as the percentage of admissions for poisoning by a single drug. Thus the incidence of deliberate self poisoning by single drugs would appear to be very high. Of the types of drug taken, a clear pattern seems to be emerging. Matthew(1966) reported that barbiturates were the most common drugs involved, followed by tranquillisers, antidepressants and hypnotics. However, since the falling out of favour of barbiturates this incidence has been widely reported to be decreasing (Reudy, 1973; Murray

et al., 1974; Petersen and Brosstad, 1977; Proudfoot and Park, 1978) although in several reports they were still the most common drug used in deliberate self poisoning (Glauser and Smith, 1975; Holland et al., 1975). It is generally believed that this decrease is largely offset by a concomitant increase in the incidence of self poisoning by psychtropic drugs and also hypnotics, this pattern being closely aligned to the changing prescribing patterns of physicians (Murray et al., 1974; Petersen and Brosstad, 1977; Proudfoot and Park, 1978). There is some evidence that the numbers of people taking deliberate overdoses of tricyclic antidepressants have increased more rapidly over the period 1965 to 1973 than has the prescribing rate for these drugs (Brewer, 1976).

It seems also that the number of people admitted after deliberate self poisoning with aspirin (usually 10-15% of self poisoning admissions) has shown a slow but marked decline since 1967 which is matched by a concomitant increase in self poisoning due to paracetomol (Proudfoot and Park, 1978). The most recent survey of this kind is that by Proudfoot and Park (1978) and this states that "Benzodiazepines, barbiturates, tricyclic antidepressants, salicylates and paracetomol are the most common drugs encountered.".

Treatment required

Matthew (1966) makes the point that less than 2% of poisons have a known antidote. As a result presentation of a patient in a casualty department after a drug overdose does not involve, initially at least, the necessity to discover what drug has been taken, and a routine procedure based on "basic therapeutic principles" comes into use. Briefly, the object of this is to

unblock airways, ensuring sufficiency of respiration; to relieve hypotension where present; to maintain adequate hydration and to control infection. This conservative management is usually sufficient for all but the most severe forms of intoxication, and a good index of the incidence of the severity of poisoning is the additional procedures required to ensure recovery.

Reudy (1973) reported that 56% of his patients required endotracheal intubation (ETT) and assisted ventilation and Murray et al. (1974) reported a similar situation where 65.5% required ETT but only 21% assisted ventilation. Burston (1969) found a much lower incidence (17% ETT and 8% assisted ventilation). 6% of Reudy's patients required actual resuscitation including a cardiac massage to prevent death as did only one of Burston's 129 patients. Tracheotomy was required in 2.7% of Murray's patients. Other procedures required included expansion of plasma volume to maintain tissue perfusion in 12% of Reudy's patients and diuresis to speed removal of drug in 14% of Reudy's patients, 33%of Murray's and 53% of Burston's. The frequency of the use of dialysis was very low: 2.8%, Reudy and 3%, Murray.

The proportion of deaths of patients admitted after self poisoning episodes seems to have declined over the last few years, although the comparability of figures is somewhat suspect. Published figures are shown in Table 1.

Seasonal variations in admission rate

The possibility of seasonal variations in admissions for self poisoning has been studied by Middleton et al. (1961) who concluded that there was no significant seasonal variation in

Table 1. Proportion of deaths among patients admitted after self poisoning, as quoted by various authors.

Middleton et al.	1961	3.2%
Reudy	1973	5.6%
Murray et al.	1974	0.5%
Holland et al.	1975	1.3%
Petersen and Brosstad	1977	1.0%
Jones	1977	0.5%

the number of admissions, but that there seemed to be a distinct fall in numbers during April, a phenomenon also noted by Kessel, McCulloch and Simpson (1963). Murray et al. (1974) plotted the pattern of admissions over 3 years to the Intensive Care Unit of Glasgow Royal Infirmary. This showed a peak at midsummer, falling off and then rising again in late autumn and early winter. Jones (1977) showed there to be little variation in admissions per month in Sheffield over a ten year period except that on the whole there were consistently more admissions from February to August than from September to January. The difference, however, was very slight. The opinion seems to be held (Jones, 1969; Sclare, 1974; Matthew, Proudfoot, Brown and Aitken, 1969) that deliberate self poisonings tend to be admitted at weekends and late at night or early in the morning, however there is little evidence for this.

Age distribution of admissions

The age distribution of self poisoning admissions is another interesting facet of this type of behaviour. Mean ages of 33 years (Reudy, 1973) and 34.5 years for men and 31.4 years for women (Ghodse, 1977) have been reported, also there appears to be a tendency for the mean age to have fallen (Kreitman, 1977). To appreciate the full impact of the statistics it is necessary to look at the age groups separately. Burrows and Harari (1974) working in Australia showed that, of all their self poisoning admissions, patients in the age range 15–19 constituted 22% and those 20–29 years 30%. Thus 52% of all admissions were patients under 30 years of age. Similar findings have been reported elsewhere. Jones (1977) reported that in his Sheffield study 70% of patients were aged below 34 years and Ghodse (1977) found that 53% were aged below 30 years and 74% below 40 years old.

All of these studies show a peak incidence of this kind of behaviour in patients in their second decade, a fact that has been noted elsewhere (O'Brien,1977; Burrows and Harari, 1974). Murray's study (1974) is atypical in that his peak admissions occurred in the 30-40 age group.

There is evidence that young persons are overrepresented in the self poisoning population. O'Brien (1977) calculated that, over the years between 1964 and 1972, the incidence of self poisoning in persons aged between 20 and 29 years has increased by 257%. When one considers that, at the same time the increase within the population of the same age group was 45% it is obvious that they are vastly overrepresented. O'Brien suggested that this was also true for the 10-19 age group. Sclare (1974) also reported an increase in the incidence of self poisoning in adolescents, and it has been suggested (Jones, 1977) that the evident increase in the overall incidence of self poisoning is largely due to this increase in admissions of the under 30s. Alderson (1974), however, reported that there had been a very great increase in admission rates for all age groups. Some reports (Burrows and Harari, 1974, Alderson, 1974) emphasise the fact that the rate of self poisoning among young women is surprisingly high and several theories (which will be examined in more detail later) have been forwarded to explain this.

Involvement of mental illness

It was assumed until fairly recently that drug overdoses were taken by people who had some kind of mental illness. This attitude was reflected in the work published in 1961 by Middleton et al., in which 87% of cases were diagnosed as having a psychiatric illness and only 5% as having made a "hysterical

gesture". In 8% of cases no firm diagnosis was made. Kessel et al. (1963) reported that only 10% of males and 17% of females had no mental illness, and in a later study (1965) Kessel reported no psychiatric illness in 22% and where psychiatric illness was present it was most often in the form of depression or of a personality disorder, as in fact was the case in the earlier (1963) study. Women tended to present with depression and men with personality disorders. Jones (1969) considered that half of the patients admitted had no definite psychiatric abnormality. 30% of patients were transferred to a psychiatric unit. Those not diagnosed as mentally ill were considered to have responded impulsively to stress.

Burston (1969), while not stating actual psychiatric diagnoses, reported that 44% of the patients studied were admitted to psychiatric care as in-patients voluntarily and 8% were committed to care. Of the remaining patients, 12% were discharged with psychiatric follow up and the rest (about 40% were discharged to the care of their G.P.s. It must be assumed that this latter group had no psychiatric illness. Sclare (1974) considered that only 25% of all self poisoning admissions had psychiatric problems such as personality disorder, endogenous depression or schizophrenia. The proportion of self poisoning admissions who had previously been in the care of a psychiatrist is variously reported as 14% (Middleton et al., 1961), 33% (Jones, 1977) and 41% (Burston, 1969).

It is obvious from the figures quoted that approximately one half of all deliberate self poisoning admissions are not mentally ill. Of those who do have some kind of mental illness, the diagnosis is likely to be depression or personality disorder.

Length of stay in hospital

There are few reports of the length of time spent in hospital after deliberate self poisoning. In 1963 Kessel et al. reported that a survey of patients in the Royal Infirmary, Edinburgh, showed that 7.5% did not even spend a night in hospital. 24.5% of admissions had been discharged after 24 hours and over 50% were discharged within 48 hours. A similar pattern was reported in 1969 by Jones where 27% were discharged within 24 hours and 48% within 48 hours.

(v) Summary

The major points to emerge from the studies examined so far are these:

- The number of admissions for deliberate self poisoning has increased dramatically over the last 20-30 years, although their percentage of total admissions has probably remained stable.
- Terminology must be used with care so that possibly false motives are not attributed to the act.
- The collection of official statistics makes an overall assessment of the problem difficult.
- The incidence of overdose in women is approximately twice that in men.
- Patterns of prescribed-drug overdose have tended to follow changes in prescribing habits. Patterns of overdose from over-the-counter analgesics show that aspirin and paracetomol now share equally the proportion of admissions once attributable to aspirin alone.

- The death rate from overdose has decreased and is now around 5%.

- Supportive therapy is the norm in treatment of overdose and only a small percentage of patients need more drastic treatment.

- There is little seasonal variation in overdose admissions though April seems to have fewer than average.

- About half of all admissions are of patients below 30 years of age. This is an overrepresentation of this age group compared with the general population. Women patients tend to be younger, on average, than men.

- About half of the people admitted after a deliberate overdose are not mentally ill. Where mental illness is diagnosed it is most frequently depression or personality defect.

- The length of stay in hospital after an overdose episode tends to be less than 48 hours.

Having drawn a general picture of the incidence and consequences of deliberate drug overdose, it would now be appropriate to investigate as far as possible the factors contributing to this type of behaviour.

3. Factors Contributing to Deliberate Drug Overdoses

(i) Social factors

Kessel (1965)pointed out a relationship between high self poisoning rates and "indices of social disorganisation". This involved a significant association with overcrowding, with the proportion of people "out of the family setting", in hostels and lodgings and with areas (in Edinburgh) of high criminal residence. However he found no significant correlation with social isolation. He summarised the conditions from which the

majority of overdose patients came as "living in overcrowded, poor surroundings, living in bad social conditions...". This, unfortunately, tends to give the impression that the problem is a simple one which could be helped by an improvement in living conditions - an impression of social problems widely held at that time and giving rise to the programmes of slum clearance and improvement of housing over the last two decades, which many thought would put an end to "social deprivation". Although the dubious "improvements" made in housing since then (with the possible exception of the erection of multistorey blocks of flats) stemmed from a genuine desire to improve people's living conditions, the problem of deliberate self poisoning in urban areas has not eased but worsened as was illustrated earlier.

Kessel went on to give a rank order of "precipitating factors" leading to overdose episodes. The major factor for males tended to be marital disharmony, drinking problems, financial difficulties, unemployment and kin disharmony, in that order. For women the emphasis was slightly different, although marital disharmony, financial difficulties and kin disharmony were again among the major factors. He pointed out that a quarrel was often the immediate spark of an episode of self poisoning but that the underlying problem was usually the lack of emotional support between spouses, leading to a sense of isolation.

(ii) Desire to die

Kessel (1965) stressed that very few overdose patients, having recovered physically, still maintained that they would prefer to be dead, and that the concept of "attempted suicide"

was an inaccurate description of behaviour in four fifths of his patients. He also considered that most patients were aware at the time of taking the overdose that they would survive long enough to ensure rescue and that they were "comparatively safe". He stressed the important view that the index of endangerment of life was not correlated with the need for psychiatric treatment and should not be used as a guide in referrals. The question of the intention of the patient to take his own life is an important issue. Stengel (1963) pointed out that "attempted suicides" were committed not in a mood of clearly wishing to die but rather with an ambivalent attitude of not caring about the outcome. In an earlier work (1952) he describes these patients as "Janus-faced", looking one way towards death and the other towards life. This may have been the case some years ago but more recent studies are emphasising the manipulative nature of overdoses and it seems that Kessel's original assertion that the majority of deliberate drug overdose patients have every intention of surviving the act is correct. Such studies include that by Ovenstone (1973), where a population of suicides were compared with a population of "attempted suicides". There was a clear differentiation between the two when sex, age and psychiatric disturbance were considered. The means were also clearly defined,"attempted suicides" almost exclusively taking drug overdoses while coal gas and other means of self injury were used to commit suicides. Ovenstone also pointed out the similarities in the populations, including that of social disorganisation, and the fact that there is an overlap group which, when compared with the "attempted suicide" group, consisted of a large number of barbiturate overdoses. Kennedy, Kreitman and Ovenstone (1974) have discarded the term "attempted suicide" and substituted "parasuicide" to emphasise the point that,

although in some respects the two are similar, they are different patterns of behaviour. The point is again made, however, that the populations overlap, as illustrated by the fact that one percent of the parasuicides studied had committed suicide within one year.

That this apparent lack of suicidal intent should not be used as a criterion in deciding psychiatric referrals has been mentioned previously and is restated by Kennedy and Kreitman (1974), however the practice of referring all deliberate overdose admissions is condemned by Blake and Mitchell (1978) on the basis that the medical ward staff may feel that it is "absolved from ascertaining those social and personal details that it obtains from other patients" and that this can lead to nursing and medical staff feeling that "they have no part to play in his management". They also point out that there is no agreed way of handling patients other than those who clearly require admission to a psychiatric hospital. For this Blake and Mitchell produce figures for two psychiatric teams, one of which dismissed with no further psychiatric or social intervention 51% of its referred cases and another for which a similar figure was 4%. They call for a re-examination of the assumption that "every overdose is a cry for skilled psychiatric help" and suggest that the physician should be capable of deciding which patient requires this type of assistance. It is obvious that if this were to be the case, there would have to be some kind of criterion used to decide which patients were referred and which not and it seems likely that, with such a complex problem, the apparently useful index of "suicidal intent" would come into use and we would be back to the situation already condemned by leading workers in the field.

(iii) The importance of interpersonal relationships

The involvement of marital disharmony in drug overdose has been pointed out by, among others, Mitchell and Lawson (1974) and Lukianowicz (1972). This concept widens into one of interpersonal relationships where it is not necessarily the relationship with a marital partner that is at the forefront but where there is, as Mitchell and Lawson described it, a "difficult relationship with an important figure in the patient's life ". This type of problem is highlighted by a study of drug overdose in adolescents carried out by White (1974) who emphasised the "high incidence of parental deprivation" either physical or emotional. Thus it seems that the attitude that deliberate drug overdose is a "cry for help", while still accurate in many cases, must be modified somewhat. In considering his adolescent patients, White remarked upon the resentful attitude of many when offered help. He considered that the "cry for help" was not necessarily being directed towards "helping agencies" but towards the other members of the troubled interpersonal relationships. He expressed this as a wish to "modify the outlook" of those close to them. This concept is also described by Bancroft, Skrimshire and Simkin (1976) who, describing a person's first deliberate drug overdose considered that this type of behaviour is "...often precipitated by emotional crises in close relationships and such acts may be understood as attempts to influence the other person or express anger...". White (1974) also mentions the acting out of frustrations as an underlying factor.

(v) The "stress" factor

It is clear that the "epidemic" of drug overdoses seen in

recent years is a response by the individuals concerned to stress, whether this be caused by poor housing conditions, marital disharmony or the break-up of adolescent relationships, and that some people in certain situations will, if the underlying cause of the overdose is not relieved or if relieved it returns, repeat the behaviour. Bancroft et al. (1976) considered that the practical consequences of a first overdose may increase the likelihood of a repeat episode to reproduce these consequences. They concluded this from a study of the reasons given by patients for taking an overdose where they found a positive relationship between the choice of "seeking help" as a reason for overdose and the presence of "previous attempts". Bancroft and Marsack (1977) recognised three types of repeater:

a) The chronic repeater, moving from one crisis to another with overdosing a habitual method of responding
b) Bursts of repeats extending over a long period of stress
c) The "one-off" overdoser who takes an overdose at the time of a severe crisis and then repeats rarely

The concept of overdose as a response to stress was first mentioned by Jones (1969) and expanded by Lukianowicz (1972) who mentioned that his patients, as a result of the overdose/attempted suicide had removed themselves from the stressful environment and had won them "rest and some kind of holiday". Kennedy (1974) considers that, among other reasons, overdoses are taken to obtain temporary oblivion, and that people taking drug overdoses have a low tolerance to stress. Mitchell and Lawson (1974) also suggested that people on the whole were becoming less tolerant of unhappiness, and Rohn, Sarles, Kenny, Reynolds and Herald (1977) suggest that adolescents who attempt suicide may be less able to withstand adversity and become apt

to attempt suicide as "alternative coping mechanisms fail". White (1977) suggested that modern socio-cultural attitudes decreased the individual's capacity for sustaining anxiety and that overdosing was a form of "shutting off", however temporarily, the anxiety.

However far from the immediate problem such ideas may seem, we have to consider the old adage that "prevention is better than cure" and that an understanding of the underlying problems may be the best way to tackle the symptom. Burrows and Harari (1974),while regretting that the present evidence offers few solutions, suggest that further research may be necessary into the effects of "urbanisation, changing social structures and roles, mobility, isolation and loss of social role" in an attempt to understand the modern epidemic of deliberate self poisoning.

4. Facilities for Treatment

(i) Atkins report

In 1962 a report entitled "Emergency Treatment in Hospital of cases of Acute Poisoning" was published as the Report of the Subcommittee of the Standing Committee of the Central Health Services Council - the "Atkins report" (H.M.S.O., 1962). This made a number of recommendations which, while not directly applicable to deliberate self poisonings, include them in the general term "acute poisoning". The recommendations are outlined below along with any action which may have been taken by 1968:

- All casualty departments should have adequate works of reference on poisoning and a handbook on the immediate treatment of poisoning - these were available to many but not all

accident and emergency departments.

- One hospital in each area should be designated as a receiving centre for cases of poisoning and should have an adequate laboratory service - centres had been nominally designated but the limit of their active functioning was variable. Poisonings tended to be received at any accident and emergency centre whose laboratory facilities were often inadequate.

- Centres should be in the charge of a physician specially interested in poisoning - this recommendation has not been acted upon.

- Specialised techniques, e.g. dialysis, should be carried out only at these centres - these techniques were available to all regions where needed.

- An information unit should be set up with central co-ordination - this was operating in 1968.

- There should be discussions about the setting up of a single centre for academic research on toxicology - there was only a centre for experimental toxicology in 1968 and no unit for clinical toxicological research.

- A committee should be set up to consider identification of tablets - this had made limited progress.

(ii) Hill report

Over the few years after the Atkins report, the incidence of poisoning was increasing and there was a need to review the situation. As a result of this, another committee, chaired by Professor Sir Denis Hill was given the task of looking further into the problem. Its remit was "To review the arrangements for treatment in hospital of patients suffering from acute

posoning by drugs and certain other domestic substances, and their aftercare." Their report (H.M.S.O., 1968) made several recommendations:

- There should be a reclassification of categories of drugs so that the then new psychotropic drugs would not go under general headings.
- Records should be kept of ambulance journeys, distance travelled, the time taken, number of deaths in transit and emergency measures taken on route.
- There should be training of staff and equipment of ambulances to deal with poisoning. There should be clear directions as to which hospital the patient should be taken to. The patient should, where possible, be accompanied by relatives with any information or remaining bottles of tablets.
- All accident and emergency centres should be equipped for child and adult poisonings.
- Poisoning Treatment Centres should be established, equipped, staffed and designated. At least one consultant interested in poisoning should be available to advise.
- Every Centre should have laboratory facilities able to detect the major poisons at short notice through 24 hours.
- All cases of deliberate self poisoning should go to the Regional Poisoning Treatment Centre. The suggestion of "accidental poisoning" should not be accepted without enquiry. All cases of deliberate self poisoning should have psychiatric and social help.
- Designated hospitals should have psychiatric units able to provide seven day cover to ensure that medical beds are not

blocked by patients waiting for psychiatric referral.

- Specialised centres for research into new methods of treatment and training should be set up.
- There should be a special study on prevention.
- All medicines in solid dosage form should be readily identifiable, and containers should be labelled.

(iii) Regional Poisoning Treatment Centre, Edinburgh

Prior to these recommendations a Regional Poisoning Treatment Centre had already been evolving at the Royal Infirmary, Edinburgh for 90 years (Matthew et al., 1969). At the time, this was a self-contained unit of twenty beds and one single room for severe illness. The centre was connected by intercom with the Accident and Emergency Department, the anaesthetic department and toxicology laboratory. The medical staff consisted of one consultant physician, a registrar and a senior house officer. A resident house officer helped to provide night cover. All these had other duties and helped to run the Scottish Poisons Information Bureau. Psychiatric assessments were made by staff from the local mental hospital on full time secondment for 6 month periods assisted by research fellows and an emergency rosta. There was a full time social worker and part time health visitor and mental health officer. The laboratory was staffed on an on-call basis and could carry out most of the major analytical techniques. Since 1967 medical, laboratory and psychiatric information was recorded on code sheets and transferred to punch cards so that statistics could be generated. It was believed that, for morale reasons, all members of the staff should have other duties and the junior nursing staff should

change duties regularly. Without any available psychiatric ward it was necessary to rely heavily on local mental hospitals being able to quickly admit any patients requiring further in-patient psychiatric help.

(iv) Attitudes of medical and nursing staff

About ten years after the Hill report, it is difficult to see much evidence of the adoption of the proposals of it and a possible cause of this inaction could be the attitude of doctors and nurses themselves towards patients admitted after a self poisoning episode. A typical comment made to the present author in the course of this research came from a sister in charge of a general medical ward prior to the setting up of the R.P.T.C. (Regional Poisoning Treatment Centre) at Dudley Road Hospital, Birmingham. When asked of the whereabouts of an overdose patient so that interviewing could take place, the sister became very angry and replied that she had "...twenty four sick patients to worry about..." and had not got "...time to worry about her.". This was not an isolated incident and several times it seemed that nursing staff resented the attention demanded by these patients. This type of attitude is not confined to nursing staff and although the concept of the "cry for help" has been known for some years, it seems that the help and sympathy sought is rarely forthcoming from medical and nursing staff. Patel (1975) studied the attitudes of physicians and senior nursing staff towards a variety of common illnesses and self poisoning.

The senior medical staff and most of the junior medical staff were of the opinion that all or most self poisoning cases should be admitted to hospital, but the nursing staff disagreed.

Generally staff considered that these patients were not personally satisfying to treat and doubted that the patients themselves benefitted from their stay in hospital. The general feeling was that all or nearly all cases resulted from social rather than psychiatric problems and also that these patients should be treated in specialised wards, but that few of them required admission to psychiatric hospitals. 13 out of 14 consultants disagreed with the statement that a large number of self poisoning patients did not require psychiatric evaluation. The junior staff nurses agreed with it. These groups were also more likely to express more unfavourable attitudes towards self poisoning patients than towards (e.g.) myocardial infarction patients.

Patel concluded that these differences in opinion were due to the fact that junior medical staff and nursing staff have a greater degree of contact with self poisoning cases especially when they are initially admitted, often drunk, abusive and unco-operative. They are also the staff who have to perform the unpleasant task of administering gastric lavage to these patients.

Barber, Hodgkin, Patel and Wilson (1975) expanded this study of attitudes to fourth year students, final year students, house physicians and medical social work students. The fourth year students were given the opportunity of not only seeing the self poisoning patients in hospital but also of interviewing the family doctor and visiting the patients at home. In the words of the authors, there was "...a planned and detailed exposure of the behavioural aspect of the incident of self poisoning...".

When the attitudes of the fourth year students after this exposure were compared with those before it, there was a reduction

in the expression of hostile feelings towards self poisoning although the small numbers involved could not be assessed statistically. It was also found that the opinions of this group were more like those of the medical social workers than their own medical colleagues. The authors considered that the unfavourable attitudes shown by the other medical staff may have been related to the emphasis given in physicians' training to organic aspects of illness rather than behavioural aspects as stressed in medical social work training.

(v) Prevention of self poisoning

The prevention of self poisoning is itself a complicated subject. It can be primary - prevention before an initial overdose admission - or secondary - prevention of the repetition of a previous overdose. Literature on prevention of deliberate overdose is notably scarce, seeming to appear under the heading of "suicide prevention" (Barraclough, Jennings and Moss, 1977; Bridge, Potkin, Zung and Soldo, 1977) and, if one talks about "suicidal behaviour" alone, what of the patients, largely overdose patients, who fall into Kennedy et al's (1974) category of "parasuicide"? Are those patients likely to benefit from the same treatment as potential suicides? If, as has been suggested, the primary cause of most initial overdosing is a breakdown of interpersonal relationships, can the intervention of a third party really help to relieve the problem in secondary treatment and is it at all possible to identify a population at risk and intervene at a primary treatment level to prevent episodes of self poisoning? Also, does the intervention of social work help, for example, with a financial problem only lead to repetition of the self poisoning behaviour when the individual meets the same stressful situation later? Would

a decreased availability of drugs help to promote a more positive reaction to problems or would the people who would formally have taken an overdose to temporarily escape from their situation find an alternative (and possibly more lethal) way of achieving this?

A study carried out by Jones (1977) showed that patients tended to take "the first thing they could get hold of" and that in a third of cases the drug taken belonged to a friend and a further third took drugs available without prescription. There is little that can be done to prevent people from hoarding drugs and education is of little help here, as an examination of any doctor's or pharmacist's bathroom cabinet would show! If, as suggested, the choice of drug is a matter of convenience, it is unlikely that restricted prescribing of drugs would have much effect, especially when one considers the large quantities of aspirin or paracetamol freely available over the counter of any pharmacy and the relative convenience and economy of buying large rather than small packs. Would restriction of sale of analgesics to small packs, were this possible, have any preventive effect? Would these types of restrictions be valuable in themselves or would this be simply a case of treating the symptom rather than the disease?

Unfortunately, few efforts have been made to answer (or even ask) these questions. Kennedy (1972) attempted to evaluate the success of psychiatric referral of patients on the rate of recidivism among self poisoning patients treated at R.P.T.C., Edinburgh. He concluded that the type of 'crisis intervention' offered by their team at Edinburgh was effective in secondary prevention. He found that repetition was three times as common in a group either given treatment at a later date elsewhere or

given no treatment at all than in the group seen immediately after physical recovery by the ward psychiatrist. He expressed the view that G.P.s in Ediburgh should be encouraged to refer all patients regardless of the physical severity of their overdose, to the R.P.T.C.. However, he also stressed that repetition after discharge from the unit was still high at 12%, and that there was no room for complacency and a definite need for "developing and evaluating new methods of treatment and aftercare".

5. Deliberate Self Poisoning with Salicylate Analgesics

(i) Morbidity

As stressed previously (2 (iii)) statistics concerning morbidity due to drugs overdose are difficult to isolate. How, therefore, is it possible to ascertain, on a national scale, the problem of salicylate analgesic overdose?

The O.P.C.S. publishes a yearly Hospital In-Patient Enquiry (H.I.P.E.) which brings together information about the treatment of patients in England and Wales. It gives tables showing the numbers of discharges and deaths from all causes including "adverse effects of drugs". This category is the nearest one can get to official recording of the incidence of deliberate drug overdoses, however, it also includes accidental poisonings and allergies or idiosyncratic reactions to drugs. "Salicylates" have the code number 965.1 in the International Classification of Diseases (I.C.D.) and Table 2 shows the number of discharges and deaths due to adverse effects of category 965.1. A multiplying factor is used to get absolute figures as the numbers

Table 2. Discharges and deaths due to adverse effects of salicylates from non-psychiatric hospitals in England and Wales.

Year	Sample Number	Multiplying Factor	Actual Number
1969	1,347	10.793	14,538
1970	1,305	11.065	14,440
1971	1,313	11.073	14,572
1972	1,423	10.737	15,279
1973	1,430	10.619	15,185
1974	1,470	10.974	16,176

quoted are from samples only.

The table shows that discharges and deaths due to adverse effects of salicylates increased by 10% between 1969 and 1974.

(ii) Mortality

More specific statistics are available concerning deaths from drugs overdose. These are published yearly by O.P.C.S. in "Mortality Statistics; accidents and violence" as:

"Deaths by poisoning - Suicide"

"Deaths by poisoning - Accident"

"Deaths by poisoning - Intention uncertain"

Reservation about which categories deliberate drug overdoses are included in have been expressed earlier (Adelstein and Mardon, 1974) and to avoid any further discussion, only those deaths officially described as "suicidal" have been used here, although it is accepted that this may result in underestimation of the problem. Deaths due to suicidal poisoning are classified according to the "poison" stated on the death certficate. When more than one substance is mentioned, no assumption is made by O.P.C.S. about the contribution of each to death from the order in which the drugs were written on the certificate. For the purpose of this study, however, it was assumed that the first drug mentioned was the "principal poison", and where this fell into the category "salicylates" the figures were extracted and are quoted in Table 3. Salicylates were mentioned in many more cases where they were a "secondary poison", and as a result the figures in Table 3 do not represent all cases where salicylates were involved. Table 3 shows that of deaths due to deliberate self poisoning between 9 and 13% were due to salicylates.

Table 3. Deaths from suicidal poisoning with salicylates and all other drugs in England and Wales.

Year	Total Drugs	Salicylates	% of Total
1966	1,738	217	12.49
1967	1,840	184	10.00
1968	1,926	197	10.23
1969	1,922	176	9.16
1970	1,787	162	9.07
1971	1,874	201	10.88
1972	1,771	166	9.37
1973	1,741	173	9.94
1974	1,875	208	11.09
1975	1,678	211	12.57
1976	1,595	180	11.29

6. The Purpose of the Study

(i) Aims

In December 1975 a study was initiated to investigate certain aspects of deliberate self poisoning, including a special study on the incidence and characteristics of deliberate self poisoning with salicylate analgesics.

The aims of the study were several. It was intended to obtain general information about the incidence of deliberate self poisoning in part of Birmingham so that comparisons could be made with similar studies carried out elsewhere. It was also hoped that by studying the factors contributing to drug overdoses, some conclusions might be drawn which might help to define a "population at risk" which could be of use in prevention. It was also considered worth investigating more closely that proportion of drug overdoses involving salicylate analgesics as this, along with paracetomol, is the major category of "over the counter drugs" involved in self poisoning. There was also the feeling that the extent of clinical information gathering from overdose cases is generally limited and this study was partly an attempt to create a "pool" of comprehensive clinical knowledge about this type of patient. It was hoped that this study might give some indication of possible measures that could be taken to reduce the problem of deliberate self poisoning.

(ii) Objectives

- To interview as many as possible of the patients admitted to Dudley Road Hospital after a self poisoning incident

during the available period.

- To obtain from these patients detailed information about the circumstances surrounding their overdoses.
- To supplement this information with clinical data concerning the overdoses, as recorded in the patients' hospital records.
- To encode this information in such a way that it might be analysed by computer.
- To carry out this analysis and form conclusions about the overdose population generally and more specifically about those patients who had taken salicylate analgesics.
- To form the basis of a detailed information collecting and analysis service for the West Midlands Regional Poisoning Treatment Centre.

1. The Origin of the Study

(i) Background

Dudley Road Hospital, (D.R.H.) Birmingham, is a district general hospital of 1,000 beds, set in an "inner city" area among Victorian terraces and redeveloped areas of council housing including many high rise blocks of flats. Its catchment area includes areas of high immigrant population and also more prosperous suburbs such as Edgbaston, as well as areas with a high student population and has a total population of approximately 500,000. Emergency cases are taken from across the city.

Patients arriving at D.R.H. after a deliberate self poisoning episode are brought or bring themselves to the Accident and Emergency (casualty) Department. Their condition is assessed by the medical staff on duty at the time, and they are often given a gastric lavage or an emetic. If willing they are then admitted to a ward for further treatment or observation. Prior to the setting up of the West Midlands Regional Poisoning Treatment Centre (R.P.T.C.) such admissions were sent to whichever ward had beds available and thus cases could be scattered between several of the 30 wards.

The R.P.T.C. was opened in April 1977. It is a unit of 6 beds and receives all self poisoning cases admitted to the hospital. It will also receive cases transferred from other hospitals, and aims to fulfil the recommendations of the Hill report (see Page 32).

(ii) Recording of information

In order that comprehensive records might be kept of the incidence of self poisoning and its treatment, a recording system was required which enabled information to be quantified and manipulated to provide easily accessible statistics. Much of the information necessary, e.g. the treatment given, was available from the patients' hospital records, however, these "notes" did not show the depth of detail required. It was, therefore, necessary to interview patients directly and since interviewing would be carried out by various individuals, some form of structuring was required to minimise interviewer variability and to provide easily quantifiable responses. Thus it was decided to use a questionnaire to collect the various pieces of information together.

2. Pilot Study

(i) Questionnaire

The questionnaire used at this stage is shown in Appendix 1.

The pilot study was carried out in order that any problems likely to be encountered during interviewing could be overcome at an early stage. The simple style of the questionnaire ensured that its questions were open-ended to allow the maximum range of responses to be recorded. This was done so that the coding of the final questionnaire might be as comprehensive as possible, although this meant that responses in the pilot study would be more difficult to quantify.

The pilot study also served to ascertain whether responses answered the questions asked by the questionnaire and whether, as a result, any of the questions needed rewording. Most of the questions were "factual", however some, such as those about "violence", "debts" and "criminal record" relied on the judgement of both patient and interviewer.

The information was obtained from the patients during an interview carried out by the author or a member of the medical staff, usually the day after their admission. The form of the interview was semi-structured, using the questionnaire as a base, but allowing a certain amount of "free association". Newman (1957) wrote of free association "... the basic idea is that if a person gives up the usual logical controls he exercises over his thoughts and says whatever comes into his mind at the moment, in the presence of a skilled listener, unconscious feelings and thoughts can be discovered. This is the product of a free flowing chain of words or ideas, which are consciously or unconsciously associated with one another, so that one calls up the next and so on. This idea has led to depth or detailed interviewing in marketing research, as a means of giving the respondent favourable conditions for telling of his ideas and feelings..."

(ii) Results of the Pilot Study

The figures presented here are the result of interviews with self poisoning patients admitted to D.R.H. during the period December 1975 to August 1976. They did not represent every self poisoning admission, but only those from whom the information could be obtained before their discharge.

Efforts were made to obtain as complete a set of results as possible, however in some cases certain facts (e.g. the source of drug taken) were not available.

During the study, 204 different patients were interviewed. Of these, two males repeated the act once each, as did four females; two females repeated twice, making a total of 214 episodes of deliberate self poisoning.

There were almost three times as many women as men admitted: 58 males:156 females.

Age of Patient - See Table 4, Figs. 1 and 2

The peak range for self poisoning admissions was quite distinctly 15-25 years. The incidence fell gradually with age with a small peak around 40-45 and another for the over 60s.

When male/female figures are compared, it can be seen that the female peak is slightly earlier than the male; 15-20 as opposed to 20-25.

Drugs Taken - See Table 5

The drugs most frequently taken were sedatives and tranquillisers. These were involved in twice as many incidents as the next group, the antidepressants. These were followed closely by aspirin and other analgesics, then barbiturates, hypnotics and paracetamol.

The patterns varied only slightly between the sexes. Sedatives and tranquillisers came at the top of the list for both men and women. Antidepressants and aspirin were also

Table 4. Pilot Study Results: Number of patients in each age group admitted after deliberate self poisoning.

Age Group	Males	Females	Totals
< 15	-	10	10
15–20	6	42	48
21–25	19	21	40
26–30	10	12	22
31–35	5	16	21
36–40	3	12	15
41–45	5	12	17
46–50	4	5	9
51–55	1	6	7
56–60	-	3	3
> 60	3	9	12
Total	56	148	204

Figure 1. Pilot Study: Distribution of age groups among self poisoning patients.

Figure 2. Pilot Study: Frequency polygon showing percentages of patients in each age group for both sexes.

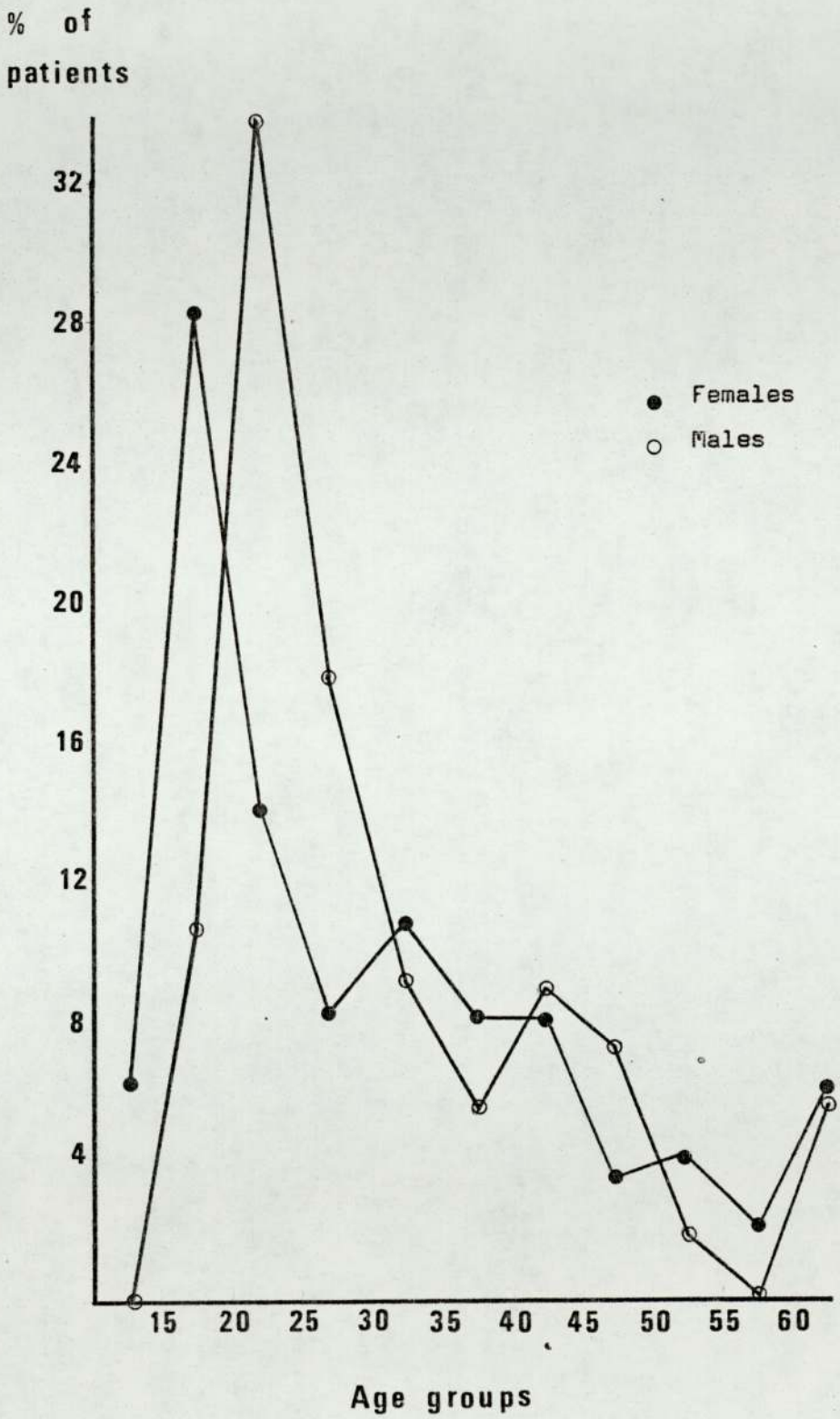


Table 5. Pilot Study Results: Types of drug taken by patients admitted after deliberate self poisoning episodes.

Type of Drug	Males	Females	Totals
Antidepressants	6	14	20
Hypnotics	-	11	11
Sedatives and Tranquillisers	9	29	38
Barbiturate Sedatives	7	8	15
Aspirin	6	12	18
Paracetamol	1	2	3
Other analgesics	1	16	17
Combinations of drugs	21	43	64
Other drugs	7	21	28
Total	58	156	214

taken just as often by men and women. More men than women used combinations of drugs.

Previous Overdoses - See Table 6

A slightly higher proportion of the women admitted to ever having taken a previous overdose: 23.7%:16.1%. Women tended to have overdosed only once previously although 10.7% of the men had taken several previous overdoses.

Psychiatric Diagnosis - See Table 7

The condition most often diagnosed was depression; 23.6% of all cases, and by far the most common diagnosis in both sexes. In men the next most common diagnosis was alcoholism/addiction (14.3% of males) and in women the next most common was schizophrenia (11.5% of females).

Source of Drug - See Table 8

The majority of patients had taken medicines prescribed for themselves (58.0%). 11.2% of patients used drugs prescribed for other members of the family and a similar proportion bought drugs specifically for the act. 8.9% of all self poisoning admissions took drugs from a combination of sources. Patterns were similar for men and women.

Ethnic Origin - See Table 9

Three quarters of all self poisoning patients were Caucasians of English/Scottish/Welsh origin. Next most common were Asians, followed by Irish and West Indians. The patterns were broadly similar for men and women.

Table 6. Pilot Study Results: Previous overdoses taken by patients admitted after deliberate self poisoning.

Previous Overdoses	Males	Females	Totals
None	40	106	146
One	9	35	44
Two	1	6	7
Several	6	1	7
Total	56	148	204

Table 7. Pilot Study Results: Psychiatric diagnoses of patients after deliberate self poisoning.

Diagnoses	Males	Females	Totals
Depression	15	33	48
Personality defect	6	14	20
Psychotic disorder	7	10	17
Schizophrenia	3	17	20
Alcoholism/Addiction	8	3	11
Anxiety	1	3	4
Mental Subnormality	1	1	2
No formal diagnosis	15	67	82
Total	56	148	204

Table 8. Pilot Study Results: Source of drug taken in episodes of deliberate self poisoning.

Source of Drug	Males	Females	Totals
Family Stock	2	9	11
Prescribed for family	7	17	24
Prescribed for self	29	95	124
Purchased for overdose	8	16	24
Stolen	-	1	1
Combination of sources	6	13	19
Other/Unknown	6	5	11
Total	58	156	214

Table 9. Pilot Study Results: Ethnic origins of patients admitted after deliberate self poisoning.

Ethnic Origin	Males	Females	Totals
Caucasian: U.K.	47	105	152
Irish	2	13	15
Others	-	1	1
Asian	4	16	20
West Indian	2	12	14
Others	1	1	2
Total	56	148	204

Time of Admission - See Table 10, Figs. 3 and 4

There appeared to be a definite peak of admissions around 12 noon followed, after a slight fall, by a slow build up to a larger peak at 8 p.m. The frequency of admissions then fell until 4–5 a.m. By far the majority of admissions occurred between 1 p.m. and 2 p.m. with a large block between 3 p.m. and 9 p.m.

Both sexes showed a peak of admissions between 1–2 p.m., and male admissions also peaked between 7–9 p.m. The majority of admissions for both sexes occurred between 3 and 9 p.m.

Day of Admission - See Table 11

Due to patients discharging themselves over the weekend before they could be interviewed, it is possible that Saturday and Sunday admissions may be underestimated.

It appeared that Tuesday was the most popular day for self poisoning admissions, followed by Monday and Friday.

Patterns were broadly similar for men and women except that Thursdays seemed more popular with men than women.

Precipitating Reason - See Table 12

The precipitating reason was either not known or vague in one third of all admissions. In 32.0% of all admissions, psychiatric illness was considered a precipitating reason.

In male patients, psychiatric illness accounted for the act in 41.4% and girlfriend problems 10.3%. In female patients, psychiatric illness accounted for 28.9% of

Table 10. Pilot Study Results: Times of admission of patients admitted after deliberate self poisoning episodes.

Time (00 Hours)	Males	Females	Totals
00–01	1	8	9
01–02	2	7	9
02–03	–	7	7
03–04	4	3	7
04–05	2	3	5
05–06	–	–	–
06–07	–	1	1
07–08	2	–	2
08–09	–	2	2
09–10	1	4	5
10–11	4	4	8
11–12	3	8	11
12–13	2	13	15
13–14	3	6	9
14–15	2	4	6
15–16	–	12	12
16–17	4	8	12
17–18	4	10	14
18–19	3	12	15
19–20	6	10	16
20–21	6	10	16
21–22	3	7	10
22–23	2	10	12
23–24	3	6	9
Unknown	1	1	2
Total	58	156	214

Figure 3. Pilot Study: Frequency polygon showing the distribution of admissions over the 24 hour period.

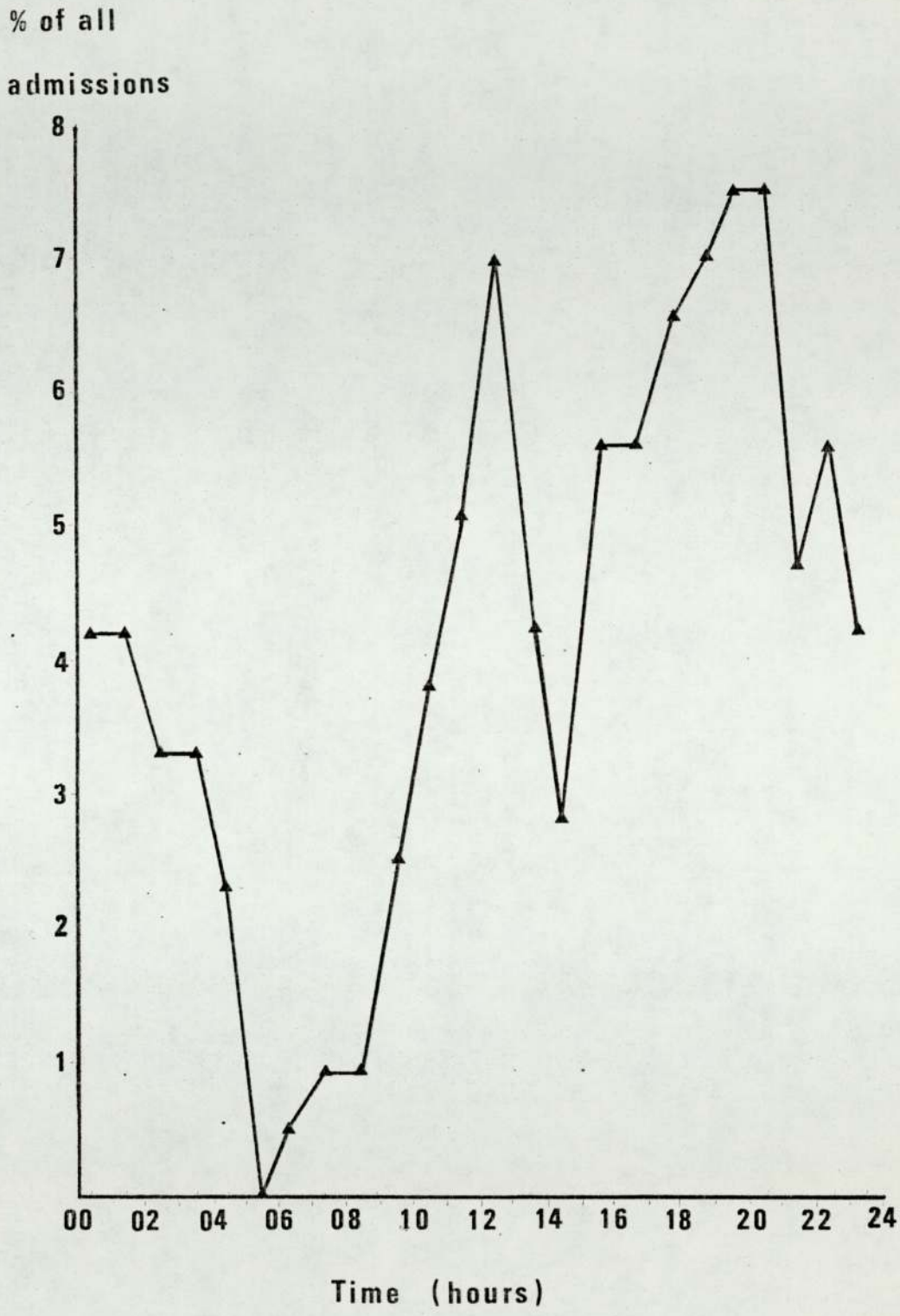


Figure 4. Pilot Study: Frequency polygon showing the distribution of admissions over the 24 hour period for both sexes.

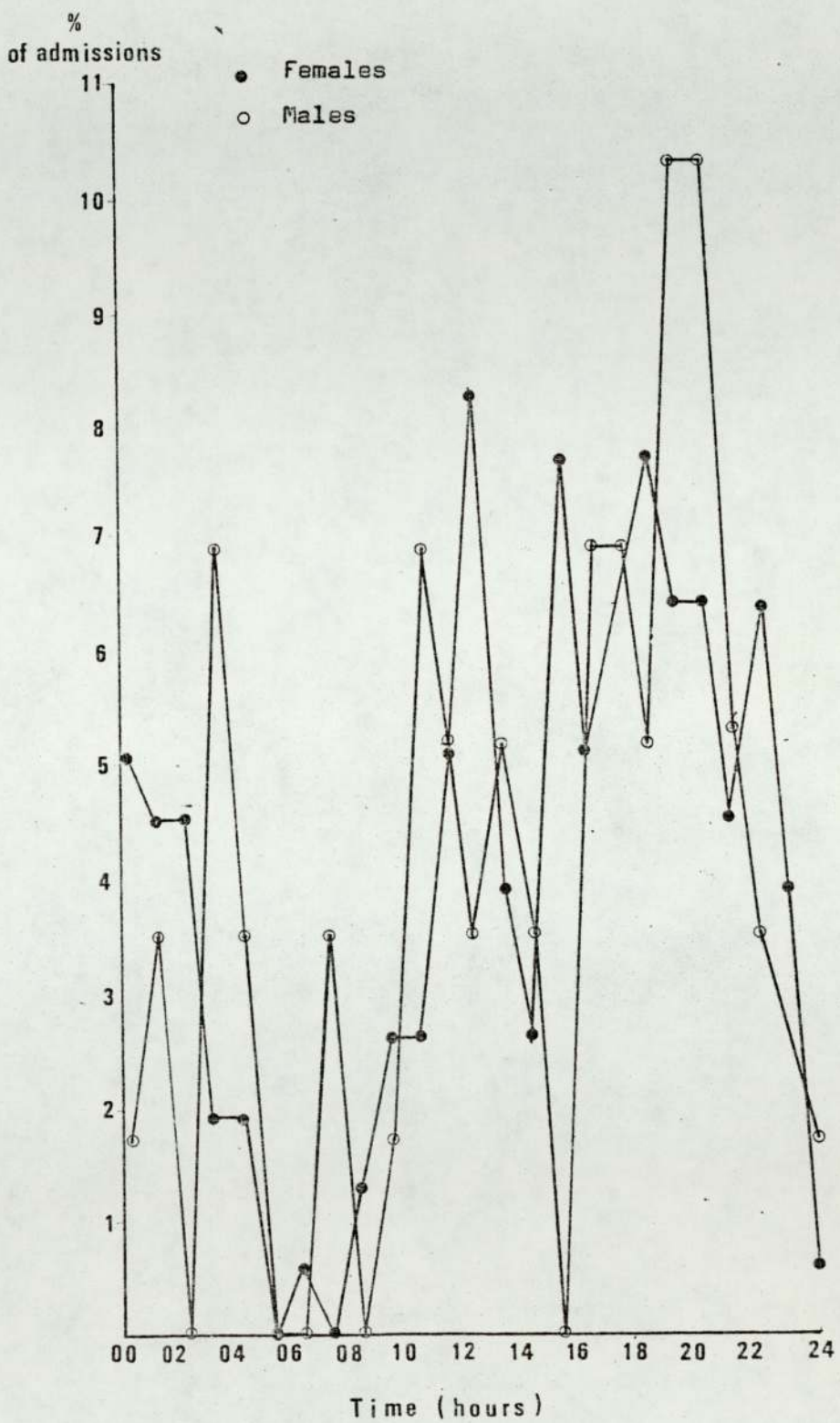


Table 11. Pilot Study Results: Days of admission of patients admitted after self poisoning episodes.

Day	Males	Females	Totals
Monday	8	26	34
Tuesday	14	31	45
Wednesday	4	22	26
Thursday	11	17	28
Friday	7	25	32
Saturday	8	19	27
Sunday	6	16	22
Total	58	156	214

Table 12. Pilot Study Results: Precipitating reason for deliberate self poisoning episodes.

Reason	Males	Females	Totals
Psychiatric illness	24	45	69
Marital disharmony	3	25	28
Boy/Girlfriend problems	6	8	14
Parental problems	2	19	21
Unemployment	2	1	3
Chronic illness	4	2	6
Police trouble	-	2	2
School problems	-	2	2
Vague/Unknown	17	52	69
Total	58	156	214

admissions and marital problems 16.0%. Almost four times as many women as men gave disagreements with parents as the precipitating reason for their overdose.

(iii) Conclusions

Although the pilot study questionnaire gave some quite comprehensive results, it was apparent that the wording of several questions needed to be more specific and that some additional questions concerning the clinical details were required. As a computer analysis was envisaged, a coding system had to be designed for the most likely responses to any question. Taking all of these factors into consideration, the "Overdose Admissions - Coding Sheet" was designed, a sample of which can be found in Appendix 2.

3. Expanding the Survey

(i) Overdose Admissions Coding Sheet

This was designed not only for the purpose of this study but to form the basis of the input of statistics about self poisoning patients admitted to Dudley Road Hospital. As such, some of the more detailed clinical information is irrelevant to this study and will not be discussed.

Due to the deliberately detailed nature of the information obtained from the pilot study, it was possible to transfer this to the new coding sheets when these became available. Further interviews took place using the new coding sheets as a basis.

(ii) Analysis of the Results

All completed coding sheets were taken to Aston University Computer Centre where the information was punched onto cards. Two 80 column cards were used for each case, both of which were identified by the case number of the patient and the date of admission. An initial sorting procedure was carried out to eliminate any duplication.

The analysis of the information was carried out using the "Statistical Package for the Social Sciences" (S.P.S.S.) suite of statistical procedures. This package was designed at Stanford University (U.S.A.) and has been modified and expanded and the version used here was S.P.S.S. H, Version 5. The computer used for analysis was the I.C.L. 1904S.

1. The General Overdose Population

(i) Duration of the study and incidence of self poisoning

The results shown below are the results of the analysis of information collected between December 1975 and May 1978. During this period, 1363 incidents of self poisoning were referred or referred themselves to Dudley Road Hospital. of these, 201 either refused admission or were transferred to other hospitals, and the remaining 1162 were admitted for observation or further treatment. Of those admitted, 1009 were interviewed for the purpose of this study. Fig. 5 shows the distribution of admissions for the various Health Districts within the Birmingham Area Health Authority area.

(ii) Demographic information

Sex

655 (65%) admissions were female patients and 352 (35%) were male. 2.5% of the female patients were pregnant. Sex was not recorded in two cases.

Age

The mean age of admissions was 30 years; the maximum 91 years and the minimum 11 years. Table 13 and Fig. 6 show the distribution of age groups, adjusted to account for missing information. The mean age for female admissions was slightly lower than that for males (30.22:32.33). Table 14 and Fig. 7 show the distribution between age groups for each sex.

Figure 5. Distribution of origins of self poisoning patients between the various Health Districts within the Birmingham Area Health Authority area.

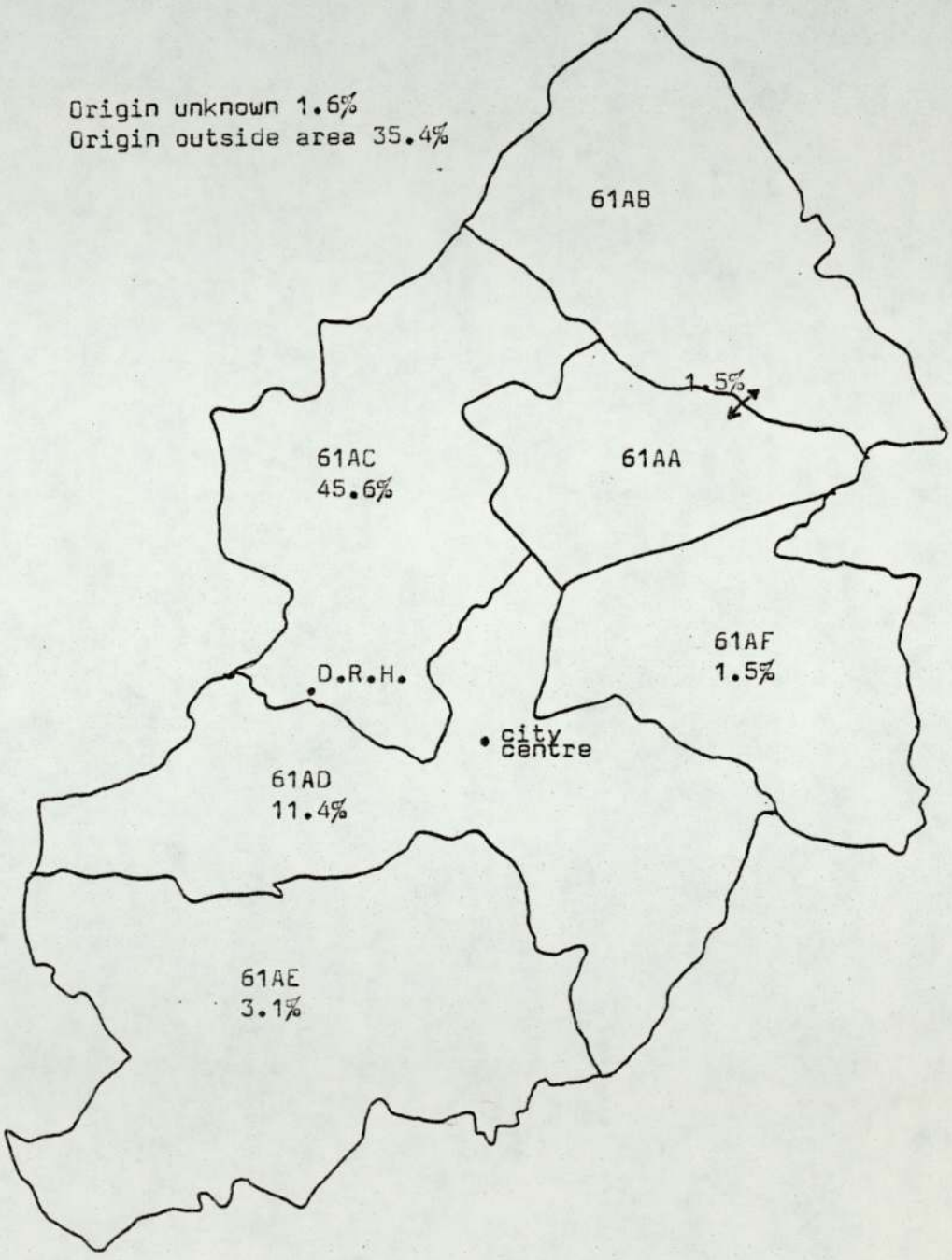


Table 13. Number of patients in each age group admitted after deliberate self poisoning.

Age group	Number	Adjusted Frequency %	Cumulative Adj. Frequency %
< 15	43	4.3	4.3
15–19	202	20.1	24.4
20–24	200	19.9	44.3
25–34	236	23.5	67.8
35–44	147	14.6	82.4
45–54	86	8.6	90.9
55–64	57	5.7	96.6
65–74	25	2.5	99.1
> 75	9	0.9	100.0
N/A	4	-	100.0

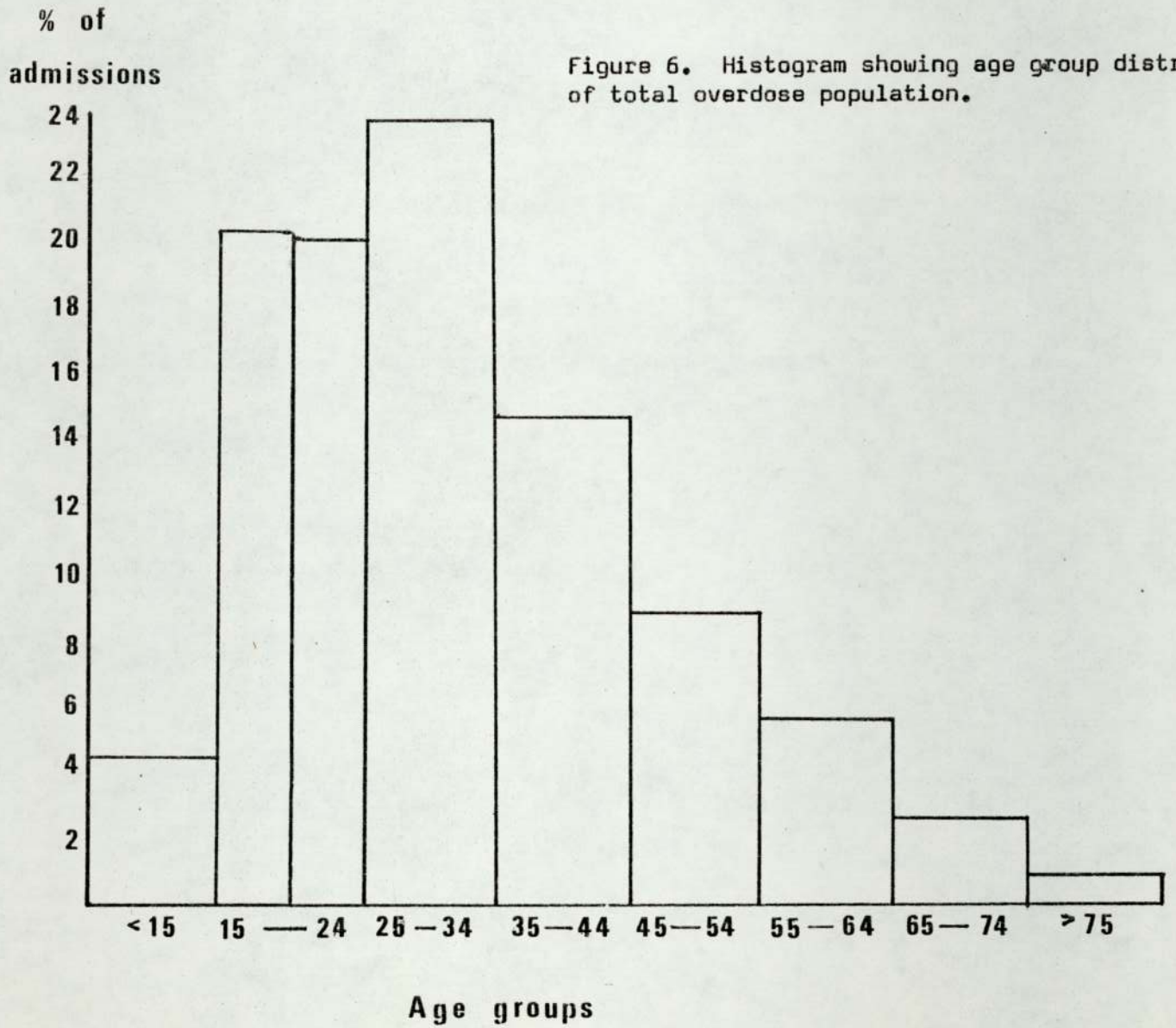


Figure 6. Histogram showing age group distribution of total overdose population.

Table 14. Proportions of each sex in each age group of self poisoning admissions.

Age group	Adj. Freq. % Females	Cum. Adj. Freq. % Females	Adj. Freq. % Males	Cum. Adj. Freq.% Males
< 15	5.2	5.2	2.6	2.6
15–19	24.0	29.2	12.6	15.2
20–24	19.3	48.5	20.9	36.1
25–34	20.9	69.4	28.4	64.5
35–44	13.0	82.4	17.8	82.2
45–54	8.0	90.4	9.7	92.0
55–64	6.4	96.8	4.3	96.3
65–74	2.3	99.1	2.9	99.1
> 75	0.9	100.0	0.9	100.0

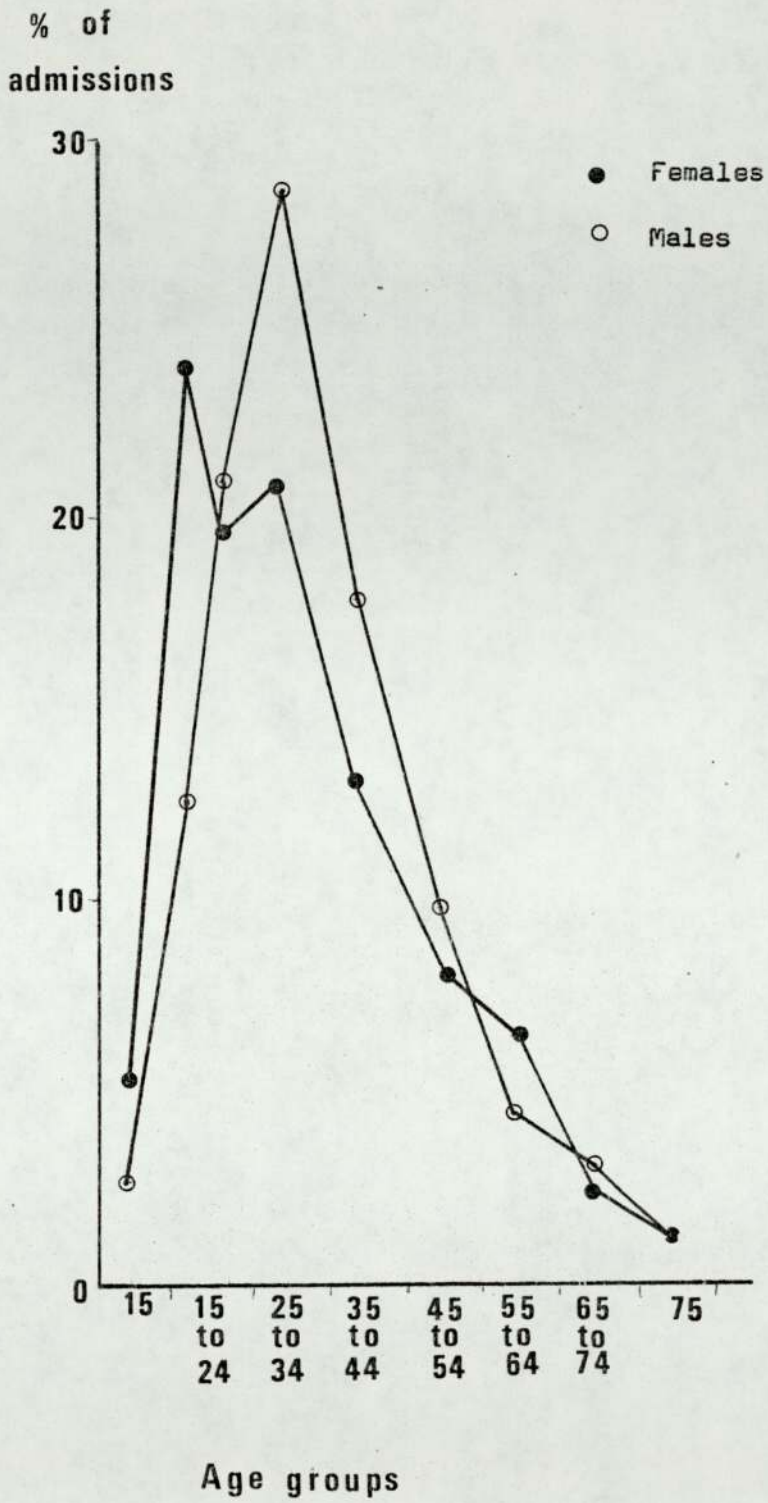


Figure 7. Frequency polygon showing percentages of patients in each age group for both sexes.

Social class

63.8% of all admissions came from social classes 4 and 5. There was no obvious difference in distribution between the sexes (see Table 15). 5.1% were classified as students.

Civil status

36.1% of all self poisoning admissions were married and 43.5% were unmarried.

Misuse of drugs

In 95.8% of admissions there was no evidence of misuse of drugs. In only 61 cases out of the 1009 was there any doubt or any positive evidence of regular misuse of drugs. Of total admissions 2.6% showed evidence of habitual oral misuse and 1.2% showed evidence of I.V. misuse. There were only 4 cases of evidence of marijuana misuse.

Alcohol and nicotine use

Teetotallers and normal social drinkers made up three quarters of the overdose population. 17.6% were heavy social drinkers and 9% were chronic alcoholics. Almost 40% of the overdose population did not smoke at all.

Problems within the family

The majority (57.2%) of the overdose admissions admitted to no obvious problems within the family. The most frequent single problem was marital disharmony which was cited in 11.5% of admissions or 28.0% of cases where a problem was defined. Psychiatric problems were cited in 4.0% of all

Table 15. Distribution of self poisoning admissions between the social classes.

Social class	% Females	% Males	% Total
1	1.2	0.9	1.1
2	4.5	1.8	3.5
3	13.7	17.1	15.0
4	17.9	21.2	19.0
5	47.5	40.3	44.8
6	9.4	15.0	11.5
7	5.8	3.8	5.1

admissions and "alcohol problems" in 3.2% of cases.

Overcrowding at home

This information was only available in 90% of cases. Of these 11.3% stated that their living conditions were overcrowded.

Violence within the last year

In 85.0% of the cases where this information was available there had been no violence either against or by the patient. In 8% of cases, there was evidence of violence against the patient and in 4.9% of cases the patient had been violent him/herself. In 1.8% of cases, violence had been used both against and by the patient.

Debts

In 14.6% of all cases, this information was not available Of cases where it was available, 93.2% would admit to no debt of any kind. 2.1% admitted to rent arrears and 3.9% to other debt (not specified). The threat of legal action due to debt of any kind did not feature to any significant extent, (0.7% of cases).

Criminal record

In 13.2% of cases this information was not available. Of cases where it was available, 90.8% did not admit to any criminal record. The remaining 9.2% had either been convicted in the past or had procedings taking place currently against them.

Present household

Where this information was available, 62.4% lived with either the spouse or the parents and 10.9% lived with other relatives or friends. 10.2% lived alone and 5.6% lived in lodgings or a hostel.

Separation from mother

This information was available in 80.2% of cases. Of these, only 7.5% had been separated from their mothers before the age of fifteen.

Separation from father

This information was available in 79.7% of cases. Of the these, 8.3% had been separated from their fathers before the age of fifteen.

Previous self poisonings admitted to hospital

In 67.7% of cases there was no evidence of any previous admissions after self poisoning incidents. 17.0% had one previous admission and 5.3% had two previous admissions. 10.0% had been admitted previously on several occasions. When males and females were considered separately, it was found that there was little difference in the numbers of previous overdoses between the sexes. This is shown in Table 16.

Most recent admission to hospital after an overdose

Approximately one half of those admitted previously after an overdose had been admitted within the six months prior

Table 16. Previous overdoses admitted to hospital for both male and female patients.

Number of previous overdoses admitted	% of Female patients	% of male patients
1	68.7	66.0
2	16.7	17.5
3	5.6	4.7
4	3.4	5.0
5–10	2.2	1.8
>10	-	0.3

to their admission during the study. Approximately one sixth had been admitted previously within the month prior to their admission during the study.

Previous psychiatric treatment

Of the total overdose admission population, 23.4% had, at some time, received inpatient psychiatric treatment, and 25.9% had received outpatient psychiatric treatment within the previous year.

Self poisoning within the family

95.4% of all cases did not admit to any contact with friends or family who had taken an overdose. 3.9% had an immediate family member who had overdosed, and 0.6% had a friend who had overdosed.

Suicide within the family

98.7% of all cases had had no contact with friends or family who had committed suicide. 1.2% had experienced a suicide within the immediate family.

(iii) Information concerning the overdose admission

Day of week

There appeared to be slightly more admissions on Mondays and Tuesdays with Monday the busiest day for admissions. Table 17 shows that there was little variation between the sexes with regard to the pattern over the week. The only exception was that the largest proportion of male patients were admitted on Thursdays.

Table 17. Overdose admissions per day for both sexes.

Day	% of Female patients	% of male patients
Monday	15.9	15.3
Tuesday	15.4	14.5
Wednesday	14.4	13.4
Thursday	13.4	16.2
Friday	13.3	13.6
Saturday	12.8	13.6
Sunday	14.8	13.4

Time of day

23.0% of all admissions were between 6 p.m. and 10 p.m.. The next busiest period was 10 p.m. to 2 a.m. during which 21.8% of patients were admitted. In the 12 hours between 2 p.m. and 2 a.m. 64.7% of all patients were admitted; twice as many as between 2 a.m. and 2 p.m.. Table 18 and Fig. 8 illustrate that there was little difference between the sexes with reference to admission times except that the peak for female admissions was during the period 6 p.m. to 10 p.m. and the male admissions peak was between 10 p.m. and 2 a.m..

By whom the patient was referred to D.R.H.

Of cases where this information was available (98.2%), 77.0% were admitted to D.R.H. after the intervention of a third party. 28.7% of all cases were admitted after a 999 call; by far the most common form of reference for admission. 7.4% were referred to D.R.H. by their G.P., 23% arrived at the casualty department unaided or unaccompanied. Only 0.2% were referred by the Samaritans organisation.

Reason for admission

Of all cases where this information was available (99.8%), 91.5% were classified as intentional self poisoners, and in 5.0% of cases the intention was unclear. 0.7% of cases were considered to have overdosed after taking drugs for "kicks" and only 2.0% were classified as accidental self poisonings.

Evidence that poison was taken

There was toxicological evidence that an overdose had taken place in 69.7% of cases, and evidence from clinical

Table 18. Distribution of admissions of both sexes throughout the 24 hour period.

Time of Admission	% of Female patients	% of Male patients
0200–0559	8.4	11.3
0600–0959	6.5	7.5
1000–1359	20.2	16.8
1400–1759	20.8	18.2
1800–2159	23.8	21.7
2200–0159	20.2	24.6

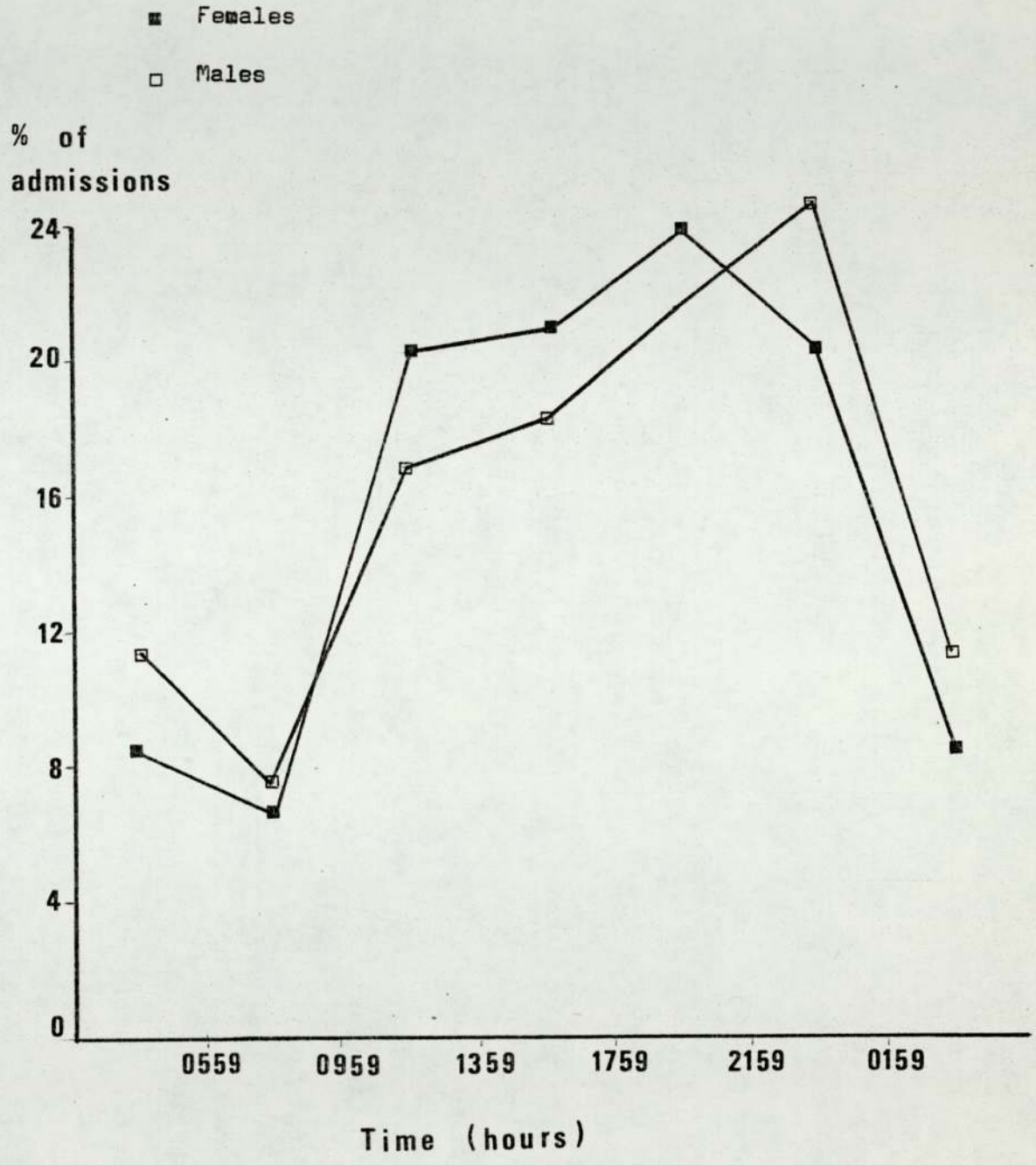


Figure 8. Frequency polygon showing the distribution of admissions over the 24 hour period.

signs in another 2.8%. The patients' own histories were the only evidence in 25.3% and in only 0.9% of cases was there no evidence of any overdose having taken place.

Number of substances taken beside alcohol

One drug only had been taken in 69.8% of cases. Two or more drugs had been taken in 29.0% and the overdose was of alcohol alone in 1.2% of cases. A slightly higher proportion of women than men took only one drug (see Table 19).

Alcohol simultaneously

Alcohol was taken at the same time as the overdose in 28.2% of cases.

Source of Drug

This information was available in 94.5% of cases. Of these, in 52.6% of cases, the drugs taken had been prescribed for the patient at some time. In 11.8% of cases the drugs had been prescribed for the patient within a week of the overdose. In 30.6% of cases, the drug had been bought "over the counter", and in 13.7% of cases they had been bought specifically for the overdose.

Length of time between ingestion and admission

Of the cases where the information was available, 45.2% of patients were admitted within two hours of ingestion of the drugs. 71.2% of all patients were admitted within four hours of the overdose. Table 20 shows that there was little difference between the sexes with regard to the length of time between ingestion of the drug and admission to hospital.

Table 19. Number of substances besides alcohol taken by male and female patients.

Number of substances	% of female patients	% of male patients
One	72.0	65.6
Two	18.3	22.8
Three	6.2	6.4
Four	1.7	2.9
Five	0.5	0.9
Six	0.2	0.3
Nil	1.2	1.2

Table 20. Time between ingestion and admission in hours for both sexes.

Time (hours)	% of female patients	% of male patients
0–2	45.4	45.0
2–4	28.2	21.9
4–6	9.2	10.9
6–8	5.2	7.9
8–12	5.4	6.0
12–18	4.3	3.3
18–24	1.6	2.0
> 24	0.9	3.0

Length of stay

48.0% of all patients stayed in the hospital for less than 24 hours, and 75.5% stayed for less than 48 hours. Table 21 shows little difference in the length of stay between the sexes.

(iv) Clinical features of the overdose population

Level of consciousness

This information was recorded in 99.8% of cases. It was apparent that 73.9% of patients were fully conscious when admitted. 12.9% of patients were Grade 1 unconscious.

Hypothermia

The presence or absence of hypothermia was recorded in 93.9% of patients. Of these only 5.3% showed any signs of hypothermia.

Other system complications

This information was recorded in 99.9% of cases. Of these, complications to other systems were noted in 2.3% of patients. Death was recorded in 2 cases; cardiovascular complications in 8 cases; respiratory complications in 5 cases; hepatic system complications in 7 cases and neurological systems complications in 1 case.

Investigative biochemistry

This information was recorded in 99.0% of cases. Of these, 39.9% involved routine biochemical investigation; 18.1% involved emergency biochemical investigation and 3.5%

Table 21. Length of stay in hospital of overdose patients of both sexes.

Length of stay	% of female patients	% of male patients
< 24 hours	47.6	48.4
24–48 hours	27.9	26.8
48–72 hours	6.9	4.9
3–5 days	11.9	13.3
5–7 days	2.0	2.9
7–10 days	2.0	2.6
10–14 days	0.8	1.2
15–28 days	0.6	0.0
> 28 days	0.3	0.0

had investigation involving blood gas determination.

Investigative toxicology

This information was recorded in 99.6% of cases. Of these, 48.2% of cases involved routine toxicological investigation and 34.7% involved emergency toxicological investigation.

Haematological investigation

This information was recorded in 99.5% of cases, of which 28.5% involved routine investigation and 10.7% emergency investigation.

Gastric lavage

This information was recorded in 99.3% of cases of which 61.9% of patients received a gastric lavage and 11.9% received ipecachuana as an emetic.

Airway

Of all cases only 4.3% required any assistance with their breathing. 1.1% of patients required an oropharyngeal tube; 1.7% required endotracheal intubation and 1.5% required endotracheal intubation and assisted ventilation.

Maintenance fluids

Maintenance fluids were required by 19.7% of patients.

Treatment for hypotension

This information was recorded in 99.4% of cases. Of these 2.3% required any treatment for hypotension.

Forced diuresis

Forced diuresis was required in 63 cases (6.2%) for salicylate intoxication and in one case for barbiturate intoxication.

Other treatment

There were no cases where peritoneal dialysis, haemodialysis or mannitol infusion were required, and only one case where charcoal haemoperfusion was required.

Specific antagonist

This information was available in 99.8% of cases. Table 22 shows which specific antagonists were required.

Psychiatric interview

This information was available in 99.8% of cases. 79.4% of these were interviewed either by a psychiatrist or a social worker. 7.3% of patients were not seen by either while in hospital but were given outpatient appointments. 3.7% were discharged by the physician with no outpatient appointment, and 6.8% took their own discharge with no outpatient appointment.

Illness diagnosis

This information was available in 96.8% of cases. Of these, 64.3% were diagnosed as having no psychiatric illness. Table 23 shows the distribution of diagnoses between the remainder.

Table 22. Use of specific antagonists in the treatment of drug overdoses at D.R.H.

Antagonist	No. of cases	% of cases
Cysteamine (Paracetamol)	16	1.6
Naloxone (Paracetamol)	1	0.1
Desferioxamine	4	0.4
Other	4	0.4

Table 23. Diagnoses of Psychiatric illness in overdose patients at D.R.H.

Diagnosis	No. of cases	% of cases
Depressive reaction	105	12.7
Depressive illness	124	10.7
Organic psychiatric disorder	30	3.1
Schizophrenia	30	3.1
Epilepsy	17	1.7
Mania	1	0.1
No psychiatric illness	628	64.3

Personality diagnosis

This information was available in 97.0% of cases. Of these, 72.8% had a normal personality. Table 24 shows the distribution of other diagnoses.

Agreed psychiatric disposal

This information was available in 98.1% of cases. No further action was taken with regard to psychiatric disposal in 45.9% of cases. 33.1% were given outpatient appointments at Dudley Road Hospital. 9.9% became voluntary psychiatric inpatients and 3 patients were detained as psychiatric in-patients.

Agreed social work disposal

This information was available in 98.1% of cases. 81.9% of these patients required no further action. In 10.6% of cases the hospital social worker was further involved and in 5.3% of cases the relevant social services centre was involved. In 0.6% of cases a health visitor became involved.

2. Subfile "Salicylates"

(i) Background

Subfile "salicylates" contained records duplicated from the main file where salicylates were the principal poison. "Salicylates", in the context of this study, represented all of the most commonly available analgesic preparations having aspirin as their main or sole constituent. During the period of the study, there were 159 incidents of self poisoning with salicylates; this represented 15.8% of the total overdose population.

Table 24. Diagnoses of personality in overdose patients at D.R.H.

Diagnosis	No. of cases	% of cases
Personality disorder	139	14.2
Alcoholism	63	6.4
Drug addiction	28	2.9
Subnormality	10	1.0
Normal personality	713	72.8

(i) Demographic information

Sex

105 (66.1%) of admissions were female and 54 (33.9%) were male.

Age

The mean age of admissions was 27.0 years and the peak age group for salicylate overdose admissions was 15–19 years. Table 25 and Fig. 9 show the distribution of agegroups for the salicylate overdose population.

Social class

66.9% of all admissions came from social classes 4 and 5. 6.6% were classified as students.

Problems within the family

59.4% of salicylate overdose patients reported no particular problems within the family. Marital disharmony was again the main single problem, being cited in 9.1% of cases. Cultural and psychiatric problems were the next most common, each of which accounted for 17.5% of admissions.

Previous self poisonings admitted to hospital

In 72.8% of cases there was no evidence of any previous admissions after self poisoning incidents. 18.4% had one previous admission.

Table 25. Number of patients in each age group admitted after deliberate self poisoning with salicylate analgesics.

Age group	No. of cases	Adjusted Frequency %	Cumulative Frequency %
15	4	2.5	2.5
15–19	49	30.8	33.3
20–24	35	22.0	55.3
25–34	36	22.6	78.0
35–44	17	10.7	88.7
45–54	13	8.2	96.9
55–64	2	1.3	98.1
65–74	3	1.9	100.0
75	0	0.0	100.0

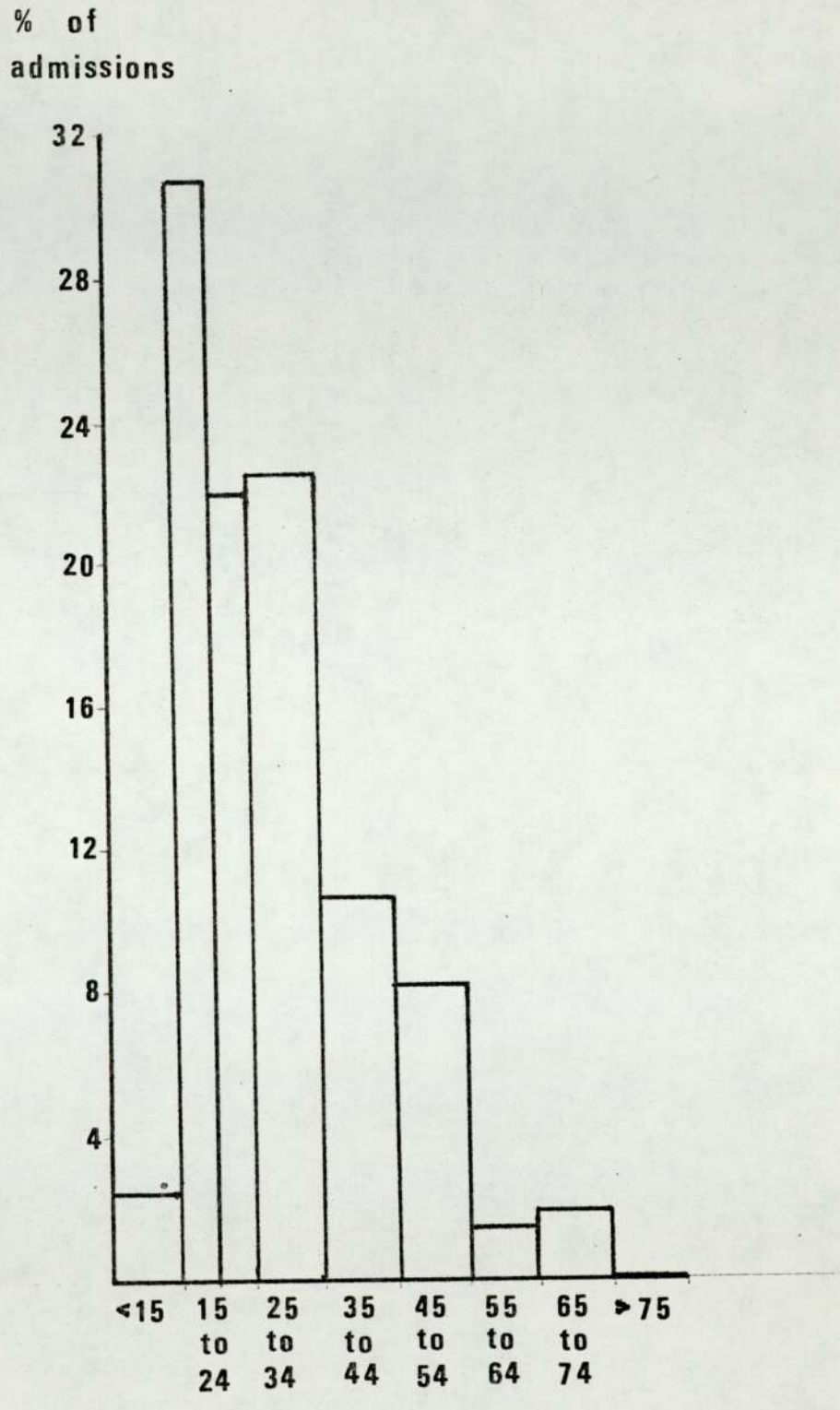


Figure 9. Histogram showing the distribution of age groups among the salicylate population.

(ii) Information concerning the overdose admission

Day of week

Table 26 shows that there was a definite peak of salicylate admissions on Tuesday and Wednesday.

Time of day

Table 27 shows that the period between 6 p.m. and 10 p.m. was the peak time for salicylate overdose admissions.

By whom the patient was referred to D.R.H.

25.1% of salicylate overdose patients brought themselves to hospital unaided or unaccompanied.

Reason for admission

95.6% of all incidents of self poisoning with salicylates were classed as intentional self poisoning. There were two cases of self injury and self poisoning and one case of the drug having been taken "for kicks". There were 3 cases where the intention of the patient was not clear.

Number of substances beside alcohol

One drug only had been taken in 79.6% of cases, and two drugs had been taken in 12.1% of cases. In two cases there was a possibility that only alcohol had been taken.

Source of drug

This information was available in 96.9% of cases. Of these, 12.9% took drugs that had been prescribed; 88.1% took drugs bought "over the counter" and 37.7% had bought the drug for the overdose.

Table 26. Distribution of salicylate overdose admissions per day.

Day	% of cases
Monday	13.0
Tuesday	17.0
Wednesday	17.6
Thursday	15.1
Friday	13.2
Saturday	11.9
Sunday	11.3

Table 27. Distribution of salicylate overdose admissions throughout the 24 hour period.

Time of admission (hours)	% of cases
0200–0559	8.9
0600–0959	7.0
1000–1359	15.8
1400–1759	20.3
1800–2159	27.8
2200–0159	20.3

Length of time between ingestion and admission

Of cases where this information was available, (94.3%), 37.7% of patients were admitted within two hours of ingesting the drug. 63.3% were admitted within four hours of ingestion.

Length of stay

53.8% of all patients left the hospital within 24 hours, and 82.9% stayed less than 48 hours.

(iii) Clinical information

Psychiatric interview

Of all cases, 77.3% were seen by either a psychiatrist or a social worker. 4.4% were not seen by either while in hospital, but were given an outpatients appointment. 2.5% were discharged by the physician without an outpatients appointment, and 3.8% took their own discharge without an outpatients appointment.

Illness diagnosis

Of all cases where this information was recorded, 70.7% were diagnosed as having no psychiatric illness. Table 28 shows the distribution of other diagnoses.

Personality diagnosis

This information was available in 99.4% of cases. Of these, 81.6% had a normal personality. 13.3% were diagnosed as having a personality disorder and 2.5% were diagnosed as subnormal. 1.3% were diagnosed as alcoholic.

Table 28. Diagnoses of psychiatric illness in salicylate overdose patients at D.R.H.

Diagnosis	No. of cases	% of cases
Depressive reaction	17	10.8
Depressive illness	17	10.8
Organic psychiatric disorder	7	4.5
Schizophrenia	1	0.6
Epilepsy	1	0.6
Mania	1	0.6
No psychiatric illness	111	70.7

Agreed psychiatric disposal

No further action was taken with regard to psychiatric disposal in 48.4% of cases. 35.2% were given outpatient appointments at Dudley Road Hospital. 8.8% became voluntary psychiatric inpatients.

Packaging of salicylates taken in overdose

Two thirds of patients who took a salicylate overdose obtained the tablets from a large volume container such as a bottle. 19.0% used tablets from small quantity containers and a further 12.0% took tablets from "pop-out" packs.

3. Discussion

It was stated earlier (Page43) that one of the aims of the study was to interview as many as possible of the patients admitted to Dudley Road Hospital after a self poisoning incident during the available period. It appears that the attempts made to do this were very successful, interviews having been carried out in 1009 cases out of a possible 1162 - a success rate of 86%. In view of the fact that many overdose patients discharge themselves within a short time of their admission the high success rate was very encouraging.

It was not the intention of this study to investigate the pattern of drugs taken in overdose, although this could be the subject of subsequent studies. The author was mainly interested in the patients who had taken salicylate analgesics and it was found that these represented 15.8% of the total overdose population. This proportion seems slightly higher than proportions reported elsewhere. Kessel (1965) reported that

aspirin overdose represented 12% of patients in his study; Jones (1977) reported a proportion of 12% of overdoses being due to salicylates and Petersen and Brosstad (1977) reported quite a low figure of 5% salicylate overdosage in their study. As the data collection continues at the West Midlands Regional Poisoning Treatment Centre it would be interesting to check this proportion periodically and to compare any trend found with the declining trend in salicylate overdose found by Proudfoot and Park (1978). Sivner and Goldberg (1978) reported that 5.7% of patients that they studied had overdosed with paracetamol and 9.6% with aspirin and aspirin-containing compound.

Sex

The female : male ratio for the general overdose population was 1.9:1 and that of the salicylate overdose population was 1.9:1 also. This was a slightly smaller ratio than that found during the pilot study (2.7:1) but compares well with the results of similar studies carried out elsewhere in Britain. (Middleton et al., 1961; Burston, 1969; Mitchell and Lawson, 1974; Murray et al., 1974 and Ghodse, 1977). It seems that the trend in Britain is for twice as many women as men to take deliberate overdoses of drugs.

Age

The mean age for the general overdose population was 30 years and that of the salicylate overdose population was 27. Figure 10 shows that, although the spread of ages was very similar, there was still a large peak in the 15-19 year age group of salicylate overdoses. The peak for the general

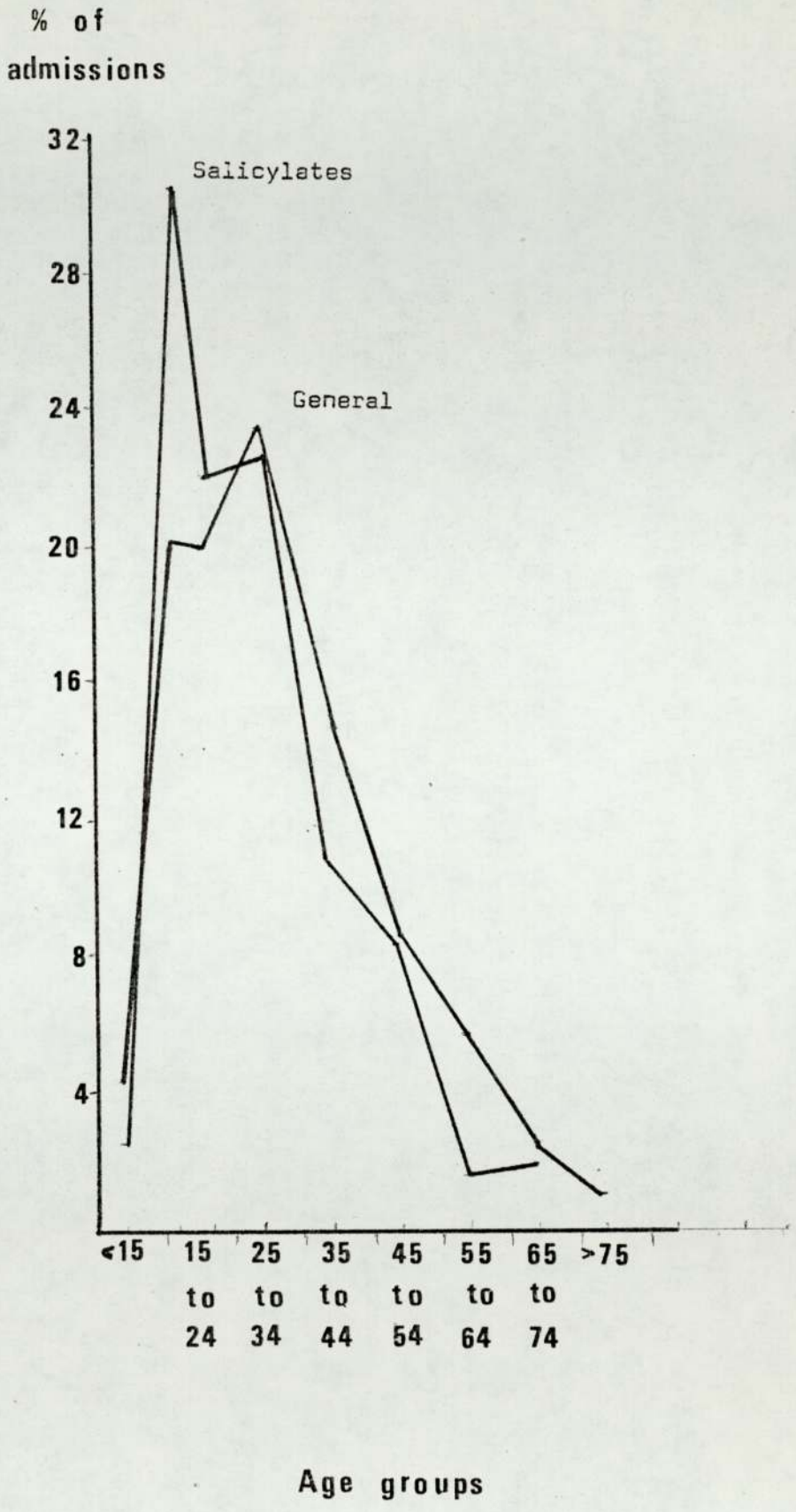


Figure 10. Frequency polygons showing percentages of patients in each age group for the general and the salicylate overdose populations

overdose population occurred in the 25-34 age group. These facts indicate that although salicylate overdosage is not restricted to younger patients, the taking of salicylates tends to be a particular problem in the adolescent section of the overdose population.

The male/female comparisons for the total overdose population as shown in Figure 6 indicate that females taking overdoses tend to be younger than males. This is also reflected in the slightly lower mean age of female overdoses - 30.22 years as opposed to male mean age of 32.33 years. A similar phenomenon was reported by Petersen and Brosstad (1977).

Social Class

It is difficult to make any comment about the relevance of the high proportions of social classes four and five in the general overdose population without reference to the population in the area of intake. Taking into account the large catchment area of the hospital and the fact that emergency admissions could be taken from anywhere within the city, such information as could be obtained about the distribution of social classes would have been of dubious benefit. Kessel (1965) reported "a seeming excess in the lower classes" but found this only true for male patients. A slightly higher proportion of the salicylate population of this study came from social classes four and five than that found in the general overdose population but again the significance of this is uncertain.

A slightly higher proportion (6.6%) of the salicylate overdose population than the general overdose population (5.1%) were classified as "students". This is possibly a reflection of the large number of adolescents taking salicylate overdoses.

Misuse of drugs and reason for admission

Of the general overdose population in only 4.2% of cases was there any evidence of regular drug abuse. This may help to dispel the association in many people's minds between the phrases "drug overdose" and "drug addiction". In conversations with the general public, the author has been surprised by the lack of awareness of society that a drug overdose problem exists entirely separately of the other major problem of drug addiction.

The preponderance of intentional (as opposed to accidental) self poisoning was underlined by the proportion of the general overdose population who either admitted to having or were considered to have taken a deliberate overdose of drugs - 91.5%. Only 2.7% of patients were considered to have overdosed accidentally or in the course of other drug abuse.

Alcohol and nicotine use

Without a means of comparing the results obtained here with the general population, their significance is limited. However, the low proportion of heavy drinkers (17.6%) compared with the number of patients taking alcohol at the same time as the overdose (28.2%) might indicate that alcohol use at the time of overdose is a significant factor. It

could be that patients behaviour was affected by the alcohol or that alcohol was taken deliberately to give "courage" for the overdose attempt.

Problems within the family

In the overdose population generally, the preponderance of interpersonal problems is illustrated by the 28.0% of patients with a defineable problem citing "marital disharmony" as a factor. A similar pattern was seen in the salicylate overdose population but the importance of "cultural problems" is also shown here. This reflects a disturbing trend noticed by physicians, nursing staff and social workers (various personal communications). The problem of the young Asian (usually female) brought up in a Western culture by conservative Eastern parents is increasing, and this type of patient is becoming a regular visitor to hospital after drug overdose attempts which are usually trivial. The problem is compounded by the limited help that can be given to adolescents who find themselves in this position.

Other social factors

Violence in the home, debts, involvement in crime and social isolation or overcrowding did not feature to any great extent as problems in the lives of the overdose patients in this study. This is in direct opposition to the findings of Kessel (1965) who found very high correlation of self poisoning with "indices of social disorganisation" in particular overcrowding, association with criminals and social isolation.

Evidence of psychiatric disturbance

The fact that one quarter of the general overdose population had, at some time, had psychiatric treatment does seem to indicate that a "mental disturbance" factor might be involved in overdose behaviour. This is probably upheld by the fact that 35.7% of patients were diagnosed as having some form of mental illness at the time of their overdose and 27.2% had some form of personality abnormality.

The incidence of mental illness in the general and the salicylate populations was very similar with depression not surprisingly the single most common diagnosis. The only difference between the two populations was the slightly higher proportion of patients with no mental illness in the salicylate population. This could be a reflection of the lower average age of this group. A similar pattern was observed with the personality diagnosis. More of the salicylate population were diagnosed as "normal personalities" and the incidence of alcoholism was lower.

It may be argued that, although mental abnormality was a factor in a third of all overdoses, this was recognisable and possibly amenable to treatment. As such, the emphasis in prevention and research could be shifted to the two thirds of the overdose population who, although psychiatrically normal, nevertheless made themselves ill by taking an overdose of drugs.

Emphasis is often placed upon the manipulative nature of this type of behaviour (Henderson, Hartigan, Davidson, Lance, Duncan Jones, Koller, Ritchie, McCauley, Williams

and Sloughis, 1977; Kerfoot, 1979; Kessel, 1965), but questions must be asked about how the patient knew that the behaviour would achieve a particular outcome. The figures concerning previous exposure to overdose behaviour show that very few patients had experience of it. It could be agreed that removing him/herself temporarily from the prevailing situation was an outcome in itself. Henderson et al. (1977) suggest that Aldous Huxley's descriptive term "chemical holiday" could be used to describe the outcome of an overdose for some patients. It could be that the long accepted maxim that an overdose was a "cry for help" is no longer applicable.

Further psychiatric or social work help was offered in all cases where it was considered that it might be of benefit to the patient. It was evident that further psychiatric assistance was possible in more cases than further social work assistance. There was little difference between the amount of follow up work done with the general and the salicylate populations. It was not within the scope of this study to assess the success of follow up procedures, however, it is generally held that the drop out rate from proffered psychiatric or social work help is very high and this could also be an indication that the taking of an overdose might simply be a form of "opting out" of difficult situations rather than an attempt to get specialist help or the reaction of a disturbed mind,

Day of week and source of drug

In comparison with the even distribution of the general overdose population over the days of the week, salicylate

overdoses showed a definite peak on Tuesdays and Wednesdays. The significance of this is somewhat vague although it could have some connection with the availability of salicylate analgesics. A high proportion of salicylate overdose patients had bought the drug specifically for the overdose and this was more likely to occur during the week when shops were open.

The fact that two thirds of salicylate overdose patients took tablets from large quantity containers is very significant and the unlimited sale of aspirins was criticised as long as fifteen years ago by Kessel (1965). Despite recent attempts to modify the legislation concerning the sale of salicylates, (motivated mainly, however, by the problem of accidental poisoning of children) it is still possible, with very little effort, to buy large quantities of aspirin and other analgesics from many pharmacies and other outlets.

Time of day

The patterns of admissions of the general and salicylate overdose patients were very similar with the hours between 6p.m. and 2 a.m. accounting for almost half of all admissions. The peak of salicylate overdose admissions was slightly more pronounced and occurred during the 6 p.m. - 10 p.m. period. This may have been a reflection of the types of problems contributing to salicylate overdose in the younger patient. Cultural problems and arguments with parents about boy/girl friends and "going out" featured regularly.

Evidence that poison was taken

The confirmation of overdose by toxicological evidence was available in two thirds of all overdose cases. This is higher than might be expected (Wright, to be published), and represents an encouraging trend as patients' or other history is notoriously unreliable when the type or amount of drug taken is requested.

Number of substances taken

The results show conclusively that patients having taken salicylate overdoses rarely take more than one drug. The occurrence of multiple drug overdose was higher in the general overdose population, and there was little difference between male and female patients in the number of substances ingested.

The 70% incidence of single drug overdose was very similar to that found by Holland et al (1975) and Glauser and Smith (1975).

Length of time between ingestion and admission

A slightly lower proportion of salicylate overdose patients than those in the general overdose population were admitted within four hours of ingestion of the drugs (63.3% as opposed to 71.22%). This could be a result of the lack of respect shown for salicylates generally. It is likely that an overdose of such a familiar drug might not cause the concern to relatives that an overdose of (for example) a tranquilliser would and the patient would not necessarily be rushed to hospital until he/she started to show symptoms of poisoning.

Length of stay in hospital

Salicylate overdose patients tended to leave hospital sooner than patients in the general overdose population. This might be taken as an indication that the salicylate overdoses seen were less severe than overdoses of other drugs but is as likely to be a reflection of the lower incidence of complications such as mental and physical illness or the relative success of treatment.

Other system complications

The incidence of complications of any sort in the general overdose population was very low and the death rate of 0.2% was lower than any reported elsewhere. (Middleton et al., 1961; Rendy, 1973; Murray et al., 1974; Holland et al., 1975; Petersen and Brosstad, 1977 and Jones, 1977).

Use of laboratory facilities

As can be seen by the proportions of patients requiring biochemical, toxicological or haematological investigation, the demands made by overdose patients on laboratory facilities were very great. Emergency investigations were often required, necessitating the use of stand-by staff. The extent of usage of facilities found in this study reinforces the need for adequate laboratory facilities expressed by both the Atkins and Hill reports.

Treatment required

The results of this study show that conservative treatment was the main feature of the clinical care of

overdose patients. Gastric lavage and administration of maintenance fluids were the measures most often employed, along with forced diuresis for salicylate intoxication. The use of specific antagonists were limited to the administration of cysteamine for paracetamol overdose. This correlates well with the findings of Sivner and Goldberg (1978).

4. Conclusions

. The female:male ratio of approximately 2:1 confirms the findings of other workers.

. Males who overdose tend to be slightly younger than females.

. There is little connection between drug abuse/addiction and drug overdose behaviour.

. The majority of overdoses admitted are intentional.

. It is possible that alcohol is a significant factor in affecting behaviour at the time of overdose.

. The problems associated with drug overdose behaviour tend to be of an interpersonal nature with marital disharmony and cultural problems a major factor, along with psychiatric abnormality in the form of depression.

. It is possible that the well worn description of overdose behaviour as a "cry for help" may be outdated and that many instances of drug overdose behaviour could be interpreted as "opting out" of stressful situations.

. The peak time of day for overdose admissions is the evening.

. Most overdose patients take only one drug.

. Overdose patients tend to stay in hospital for only a short period.

. The demands made by overdose patients on laboratory facilities, especially the emergency service, are great.

. Conservative treatment is usually sufficient to ensure satisfactory recovery.

. The mortality rate from overdoses admitted to Dudley Road Hospital is very low.

. Despite reports of declining trends in salicylate analgesic overdose, the proportion of overdosed patients who take aspirin as the sole or primary overdose drug is very high.

. Salicylate overdosed patients tend to be slightly younger than the general overdose population.

. The incidence of psychiatric disturbance in the salicylate overdose population is similar to that in the general overdose population.

. Patients taking overdoses of aspirin tend to take the tablets from large quantity containers which they buy themselves.

. Salicylate overdose patients tend to arrive at hospital later after the incident than cases of overdose of other drugs, and tend to spend less time in hospital.

. A regular follow-up study on the trends in aspirin/paracetamol overdose would be interesting.

PART B A Biochemical Study of Acute Intoxication with Aspirin

Section 1 Introduction

1. Chemical Structure

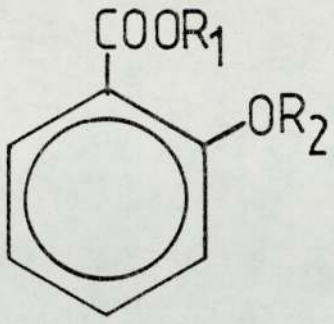
Aspirin belongs to the group of drugs collectively known as "salicylates". The parent structure of the group is shown in Figure 11.

Aspirin is a member of the chemical group "carboxylic acids" which characteristically possess the functional group -COOH. The carboxylic acids are weak acids and present usually as free acids or salts (e.g. the Na salt).

From the structure of its molecule it can be seen that aspirin is also an ester as it has the functional group -CO-O-. Esters usually present as neutral molecules. Thus aspirin can be expected to be soluble in both aqueous and organic solvents. This fact is mentioned here as the degree of ionisation of drug molecules generally is important in determining the rate at which drugs pass through biological membranes and are thus absorbed and subsequently metabolised.

In general, drugs pass through biological membranes mainly by simple diffusion. For non-electrolytes, diffusion occurs according to the lipid solubility of those molecules which are not ionised. Obviously, the proportion of ionised and non-ionised molecules of drug is dependent upon the pH of the medium and the pKa of the drug itself. The pKa is the pH of the aqueous solution in which the compound is 50% ionised and, in the case of aspirin, this is 3.5.

a)



b)

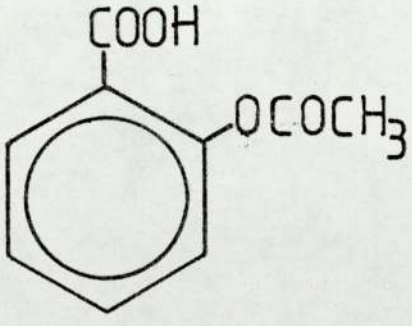


Figure 11. Parent structure of salicylate a) and structure of aspirin b).

It is generally held that the major site of absorption of aspirin is the small intestine and that conditions causing rapid gastric emptying enhance absorption of the drug. At first sight, this would seem a paradox as the very acid nature of stomach contents (pH of approximately 1 as opposed to the pH of the intestine of about 6) would favour high proportions of unionised drug and thus enhance absorption better than the conditions in the intestine. However, other factors must be considered, especially the fact that the intestine has a much larger surface area for absorption. It is also possible that aspirin passes more rapidly through the intestinal lumen than through the stomach lumen (Curry, 1977).

Levy and Leonards (1966) give an extensive account of absorption and all the factors which influence it, and as this is comprehensively treated, further discussion here is inappropriate.

2. Metabolism

The metabolism of aspirin follows a generalised pattern of a hydrolysis reaction followed by various conjugation reactions and, as such, is of a routine nature in the context of general drug metabolism. The metabolism of acetylsalicylic acid begins within minutes of its ingestion with its absorption from the stomach into the blood, and later after absorption from the small intestine.

(i) Initial hydrolysis to salicylic acid

The first step in aspirin metabolism is the hydrolysis/deacetylation shown in Figure 12.

The reaction is believed to be too rapid to be accounted for by acid/base catalysed systems and is thought to be primarily due to enzyme action.

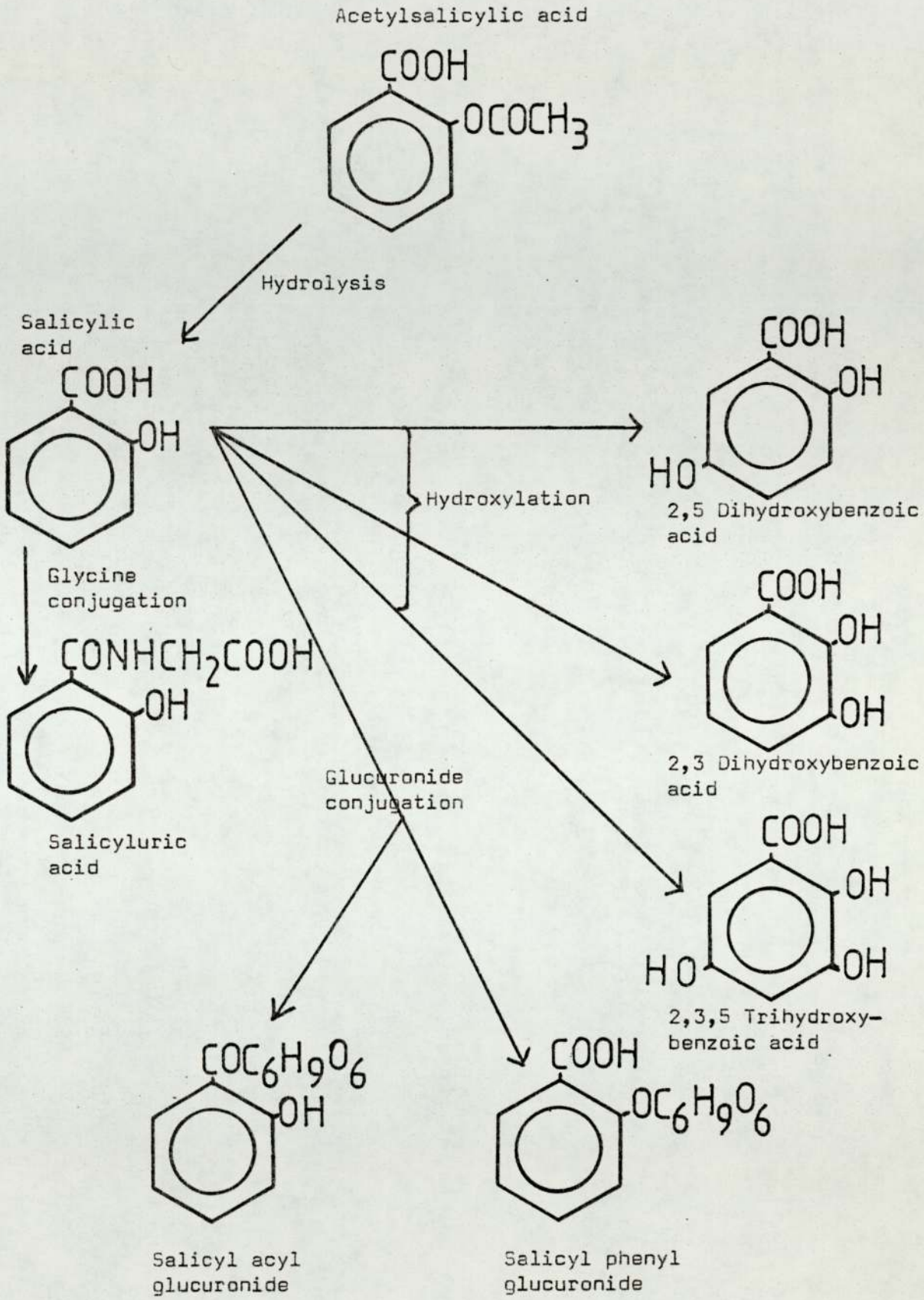


Figure 12. Routes of metabolism of acetylsalicylic acid.

The enzymes involved are arylesterases (Sechserova, Sechser, Raskova, Jecna, Elis and Vanacek, 1975, Testa and Jenner, 1976), sometimes dubiously referred to as "aspirin esterases" (Menguy, Desbaillets, Masters and Okabe, 1972) which preferentially hydrolyse aromatic esters. In general, hydrolases which metabolise exogenous compounds such as drugs often belong to the normal metabolic pathways of endogenous substances or nutrients. These enzymes are quite widely distributed in the body, occurring in liver, kidney, plasma, intestine and elsewhere. The presence of arylesterases in plasma is believed to account for 20% of the hydrolysis of acetylsalicylic acid. The remaining 80% is hydrolysed by similar enzymes in liver and, to a lesser extent, kidney (Rowland and Riegelman, 1967).

Levels of this enzyme have not been investigated in any depth, although the hydrolysis/deacetylation of acetylsalicylic acid has been known since 1943 (Vandell and Scantrim, 1943). Menguy et al. (1972) thought that differences in levels of the enzyme could account for the higher incidence of gastric ulceration with regular aspirin use in women than men reported by Chapman and Duggan (1969). They postulated that, as gastric injury results from the intact aspirin molecule rather than salicylic acid (Brodie and Hooke, 1971), people with lower levels of arylesterase may be more susceptible to gastric injury. They compared levels of arlyesterase in samples of plasma from human males and females by incubating the plasma with known amounts of aspirin under constant conditions and assaying the salicylic acid produced. Their results showed a significantly lower level of enzyme activity in female plasma. They had also showed that there is a definite sexual difference

in the activity of the enzyme in rat liver homogenates: again female rats showed lower enzyme activity.

Of the salicylic acid thus formed, 10-85% may be excreted as free salicylic acid (Alpen, Mandel Rodwell and Smith, 1951). Studies in humans and dogs (Alpen et al., 1951, Mandel, Rodwell and Smith, 1952) and in rats (Quilley and Smith, 1954, Schayer, 1950) have shown that the remainder is then further metabolised by the general metabolic routes of glucuronide formation and ring hydroxylation and also by conjugation with glycine (see Figure 12.

(ii) Glycine conjugation

The conjugation of glycine with salicylic acid was first reported in 1856 by Bertagnini (Bertagnini, 1856). Quick (Quick, 1933) remarked that structural factors indicated that the formation of salicyluric acid from salicylic acid and glycine would be expected to occur on only a small scale since the substitution of the benzene ring in the ortho position, as in salicylic acid, would tend to inhibit reaction of glycine with a carboxyl group on the benzene ring. In fact the proportion of a dose of aspirin excreted as salicyluric acid does vary due to a variety of factors to be discussed in detail later.

Glycine conjugation is an important method of detoxication of many molecules with available carboxyl groups. The enzyme system involved is found in kidney and liver, although there are species differences in the relative importance of these organs. There are also species differences in the cytoplasmic location of the system - the enzymes are variously described as "mitochondrial" and "microsomal".

The conjugation proceeds in two basic steps: first the formation of an "active" carboxyl group, then its transfer to glycine by glycine-N-acylase. The complete reaction is represented in Figure 13.

Quick (1933) noticed that, with increasing doses of salicylate, free salicylic acid excretion increased but the excretion of salicyluric acid was only slightly altered. Bedford, Cummings and Martin (1965) reported that formation of salicyluric acid was rate limited after a dose of 0.3 gm of aspirin and Levy (1965) showed a similar effect after a dose of 0.36 gm aspirin equivalent. It appeared that even at this therapeutic dose level, conjugation of glycine and salicylic acid reached a maximum, and from there proceeded by zero-oder kinetics. Limited capacity for salicyluric acid formation was again confirmed by Gibson, Zaphiropoulos, Grove, Widdop and Berry (1975). Cummings, Martin and Renton (1966) had considered this rate limitation in terms of saturation of an enzyme or transport system.

Levy (1971) pointed out that salicylurate formation is quantitively the most important route of elimination of a therapeutic dose of aspirin and that, if its capacity could be increased, relatively less free salicylic acid would remain in the system to cause adverse effects. Hippurate formation is an analogous process and produces hippuric acid from benzoic acid. It is known that administration of glycine can increase the formation of hippuric acid, however the same is not true for salicylic acid (Quick, 1933, Nelson, Hanano and Levy,1966). Thus a different rate limiting step is indicated.

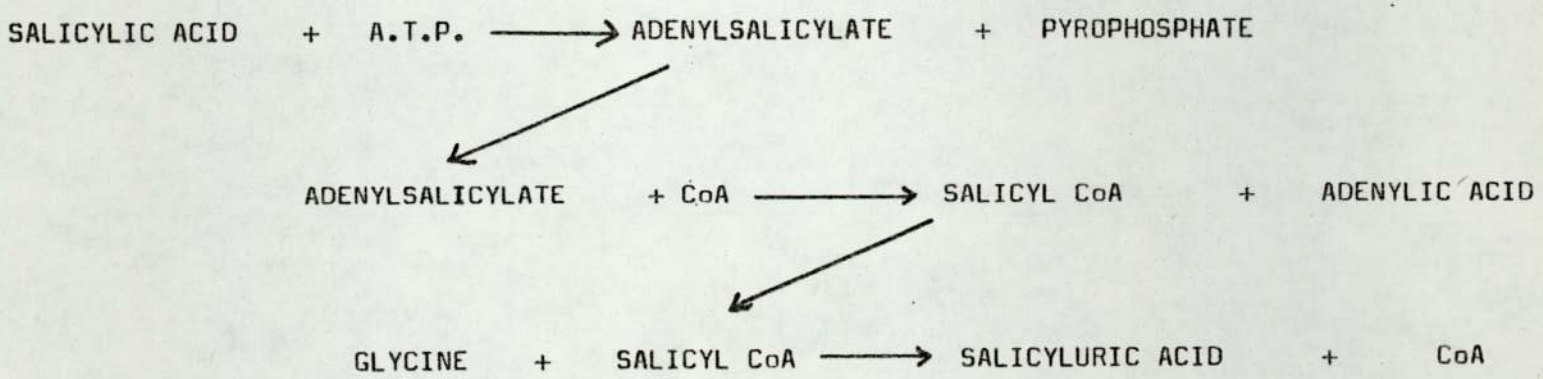


Figure 13. Representation of glycine conjugation of salicylic acid.

Forman, Davidson and Webster (1971), while demonstrating for the first time "in vitro" salicylurate formation, showed that overall this was dependent upon adenosine triphosphate, magnesium chloride, coenzyme A, glycine and the active enzyme. They also showed that the rate limiting step was the first - "activation" - step. These experiments were done using beef liver protein as the source of enzyme, and also with synthetic coenzyme A.

There appears to be a genetic factor involved in salicylurate formation. Gupta, Paulus and Pearson (1973), studying plateau serum salicylate levels in twins concluded that individual variations in plateau serum salicylate levels on the same weight adjusted dose were due to genetically determined variations in their capacity to produce salicyluric acid. This was confirmed by further experiments on twins by Furst, Gupta and Paulus (1977) who also reported induction of salicylurate formation with chronic therapy.

(iii) Glucuronic acid conjugation

Again the extent to which the conjugation of salicylic acid takes place, in this case with glucuronic acid, is variable, showing differences between species and between individuals. In man, the conjugates thus formed may account for 15-40% of a therapeutic dose of aspirin (Alpen et al., 1951). Glucuronic acid conjugation is an important general detoxication reaction. It is also important in carbohydrate metabolism as it is dependent upon a supply of glucose-1-phosphate.

Traditionally, the liver was regarded as the main, if not only, site of glucuronic acid conjugation, but Schachter, Kass

and Lannon (1959) reported that the carboxyl and phenolic groups are conjugated "in vitro" with glucuronic acid by slices of guinea pig intestine, liver, kidney, urinary bladder, lung and spleen. In the rabbit and hamster the intestine was more efficient than liver.

The mechanism of formation is a two-stage process. There is synthesis of a coenzyme donor, followed by transfer of the glucuronyl moiety from this to the substrate. This is more fully represented in Figure 14 . Microsomal UDP-glucuronyl transferase enzyme systems guide the transfer of the glucuronyl group to the acceptor molecule.

For a drug to undergo this kind of reaction it obviously must have an available -OH group. It is also obvious that salicylic acid has two of these groups and thus two possible sites for acceptance of the glucuronyl group. The two metabolites thus formed are termed "acyl" glucuronide, when the glucuronyl group attaches to the carboxylic -OH, and "phenolic" glucuronide when it attaches to the phenolic -OH.

As with salicyluric acid formation, there appears to be a saturation of glucuronide formation with differing doses. Levy, Tsuchiya and Amsell (1972) reported a study showing a limited capacity in man for salicyl phenolic glucuronide formation which was evident in the therapeutic dose range. This was not the case with the acyl glucuronide. It is difficult to see, on the basis of existing knowledge about the mechanisms of acyl and phenolic glucuronide formation, where this differentiation could take place, unless a different transglucuronidase is responsible for the respective reactions.

Figure 14. Representation of glucuronide conjugation of salicylic acid (continued overleaf).

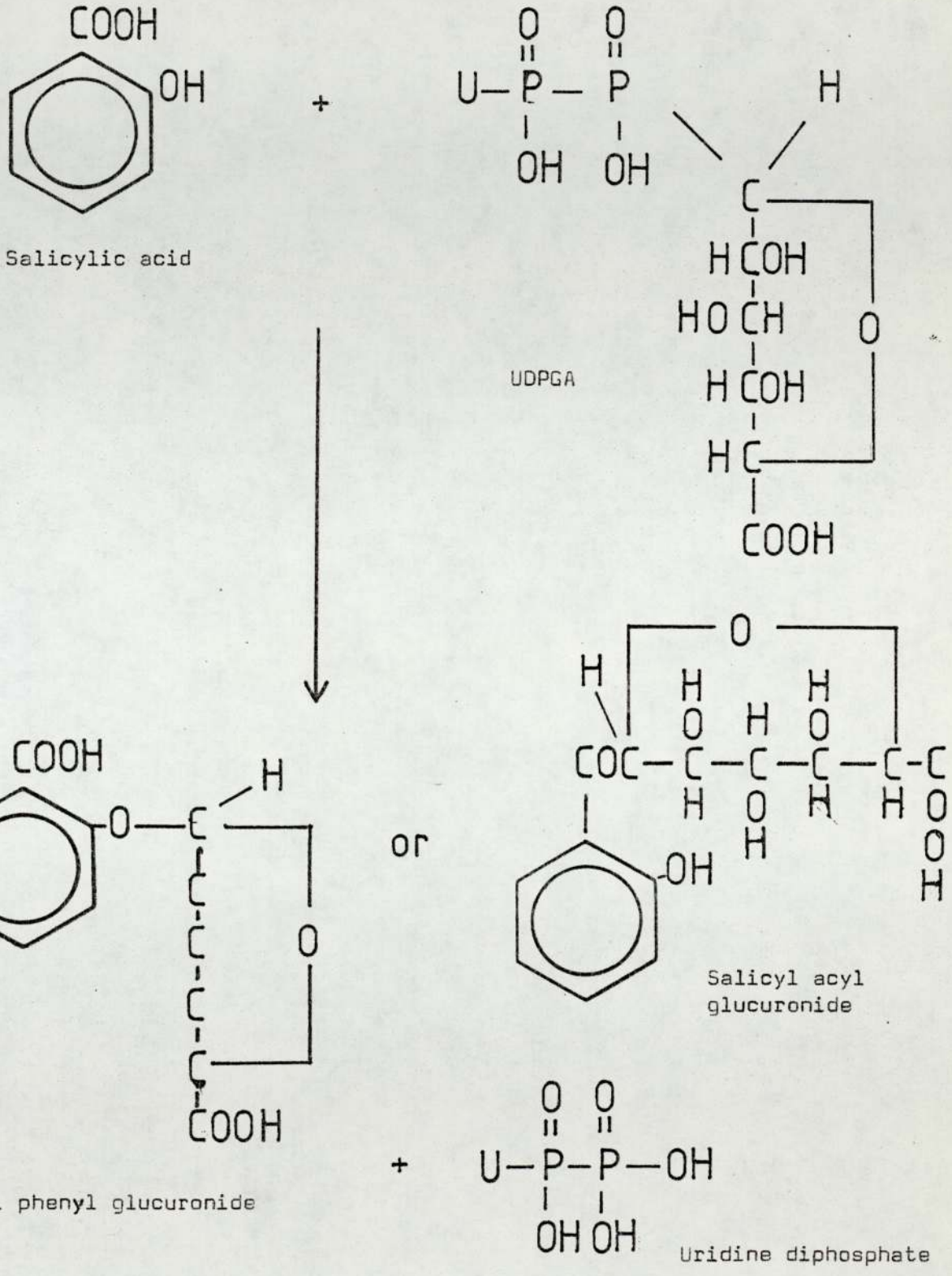
Glucose-1-phosphate + Uridine triphosphate (UTP) $\xrightarrow{\text{Uridyl transferase}}$

Uridinediphosphate-α-D-glucose (UDPG) + Phosphate

and

UDPG + 2NAD $\longrightarrow$ Uridinediphosphoglucuronic acid (UDPGA) + $NADH_2$

Figure 14 (contd.)



An important clinical aspect of glucuronide formation is the possibility of competitive inhibition of metabolism of simultaneously administered drugs sharing this pathway of metabolism. Levy and Procknal (1968) reported a marked decrease in the formation of glucuronides of co-administered salicylamide and sodium salicylate at therapeutic dose levels. They considered that the interaction may result from competition for UDPGA or UDP glucuronyl transferase with the possible contribution of the uncoupling effect of salicylate on oxidative phosphorylation.

This effect is not really surprising since enzyme systems which have been shown to be saturable (Levy and Procknal, 1972) are involved. However, it does have serious clinical implications in that intensity and duration of pharmacological effects of the drugs may be altered.

The importance of this phenomenon is increased when it is considered that the mutual inhibition previously cited is between salicylamide and salicylate; two compuunds that are frequently found in combination in proprietary products such as Anadin, Beecham's Powders and Phensic. Levy (1971) points out that interactions such as this could be used as investigative tools in pharmacokinetics and that it may be possible, on consideration of such phenomena, to increase the effectiveness and/or therapeutic ratio of medicines containingthese types of compound.

Another important factor is that salicylate-containing analgesics are frequently taken in overdose and it is uncertain at present, what effect this mutual inhibition might have on the course of clinical symptoms in the poisoned patient.

Gibson, Zaphiropoulos, Grove, Widdop and Berry (1975) suggested that there are inter-subject variations in glucuronide producing capacity at higher doses, and that these differences may affect plasma salicylate levels.

Klinenberg and Miller (1965) have also reported interaction of aspirin and corticosteroids. They considered that glucuronide formation was involved but that mutual interference in urinary excretion was also a factor. Tsuchiya and Levy (1972) suggest that our increased knowledge of the salicylate pharmacokinetics should permit the formulation of optimum dosage regimes for therapy using salicylates. This is especially important in the large field of chronic therapy in inflammatory disease.

(iv) Hydroxylation

The products of the hydroxylation of the phenol ring of salicylic acid are generally held to be minor metabolites found only in trace quantities.

They include 2,5 dihydroxybenzoic acid (gentisic acid), 2,3 dihydroxybenzoic acid and 2,3,5 trihydroxybenzoic acid (see Fig. 12).

(v) Clinical implications of metabolic route

It would be appropriate at this point to reemphasise the clinical implications of various aspects of salicylate metabolism, beginning with the sexual differences mentioned earlier. It was noted that there exists a definite sexual difference in the activity of aryl esterase in humans and rats (Menguy et al., 1972). Sechserova, Sechser, Raskova, Jecna, Elis and Vanacek (1975) enlarged upon this by post-

ulating that sexual differences in metabolism of salicylates may not be limited to differences in arylesterase activity. Their work with calves and mice showed a higher toxicity of salicylates in females although, at that stage, they were not in a position to decide whether the difference was due to primary genetic differences or to differences in sex hormones. It would be interesting to know how, if at all, this difference could affect the prognosis for the patient poisoned by salicylate.

We have also seen how some routes of metabolism are readily saturated at even therapeutic levels. Levy (1976) points out succinctly the clinical implications of non-linear pharmacokinetics: "...(a) the time required to eliminate a given fraction of a dose increases with increasing dose, (b) the steady state salicylate concentration in plasma increases more than proportionally with the dose rate, and (c) the time required to attain steady state salicylate concentrations increases with increasing dose...". It is obvious that as dose levels approach those seen during deliberate self poisoning , these considerations become even more important, as they do when high doses of salicylates are chronically administered, for example in rheumatiod arthritis (Levy and Tsuchiya, 1972).

3. Intoxication

(i) Symptomatology

It has been appreciated for many years that there is poor correlation between the amount of salicylate ingested and the severity of toxic effects, and also between the amount

of circulating salicylate and the severity of symptoms. As an example of the type of discrepancy seen, it has been observed (Done, 1960) for a patient to die with a salicylate blood level of 15 mg/100 ml(often seen with chronic salicylate therapy) but for another patient to remain assymptomatic with a blood level of 50-60 mg/100 ml. This fact often makes prognosis difficult.

The symptoms of salicylate overdose include gastric irritation, impairment of prothrombin time, tinnitus (ringing in the ears) and hyperventilation - a rapid and deep breathing which could lead to a respiratory alkalosis: a disturbance of carbohydrate metabolism which could lead to a metabolic acidaemia and an increase in metabolic rate which could cause hyperpyrexia.

These symptoms arise as a result of the interference of a large dose of salicylate with various aspects of normal physiology, most of which control the acid/base balance of the body. Thus the characteristic feature of a salicylate overdose is a severe acid/base imbalance, along with a varying degree of central nervous system involvment, depending on the severity of the overdose.

(ii) Central nervous system effects of salicylate intoxication

Hyperpnoea

Hyperpnoea is possibly the most obvious symptom of salicylate overdose, often making the obtaining of a history from the patient difficult as he or she is being forced to breathe deeply and rapidly and is in some discomfort.

It was known that hyperventilation was a central effect of salicylates as long ago as 1955 when Tenney and Miller (Tenney and Miller, 1955) showed that salicylate in the vicinity of the respiratory neurones of the dog stimulated ventilation. The increase was dramatic and independent of chemoreceptors and the vagus nerve. This fact was confirmed by Brem, Pereli, Gopalan and Miller (1973) who conducted a series of experiments on dogs in which they showed that salicylates, peripherally administered, crossed the blood/brain barrier and acted directly on the respiratory neurones to stimulate respiration. Buchanan, Kundig and Eyburg (1975) suggested that the salicylate is transported to the medulla via the cerebrospinal fluid (csf).

Stimulation of respiration is the cause of the respiratory alkalosis seen in many patients, the degree depending on their age and their sensitivity. There seems to be a difference in the prevalence of respiratory alkalosis in different age groups. Arora, Sridharan, Schacht and Martin (1974) consider it to be more pronounced in children than in adults and infants. Buchanan et al. (1975) stated that it was either uncommon or short lived in infants but that its incidence increased between the ages of 4 to 12 years when what they call an "adult pattern" emerged. They state that respiratory alkalosis occurred in adults although it was sometimes supervened by other effects to be mentioned later. There was no apparent explanation for these age differences, or the apparent inter-individual differences in susceptibility to this effect.

Respiratory alkalosis results from the increased expulsion of alveolar carbon dioxide which in turn results in a reduced partial pressure of carbon dioxide in the blood. This causes a fall in the hydrogen ion content of the blood. It also has several other side effects. The kidneys attempt to compensate for this by increasing the excretion of bicarbonate, reducing plasma bicarbonate and thus the buffering capacity of the plasma, and at the same time causing diuresis. There is also a movement of potassium into cells causing a possible hypokalaemia.

There is also evidence (Tenney and Miller, 1955) that high levels of salicylate increase the sensitivity of the medullary respiratory centre to carbon dioxide, thus stimulating respiration further and adding to the alkalosis. The alkalosis serves to ionise further any free salicylate and thus decrease the amount available to be absorbed into the central nervous system.

Other effects

There are other symptoms of the effects of salicylate on the central nervous system including disorientation and tinnitus and in serious poisoning, convulsions and coma. These appear as a result of a general CNS disfunction.

In an attempt to define the precise mechanism involved, Thurston, Pollock, Warren and Jones (1970) examined the effects of salicylate on brain glucose in mice. They found that there was a reduction in brain glucose although plasma glucose levels were normal. They also found that concurrent I.P. administration of glucose with the salicylate caused an

improvment in the condition of the animals and their chances of survival. They suggested that, in salicylate poisoning, the glucose supply to the brain may be inadequate although plasma levels could be normal. Hill (1973) reported that there might be a critical level of salicylate in brain after which death is likely.

Woods, Stubbs, Johnson and Alberti (1974) reminded us that salicylates have been used as hypothermic agents although the mechanism of this effect had never been elucidated. Their experiments on perfused rat liver sought to correct this, and it seems that the CNS effects might be the results of the general action of salicylates on oxidative phosphorylation to be discussed in more detail later.

It appears then that the CNS dysfunction seen in salicylate overdose may be connected with inadequate brain glucose levels as gluconeogenesis is inhibited by a fall in high energy phosphate levels as a result of uncoupling by salicylate of oxidative phosphorylation.

(iii) Peripheral effects of salicylate intoxication

Most discussions of salicylate intoxication mention the occurrence of acidaemia. Tenney and Miller (1955) considered that this was a reflection of respiratory acidosis due to impaired ventilation. However Proudfoot and Brown (1969) reported a study of salicylate poisoning in adults which showed clearly that the acidaemia was associated with a metabolic acidosis of a separate origin.

It is generally held (Proudfoot and Brown, 1969; Winters, 1959; Bender, 1975; Woods et al., 1974; Smith, 1966; Done,

1963) that metabolic acidosis results from an accumulation of organic acids as a result of interference with several metabolic pathways. The organic acids implicated are lactate and pyruvate (Thurston et al., 1970; Woods et al., 1974) malate, fumarate and citrate (Smith, 1966) and β-hydroxybutyrate and acetoacetate (Bender, 1975), although the mechanisms involved are still not fully understood.

Inhibition of gluconeogenesis

When considering CNS effects of salicylate overdose, it was mentioned that the uncoupling of oxidative phosphorylation and the consequent drop in high energy phosphate levels inhibits gluconeogenesis. That salicylates uncouple oxidative phosphorylation in isolated mitochondria was shown by Brody (1956). Oxidative phosphorylation is the process by which the energy obtained from food breakdown products is eventually trapped within "high energy" phosphate bonds of adenosine triphosphate (ATP). This energy can be released later by hydrolysis to provide energy for various purposes including synthetic reactions such as the production of glucose from substrates - gluconeogenesis. "Uncoupling" of this process simply means that the link between the two steps of oxidation and phosphorylation required to form ATP is broken and ATP production from this source is diminished.

The substrates for gluconeogenesis are primarily lactate and alanine, and inhibition by salicylate of this process was shown by Woods (1974). The substrates might be expected to accumulate under these circumstances and measurements of lactate and alanine levels in salicylate overdose may clarify this point. However Woods (1974) also stated that "No reliable

measurements have been reported of blood lactate...concentrations in cases of salicylate intoxication...". Thurston et al. (1970) reported that lactate levels in brain and liver of mice were increased after I.P. injections of salicylate, and Buchanan and Rabinowitz (1974) found "mildly elevated" arterial lactate levels in poisoned infants.

Stimulation of glycolysis

The uncoupling effects of salicylates will also stimulate glycolysis which would be expected to increase levels of pyruvate, and here another effect of salicylate becomes important. It has been known for many years (Buchanan, 1975) that salicylates inhibit tissue dehydrogenases and aminotransferase enzymes. These enzymes are responsible for the conversion of pyruvate to lactate and alanine and their inhibition would imply an accumulation of pyruvate. Thurston et al. (1970) reported no change in brain and liver pyruvate in their salicylate intoxicated mice and Buchanan and Rabinowitz (1974) reported normal pyruvate levels in poisoned infants. The excess pyruvate might be expected to be converted into acetylcoenzyme A and excess of this could increase the levels of ketone bodies such as hydroxybutyrate and acetoacetate. Winters (1959) considered that this ketosis, more commonly seen in infantile therapeutic overdose accounted for this rise in organic anions and it is a fact that infants are more likely to present metabolic acidosis than all but the most severely poisoned adults (Arora et al., 1974). The excess acetylcoenzyme A could normally be taken up by the tricarboxylic acid cycle, however, the inhibitory effect of salicylate on dehydrogenase and aminotransferase enzymes mentioned earlier

would tend to prevent this. The inhibition of these systems might also cause accumulation of intermediate anions such as malate, fumarate and citrate, but there is no evidence that this does in fact happen.

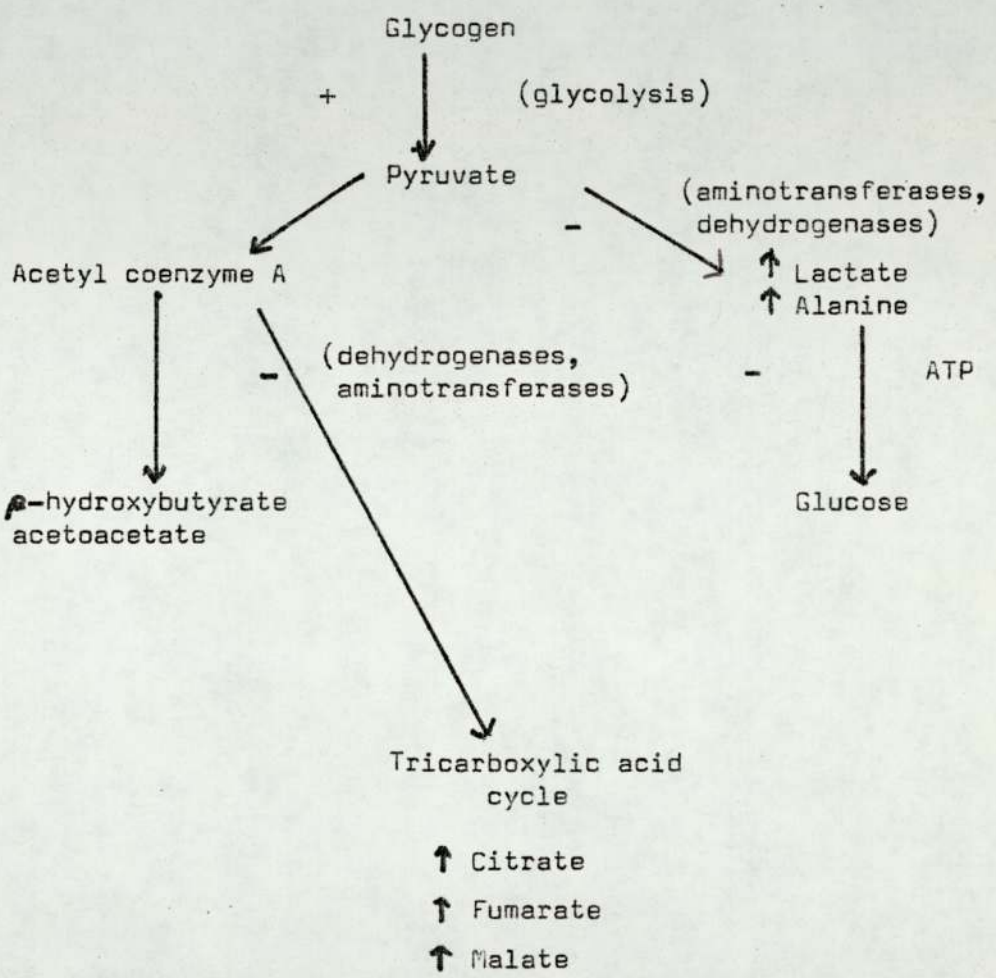
The complicated picture of salicylate interference with carbohydrate metabolism and the consequent metabolic acidosis is summarised in Fig. 15.

It seems that there is empirical evidence for accumulation of certain organic anions, however this does not appear to occur on such a large scale for any individual compound to in itself cause the acidaemia, and the source of this symptom of salicylate overdose remains largely a matter for conjecture.

We have seen, then, that there are two major features of salicylate overdose; a respiratory alkalosis and a metabolic acidaemia. The two would appear to be mutually exclusive, but the picture is more complicated than this.

Stimulation of respiration and consequent alkalosis is, as mentioned previously, one of the first events in the course of poisoning. However, the metabolic acidosis described may supervene to various degrees depending upon age, so that most patients show a mixed disturbance (Proudfoot and Brown, 1969). To summarise this situation, it seems that in infants, (birth to 4 years) the acidaemia becomes a major feature (Arora et al., 1974; Buchanan, 1975). In children (4 to 12 years) the alkalosis is more pronounced than the acidaemia (Arora et al., 1974; Buchanan, 1975) as it is in adults (Arora et al., 1974; Proudfoot and Brown, 1969; Buchanan, 1975) although Proudfoot and Brown (1969) and Arora et al. (1974)

Figure 15. Model of salicylate interference with carbohydrate metabolism.



+ represents a stimulatory effect of salicylates

- represents an inhibitory effect of salicylates

↑ represents possible accumulation of substances thus marked

note that some adults do show a predominant acidaemia. Proudfoot and Brown (1969) suggest that this anomalous reaction may be a result of an abnormal response by some individuals to give an overwhelming increase in anions.

(iv) Correlation between blood levels and clinical severity of intoxication

One of the main problems in the management of salicylate poisoning is the poor correlation between blood levels of salicylates and the clinical severity of poisoning mentioned previously. Consequently there is no reliable index of the likely severity of a poisoning episode.

Done (1960) attempted to overcome this difficulty by taking into account the length of time elapsed since the salicylate was ingested. He suggested two ways that this could be done, the first being a calculation to arrive at a theoretical salicylate level at zero time. This is largely discredited now as it was based on the assumption that salicylate excretion was a first order reaction and this has since been shown to be incorrect (Levy, 1965). He also constructed a nomogram which, given the salicylate level at a known time after ingestion, could indicate the likely severity of poisoning. Unfortunately both methods tend to oversimplify an extremely complicated picture. It assumes primarily that blood levels of salicylate directly reflect tissue levels which, it will be seen later, is not necessarily the case.

We are left with the question of what actually causes the death of a patient poisoned with salicylates. Proudfoot and Brown (1969) concluded that "Acidaemia is shown to be

associated with impaired consciousness and to carry a grave prognosis...". Their study concerned adults but it is well known that infant mortality rates from salicylate overdose are higher than those of adults and when this is considered along with the greater prevalence of acidaemia in infants, this kind of acid/base imbalance is certainly implicated in severe toxicity.

Proudfoot and Brown (1969) suggested that this, along with the fact that impaired consciousness has been reported with normal or high blood pH might indicate that it was not so much the status of the blood that was important as the status of the CSF. More recent reports concerning the direct action of salicylates on the central nervous system, both in lowering glucose levels and on the respiratory system, indicate the mechanism that might be involved.

For example, Hill (1973) states that patients who die from salicylate overdose show symptomsof a primary central nervous disfunction, the mechanism of which has been described earlier. He found that when rats were given a lethal dose of salicylate, they died with a wide range of blood levels (45–195 mg/100ml) but more important, their brain levels showed little variation. He suggested that death occurred when a critical brain level was reached.

For salicylate to enter the brain it has to cross the "blood/brain barrier" (BBB), which gives preferential passage to lipid soluble molecules. Thus only unionised molecules of salicylate will be able to cross. Here can be seen the importance of the acid/base balance, as an acidaemia will tend to increase the proportion of salicylate molecules which

are unionised. At the normal pH of 7.4, salicylic acid is 99.996% ionised, however a drop in pH to 7.2 will double the proportion of unionised molecules from 0.004% to 0.008% (Hill, 1973). Thus acidaemia could facilitate the entry of salicylate into the CNS and exacerbate the toxic effects discussed previously.

(v) Treatment of salicylate intoxication

The aim of treatment of any illness is initially to maintain life, arrest or reverse the progression of the most serious symptoms and to remove the cause of those symptoms. The symptoms of salicylate overdose have been discussed in detail in a previous section and the aim of this section is to summarise the main methods of treatment used to deal with these symptoms and to remove from the body their cause:- excess salicylate. It is clear that most workers consider the early correction of electrolyte imbalance as the most important course of treatment. Arora et al.(1974) stress the importance of adequacy of respiration and the state of hydration of the patient and Buchanan (1975) considered that the return of the blood pH to normal in infants is imperative. Trapnell (1976) considered that the correction of acid/base balance "requires major emphasis" and Bender (1975) suggested that in the correction of acid/base balance and fluid/electrolyte balance, rehydration was usually adequate. However, these measures alone would be pointless unless other steps were also being taken to prevent further reabsorption of or to accelerate excretion of salicylate from the body. If the diagnosis of salicylate overdose is complicated by a state of unconsciousness, a history of renal insufficiency, evidence

of kidney malfunction, extremely high blood levels of salicylate or a severe acidosis (Buchanan, 1975; Trapnell, 1976; Bender, 1975) it may be that more drastic regimes of treatment rather than the conservative measures suggested above may be indicated. These may include some forms of diuresis, dialysis, haemoperfusion or even exchange transfusion.

Initial Treatment

Symptoms looked for in salicylate overdose have been described in detail in a previous section. When a suspected overdose patient arrives in a casualty department the usual observations of pulse rate, blood pressure, respiratory rate and state of consciousness are made, along with an attempt to obtain a history of the episode. Other signs looked for in suspected salicylate overdose include deafness/tinnitus, epigastric pain, presence or absence of flushing, sweating and hyperventilation. Wherever poosible a sample of blood and/or urine is obtained for analysis. If the time elapsed since ingestion is less than about 4 hours (Bender, 1975) it is likely that there may be unabsorbed salicylate still present in the stomach and intestines and an attempt is made to remove this. The most usual method is by gastric lavage or emesis, or by the administration of activated charcoal which "mops up" any drugs still present in the stomach and upper digestive tract.

Gastric lavage is carried out by means of a Jaques tube which is introduced into the stomach and then washed out using water or other suitable solutions. Ipecachuana, although having been accused of being slow acting, may be preferable as formed tablets are more easily recovered

(Bender, 1975). Stomach contents, as opposed to washout fluids, also give a more accurate idea of the quantity of salicylate present on analysis as they are not artificially diluted.

Further treatment

After the gastric lavage, which normally takes place in the casualty department, the patient is usually formally admitted to a ward for observation. The need for further actionother than observation depends on the salicylate level in the plasma, although, as already stated, plasma levels may not be the best guide to the likely severity of poisoning but until a better method of assessment is developed, it will still be used.

Beveridge, Forshall, Munro, Owen and Weston (1964) considered that salicylate intoxication was confirmed by levels in the plasma of over 40%. If levels were greater than 100% haemodialysis was recommended. Similarly, if the patient had a history of renal insufficiency or was in a coma or circulatory collapse had occurred. If intoxication was confirmed but was less severe than this, they recommended the use of alkaline diuresis or osmotic diuresis using (for example) mannitol. A similar view was taken by Brem et al. (1973) who recommended peritoneal dialysis or haemodialysis in cases where normal excretion was prevented due to poor kidney function. They also pointed out that the initial hyperventilation seen in salicylate intoxication is characterised by a respiratory alkalosis which limits the passage of salicylate to the brain and helps to promote its excretion by the normal kidney. Hill (1973) also takes up this point, stating that

"...alkalaemia is a desired state". He recommended that this should be maintained by the administration of bicarbonate. The lowering of plasma levels of salicylate by bicarbonate was noted by Coombs, Warren and Highley (1945).

The use of carbonic anhydrase inhibitors

Hill (1973) cautioned, as did Buchanan (1975) against the use of acetazolamide to alkalinise the urine. Acetazolamide is a carbonic anhydrase inhibitor which acts on renal cell carbonic anhydrase to alkalinise the urine and thus prevent reabsorption of salicylate and aids its excretion.

The disadvantage with this compound is that it also acts on erythrocyte carbonic anhydrase and thus affects the red cell transport of CO_2 leading to a respiratory acidosis - a dangerous state as this favours the uptake of salicylate into the CNS and can thus increase toxicity, as shown in animals (Brem et al., 1973).

Liddell and Maren (1975) claim to have overcome this problem by the use of benzolamide which acts in the same way as acetazolamide but is highly concentrated in the kidney at plasma concentrations too low to affect red cell carbonic anhydrase. However, it seems that suspicion of these compounds has not been alleviated as bicarbonate is still preferred for the process of alkaline diuresis (Trapnell, 1976). Agents which augment diuresis by a direct osmotic effect are sometimes used in conjunction with alkaline diuresis. These include mannitol, furosemide and THAM (tris(hydroxymethyl)-aminomethane, however their effects are very rapid and their use must be monitored carefully to prevent fluid and electr-

olyte imbalance (Trapnell, 1976; Editorial, 1972).

Major intervention

It is rare that a case of salicylate poisoning does not respond to the treatments discussed so far (Arora et al., 1974; Editorial, 1972). However, when this is the case, there are several possible alternatives involving the extra-corporeal removal of the drug from the circulation. This is usually only considered if the patient's life appears to be in danger or if renal failure precludes the use of diuresis (Beveridge et al., 1964; Trapnell, 1976; Editorial, 1972). The methods used are haemodialysis, haemoperfusion, peritoneal dialysis or exchange transfusion.

Hill (1973) reports that both haemodialysis and charcoal haemoperfusion are effective in removing salicylate from the blood of laboratory animals but states that whereas haemodialysis has been widely used in man, haemoperfusion has not been clinically applied to any great extent. However, he considers that its efficiency in removing salicylate and its relatively low cost might cause it to be more widely used in the future. Arora et al. (1974) considered that, when one of these procedures becomes necessary, haemodialysis should be the one of choice. Bender (1975) stated that exchange transfusion, although occasionally reported, is rarely used as it is less effective than other methods and requires a large volume of blood, making it more applicable in paediatric poisoning. However Buchanan (1975) considered it impractical in the infant. An advantage is that equipment is usually available in most institutions (Bender, 1975).

Peritoneal dialysis was considered by Bender (1975) to be fairly effective and availability of equipment was also a factor in its favour. However he considered it less effective than haemodialysis in removing salicylate from the blood. He recommended the use of ion exchange resins rather than charcoal as the latter is prone to platelet absorption and the fragmentation of charcoal particles back to the blood. Although an effective method, its use is limited to adults and older children by virtue of the fluctuating blood volumes found in infants, and also the lack of availability of equipment and personnel prevents it from being used generally (Bender, 1975). Trapnell (1976) considered that peritoneal dialysis was the procedure of choice.

Summary

In summary, the treatment of salicylate intoxication consists of a "first aid" procedure to remove any unabsorbed salicylate by emesis or gastric lavage, followed by an alkaline diuresis in moderate intoxication and in cases of renal failure or extremely severe intoxication, extra-corporeal removal of salicylate by the most effective available method.

4. Analysis of aspirin and its metabolites in body fluids

There are many different methods by which aspirin and/or its metabolites may be assayed in body fluids and these fall into most of the categories of analysis for drugs in general.

(i) Colourimetric methods

Although aspirin has been in use for over a century as an analgesic, assay methods for it and its metabolites, until

the last three to four decades were imprecise. Brodie, Udenfriend and Coburn (1944) described a method for estimating the level of salicylic acid in plasma by extraction with ethylene dichoride from acidified plasma then returning it to an aqueous phase as a coloured ion complex which is determined colourimetrically. It seemed a simple method that could be adapted for routine use for plasma concentrations of therapeutic levels. They stated, however, that the degree of specificity depended upon the presence or absence of metabolic products (e.g. salicyluric acid) which constituted a source of error. Although this error was small in plasma it could cause a large error in urine samples due to the relatively high proportions of salicyluric acid. Other metabolites did not interfere. An alternative procedure using carbon tetrachloride as the extracting liquid was suggested but they pointed out that a standard curve based on plasma must be used in conjunction with any assays.

Trinder (1954) published a "rapid method for determination of salicylates in CSF, plasma and whole blood". This was based on a reagent containing ferric nitrate, mercuric chloride and hydrochloric acid. He used this reagent to make quantifiable recoveries of salicylate from body fluids. Trinder pointed out that as colour reactions are generally of low specificity, they are prone to interference from other ions, but reminded readers that the method had the benefit of speed, a single determination taking around five minutes to perform.

During their investigations into the metabolism of salicylic acid, Roseman and Dorfman (1951) described a colourimetric method for determining gentisic acid (2,5 dihydroxy-

benzoic acid, a metabolite of salicylic acid) in serum, urine and tissues. This method involved extraction with ether then re-extraction with bicarbonate followed by a colour reaction with Folin-Ciocalteu phenol reagent. They recognised the interference of uric acid which gave high blanks with urine samples. Ranges of confidence were 4-20 mg% for urine and 5-100 mg% for blood and showed 95% and 93% recoveries respectively.

Among other colourimetric methods of detecting salicylate is that described by Johar (1974). He reported a colour reaction of salicylate with the uranyl ion in aqueous solution to form an orange-red complex. It did not occur with acetylsalicylic acid. Although it appears that his interest lay in detecting uranium rather than salicylate he remarked that as the reaction was specific, it could form the basis of salicylate detection. So far this has not been taken up.

(ii) Chromatographic methods; column, paper and ion exchange

Bray, Thorpe and White (1950) described the identification of hydroxylated and glycine conjugated hydroxybenzoic acids including two metabolites of aspirin (2,3 dihydroxybenzoic acid and 2,5 dihydroxybenzoic acid) by paper chromatography. Although the method was only qualitative as described, they stated that rough quantification (to within 15%) could be made by comparing size and intensity of the assay spots with standard spots.

Several years later Tsukamoto, Kato and Tatsumi (1957) and Tsukamoto, Kato and Yoshida (1964) used paper chromatography to isolate the ether and ester glucuronide conjugates

of salicylic acid but again no effort was made to quantify the amounts present. El-Darawy and Mobarak (1974) described three possible chromatographic techniques whereby salicylic acid, p-aminosalicylic acid, salicylamide, acetylsalicylic acid and phenyl salicylate could be detected and identified. These were a) Polyamide thin layer slides, giving good resolution in a short time, the technique being simple and reproducible for routine use; b) Ion exchange chromatography, this having the advantage of rapid solvent front movement; c) Cellulose cation exchange paper which showed a clear difference in rf values. However, again the methods described were purely qualitative and while of use as drug screening techniques are useless for quantitative analysis.

(iii) Fluorimetric methods

Mandel, Cambosos and Smith (1954) confirmed the presence of aspirin and salicylic acid in plasma using the fluorescence of these compounds under certain wavelengths of light. However, the quantification was carried out by a colourimetric method using the coloured ion complex formed with the ferric ion. A few years later, Schachter and Manis (1958) carried out a similar procedure for separating salicylate and its metabolites but used a fluorimetric method to quantify the amounts present. The limits of detection were very low compared with those of existing methods (it was possible to detect and measure 1 M/l salicylic acid) and the method had the advantage of being able to estimate the concentrations of metabolites in the presence of a large excess of salicylic acid. The method could detect salicylate, salicylurate and acyl and phenyl glucuronides. Lange and Bell (1966) also

investigated this method with a view to separating and determining acetylsalicylic acid and salicylic acid in blood, but they consider the method unsuitable for routine clinical analysis because of the critical time factor between collection of the blood samples and the extraction of acetylsalicylic acid. Blood levels of 0-16 mg/100 ml were assayed and an advantage of the method is that only small samples (0.1 ml) need to be taken.

An automated fluorimetric method for determining total salicylate in body fluids and tissue extracts was reported by Hill and Smith (1970). This also had the advantage of requiring only small samples, the method involving the separation of salicylate from the fluids by dialysis and subsequent determination by the fluorescence of the compound at an optimum wavelength. Concentrations measured in human blood were of the range 0.7-3.8 mg/100ml with a reoovery of 97-99%. Hill and Smith did not recommend the use of the method for determining levels of salicylate in urine but considered it a sensitive and convenient method for blood which could also be modified to measure acetylsalicylic acid, salicyluric acid and salicylamide.

(iv) Gas-liquid chromatography

Gas-liquid chromatography (G.L.C.) has, in the last few decades become an increasingly popular method for identifying and assaying drugs, mainly due to its specificity and to the development of sophisticated electronic ancillary apparatus such as the integrator which help to make accurate representations of the amount of a substance passing through a detector. It is inevitable, therefore, that several G.L.C. methods

have been reported for the assay of salicylates in body fluids.

Nikelly described the determination of aspirin by gas-liquid chromatography (1964). This method was devised for the assay of aspirin tablets and thus no extraction from any medium was required. Tablets were crushed and dissolved in chloroform and this mixture was injected directly onto a column of Carbowax/isopthalic acid on glass beads at 125°C. Analysis time was 10 minutes and gave a precision of 1%. The limit of detection was 10^{-11}gm.

One of the early problems encountered in this kind of analysis was the tailing of peaks due partly to the low vapour pressure of salicylates and partly to the interaction of the -OH groups with column packing materials. Attempts to over come this problem were made by reacting the salicylate molecules with large anhydride molecules similar to that used by Rowland and Riegelman (1967). They described a method for determination of acetylsalicylic acid and salicylic acid in plasma. This necessitated an extraction step using ether as a solvent. An aliquot of extract was then concentrated and reacted with a silanising agent. This served the dual purpose of masking the -OH group and increasing the vapour pressure. Acetylsalicylic acid was identified by injection of some of the "silyl" derivative onto a column of Gas ChromQ/ DC QF 1 at 125°C along with a known concentration of dibutyl maleate as an internal standard. Salicylic acid was determined by re-extraction of some of the original ether extract with phosphate buffer and measurement of its fluorescence. Thus small amounts of acetylsalicylic acid could be detected

in the presence of vast excesses of salicylic acid.

Watson, Crescuolo and Matsui (1971) reported a method of simultaneous determination of levels of acetylsalicylic and salicylic acids by G.L.C.. This involved the conversion of the drugs to methyl esters and their isothermal elution from a column of OV-17 on Diatoport along with appropriate internal standards. Recoveries of 99.7% with standard deviations of 0.58% were reported. Again the method was devised for assay of tablets and no extraction procedures were described. Patel, Perrin and Windheuser (1972) also devised a method for assaying pharmaceutical preparations containing acetylsalicylic and salicylic acids, this time using silylated preparations.

Thomas, Solmonraj and Coldwell (1973) reported their method for determining salicylates in body fluids. The plasma or urine samples were deproteinised and extracted at pH 2 with ether. Silyl derivatives were formed and injected onto a column of OV-17/Gas Chrom Q using flame ionisation detection and an integrator to measure peak heights. The time for elution at 150°C was 80 minutes for both aspirin and salicylic acid and for aspirin alone was 20 minutes. The precision was 1% for concentrations of higher than 1 mg/100 ml and the limits of detection for salicylic acid were 0.1 mg/100 ml and for aspirin were 0.2 mg/100 ml.

Walter, Biggs and Coutts (1974), when reporting their method again stressed the need for derivatisation to mask polar groups to increase volatility and to decrease tailing. They reduced the elution time for silylated derivatives

of aspirin and salicylic acid to 10 minutes run on a column of Chromosorb G with OV25 as the stationary phase at 160 C.

Rance, Jordan and Nichols (1975) conducted a clinical study to determine the relative time taken for the aspirin of three commercially available formulations to appear in the blood. They extracted samples, obtained from volunteers dosed with the aspirin formulations, with ether, evaporated these to dryness and formed silylated derivatives. These were then injected onto a column of OV17 on CQ and the temperature programmed to rise from 160-200°C. Retention time for aspirin and salicylic acid derivatives and their respective internal standards was 20 minutes. Limits of detection for salicylic acid, aspirin and salicylamide were 0.02-0.05 mg/100 ml.

5. The Purpose of the Study

(i) Aims

It was intended to investigate as far as possible the metabolism and kinetics of salicylate in patients who had taken an overdose of aspirin, and to compare this with the metabolism and kinetics of aspirin at therapeutic dose levels as reported elsewhere and as found in a volunteer study carried out by the present author.

(ii) Objectives

. To collect and analyse for salicylate and its metabolites samples of blood and urine collected from normal volunteers who had taken a therapeutic dose of aspirin.

. To collect and analyse for salicylate and its metabolites samples of blood, urine and cerebrospinal fluid collected from patients who had taken an overdose of aspirin.

1. Introduction

The problem was to develop an assay method which could be used to estimate the quantities of salicylates and their metabolites present in the plasma, urine and cerebrospinal fluid of patients admitted to hospital following an acute overdose of salicylate analgesics.

The method devised must:-

Be capable of detecting and measuring concentrations of salicylate as low as 5 mg/100 ml of body fluids and lower concentrations of metabolites.

- Be able to distinguish between salicylates and their metabolites.
- Be reasonably accurate; an accuracy of 10% would be acceptable.
- Be able to be used on blood/plasma, urine and cerebrospinal fluid.
- Be economic in its requirement of the amount of source material, as a series of samples of blood (for example) may be required from one patient, the total sample required should not exceed about 10 mls. Similarly as the amount of CSF withdrawn by lumbar puncture must be limited, the total sample of CSF required should not exceed 5 mls.

Other points to be taken into consideration in devising a method of analysis were:-

- Due to the fact that the samples could not be analysed at the hospital but some distance away, it was anticipated that difficulties might occur in the storage of plasma which would result in hydrolysis of aspirin to salicylic acid before the samples could be analysed. This, plus the fact that patients are normally admitted a matter of hours after ingestion made it highly unlikely that aspirin could be assayed with any accuracy. Thus it seemed reasonable to assume that, by the time samples were analysed most, if not all, of any aspirin

present would have been hydrolysed to salicylic acid. It did not seem feasible, therefore to attempt to assay aspirin.

• Although the methods were to be devised for research use alone and were not bound by any of the necessary strictures placed on routine toxicology laboratories, such as speed and the ability to process batches of sample in a relatively short time, in devising the methods it was necessary to take into consideration the large number of samples that would be obtained in, for example, 2 hourly sampling over 24-48 hours of both plasma and urine. Thus the method had to be reasonably straightforward and take a reasonable length of time per sample. A batch process would, therfore, be ideal for the analysis of samples both from volunteers and patients.

2. Total Salicylates

For the purpose of getting an overall picture of the concentration of salicylates in the body, an estimation of "total salicylates" is useful. This would not only give the concentration of salicylic acid but also that of its metabolites and would thus serve the additional purpose of giving a check on the amounts of these found by any other method used.

A review of the published methods for estimation of salicylates was given in Section 1. Of those considered, that of Trinder has been used routinely in hospital toxicology laboratories for many years and has established a reputation for speed, accuracy and respectability. Thus it was considered as a possible method for estimating "total salicylate" in this study. Trinder's method (1954) is a colourimetric method based on the reaction of the phenolic hydroxyl group with ferric ions, and, as such, would be expected to detect salicylic acid and its metabolites but not acetyl salicylic acid. To test this, an initial experiment was done:

Method 1 - Trinder (1954)

M1.1

Aim: To check the reaction of Trinder's reagent with acetylsalicylic acid , salicylic acid and their metabolites.

Materials: Trinder's reagent: Mercuric chloride
N-Hydrochloric acid
Ferric nitrate

Salicylic acid - 5 mg/100 ml stock solution
Acetylsalicylic acid " "
Salicyluric acid " "
2,3 Dihydroxybenzoic acid " "
2,5 Dihydroxybenzoic acid " "
2,3,4 Trihydroxybenzoic acid " "

Method: The Trinder's reagent was made up by heating 40 gm mercuric chloride in 800 ml of distilled water. This was cooled and 120 ml hydrochloric acid added. 40 gm Ferric nitrate was dissolved in a little distilled water and added and the entire solution made up to a litre. 1 ml of each of the salicylate solutions was taken and mixed with 5 ml of Trinder's reagent. Optical densities were read 30 mins. afterwards at 540 mμ in an E.E.L. spectrophotometer.

Results: Optical densities are shown in Table 29. Colour complexes were formed by all solutions except acetylsalicylic acid.

Discussion: The results were as expected if the colour reaction depend upon the phenolic hydroxyl group. On this basis, it can be predicted that, of the two remaining metabolites, the acyl glucuronide would react to give a coloured complex but the phenolic glucuronide would not. The conclusion is that "total salicylate", when measured

Table 29. Optical densities given by reaction of salicylate solutions with Trinder's reagent measured at 540 mμ.

Solution 5 mg/100 ml	Optical Densities	Mean O.D.s
Acetylsalicylic acid	1.0 0.0	0.50
Salicylic acid	11.6 9.2	10.40
Salicyluric acid	9.5 8.8	9.20
2,3 Dihydroxybenzoic acid	7.3 10.0	8.70
2,5 Dihydroxybenzoic acid	8.7 9.2	8.95
2,3,4 Trihydroxybenzoic acid	11.2 14.0	12.70

includes salicylic acid, salicyluric acid, 2,3 dihydroxybenzoic acid, 2,5 dihydroxybenzoic acid, 2,3,4 trihydroxybenzoic acid and salicyl acyl glucuronide. Having established what is measured by this method it was also necessary, in assessing its potential usefulness, to determine the limits of accuracy. To do this, solutions of known concentration were assayed in M1.2.

M1.2

Aim: To determine the accuracy of Trinder's method in assaying levels of sodium salicylate in aqueous solution.

Materials: Trinder's reagent (as M1.1)
Sodium salicylate - 200 mg/100 ml stock solution

Method: The stock solution was diluted to give solutions containing 1,5,10,20,30,40,50,60 mg/100 ml respectively. 1 ml of each of these solutions was mixed with 5 ml of Trinder's solution using 1 ml of water as a blank. Aliquots of each were read at 540 mμ in an E.E.L. spectrophotometer immediately after mixing, after 30 mins., and after 60 mins..

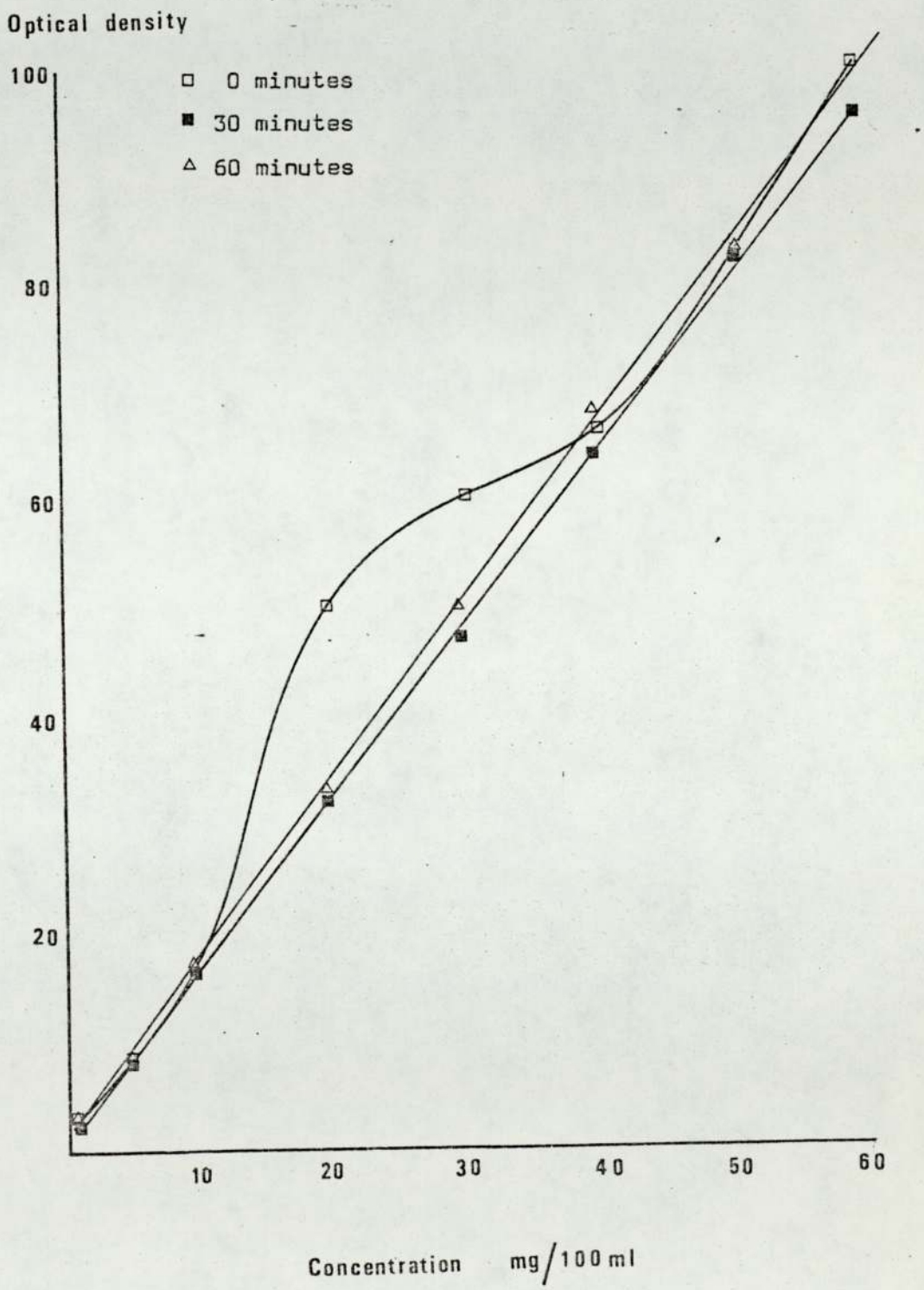
Results: Optical densities are given in Table 30 and plotted graphically in Fig. 16.

Discussion: The results show that, when read 30-60 minutes after mixing, the optical density resulting from the reaction of sodium salicylate with Trinder's reagent is directly proportional to the concentration of salicylate between 5 and 60 mg/100 ml.. These results were obtained using aqueous solutions and, to fulfil the criteria cited in the introduction, it was necessary to establish that the method could be used for plasma, urine and cerebrospinal fluid, and

Table 30. Optical densities of various concentrations of sodium salicylate solution after reaction with Trinder's reagent and measured at 540 mμ.

Concentration mg/100 ml	Optical Densities Read immediately	After 30 minutes	After 60 minutes
1	2.5	2.0	2.5
5	8.5	8.0	8.5
10	16.0	16.0	17.0
20	50.0	32.0	33.0
30	60.0	47.0	50.0
40	66.0	64.0	68.0
50	82.0	82.0	83.0
60	>100.0	95.0	>100.0

Figure 16. Optical densities of various concentrations of sodium salicylate solutions read at 540 mμ 0, 30 and 60 minutes after mixing with Trinder's reagent.



also to find out what percentage recovery of added salicylate could be expected from these fluids. To do this, samples of salicylate-free fluids were obtained and known concentrations of salicylates added to them. Due to the difficulties involved in obtaining cerebrospinal fluid, this was only done for plasma and urine in M1.3.

M1.3

Aim: To establish the percentage recovery of known amounts of salicylic acid added to salicylate-free plasma and urine.

Materials: Trinder's reagent (as M1.1)
Stock solution salicylic acid in ether - 100mg/100 ml
Salicylate-free plasma and urine

Method: Aliquots of 0.1 ml, 0.25 ml, 0.5 ml of stock solution were taken and placed in centrifuge tubes and evaporated to dryness in a stream of nitrogen. The salicylic acid remaining was taken up in samples of salicylate-free plasma and urine to give levels in these samples of 10 mg/100 ml, 25 mg/100 ml, and 50 mg/100 ml. Each sample was triplicated to ensure accuracy. Blanks of 1 ml plasma and 1 ml urine were used. 5 ml Trinder's reagent was added to each and the tubes gently agitated. Where necessary they were then centrifuged. Aliquots of each were read at 540mμ in an E.E.L. spectrophotometer after 30 minutes.

Results: Optical densities resulting from the above procedures are shown in Table 31.

Discussion: The results show that, on the whole, the method was quite accurate in that recoveries of over 93.0% could be obtained even at concentrations of 50 mg/100 ml in both plasma and urine. It would seem that this method for assaying "total salicylates" conforms to

Table 31. Percentage recovery of added salicylic acid from plasma and urine using Trinder's method.

mg/100 ml Conc. SA added	Optical Density	mg/100 ml Conc. SA found	% Recovery
Plasma			
10	17.0	11.0	110.0
10	18.0	11.5	115.0
10	17.3	11.2	112.0
25	40.0	25.2	100.8
25	34.0	21.5	86.0
25	39.0	24.5	98.0
50	70.0	44.0	88.0
50	75.0	47.5	95.0
50	77.0	48.7	97.4
Urine			
10	13.3	8.5	85.0
10	16.3	10.5	105.0
10	17.2	11.0	111.0
25	42.0	26.5	106.0
25	44.2	28.0	112.0
25	37.0	23.4	93.6
50	68.0	42.8	95.6
50	74.0	46.7	93.4
50	78.0	49.4	98.8

the criterion of 10% accuracy for both high and low concentrations of salicylates.

Conclusions

- The method of Trinder published in 1954 for "total salicylates" in fact measures salicylic acid, salicyluric acid, 2,3 and 2,5 dihydroxybenzoic acids, 2,3,4 trihydroxybenzoic acid and salicyl acyl glucuronide.

- It is accurate to within 10% for plasma and urine concentrations between 10–50 mg/100 ml.

- It is possible with this method to measure concentrations of salicylate as low as 5 mg/100 ml.

- The method has the desired qualities of speed (apart from the the 30 minute wait, a single determination takes 5 minutes) and capbility of coping with a large quantity of samples in a batch.

- It appears then that the method is suitable for checking the level of "total salicylate" in body fluids.

3. Salicylic acid

It was stated previously that it was necessary for any assay method chosen for use in later studies to be capable of distinguishing and analysing separately the individual metabolites of aspirin. As the major of these, salicylic acid levels were extremely important and thus salicylic acid was the first metabolite to be considered.

As mentioned previously, colourimetric assays are generally unspecific, those for salicylic acid depending usually on a complexation reaction via the phenolic -OH group, and thus reacting with any other

similar compounds present including metabolites of salicylic acid (this being the basis of the "total salicylates" method of Trinder (1954)). There is similar limitation on the use of shifts in absorption spectra, the presence of aspirin interfering with salicylic acid measurement. Fluorimetric techniques tend to suffer from high background fluorescence although this can be allieviated by use of plasma or urine blanks. However, by far the most specific technique for separately estimating quantities of individual components of mixtures is gas-liquid chromatography (GLC) .

Several GLC methods have been published for the assay of salicylates in body fluids. They tend to differ only in detail, being broadly similar in outline, and were mentioned briefly in Section 1. They all entail an extraction step to remove the salicylate from the body fluids and get it to an organic phase which is either concentrated or evaporated to dryness. The salicylate is of low volatility which necessitates a derivatisation. Several types of compound are available for this procedure. The apparent increased reliability of "silylating" reagents has led to a preference for silylated derivatives over the older anhydride derivatives such as those used by Rowland and Riegelman (1976). The derivatised salicylates are then injected onto columns of a variety of lengths and diameters with a variety of different supports and stationary phases. Nitrogen was favoured as the carrier gas by all workers except Walter, Biggs and Coutts (1974) who preferred helium.

Three of these methods were investigated with a view to their use in future studies, these being the methods published by Thomas, Solmonraj and Coldwell (1973), Walter, Biggs and Coutts (1974) and Rance, Jordan and Nichols (1975). The details are summarised in Table 32.

Table 32. Summary of three of the published methods for analysis of body fluids for salicylates by gas liquid chromatography.

	Thomas, Solmonraj and Coldwell 1973	Walter, Biggs and Coutts 1974	Rance, Jordan and Nichols 1975
Column Dimensions	Single column 1.8 m long	Dual column 1.8 m x 0.4 cm	Dual column 1.8 m x 0.4 cm
Column Characteristics	5 OV17 on Gas ChromQ 100–200 mesh	3% OV25 on Chromosorb G 60–80 mesh	5% OV17 on Diatomite CQ 80–100 mesh
Operating temps. inj/oven/detector	200/150/250	240/160/240	160 - 200/250 programmed
Extraction medium	Glass distilled ether	Chloroform	Glass distilled ether
Silylating reagent	BSTFA	HMDS	BSTFA
Internal standard	p-toluic acid	Butyl benzoate	m-toluic acid
Carrier gas flow rate	50 ml/ min	55 ml/min	50 ml/min
Compounds detected	Aspirin Salicylic acid	Aspirin Salicylic acid	Aspirin Sa icylic acid

Method 2 - Thomas, Solmonraj and Coldwell (1973)

M2.1

Aim: To obtain a chromatogram for salicylic acid.

Equipment: Perkin Elmer F-11 gas chromatograph with flame ionisation detector
Glass column 1.8m x 0.4 cm (internal diameter)

Materials: OV17 stationary phase
Gas chromQ 100-120 mesh support
Salicylic acid
Potassium bisuphate
Butyl benzoate
Chloroform
Ethyl acetate
Bis(trimethylsilyl)trifluoracetamide (BSTFA)

Method: A column was prepared as follows: 1 gm OV17 was weighed and dissolved in approximately 50 ml of chloroform. 20 gm Gas chromQ was weighed out and this and the OV17 in chloroform placed in a glass flask. The flask was then attached to a rotary evaporator and the chloroform evaporated off leaving the Gas chromQ coated with 5% OV17. This was then packed into the glass column with the use of a vacuum line and vibrator. The column was fitted into the chromatograph and conditioned at an oven temperature of 300°C for 24 hours. Before use this was allowed to cool to the appropriate operating temperature. A solution of 10 mg/ml of salicylic acid in distilled water was prepared as was a 5% w/v solution of potassium bisulphate. Samples were prepared according to Table 33 and extracted, where appropriate by the following method: 0.5 ml sample and 0.5 ml potassium bisul-

Table 33. Composition of samples for M2.1

Sample number	Distilled water	Salicylic acid	Butyl benzoate
1 *	x	-	-
2 **	x	-	-
3	-	-	-
4 *	x	-	x
5 **	x	-	x
6 *	-	x	x
7 **	-	x	x

Note: * Extracted with ethyl acetate

** Extracted with chloroform

Sample 3 was BSTFA alone

Butyl benzoate was used as internal standard as no toluic acid was available

phate were placed in glass stoppered centrifuge tubes and saturated with solid sodium chloride. 5 ml of ethyl acetate containing 50 µl butyl benzoate was added. The tubes were shaken for 3 minutes and then centrifuged to separate the organic and aqueous phases. The organic phases were then removed and evaporated to dryness in a stream of nitrogen and 50 µl of silylating reagent (BSTFA) added. The stoppered tubes were placed in a water bath for one hour at 50°C. On removal, they were cooled under running water to room temperature. 1 µl of each sample was injected onto the column with a gas flow rate of 45 ml/minute and an oven temperature of 150°C.

Results: The chromatograms obtained are shown in Fig. 17. All samples, including BSTFA alone, showed a large peak at 2.65 minutes after the solvent peak. Samples 4,5,6,7 showed a peak at 5.15 minutes and samples 6 and 7 showed a peak (which was split, probably due to a dirty detector) at 3.85 minutes.

Discussion: Although the origin of the 2.65 minute peak was difficult to understand, the 5.15 minute peak would seem to correspond to the elution of butyl benzoate. Similarly, the peak eluting at 3.85 minutes would seem to be that of salicylic acid. Resolution of these peaks was not very satisfactory and would tend to suggest that operating conditions were not optimal.

M2.2

Aim: To attempt to improve the resolution of peaks by alteration of oven temperatures and gas flow rates.

Equipment: As M2.1

Materials: As M2.1

Method: Various oven temperatures and gas flows were selected.

Figure 17. Chromatograms of extracted and silylated samples variously containing distilled water, salicylic acid and butyl benzoate. Samples 1,4 and 6 extracted with ethyl acetate; samples 2, 5 and 7 extracted with ch oroform. Sample 3 was silylating reagent (BSTFA) alone.

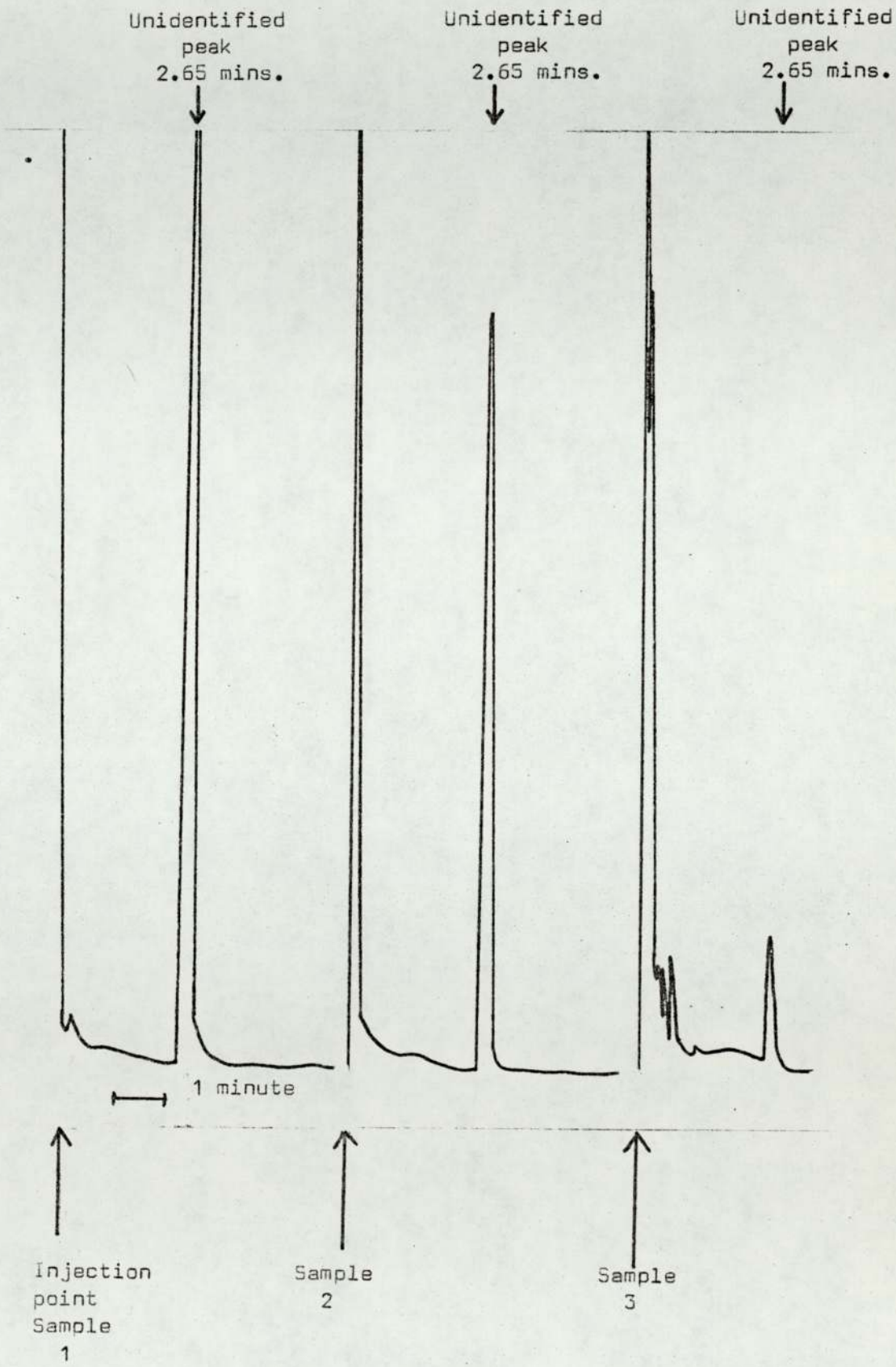


Figure 17 (contd.).

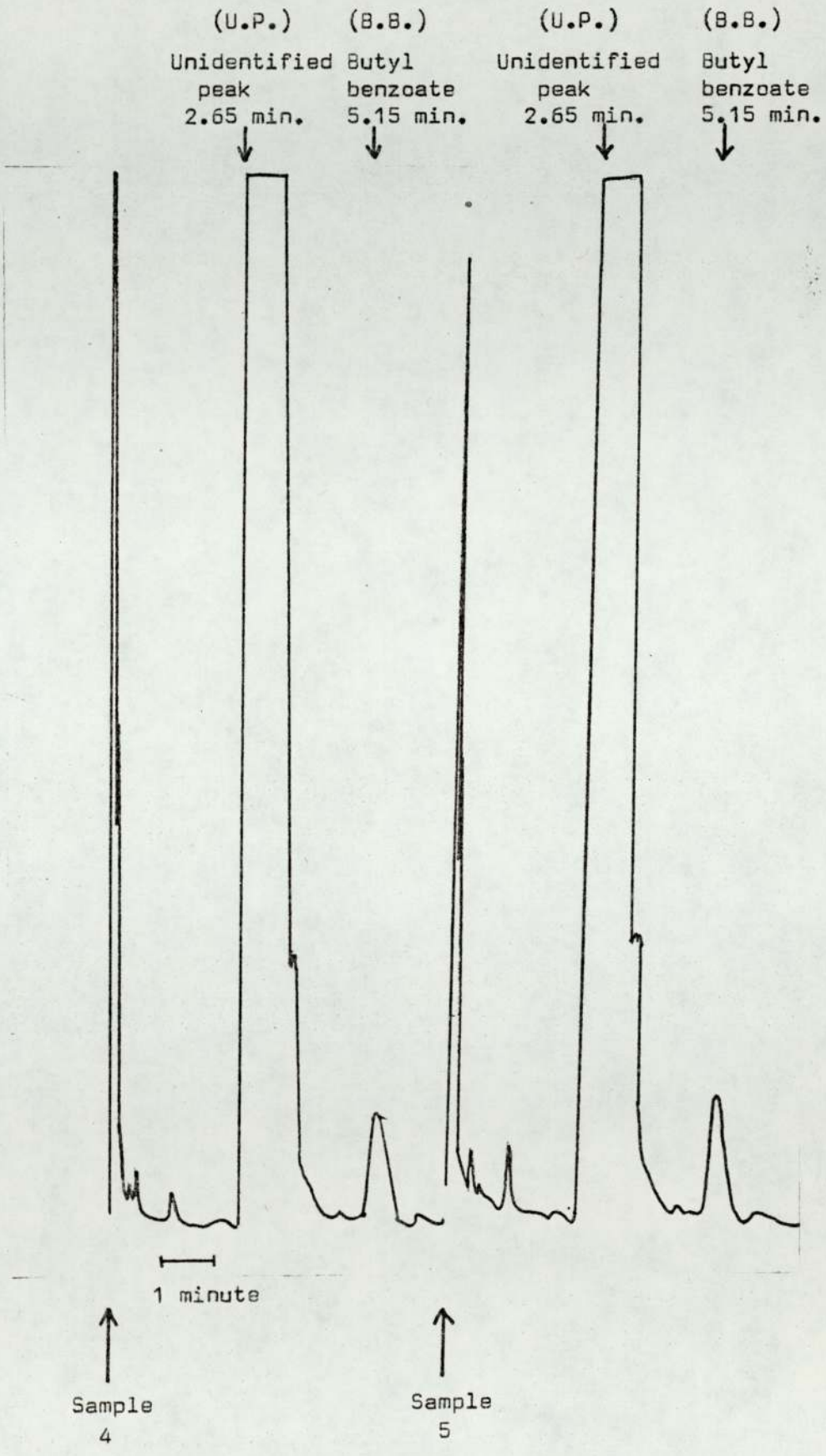
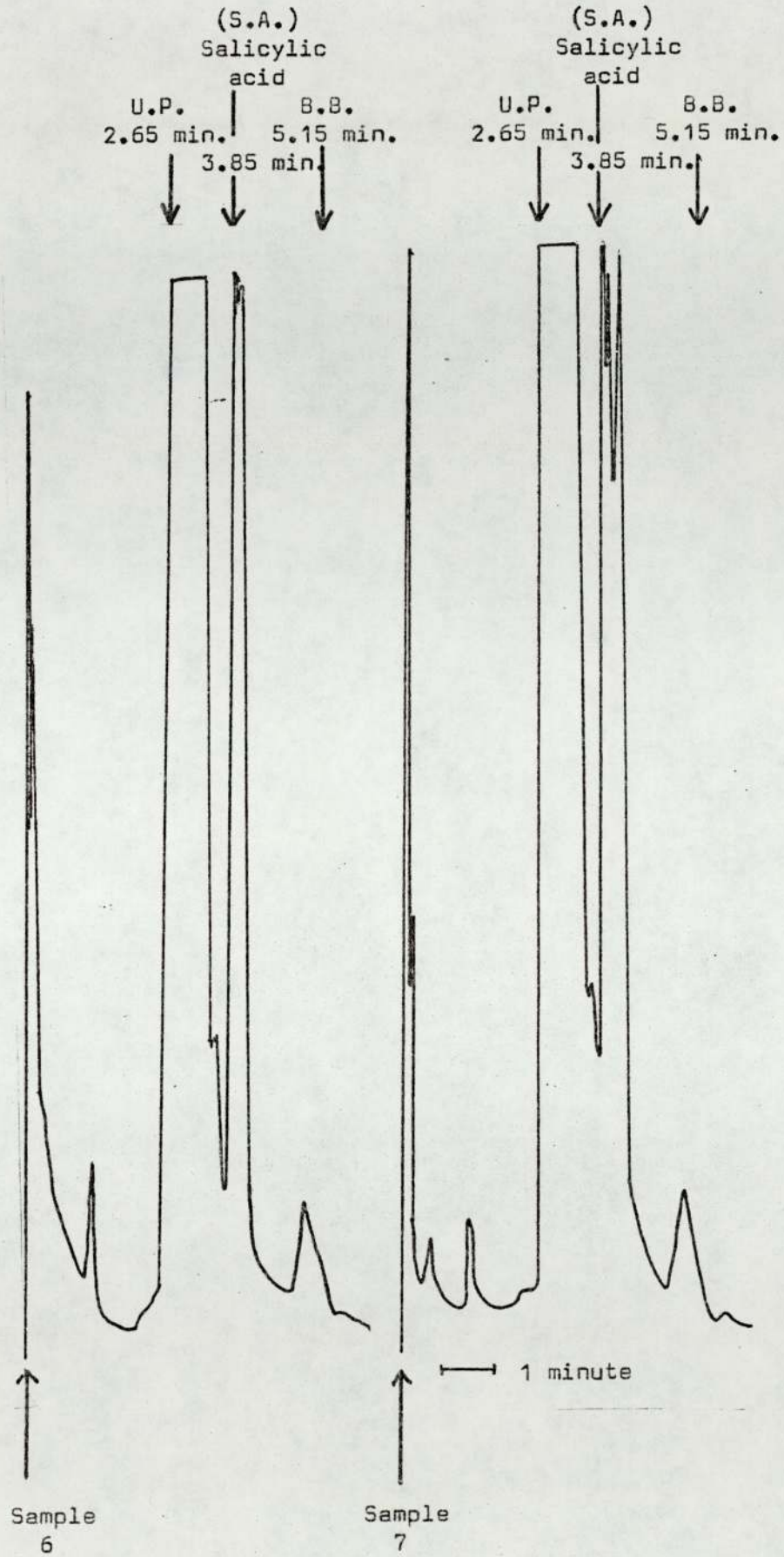


Figure 17 (contd.)



Results: The chromatograms obtained are shown in Fig. 18, 19 and 20.

Discussion: Fig. 18 shows that decreasing the oven temperature to 140°C gave only a marginal improvement in resolution and increased the retention times to 5.5 minutes (salicylic acid) and 7.45 minutes (butyl benzoate). Further decrease to 130°C gave a far better resolution of the salicylic acid peak and the large peak. However, the butyl benzoate peak had flattened out and produced a shoulder. Retention times were 9.0 minutes and 11.4 minutes respectively. Fig. 19 shows that a reduction in flow rate from 45 to 35 ml/minute had little effect on resolution and increased retention times to 4.75 minutes (salicylic acid) and 6.3 minutes (butyl benzoate). Increase of the flow rate to 50 ml/minute also had little effect on resolution but sharpened the peaks considerably. Unfortunately it also produced several smaller peaks and thus make identification of salicylic acid and butyl benzoate peaks more difficult. Fig. 20 shows the effect of an oven temperature of 135°C and flow rate of 50 ml/minute. The two peaks occurred at retention times of 2.9 minutes and 3.9 minutes respectively and were of good shape, although the large unidentified peak now occurring at 1.8 minutes was interfering markedly with their resolution.

M2.3

Aim: To obtain better resolution and possibly remove the large peak by lowering the proportion of stationary phase.

Equipment: As M2.1

Materials: As M2.1

Method: The proportion of stationary phase was lowered to 3% in a newly constructed column. The same samples as in the previous experiments were injected onto this new column.

Figure 18 a). Chromatogram of a sample containing salicylic acid and butyl benzoate, extracted with chloroform and silylated with BSTFA, injected onto a column at an oven temperature of 140°C.

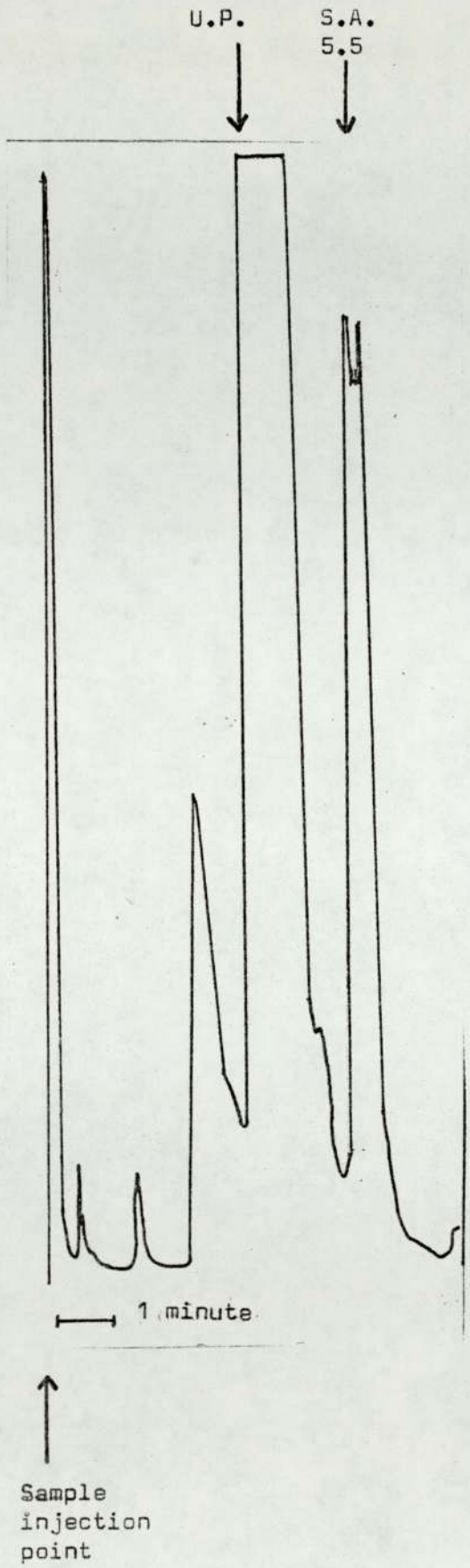


Figure 18 b). Chromatogram of a sample containing salicylic acid and butyl benzoate, extracted with chloroform and silylated with BSTFA, injected onto a column at an oven temperature of 130°C.

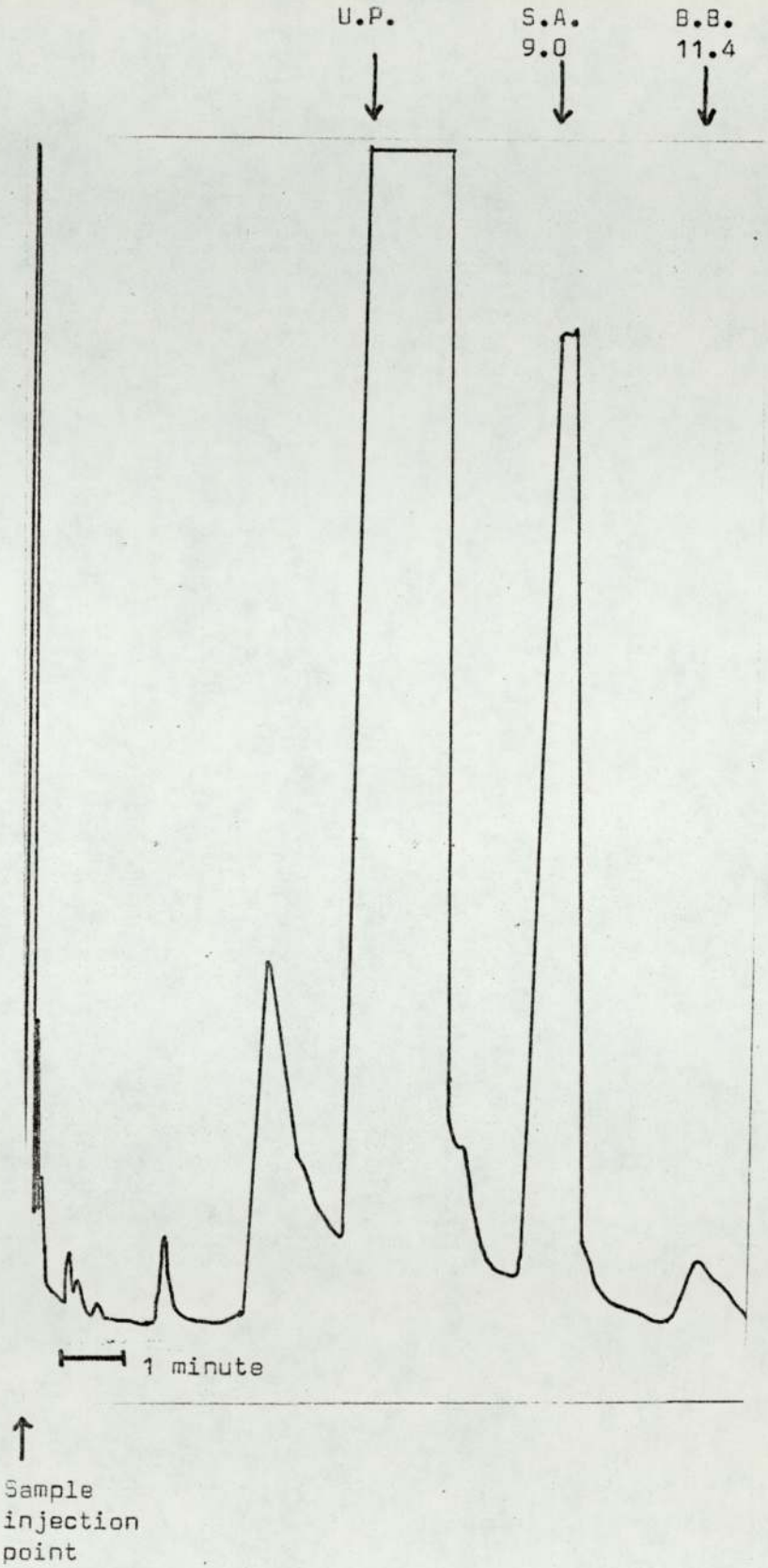


Figure 19. Chromatograms of a sample containing salicylic acid and butyl benzoate, extracted with chloroform and silylated with BSTFA, injected onto a column at an oven temperature of 150°C and a flow rate of 35 ml/minute (a) and 50 ml/minute (b).

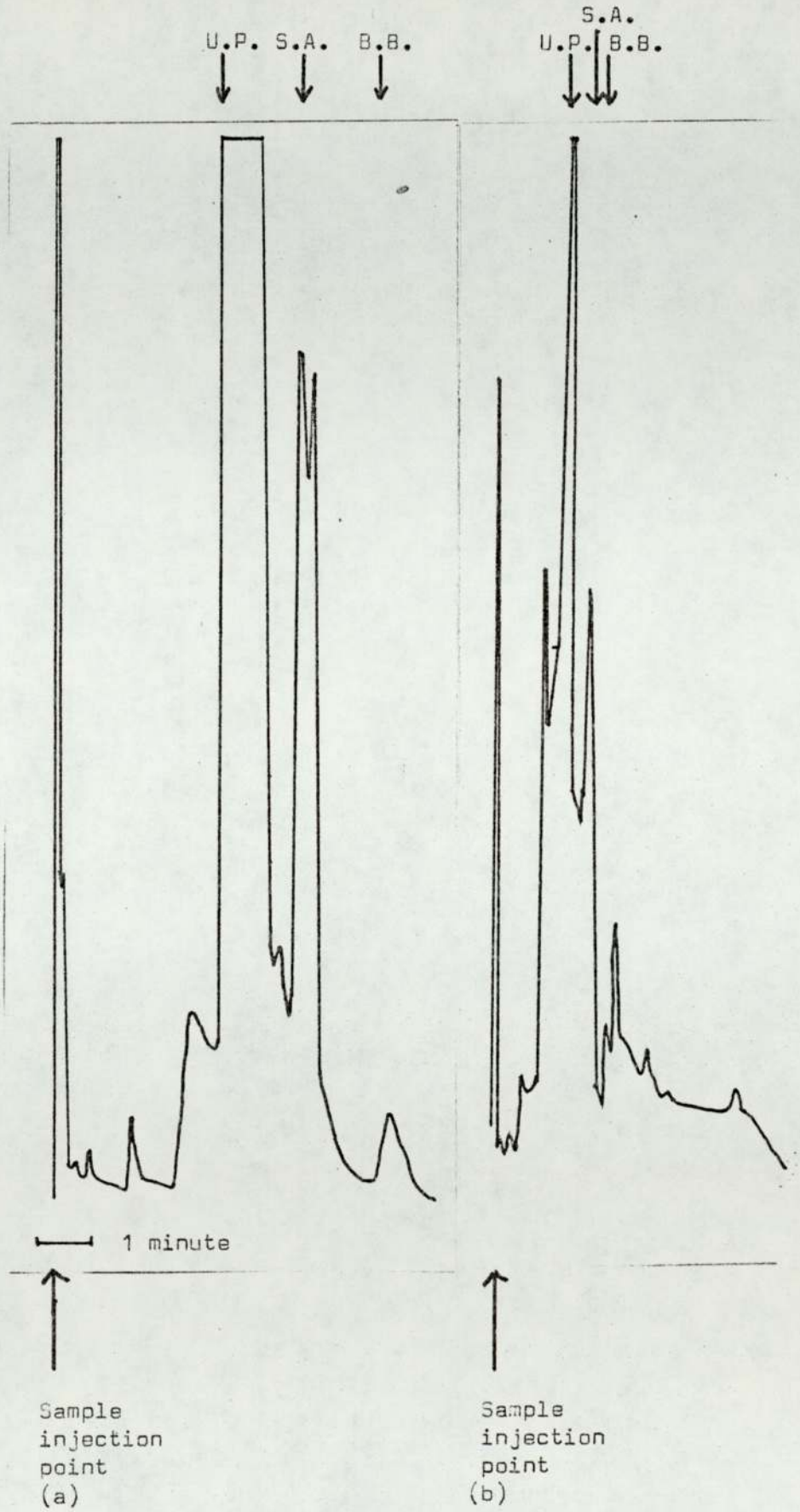
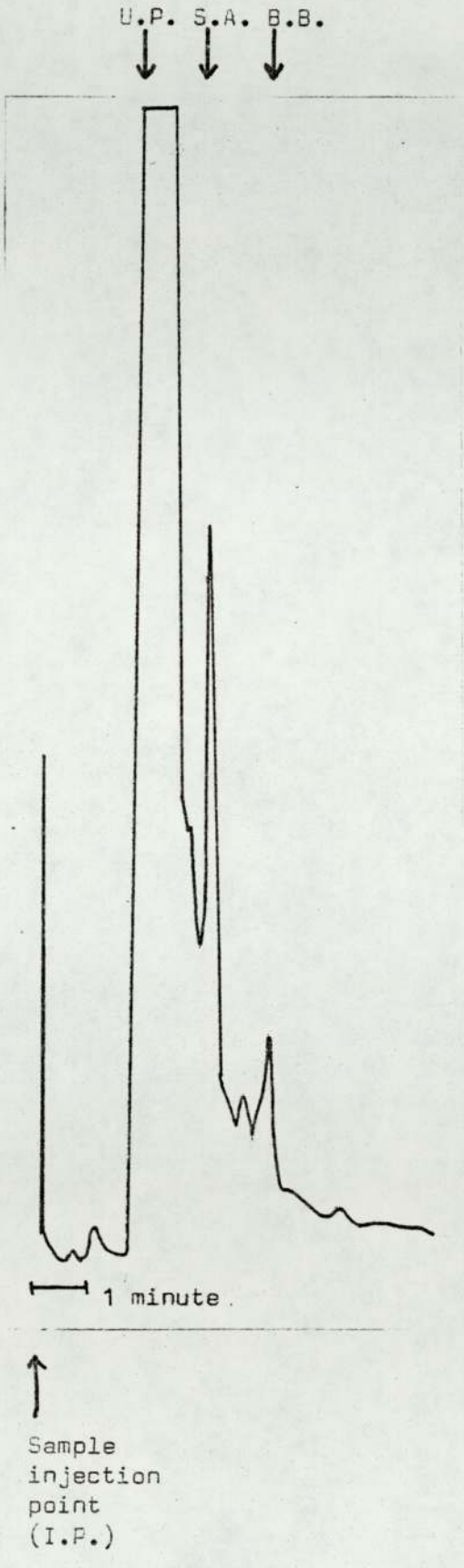


Figure 20. Chromatogram of a sample containing salicylic acid and butyl benzoate extracted with chloroform and silylated with BSTFA, injected onto a column at an oven temperature of 135°C and a flow rate of 50 ml/minute.



Results: The chromatograms obtained from these samples are shown in Fig. 21.

Discussion: There seemed to be a better resolution of the peaks and a shorter retention time. However the large peak now eluting at 0.4 minutes was again present in all samples and it was evident that this component must be a characteristic of the samples themselves. One possible source of such a peak could be the presence of moisture in the samples. However, considering this problem elsewhere (Walter, Biggs and Coutts, 1974) it is stated that "...water in the reaction mixture was slow to elute from the column...". Another confusing factor in this analysis was that it could be expected that butyl benzoate would elute from the column before the salicylic acid derivative (Walter, Biggs and Coutts, 1974). A possible alternative explanation is that the large peak could, in fact have been butyl benzoate eluting from the column. This could have been present in all of the samples if the glassware used (e.g. the injection syringe) had been contaminated. However, this seems extremely unlikely in view of the size of the peak compared with the size of that representing 10 mg/ml of salicylic acid.

Conclusions: In view of the difficulties encountered with this method, it seemed advisable to turn to one of the alternative published methods and attempt to duplicate this.

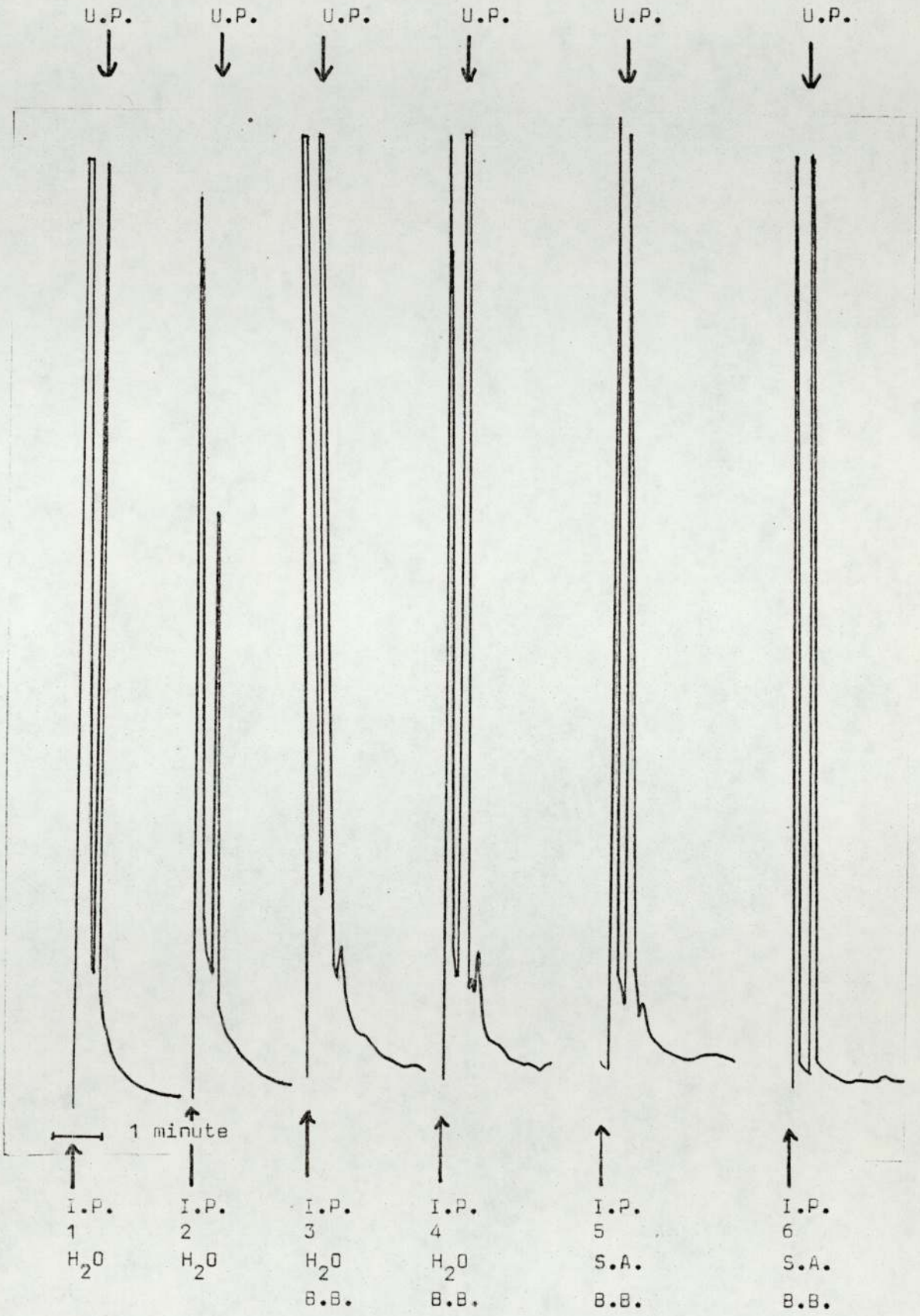
Method 3 - Walter, Biggs and Coutts (1974)

M3.1

Aim: To obtain a chromatogram for salicylic acid.

Equipment: As M2.1

Figure 21. Chromatograms of extracted and silylated samples variously containing salicylic acid, butyl benzoate and distilled water after lowering of the stationary phase level from 5% to 3%.



Materials: OV25 stationary phase

Chromosorb G 60-80 mesh support

Salicylic acid

Acetone

Hexamethyldisilazane (HMDS)

Method: It was thought advisable to begin with a basic silylation reaction before involving any extraction step, to see if an acceptable chromatogram could be obtained. Accordingly, samples were prepared according to Table 34, and silylated at room temperature for 60 minutes. 1 µl aliquots were chromatographed at an oven temperature of 160°C and a flow rate of 50 ml/minute.

Results and Discussion: Fig. 22 shows that there was a peak in sample 3 at 3.3 minutes, one in sample 4 at 2.7 minutes and one in sample 5 at 3.3 minutes with a shoulder at 2.6 minutes. This would seem to indicate that the butyl benzoate eluted at 3.3 minutes and a salicylic acid derivative at 2.7 minutes and that their resolution was inadequate. This is puzzling, as with Method 2, as the butyl benzoate would be expected to elute before the salicylic acid derivative. That this does not occur could be explained if the derivative were not in fact the trimethylsilyl,2-trimethylsiloxybenzoate derivative chromatographed by Walter, Biggs and Coutts (1974), but 2-trimethylsiloxybenzoic acid which has a lower molecular weight (282 as opposed to 210) and a shorter retention time. Their respective structures are shown in Fig. 23. It is possible, if this were true, that in this case, the silylation reaction was perhaps not running to completion, and only the monosilylated derivative was being formed. Previously, when split peaks were observed (Figs. 18 and 19) this could be explained in a similar way in that the split peaks could have been due to incomplete silylation.

Table 34. Composition of samples for M3.1

Sample number	Acetone 100 µl	HMDS 100 µl	Butyl benzoate 50 µl	Salicylic acid 150 µg
1	x	-	-	-
2	x	x	-	-
3	x	x	x	-
4	x	x	-	x
5	x	x	x	x

Figure 22. Chromatograms of extracted and silylated samples variously containing acetone, HMDS, salicylic acid and butyl benzoate, injected onto a column at an oven temperature of 160°C and a flow rate of 50 ml/minute (M3.1) (continued overleaf).

Figure 22 (contd.)

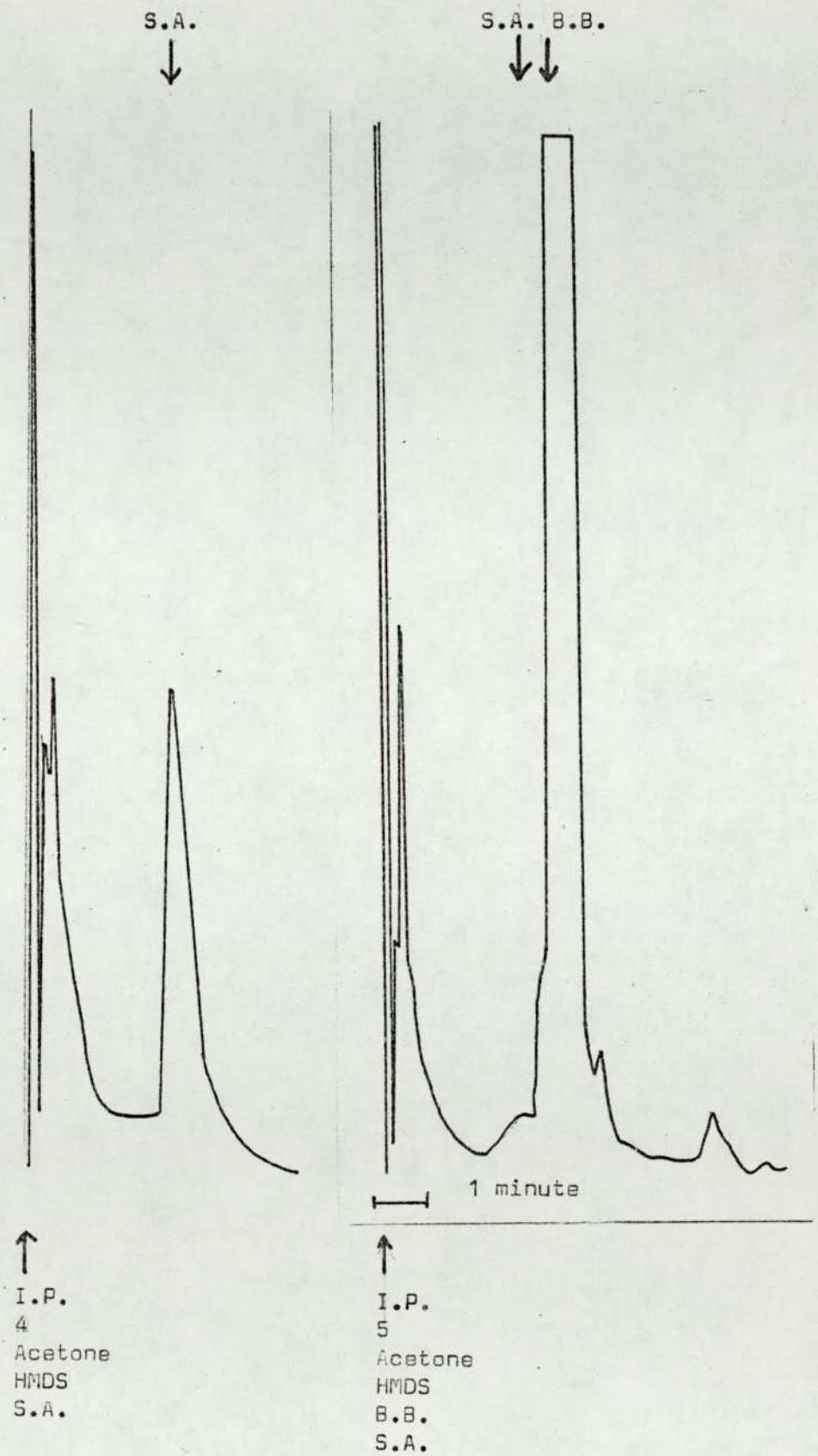
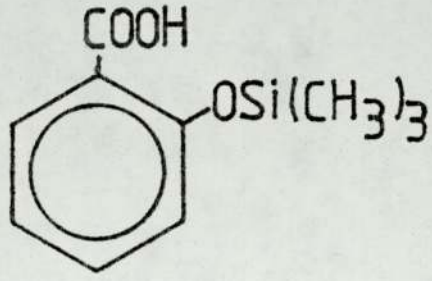
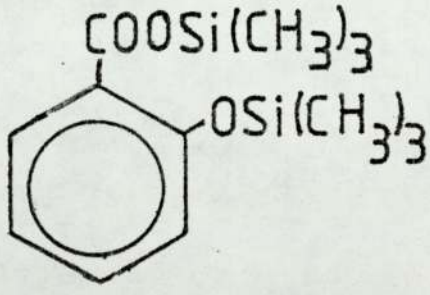


Figure 23. Structures of the two possible silyl derivatives of salicylic acid.



a) 2 trimethylsiloxybenzoic acid



b) trimethylsilyl,2 trimethylsiloxybenzoate

M3.2

Aim: To observe the effects of an alternative silylating reagent and column.

Equipment: As M2.1

Materials: As M3.1 except:-

Bistrimethylsilylacetamide

OV17 stationary phase

Gas ChromQ 100-120 mesh

Method: Samples were prepared according to Table 35. 1 µl aliquots were injected onto the new column at an oven temperature of 145°C and with a flow rate of 30 ml/minute.

Results and Discussion: The chromatograms obtained are shown in Fig. 24. They show clearly that no peaks were obtained from samples 1 and 2. A single peak at 2.4 minutes was obtained from sample 3 and another single peak at 3.2 minutes from sample 4 while sample 5 gave a chromatogram showing both of these peaks. The resolution was very good. It is apparent that the 2.4 minute peak corresponded to butyl benzoate and the 3.2 minute peak to salicylic acid. The conditions had combined to give well shaped and sharply resolved peaks with no extraneous peaks to confuse the analysis.

Method 4 - Rance, Jordan and Nichols (1975)

At this point the opportunity arose to visit the Drug Metabolism Section of Reckitt and Colman Ltd., Hull and investigate the usefulness of the method of analysis of body fluids for salicylate developed by Rance, Jordan and Nichols (1975).

Table 35. Composition of samples for M3.2

Sample number	Acetone 100 µl	BSA 100 µl	Butyl benzoate 50 µl	Salicylic acid 150 µl
1	x	-	-	-
2	-	x	-	-
3	x	x	x	-
4	x	x	-	x
5	x	x	x	x

Figure 24. Chromatograms of extracted and silylated samples variously containing salicylic acid and butyl benzoate and injected onto a column at an oven temperature of 145°C and and a flow rate of 30 ml/minute (M3.2)

M4.1

Aim: To obtain a chromatogram for salicylic acid.

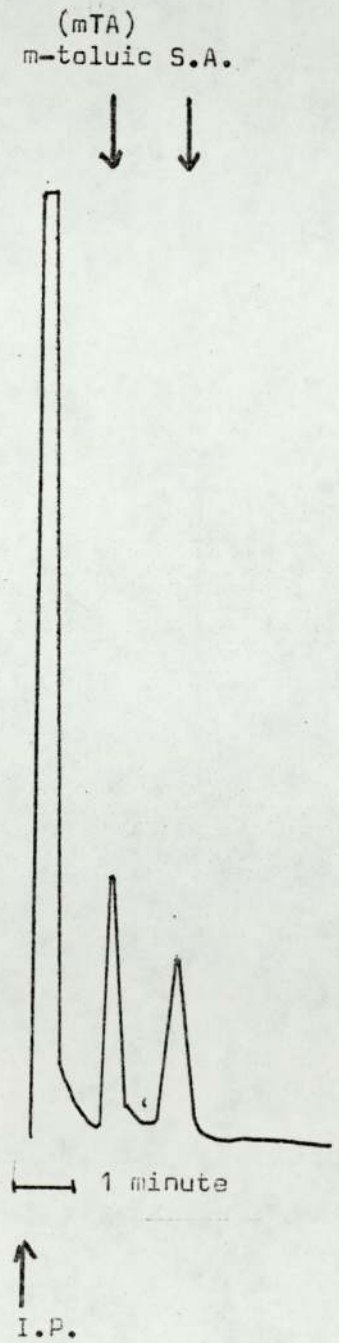
Equipment: Hewlett-Packard 5700A gas chromatograph with dual flame ionisation detectors
Glass column 1.8m x 0.4 cm (internal diameter)
Hewlett-Packard 3370B integrator
0.5 dram glass vials

Materials: OV17 stationary phase
CQ 80-100 mesh support
Salicylic acid
m-toluic acid
Glass distilled ether
BSTFA

Method: Columns were prepared by packing manually with 5% OV17 on Diatomite CQ. They were left to condition overnight at 250°C. Solutions containing 10 mg/100 ml salicylic acid and 10 mg/100 ml m-toluic acid were made up using glass distilled ether. Initially, 1 ml of each of these was placed in a single half dram glass vial and evaporated to dryness under oxygen. The residue was then shaken with 40 µl of BSTFA and incubated for one hour at 50°C. A 2 µl aliquot was injected onto the pre-conditioned column at an oven temperature of 190°C and a carrier gas flow rate of 50 ml/minute.

Results: The chromatogram obtained is shown in Fig. 25. It shows two clear peaks, the first being that of m-toluic acid derivative and the second that of the salicylic acid derivative.

Figure 25. Chromatogram obtained by silylation of a mixture of m-toluic acid and salicylic acid and injection of the sample onto a column at an oven temperature of 190°C and a flow rate of 50 ml/minute (M4.1)



M4.2

Aim: To construct a standard curve of salicylic acid concentration against peak height ratios.

Equipment: As M4.1 plus

15 ml centrifuge tubes

Materials: As M4.1

Method: 1 ml aliquots of the m-toluic acid solution were placed in the centrufuge tubes along with 1.0, 2.5, 5.0, 7.5, 10 ml, respectively of salicylic acid solution. The volumes were reduced by evaporation as before until they could be transferred to the half dram vials and evaporated to dryness. 40 µl BSTFA was added to each and they were incubated for 60 minutes at 50°C. 2 µl aliquots of each were injected onto the column.

Results and Discussion: The chromatograms obtained are shown in Fig. 26. Fig. 27 shows the integrator readout from which the peak areas were calculated. The plot of these against salicylic acid concentration is shown in Fig. 28. The graph obtained showed a straight line relationship between the peak area ratio salicylic acid/m-toluic acid for salicylic acid concentrations of 100 µg/ml - 1000 µg/ml. This is the equivalent to plasma concentrations of 10 mg/100 ml - 100 mg/100 ml.

M4.3

Aim: To assay an "unknown" quantity of salicylic acid in plasma.

Equipment: As M4.2

Materials: As M4.2 plus

Aspirin-free plasma

Figure 26. Chromatograms obtained from samples containing 0–100 mg/100 ml of salicylic acid and a standard concentration of m-toluic acid. Other conditions as described in M4.2.

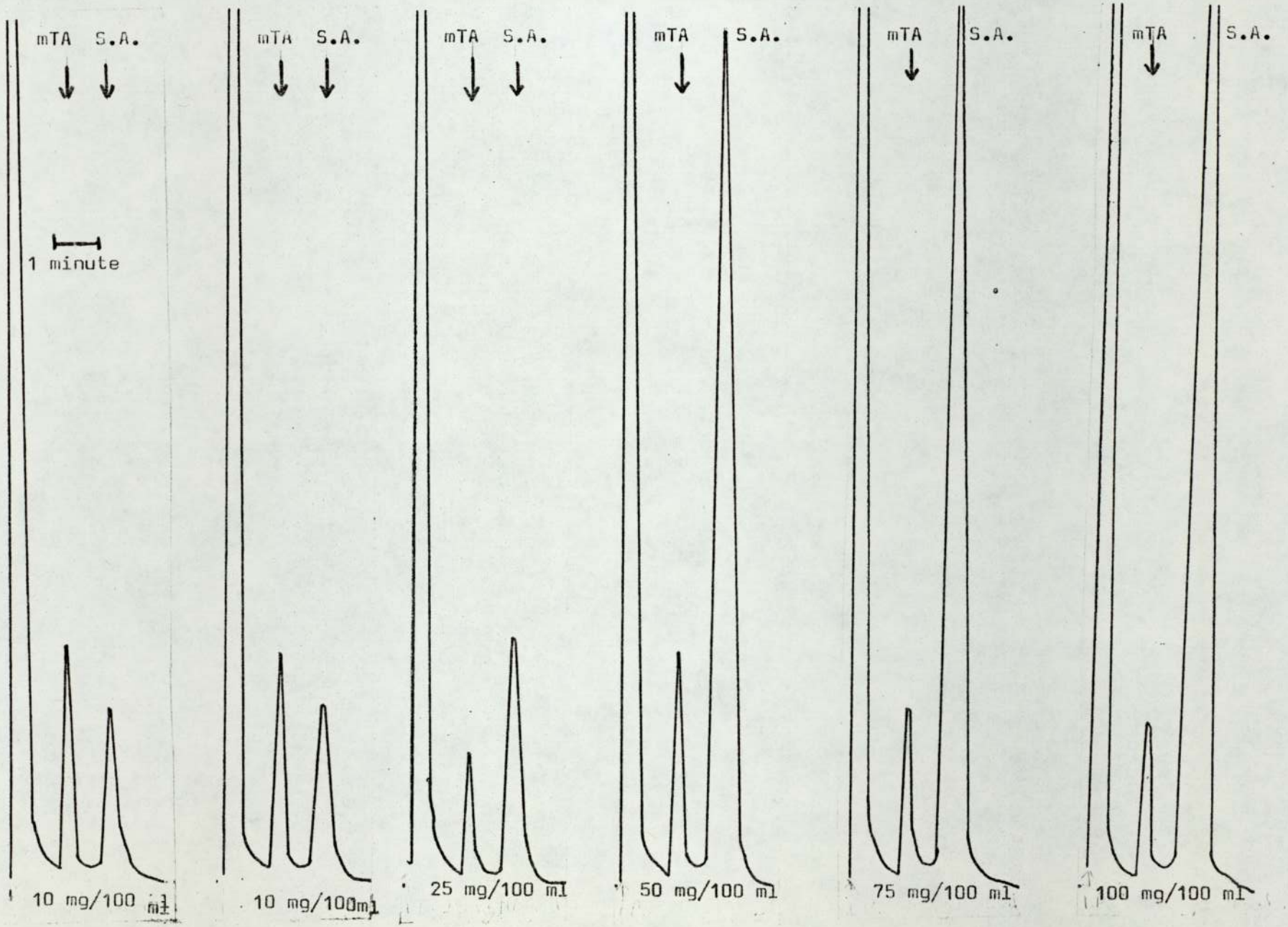


Figure 27. An example of the integrator readout from M4.2 showing the calculation of peak height ratios.

1515 (S.A. peak height)
127 (mTA peak height)

(100 mg/100 ml)

1257
1392

(75 mg/100 ml)

1068
185

(50 mg/100 ml)

2881
993

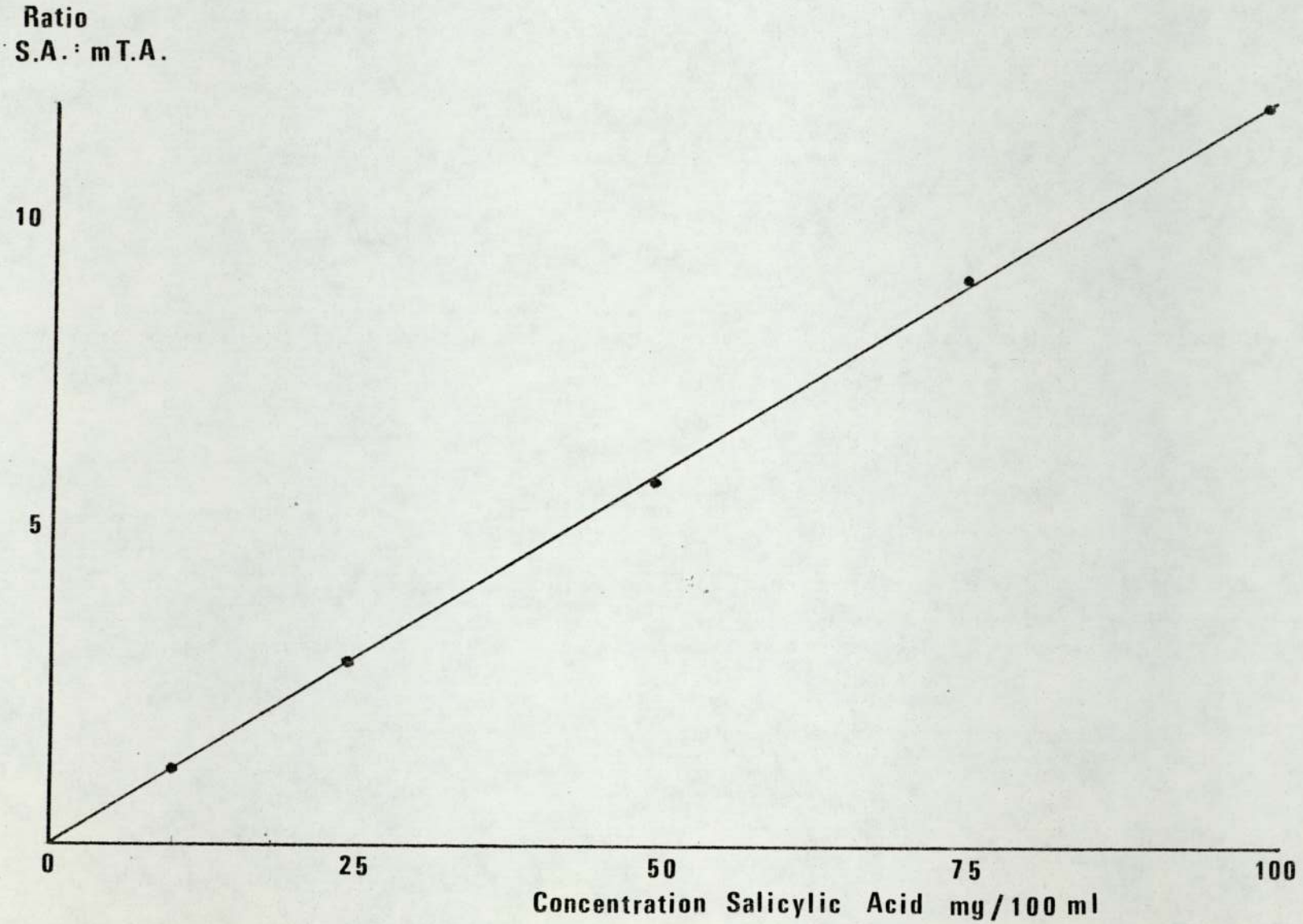
(25 mg/100 ml)

1973
1747

(10 mg/100 ml)

Calculation of peak height ratio e.g. $\frac{1515}{127} = 11.9$

Figure 28. Calibration curve showing concentrations of salicylic acid plotted against the peak height ratios of salicylic acid:m-toluic acid peaks (M4.2).



Materials: OV17 stationary phase

CQ 80-100 mesh support

Salicylic acid

m-toluic acid

Glass distilled ether

BSTFA

Method: Solutions containing 10 mg/100ml salicylic acid and a similar concentration of m-toluic acid were made up. 1 ml of each was placed in each of two half dram glass vials (duplicates) and evaporated to dryness. 40 µl BSTFA was added to each and they were incubated at 50°C for 60 minutes. 2 µl aliquots were injected onto the column which was at 190 C and the carrier gas flow rate was 50 ml/minute.

Results and Discussion: The chromatograms obtained are shown in Fig. 30. They compared favourably with those obtained at Reckitt and Colman (Fig. 25) and the duplicate samples showed similarly sized peaks. The m-toluic acid derivative eluted after 30 seconds and the salicylic acid derivative after 48 seconds.

M4.5

Aim: To repeat the above experiment (M4.2) at Aston University

Equipment: As M4.4

Materials: As M4.1

Method: Solutions of 10 mg/100 ml salicylic acid and 10 mg/100 ml m-toluic acid were made up. 1 ml of the toluic acid solution and 1.0, 2.5, 5.0, 7.5, 10.0 aliquots of salicylic acid solution were evaporated and silylated as before (M4.2). 2 µl aliquots were injected onto the column which was at an oven temperature of 190°C and

Figure 29. Chromatogram and integrator readout of a sample containing an "unknown" concentration of m-toluic acid (M4.3).

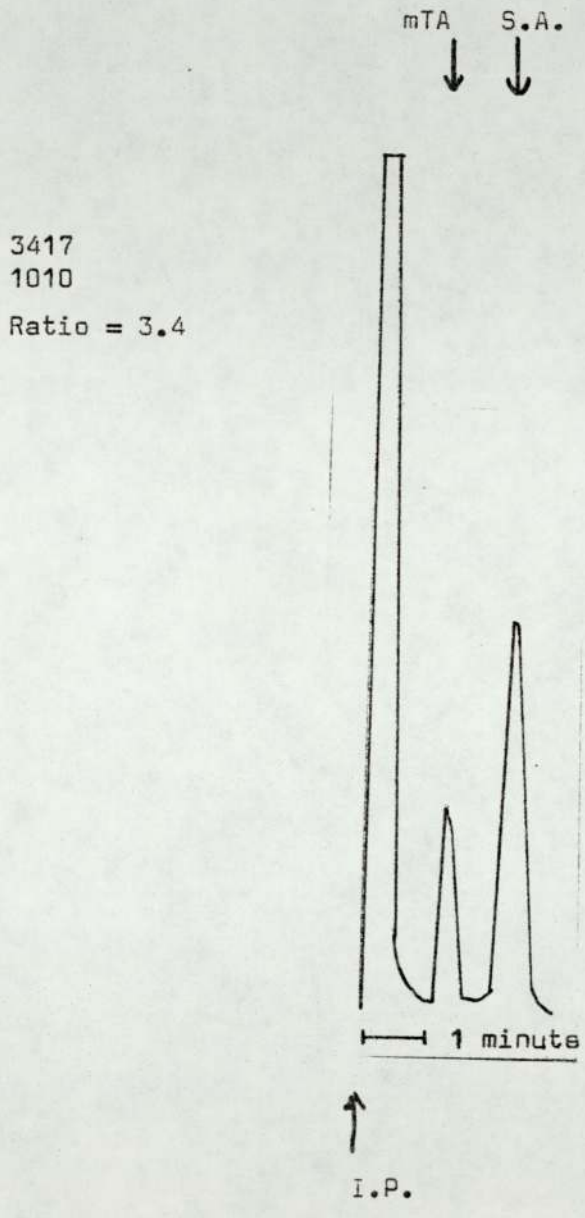
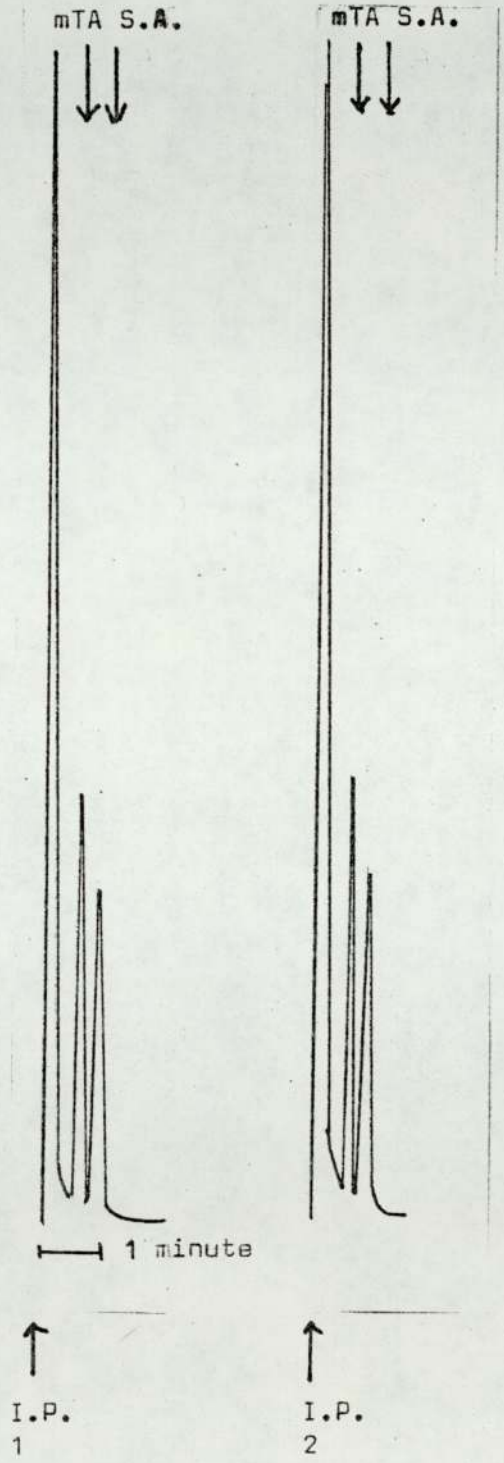


Figure 30. Chromatograms obtained by injection of duplicate silylated samples containing the equivalent of 10 mg/100 ml salicylic acid and a standard concentration of m-toluic acid. (M4.4).



Method: 3 ml of the salicylic acid solution and 1 ml of the toluic acid solution were evaporated to dryness and the residue taken up in 1 ml of plasma. This would give a plasma concentration equivalent to 30 mg/ 100 ml. To this was added 1 ml potassium bisulphate and 7 ml glass distilled ether and the mixture was thoroughly shaken in a vortex mixer for several minutes. The tube was centrifuged to separate the phases and the ether phase was then removed and placed in another centrifuge tube containing a few grammes of anhydrous sodium sulphate. This was then shaken, allowed to settle, the ether removed and placed in a third tube. The sodium sulphate was washed twice with 1 ml aliquots of ether and the washings added to the 7 mls. The ether was then evaporated until it could be transferred to the half dram vial, then evaporated to dryness and 40 µl BSTFA added. Incubation was at 50°C for 60 minutes. A 2 µl aliquot was injected onto the column.

Results and Discussion: The resulting chromatogram and integrator readout are shown in Fig. 29. When the peak area ratio calculated was compared with the calibration graph, it corresponded to a salicylic acid level of 28.7 mg/100 ml. The actual concentration was 30.0 mg/100 ml thus there was a 4% error, which seemed very encouraging.

M4.4

Aim: To repeat experiment M4.1 at Aston University

Equipment:

Perkin Elmer F-11 gas chromatograph with flame ionisation detector
Glass column 1.8 m x 0.4 cm (internal diameter)
0.5 dram glass vials
15 ml centrifuge tubes

the carrier gas flow rate was 50 ml/minute.

Results and Discussion: The chromatograms obtained are shown in Fig. 31 . It was immediately apparent that the peaks for salicylic acid were not as sharp as those obtained at Reckitt and Colman (Fig. 26), and that the shape of the peaks deteriorated after the 50 mg/100 ml sample, making accurate manual measurement of peak height difficult. Peak height calculation results are shown in Table 36 and the resulting calibration graph in Fig. 32. The graph was far from a straight line and did not pass through the origin. It was suspected that the detector was not operating effectively and was unable to cope with the more highly concentrated samples. On examination, it was found that the detector was thickly coated with silicon deposits which could have accounted for its fall off in sensitivity.

M4.6

Aim: To overcome the problem of "fouling" of the detector

Equipment: As M4.4

Materials: As M4.4

Method: The above procedure was repeated (M4.5) except that the detector was cleaned manually between each injection of sample.

Results and Discussion: The chromatogram obtained is shown if Fig. 33, and the peak height ratios are given in Table 37. These show that there was an increase in detector sensitivity, reflected in higher peak height ratios. A graph of these plotted against concentration of salicylic acid, although now going through the origin, still plateaued slightly after the 50 mg/100 ml sample - Fig.34. Since build up of silicon deposits seemed to threaten the sensitivity of the detector, and the source of these deposits was the silicon of the silyl-

Figure 31. Chromatograms of silylated samples containing the equivalents of salicylic acid concentrations between 10 and 100 mg/100 ml and the standard concentration of m-toluic acid (M4.5).

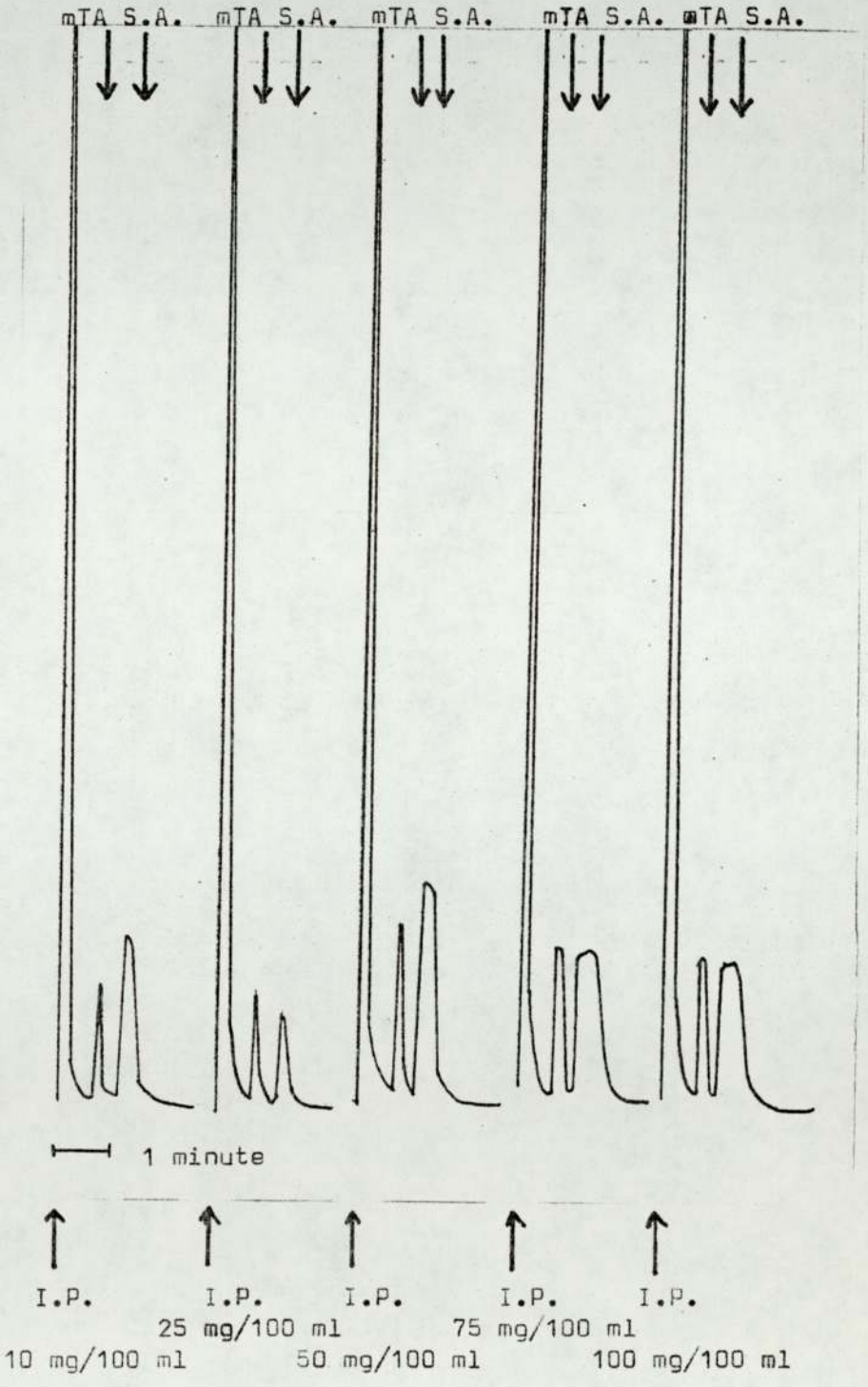


Table 36. Peak height ratios of samples containing various concentrations of salicylic acid and analysed by procedure M4.5.

Salicylic acid concentration (mg/100 ml)	Peak height ratio (Salicylic acid peak: m-toluic acid peak)
10	1.085
25	2.250
50	2.863
75	2.250
100	2.610

N.B. When peaks are sharply defined, "peak height" is usually accurate enough a measure to use in ratios. However in the chromatograms obtained in this study, peaks were not always sharply defined and therefore, in all cases, "peak height ratios" were in fact the ratios of (peak height x peak width at half height) which estimates to the actual peak area ratios. They were calculated using the formula:

$$\frac{A_u}{A_s} = \frac{h_u \cdot w_u}{h_s \cdot w_s}$$

Where:
A_u = area of unknown peak
A_s = " " standard peak
h_u = height of unknown peak
h_s = " " standard peak
w_u = width at half height of unknown peak
w_s = " " " " " standard peak

Figure 32. Graph showing the relationship between concentration of salicylic acid and salicylic acid:m-toluic acid peak height ratios as found in M4.5.

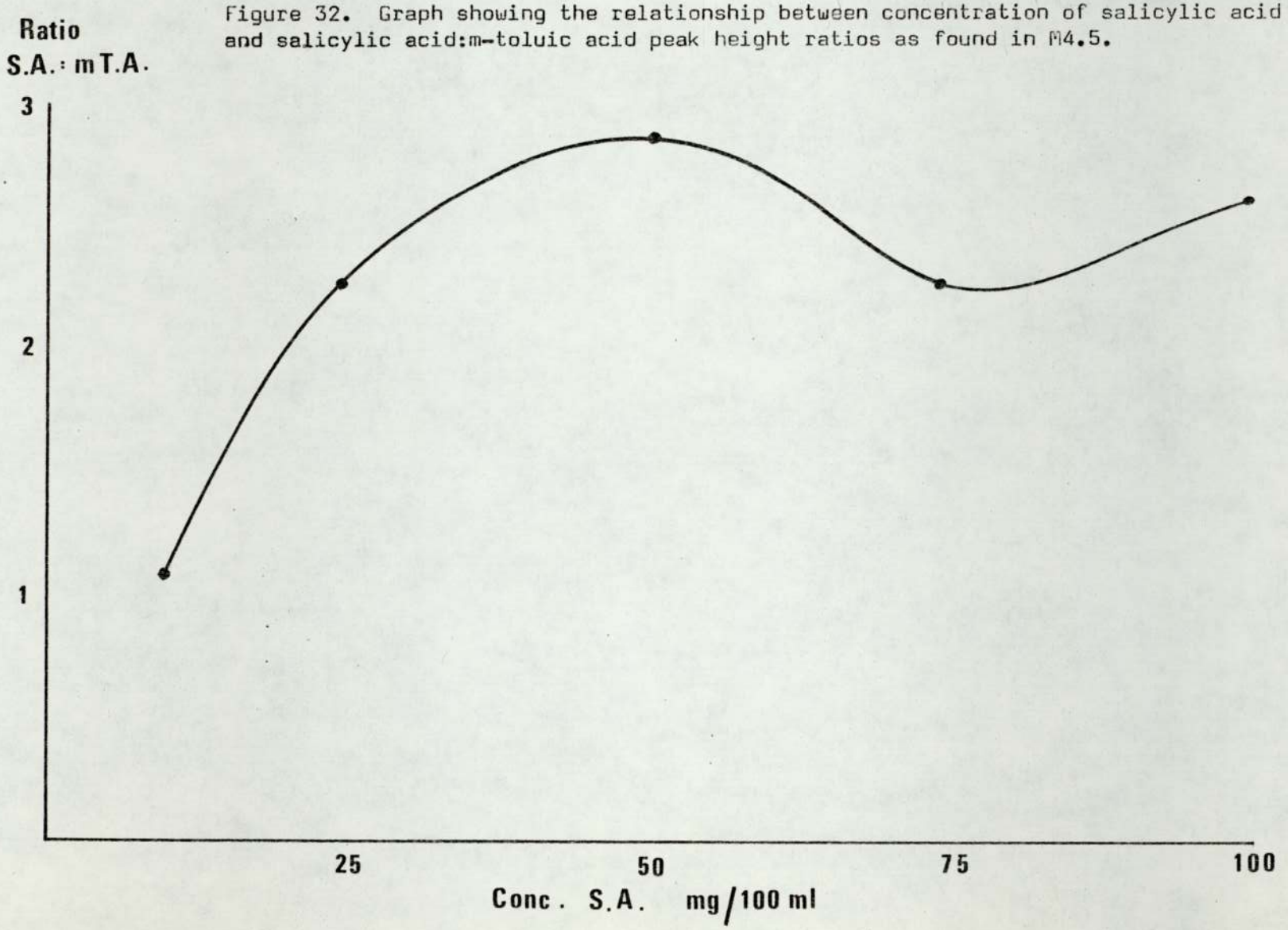


Figure 33. Chromatograms of silylated samples containing concentrations of salicylic acid between 10 and 100 mg/ 100 ml and a standard concentration of m-toluic acid (M4.6).

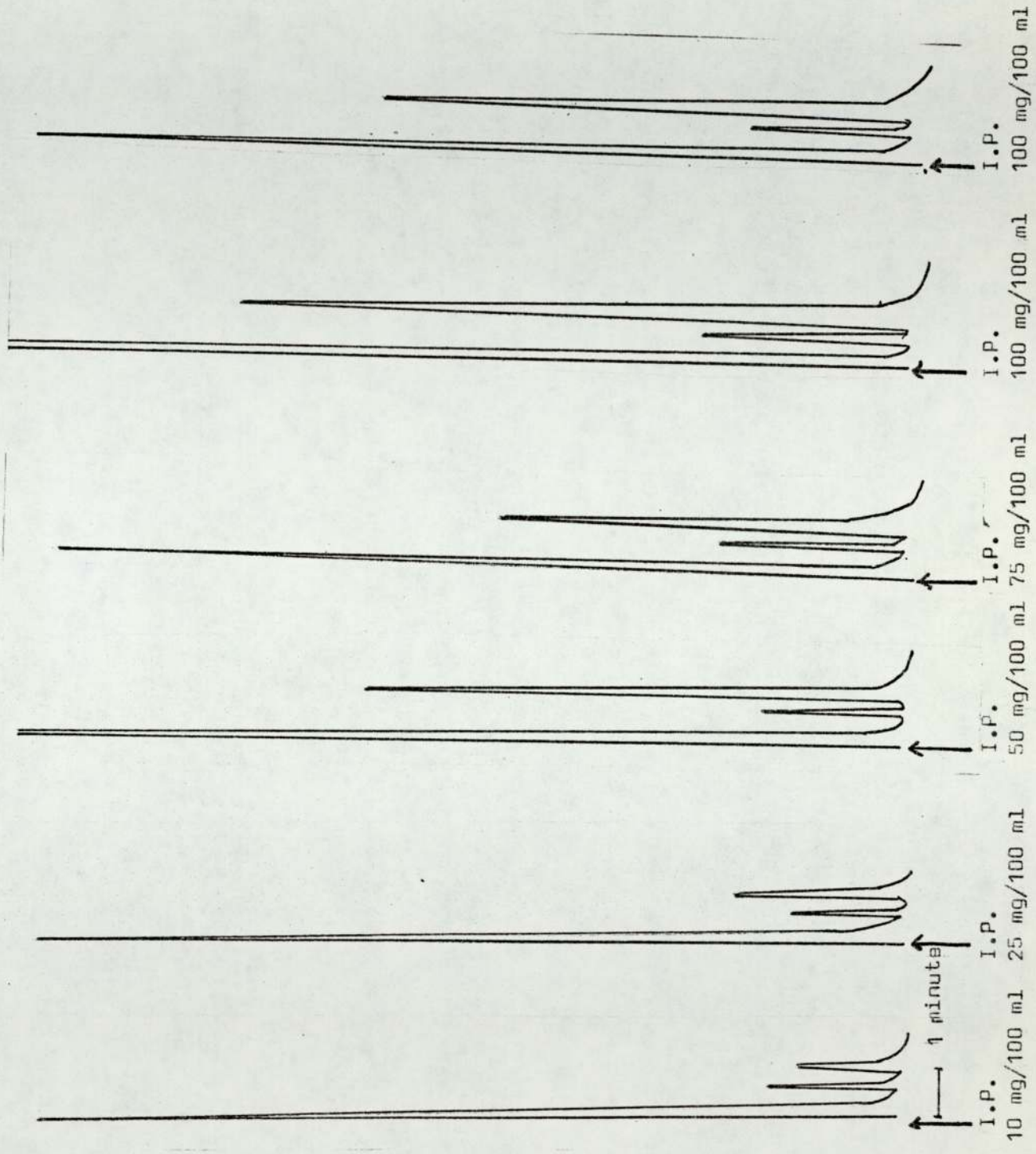
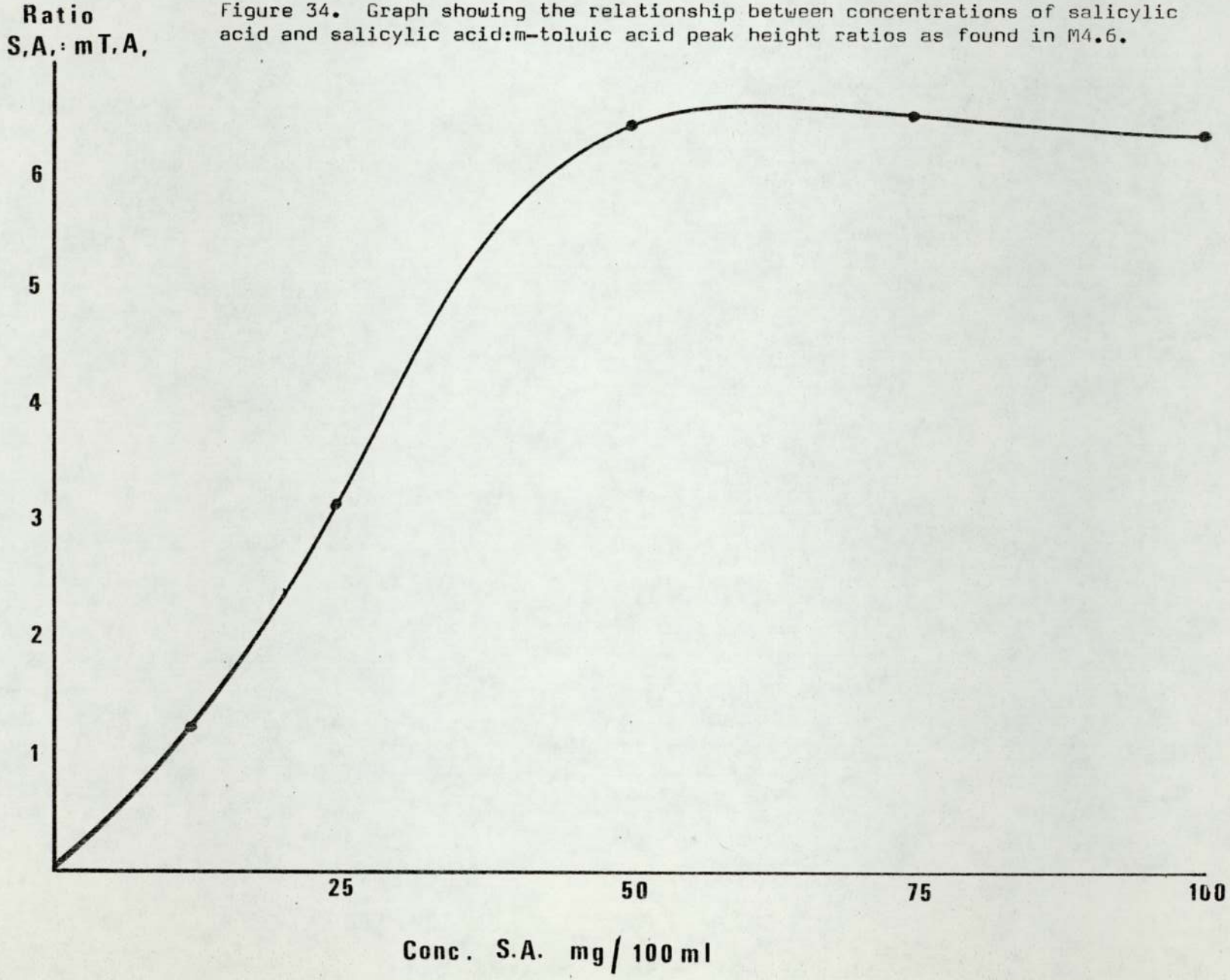


Table 37. Peak height ratios of samples containing various concentrations of salicylic acid and analysed by procedure M4.6.

Salicylic acid concentrations (mg/100 ml)	Peak height ratios
10	1.205
25	3.194
50	6.417
75	6.946
100	6.368

Figure 34. Graph showing the relationship between concentrations of salicylic acid and salicylic acid:m-toluic acid peak height ratios as found in M4.6.



ating reagent, it was considered possible that 2 µl aliquots might be too large for this particular piece of apparatus.

M4.7

Aim: To overcome the "fouling" of the detector by decreasing injected sample size.

Equipment: As M4.4

Materials: As M4.4

Method: 1 µl aliquots of the same samples as used in M4.6 were injected onto the column and the attenuation of the output adjusted accordingly.

Results: The chromatograms obtained are shown in Fig. 35 and the peak height ratios in Table 38. Graphical presentation of these ratios against concentration of salicylic acid approximated to a straight line and a line drawn between the first and last points did pass through the origin. However the relationship should be a simple straight line (M4.2) and as such this graph was not really acceptable as a calibration graph Fig. 36.

M4.8

Aim: To obtain a straight line calibration curve for concentration of salicylic acid against peak height ratio.

Equipment and Materials: As M4.4

Method: 0.5 µl aliquots of each of the previously prepared samples (M4.6) were injected onto the column three times. The means of the peak height ratios were plotted against the appropriate concentrations.

Figure 35. Chromatograms of silylated samples containing concentrations of salicylic acid between 10 and 100 mg/100 ml and a standard concentration of m-toluic acid, obtained by reducing the injected volume from 2 µl to 1 µl (M4.7).

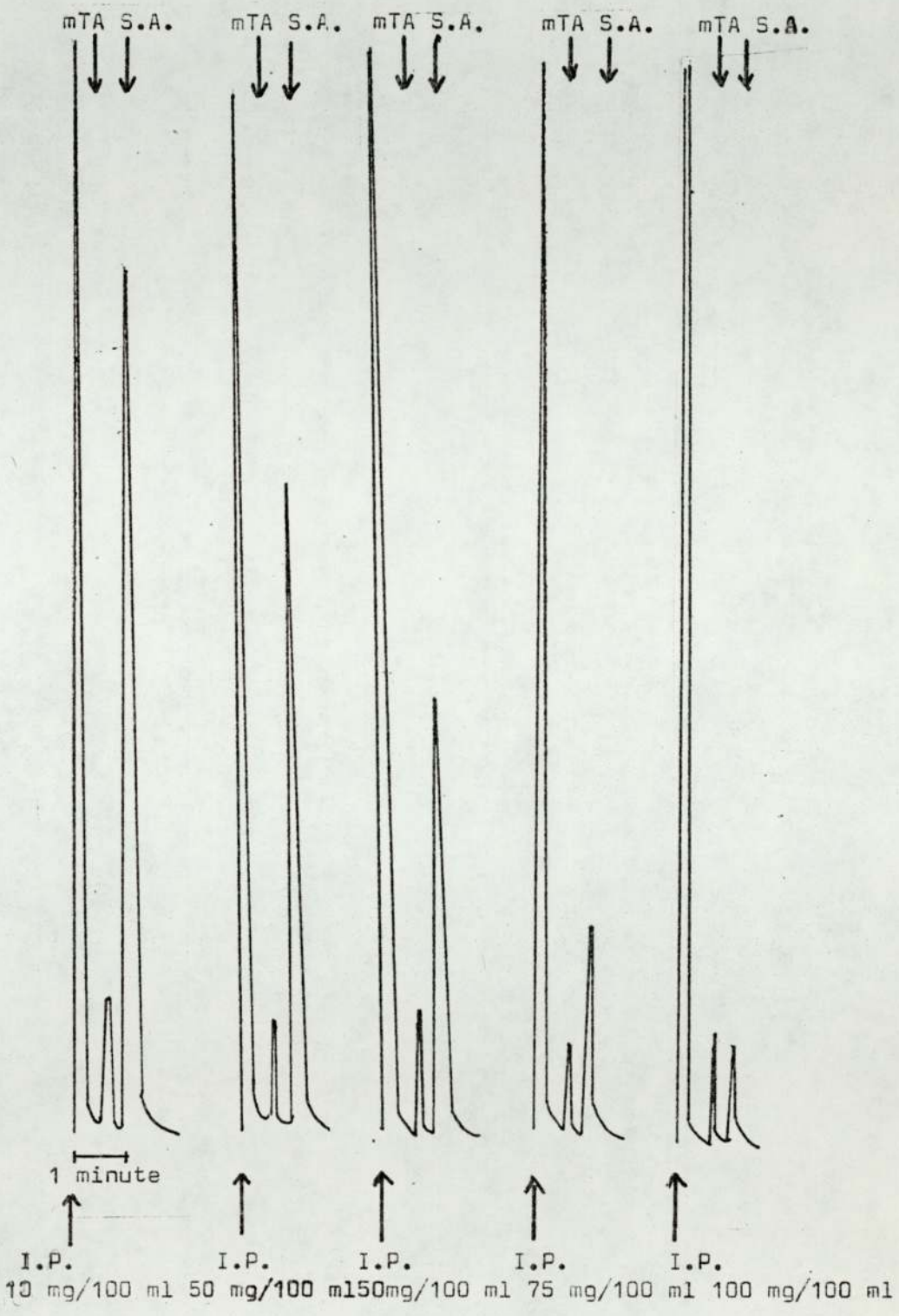
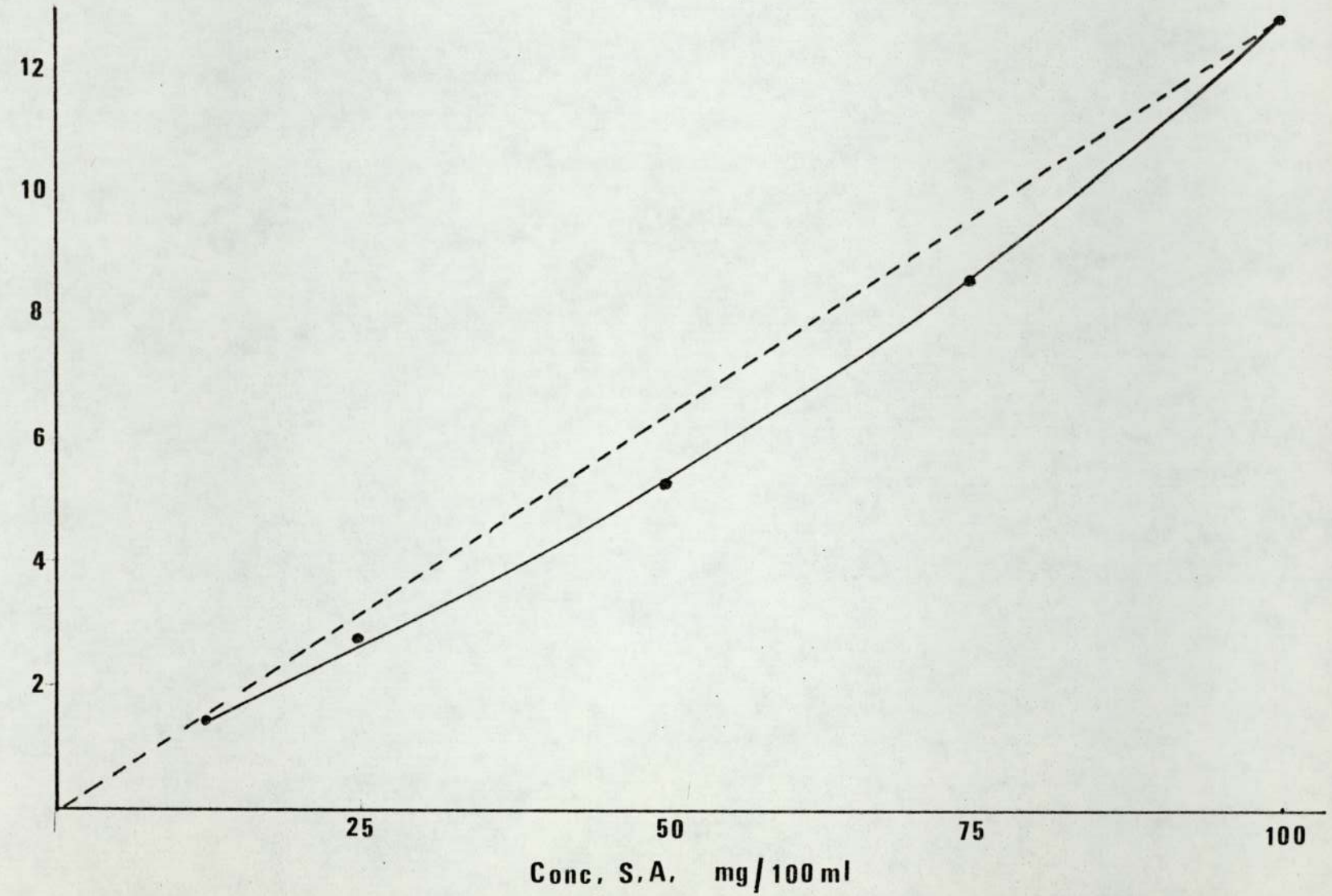


Table 38. Peak height ratios of samples containing various concentrations of salicylic acid and analysed by procedure M4.7.

Salicylic acid concentrations (mg/100 ml)	Peak height ratios
10	1.40
25	2.70
50	5.20
75	8.50
100	12.80

Figure 36. Graph showing the relationship between concentration of salicylic acid and salicylic acid:m-toluic acid peak height ratios as found in M4.7.



Results and Discussion: The chromatograms obtained are shown in Fig. 37, the peak height ratios in Table 39, and the resulting calibration graph in Fig. 38. The graph was a good straight line passing through the origin and as such could be used as a calibration graph.

M4.9

Aim: To repeat the assay of an "unknown" plasma sample to check the accuracy of the method.

Equipment: As M4.4

Materials: As M4.4 plus
Salicylic acid-free plasma

Method: A mixture of 1 ml of 10 mg/100 ml m-toluic acid solution and 3 ml of the same concentration of salicylic acid solution was evaporated to dryness and taken up in 1 ml plasma to represent a plasma concentration of 30 mg/100 ml. This "spiked" plasma sample was then thoroughly shaken with 7 ml glass distilled ether and the mixture separated by centrifugation. The ether layer was removed and placed in another centrifuge tube containing a few grammes of anhydrous sodium sulphate. This was shaken, allowed to settle and the ether removed to another tube. The sodium sulphate was washed with 2 x 1 ml aliquots of ether and these washings added to the 7 ml. The ether was evaporated until it could be transferred to the half dram vial where evaporation was completed. 40 µl BSTFA were added to the vial which was incubated at 50°C for 60 minutes. A 0.5 µl aliquot was injected onto the column.

Results and Discussion: The chromatograph obtained is shown in Fig. 39. The peak height ratio was 3.38. When this was checked against the calibration graph obtained in M4.8, it corresponded to a salicylic

Figure 37. Chromatograms of silylated samples containing concentrations of salicylic acid between 10 and 100 mg/100 ml and a standard concentration of m-toluic acid, obtained by reducing the injected volume from 1 µl to 0.5 µl (M4.8) (continued overleaf)

Run 1

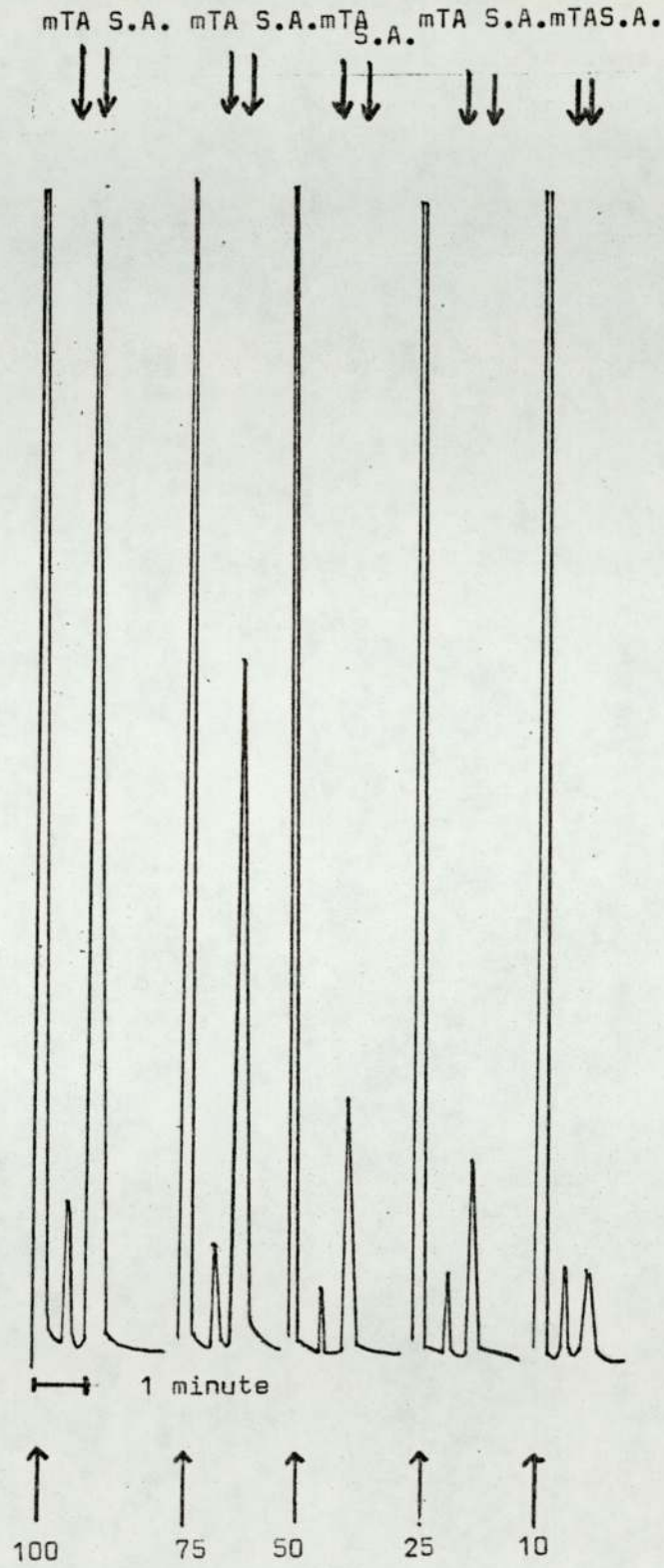


Figure 37. (contd.)

Run 2

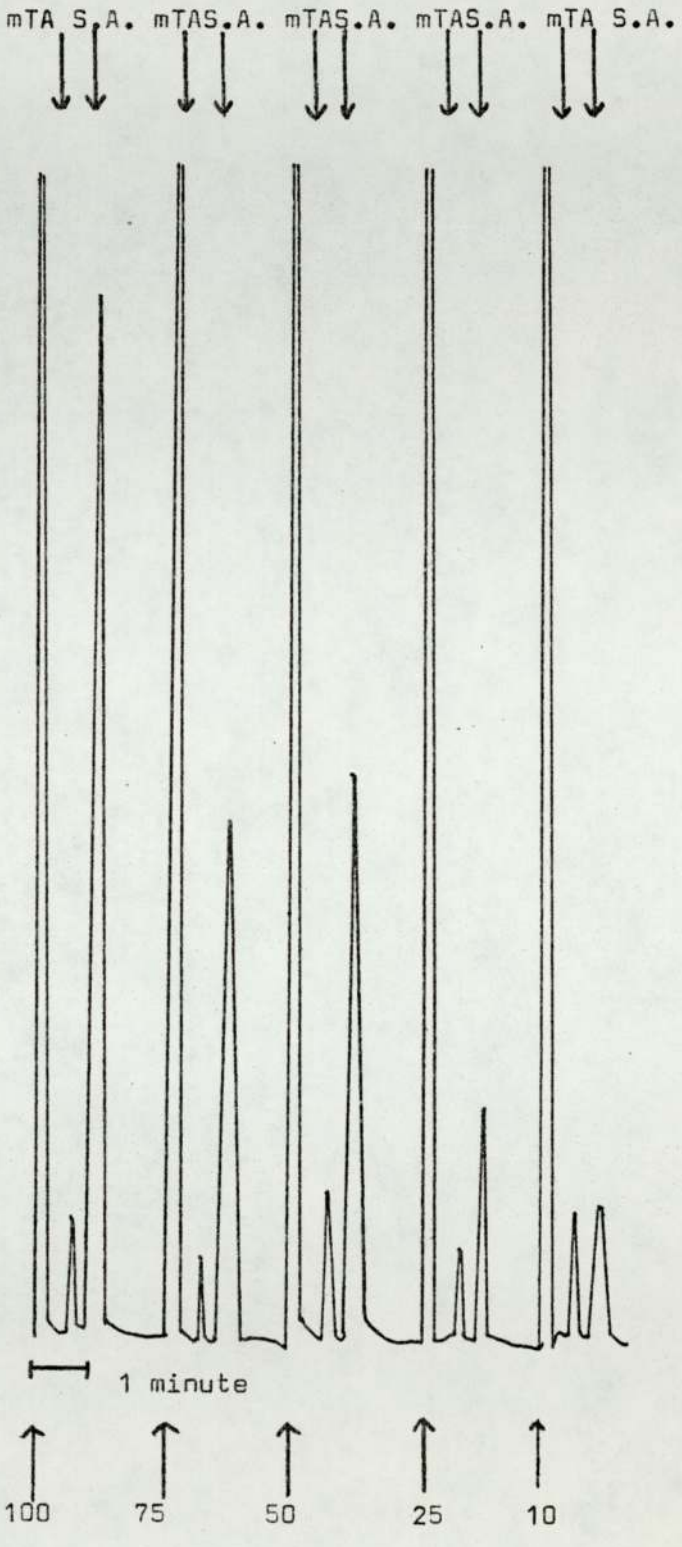


Figure 37. (contd.)

Run 3

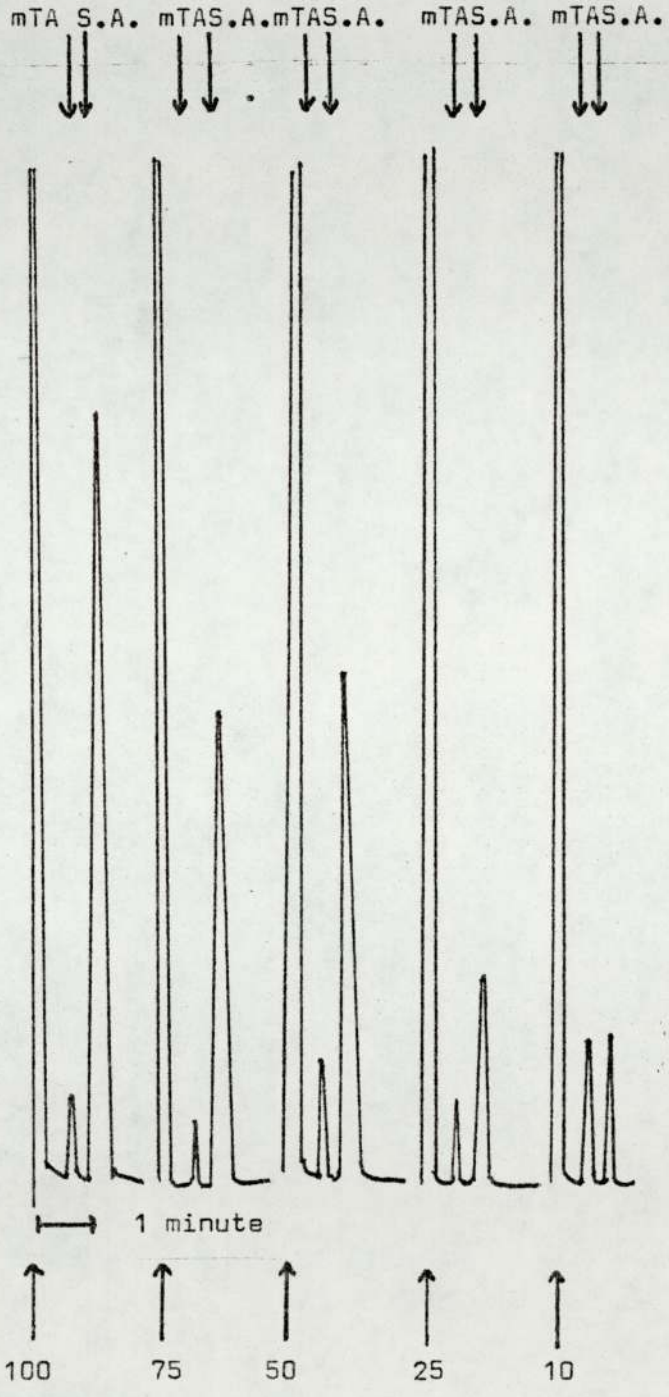


Table 39. Peak height ratios of chromatograms given by injection of 0.5 µl aliquots of samples.

Salicylic acid concentrations (mg/100 ml)	Peak height ratios			Means
	1	2	3	
10	1.21	1.45	1.28	1.31
25	2.83	3.72	3.09	3.21
50	6.15	5.46	5.57	5.73
75	7.30	9.53	8.32	8.38
100	10.82	12.22	10.11	11.05

Figure 38. Calibration graph showing the relationship between concentrations of salicylic acid of 10–100 mg/100 ml and salicylic acid:m-toluic acid peak height ratios as found in M4.8.

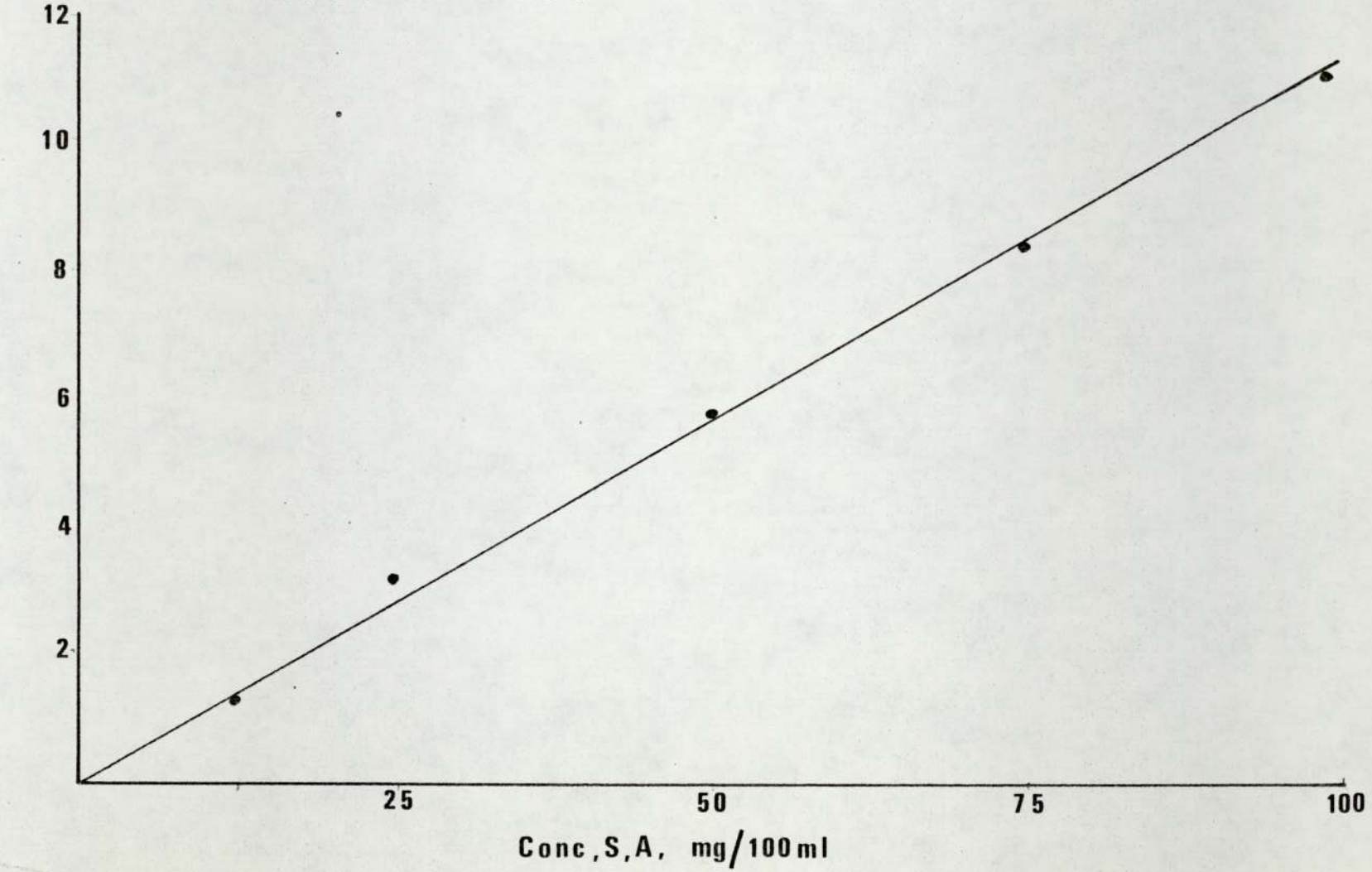
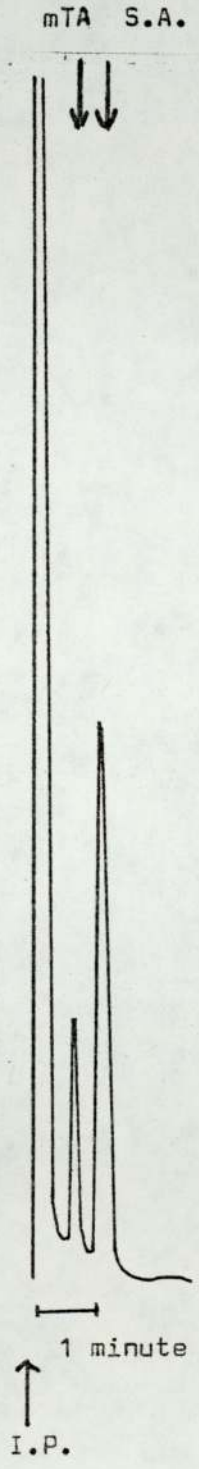


Figure 39. Chromatogram of a silylated sample containing an "unknown" plasma concentration of salicylic acid and a standard concentration of m-toluic acid.



acid concentration of 28.9 mg/100 ml. This compared favourably with the actual concentration of 30 mg/100 ml, giving an error of 3%.

M4.10

Aim: To produce a calibration graph for concentrations of salicylic acid from 1-10 mg/100ml.

Equipment and Materials: As M4.4

Method: 1 ml of the toluic acid solution and 0.1, 0.25, 0.50, 0.75, and 1.00 ml of the salicylic acid solution were mixed. They were evaporated and derivatised as previously described (M4.2) and 0.5 µl aliquots injected onto the column.

Results and Discussion: The chromatograms obtained are shown in Fig. 40 , and the peak height ratios in Table 40. These were plotted against the concentration of salicylic acid and the resulting calibration graph is shown in Fig. 41. This shows some irregularity in the lower order of concentration, but as low a concentration as 5 mg/100ml could be measured with accuracy.

Conclusions: This method appeared to be simple to reproduce and was effective in producing a chromatogram which could be used to measure concentration of salicylic acid from 5 mg/100 ml to 100 mg/100ml.

Method 5 - Levy and Procknall (1968)

This was a colourimetric method, based on a complexation with Ferric ions and was considered as a possible alternative to GLC as a method of assaying body fluids for salicylic acid.

M5.1

Aim: To assay a solution of 10 mg/100 ml salicylic acid.

Figure 40. Chromatograms of silylated samples containing concentrations of salicylic acid between 1 and 10 mg/100 ml and a standard concentration of m-toluic acid (M4.10)

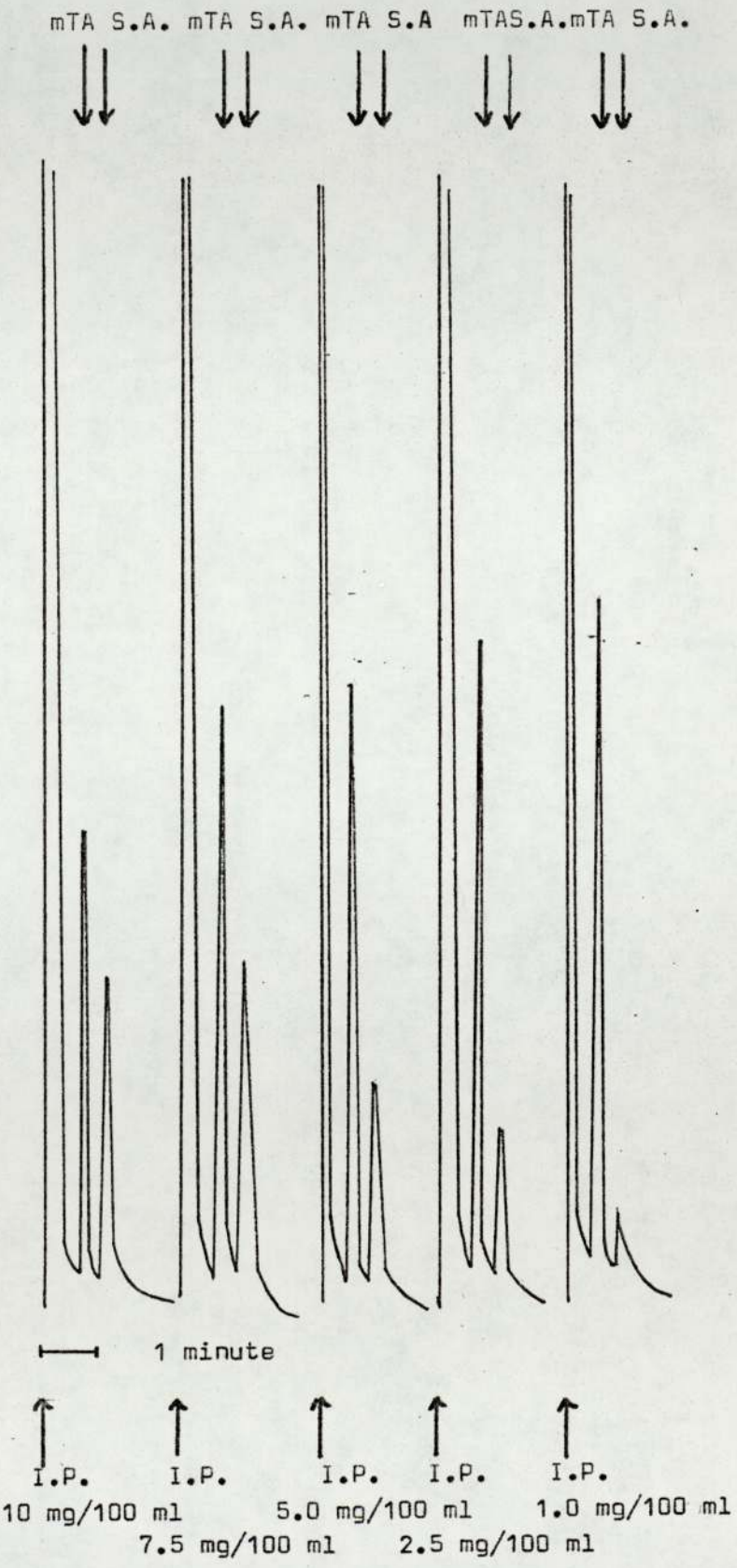


Table 40. Peak height ratios of samples containing various concentrations of salicylic acid and analysed by procedure M4.10.

Salicylic acid concentration (mg/100 ml)	Peak height ratios
1.0	2.690
2.5	2.506
5.0	6.410
7.5	10.140
10.0	13.050

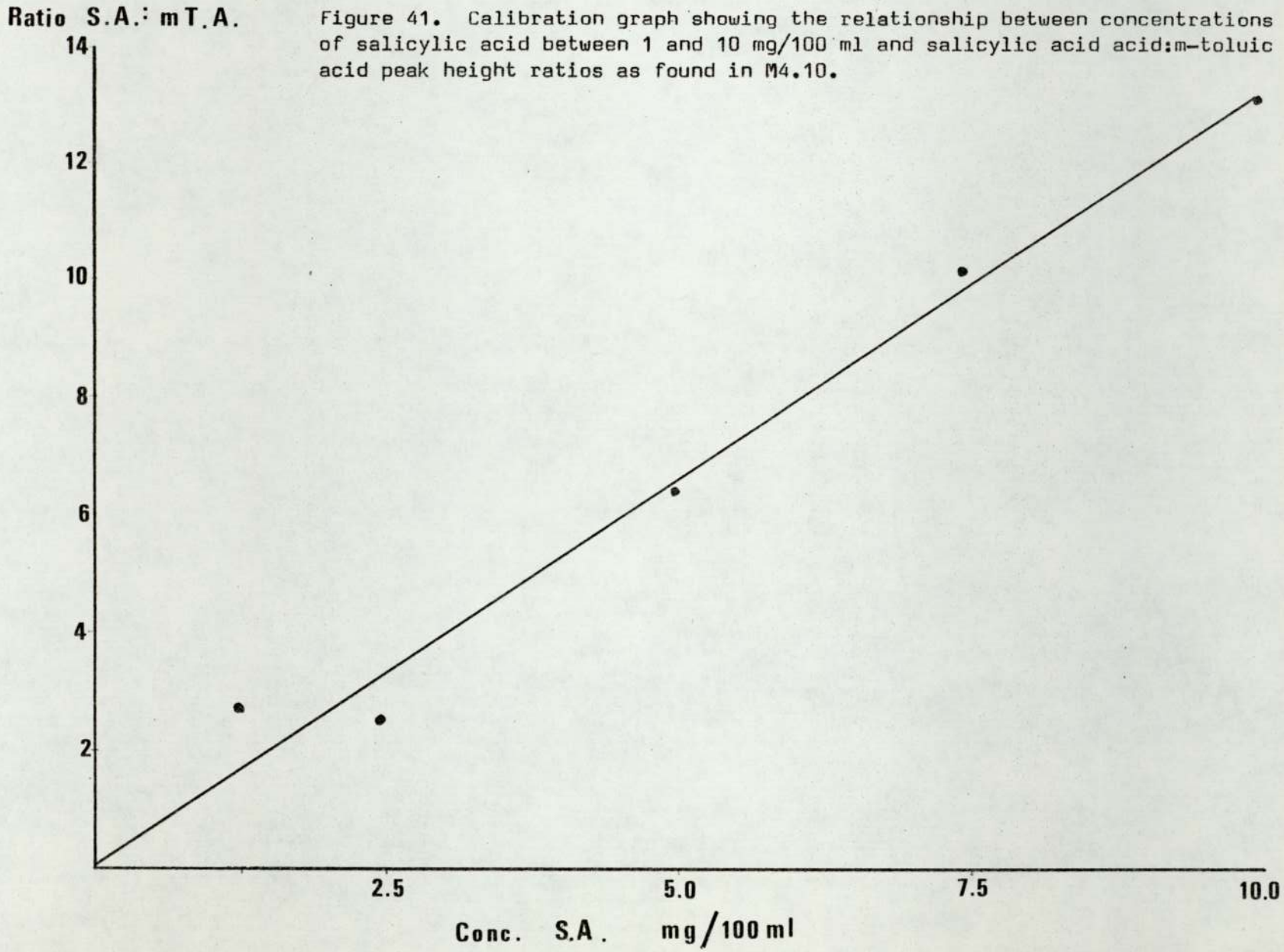


Figure 41. Calibration graph showing the relationship between concentrations of salicylic acid between 1 and 10 mg/100 ml and salicylic acid acid:m-toluic acid peak height ratios as found in M4.10.

Equipment: EEL Spectrophotometer (visible light only)

Matched glass cells

Materials: Salicylic acid solution - 10 mg/100 ml

Carbon tetrachloride

Hydrochloric acid 6N

Nitric acid 0.07N

Ferric nitrate

Method: The details of this method are shown in Fig. 42. 1 ml of the salicylic acid solution and 1 ml of distilled water were mixed with 0.5 ml 6N hydrocloric acid and 30 ml carbon tetrachloride and extracted and re-extracted by this method. The resulting aqueous phase was measured against a blank of water which had been extracted by the same method, their optical densities being measured at 530 mμ.

Results and Discussion: The optical density of the ferric nitrate phase was quite low at 8.0 compared with the blank. It was thought that such a low optical density with a reasonably concentrated solution might indicate difficulty in assaying lower concentrations accurately.

M5.2

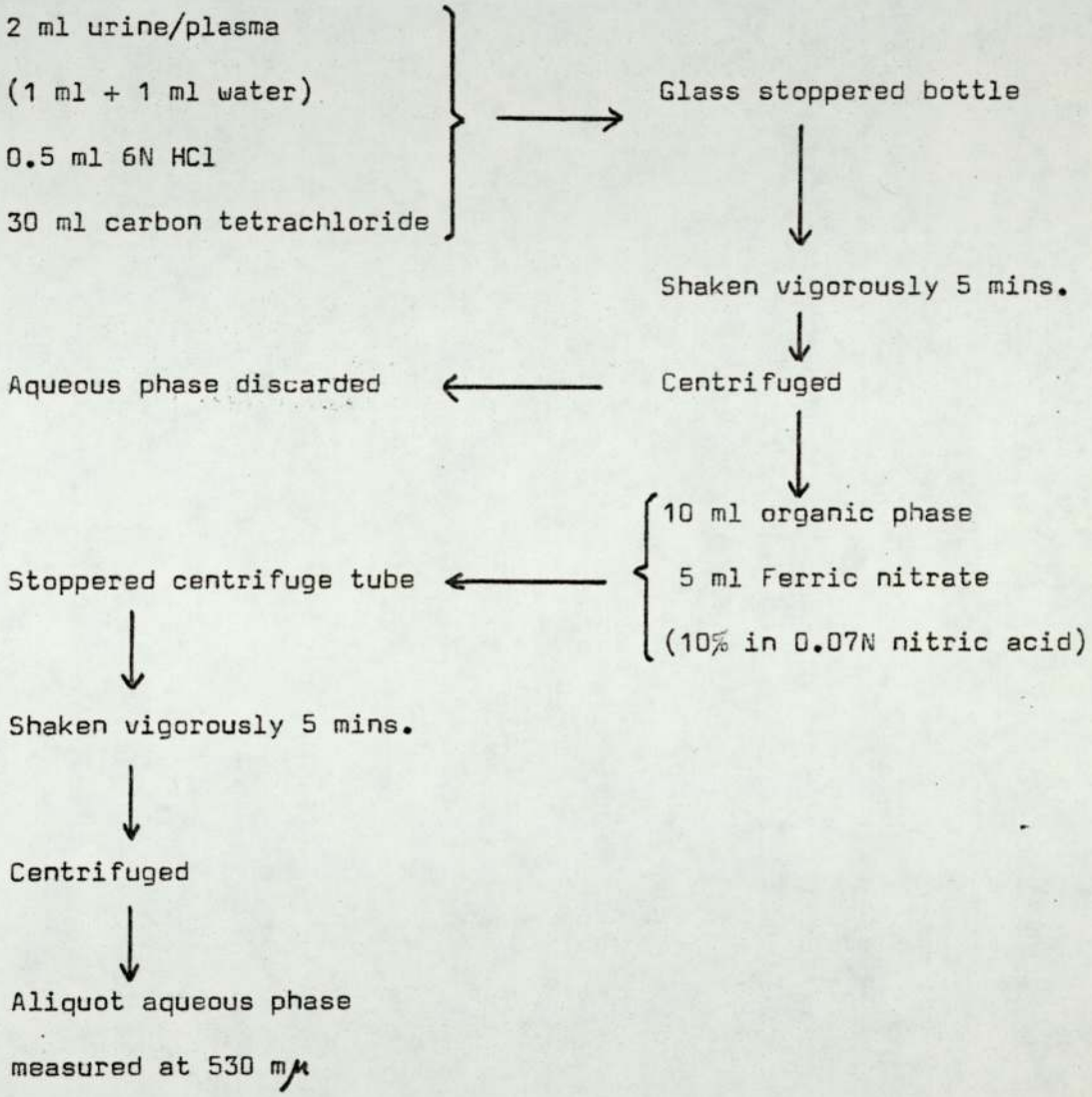
Aim: To improve the extraction procedure and produce a higher optical density.

Equipment and Materials: As M5.2

Method: The same procedure was used as in M5.1 except that two 10 ml aliquots of carbon tetrachloride were used for extraction in place of the single extraction with 30 ml of carbon tetrachloride.

Results and Discussion: The optical density of the salicylic acid-

Figure 42. Details of colourimetric method for determining concentrations of salicylic acid in urine and plasma as described by Levy and Procknall (1968).



containing sample had increased to 15.0.

M5.3

Aim: To further improve the extraction procedure.

Equipment and Materials: As M5.1

Method: The amount of organic phase was again reduced by altering the extraction procedure so that three 5 ml aliquots were used to extract in place of the two 10 ml extractions used in M5.2.

Results and Discussion: The optical density again increased to 15.8.

M5.4

Aim: To further improve the extraction procedure.

Equipment and Materials: As M5.1

Method: The 1 ml of water was omitted from the extraction mixture and the procedure used in M5.3, employing three 5 ml extractions was used.

Results and Discussion: The resultant optical density was 16.6, a further improvement.

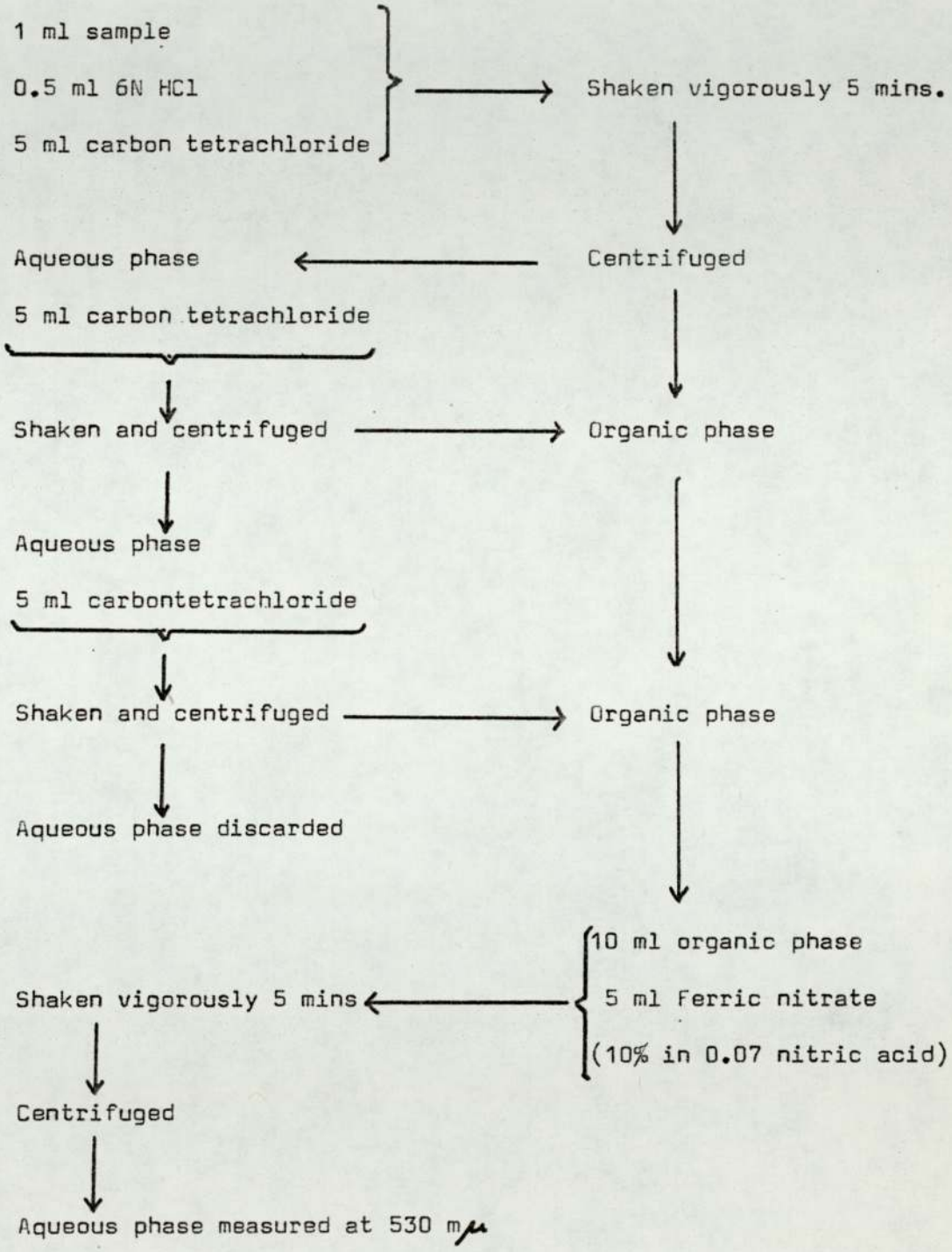
Summary: A summary of the improved method is given in Fig. 43.

M5.5

Aim: To construct a calibration curve for concentrations of salicylic acid from 0.5 - 100 mg/100 ml.

Equipment and Materials: As M5.1 plus Salicylic acid solutions containing 0.5 - 100 mg/100 ml in water

Figure 43. Details of the improved colourimetric method for determining salicylic acid in urine and plasma.



Method: Triplicate 1 ml samples of the salicylic acid solutions were analysed according to the procedure described in M5.4.

Results and Discussion: The resulting optical densities are given in Table 41. They are shown plotted against the appropriate concentrations of salicylic acid in Fig. 44. The resulting graph was a straight line but resolution at the lower end of the concentration scale was poor as shown by Fig. 45. A semi-logarithmic plot was considered and was easier to read at these lower concentrations. This is shown in Fig. 46.

M5.6

Aim: To check the percentage recovery of added salicylic acid from plasma.

Equipment and Materials: As M5.1 plus salicylate-free plasma
Salicylic acid solution 10 mg/100 ml in ether

Method: Aliquots of the ether solution were evaporated down and then taken up in salicylate-free plasma to correspond to plasma concentrations of 0.5 - 100 mg/100 ml. The samples were triplicated and subjected to the extraction procedure outlined in M5.4. The optical densities were read at 530 mμ.

Results and Discussion: The resulting optical densities are shown in Table 42. They show an average recovery from solutions containing 7.5 - 100 mg/100 ml of 89.66, however the extent of the recovery was variable between concentrations but quite consistent between the duplicates of the samples. When the optical densities were plotted against log-concentrations of salicylic acid a reasonable calibration curve resulted. (See Table 43 and Fig. 47).

Table 41. Optical densities resulting from treatment of various concentrations of salicylic acid in water with M5.5.

Salicylic acid concentrations (mg/100 ml)	Optical densities			Means	Standard Errors
	1	2	3		
0.5	3.7	3.9	3.4	3.67	± 0.145
1.0	4.0	7.0	5.6	5.53	± 0.866
2.5	7.6	7.0	8.9	7.83	± 0.560
5.0	9.4	13.1	9.8	10.77	± 1.172
7.5	12.8	14.4	-	13.60	± 0.799
10.0	15.0	16.3	15.0	15.43	± 0.433
25.0	-	38.7	30.9	34.80	± 3.899
50.0	74.0	-	66.5	70.25	± 3.749
100.0	140.0	139.0	148.0	142.33	± 2.848

Figure 44. Calibration graph showing the relationship between aqueous concentrations of salicylic acid between 0.5 and 100 mg/100 ml and the optical density at 530mμ after treatment by the method described in Fig. 43 (standard errors shown).

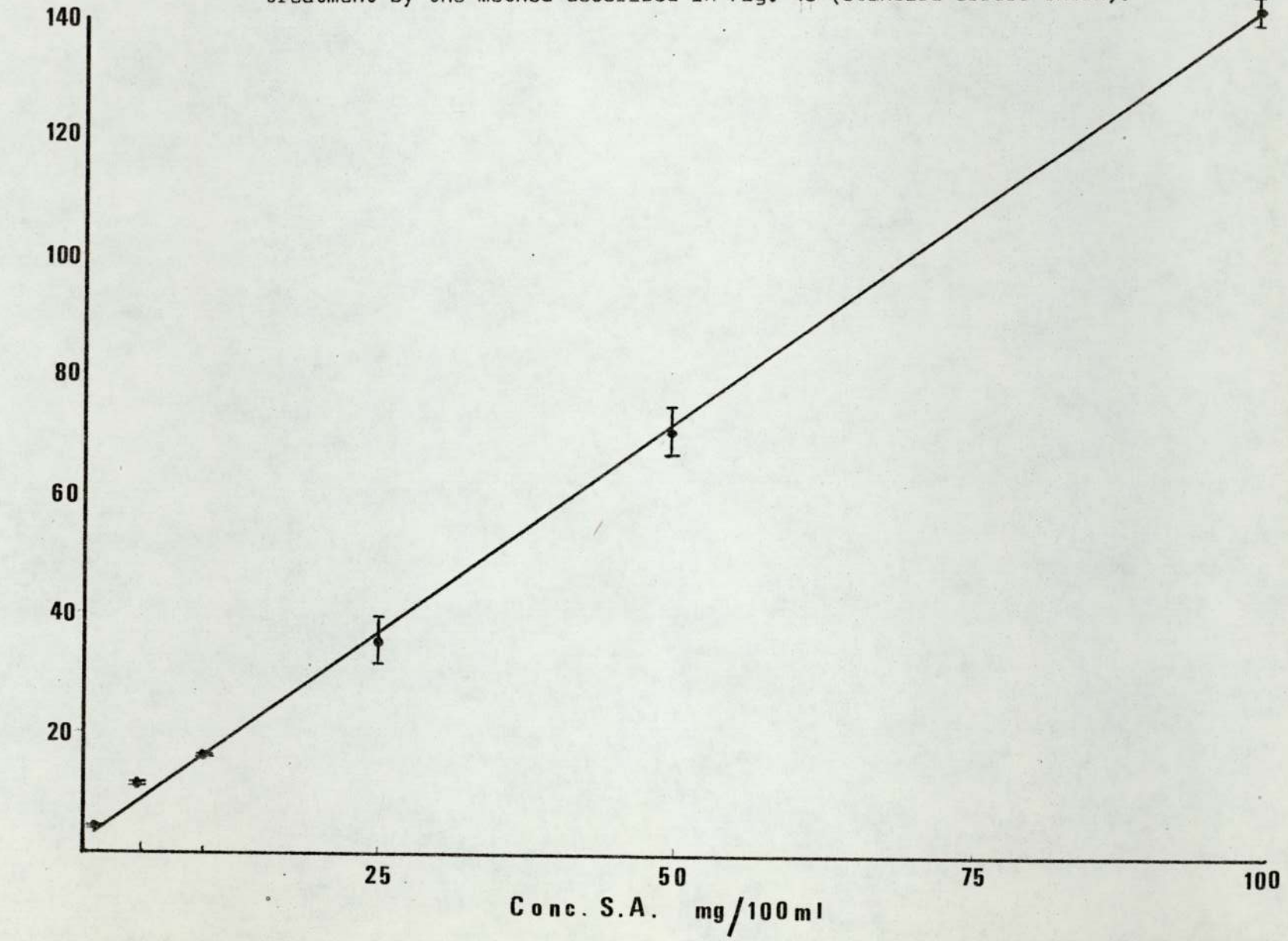


Figure 45. Calibration graph showing the relationship between aqueous concentrations of salicylic acid between 0.1 and 10 mg/100 ml and optical density after treatment by the method described in Fig. 43 (standard errors shown).

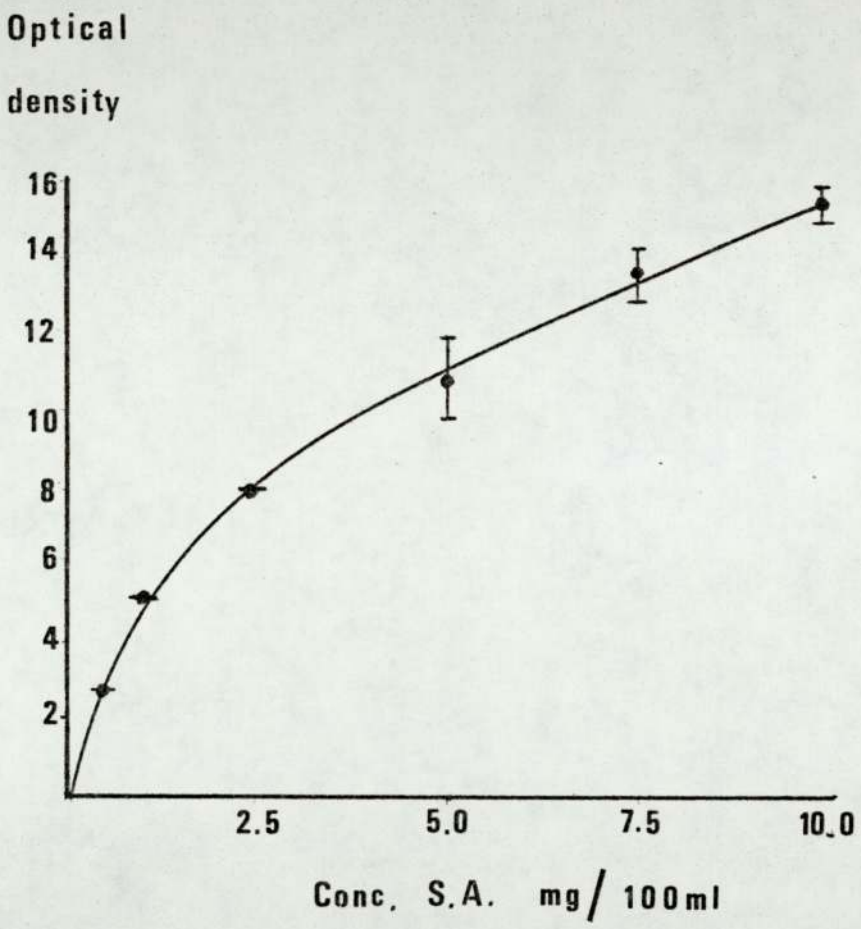


Figure 46. Calibration graph showing log. aqueous concentrations of salicylic acid plotted against optical density after treatment by the method described in Fig. 4.3 (standard errors shown).

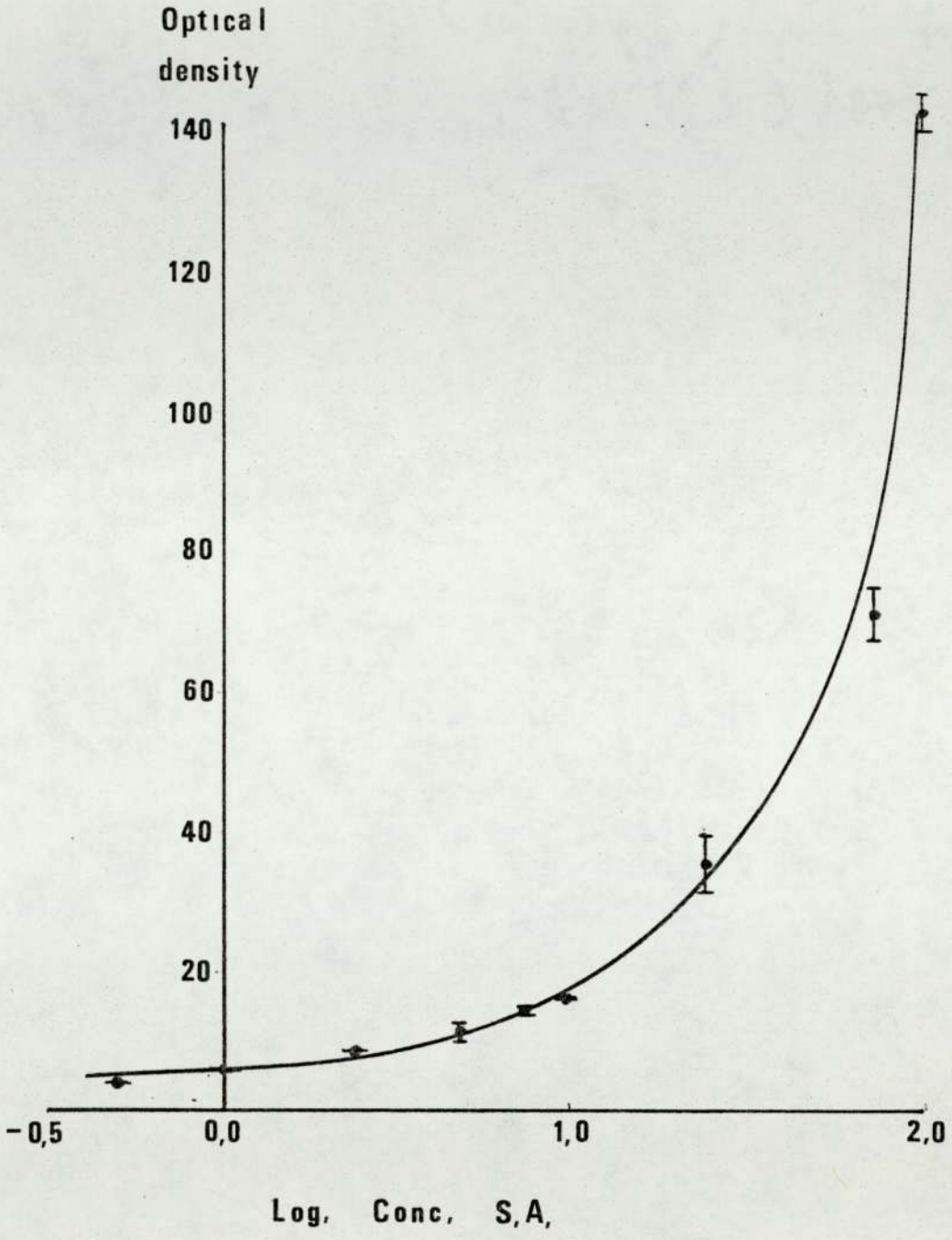


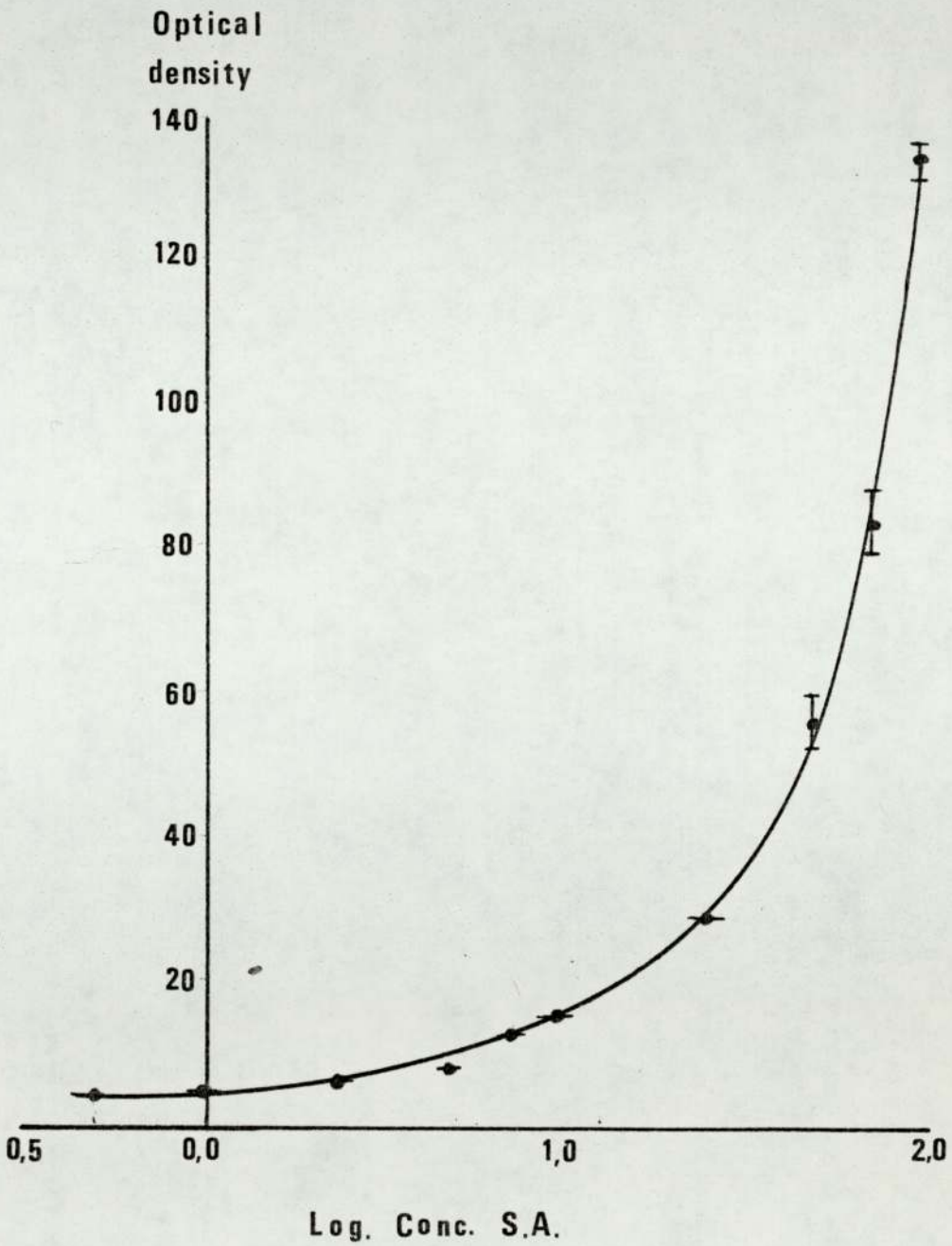
Table 42. Results of extraction of salicylic acid from plasma (M5.6).

Salicylic acid concentrations (mg/100 ml)	Optical densities				Std. Errors	Salicylic acid found (mg/100 ml)	% Recovery
	1	2	3	Means			
0.5	4.0	3.6	3.8	3.80	± 0.10	0.51	102.0
1.0	4.4	4.2	4.4	4.33	± 0.06	0.64	64.0
2.5	5.6	4.6	6.9	5.60	± 0.68	1.12	44.8
5.0	6.8	7.0	8.5	7.43	± 0.53	2.46	49.2
7.5	11.9	13.7	12.1	12.56	± 0.50	7.76	103.5
10.0	13.7	14.9	13.8	14.13	± 0.38	9.44	94.4
25.0	27.8	29.4	27.8	28.33	± 0.53	20.65	82.6
50.0	-	58.5	52.0	55.25	± 3.24	39.54	79.0
75.0	84.0	89.0	76.0	83.00	± 3.78	58.88	78.5
100.0	132.0	137.0	-	134.5	± 2.49	94.84	94.8

Table 43. Construction of calibration curve Fig. 47 by procedure M5.6.

Salicylic acid concentration (mg/100 ml)	Log. concentration (mg/100 ml)	Optical density	Standard Errors
0.5	- 0.3010	3.80	± 0.10
1.0	0.000	4.33	± 0.06
2.5	+ 0.3979	5.60	± 0.68
5.0	+ 0.6990	7.43	± 0.53
7.5	+ 0.8751	12.56	± 0.50
10.0	+ 1.000	14.13	± 0.38
25.0	+ 1.3979	28.33	± 0.53
50.0	+ 1.6990	55.25	± 3.24
75.0	+ 1.8751	83.00	± 3.78
100.0	+ 2.0000	134.00	± 2.49

Figure 47. Calibration graph showing log. plasma concentrations of salicylic acid plotted against optical density at 530mμ after treatment by the method described in Fig. 43 (standard errors shown).



M5.7

Aim: To check the percentage recovery of added salicylic acid from urine.

Equipment and Materials: As M5.1 plus salicylate-free urine
Salicylic acid solution 10 mg/100 ml in ether

Method: Urine samples were "spiked" with concentrations of salicylic acid as the plasma samples had been in M5.6. They were then extracted as in M5.4 and the optical densities read at 530 mμ.

Results and Discussion: The optical densities are given in Table 44 The mean recoveries were similar to those obtained from plasma and the optical densities showed little difference from those resulting from extraction from plasma.

Conclusions: The improved method could be used to accurately estimate concentrations of salicylic acid from 5 - 100 mg/100 ml in plasma, using Fig. 47 as a calibration curve. The similarity of optical densities obtained on extraction of similar concentrations of salicylic acid from urine did not indicate a need for a separate calibration curve for urine.

4. Metabolites

(i) Salicyluric acid

M6.1

Aim: To obtain a chromatogram for salicyluric acid.

Equipment: Perkin Elmer F11 gas chromatograph with flame ionisation detector.
Glass column 1.8 m x 0.4 cm (internal diameter).

Table 44. Results of extraction of salicylic acid from urine (M5.7)

Salicylic acid concentrations (mg/100 ml	Optical densities			Std. Errors	Salicylic acid found (mg/100 ml)	% Recovery
	1	2	Means			
1.0	4.2	4.1	4.15	0.188	0.55	55.0
5.0	8.4	7.8	8.10	0.387	2.95	59.0
10.0	13.1	-	13.10	-	8.32	83.2
25.0	28.0	-	28.00	-	20.42	81.7
50.0	52.5	54.0	53.25	0.613	38.19	76.4
75.0	76.0	80.0	78.00	1.000	55.34	73.8
100.0	116.0	128.3	122.15	1.754	85.90	85.9

Materials: OV17 stationary phase

CQ 80-100 mesh support

Salicyluric acid

BSTFA

Methanol

Method: A 10 mg/100 ml solution of salicyluric acid was made up in methanol. 100 µl of this was evaporated to dryness and 50 µl BSTFA added. The mixture was incubated at 50°C for 60 minutes. A 0.5 µl aliquot was injected onto a column of 3% OV17 on CQ support at 230°C, with a flow rate of 50 ml/minute. A blank was prepared by evaporating 100 µl of methanol to dryness, adding 50 µl BSTFA and incubating as for the other sample.

Results and Discussion: The chromatogram obtained is shown in Fig. 48. It shows two peaks, both occurring in the sample containing salicyluric acid and neither of which were present in the blank. It was thought possible that the presence of two peaks could be due to incomplete silylation. As with salicylic acid, there are two hydroxyl groups on the salicyluric acid molecule which could be points of silylation (see Fig.49). If silylation were complete, the disilylated derivative should be the only one present, and only one peak could be expected.

M6.2

Aim: To drive the silylation reaction to completeness.

Equipment and materials: As M6.1.

Method: The above (M6.1) sample was reheated to 50°C and left at this temperature overnight. A 0.5 µl aliquot was injected onto the column.

Figure 48. Chromatogram of a silylated sample of salicyluric acid injected onto a column at 230°C and a flow rate of 50 ml/minute.

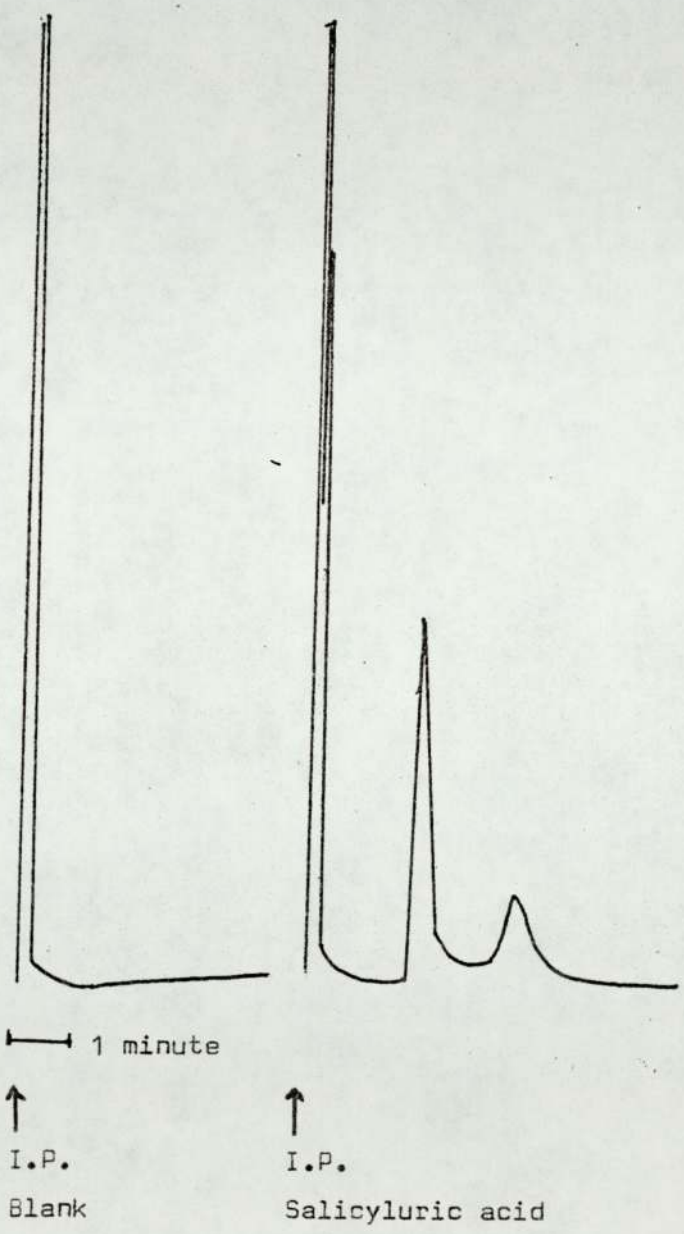
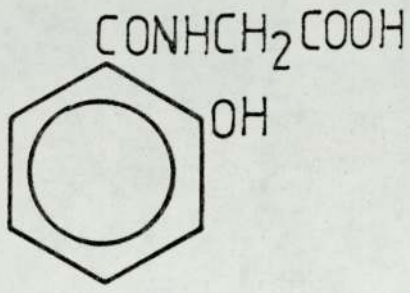
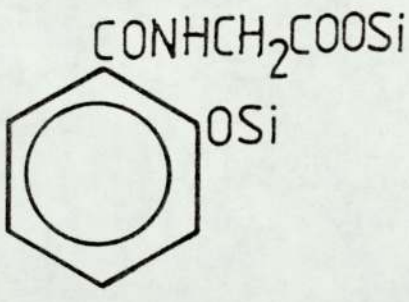


Figure 49. Structure of salicyluric acid and its disilyl derivative.



Salicyluric acid



Disilyl salicyluric acid

Results and Discussion: The chromatogram obtained is shown in Fig.50. Two peaks were still visible.

M6.3

Aim: To drive the silylation reaction to completeness.

Equipment and Materials: As M6.1.

Method: A further 20 µl of BSTFA were added to the sample used previously (M6.1, M6.2) and the mixture was heated to 70°C overnight.

Results and Discussion: The chromatogram obtained is shown in Fig.51. Again two peaks were visible and their proportions had not changed. It was thought unlikely that further silylation could be achieved and the possibility of an impurity in the salicyluric acid had to be considered.

M6.4

Aim: To obtain a chromatogram for a fresh sample of salicyluric acid.

Equipment and Materials: As M6.1 plus a fresh sample of salicyluric acid.

Method: A derivatisation of the fresh salicyluric acid was carried out as in M6.1 and a 0.5 µl aliquot was injected onto the column after silylating at 50°C for 60 minutes.

Results and Discussion: The chromatogram obtained is shown in Fig.52. It again showed two peaks although the proportions of each component had changed, the second peak now being the larger of the two.

M6.5

Aim: To test the two samples of salicyluric acid for any obvious impurities.

Figure 50. Chromatogram of a silylated sample of salicyluric acid heated to 50°C overnight and injected at 230°C and 50 ml/minute.

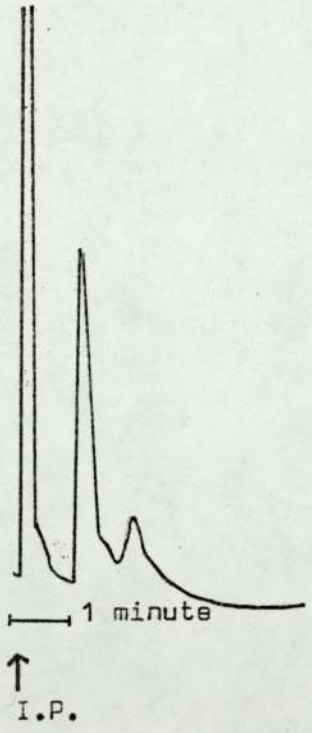
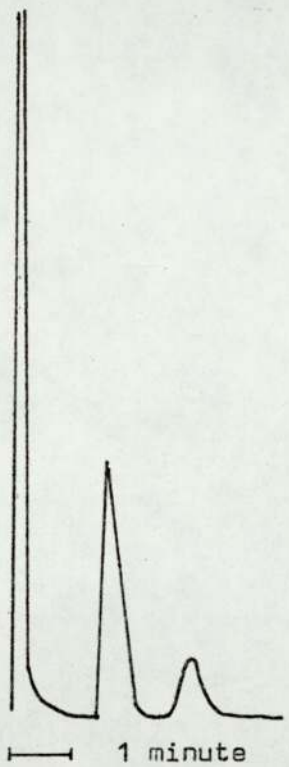
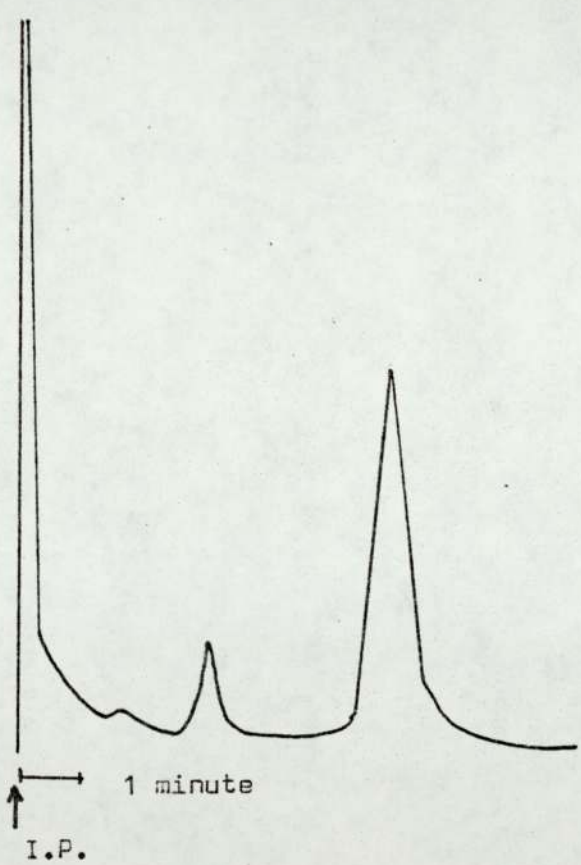


Figure 51. Chromatogram of a silylated sample of salicyluric acid heated to 70°C overnight and injected at 230°C and 50 ml/minute.



I.P.

Figure 52. Chromatogram of a fresh sample of salicyluric acid after silylation at 50°C for 60 minutes and injection at 230°C and 50 ml/minute.



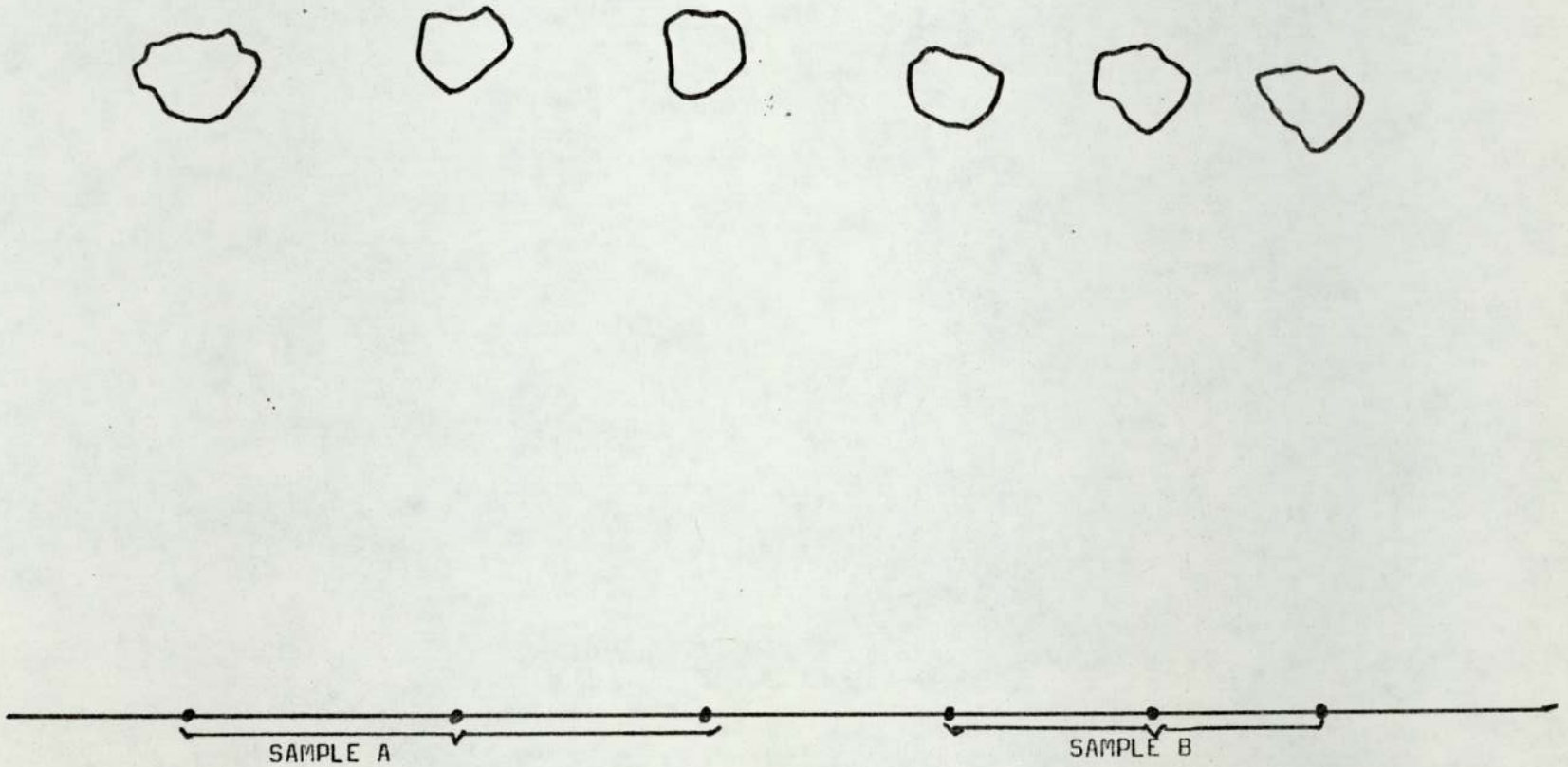
Equipment: Thin layer chromatography
chamber/plate set
Pre-prepared thin layer plate

Materials: 10^{-3}M solutions of salicyluric acid samples
Formic acid
Benzene
Ether
Methanol
Ferric chloride solution

Method: Three 10 µl spots of each salicyluric acid solution were placed onto the silica gel plate. A solvent system of Formic Acid: Benzene: Ether: Methanol in the proportions of 18:20:60:1 was made up, the equipment set up and the system allowed to run for two hours. The place was then dried and inspected at 254 nm. It was then sprayed with Ferric Chloride solution and dried.

Results and Discussions: Single fluorescent spots were observed in each sample. The plate showing the same spots visible after spraying with Ferric Chloride is illustrated in Fig.53. There appeared to be no obvious difference between the two samples either in the shape, size or number of spots seen, and no obvious impurities in either. It would seem that the appearance of the two components after silylation was a result of the silylation process itself. Since these experiments were carried out, Laycock (unpublished) has investigated this phenomenon further. From initially unsuccessful attempts to silylate salicyluric acid, he concluded that the difficulty lay not in the formation of mono - or di - silylated derivatives but in the formation of tri - silylated derivatives. He postulated that the trimethylsilyl group

Figure 53. Reproduction of a paper chromatogram of two samples of salicyluric acid in a Formic acid:Benzene:Ether:Methanol solvent system.



also reacted with the amino group (see Fig. 54 (a)). However this bond was unstable and, especially in the presence of moisture, hydrolysed to give the disilylated derivative (Fig. 54 (b)). When samples were prepared in a completely inert mixture and injected immediately onto the column, only one peak was visible. If hydrolysis was allowed to take place, (for example by leaving the reaction mixture open to the atmosphere) a chromatogram showing two peaks was obtained on injection. The disilylated derivative more stable and more polar and had a longer retention time than the trisilylated derivative. To prevent the formation of the unstable derivative and its associated problems, Laycock suggested reaction of the amino group with trifluoraceticanhydride (TFA) before silylation to give the derivative shown in Fig.54 (c). However, his attempts to do this have been, to date, unsuccessful.

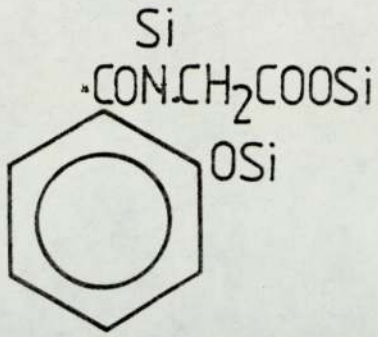
Method 7 - after Levy and Procknall (1968).

M7.1

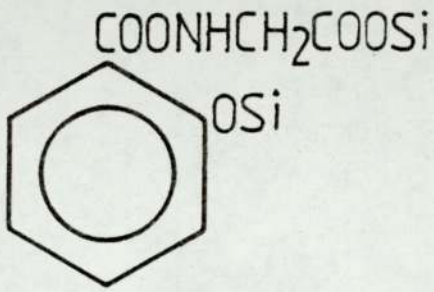
Aim: To extract salicyluric acid quantitatively from aqueous solution by using an improved version of the method described by Levy and Procknall (1968).

Equupment: EEL Spectrophotomer (visible light only)
Matched glass cells

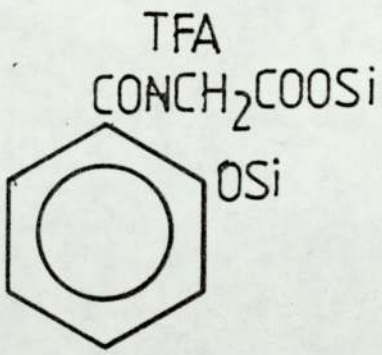
Materials: Salicyluric acid solution (100 mg/100 ml in water)
Dichloroethant
Hydrochloric acid 6N
Nitric acid 0.07N
Ferric Nitrate
Distilled water



a) Trisilyl derivative of salicyluric acid



b) Disilyl derivative of salicyluric acid



c) Possible structure of the product of reacting salicyluric acid with trifluoracetic anhydride and BSTFA.

Figure 54. Structures of possible silylated and acetylated derivatives of salicyluric acid.

Method: Aliquots of the stock salicyluric acid solution were taken and made up to 1 ml with distilled water to represent a concentration range of 0.5 - 100 mg/100 ml. These samples were then treated in the manner described in Fig.43 with the substitution of dichloroethane for carbon tetrachloride.

Results and Discussion: The resulting optical densities are shown in Table 45, and are plotted against log.concentrations of salicyluric acid in Fig.55. The extraction was consistent and standard errors were small. Thus salicyluric acid can be quantitatively extracted from water using the above method.

M7.2

Aim: To assess the percentage recovery of salicyluric acid from plasma.

Equipment: As M7.1

Materials: As M7.1 except

Salicyluric acid solution in methanol

Salicyluric free plasma

Method: Aliquots of the methanol/salicyluric acid solution were evaporated and the residue taken up in salicylate free plasma to represent plasma concentrations of 0.5 - 100 mg/100 ml. These samples were then extracted as in Fig.43 using dichloroethane in place of carbon tetrachloride.

Results: The optical densities are shown in Table 46, and are plotted against log. - concentration of salicyluric acid added in Fig.56. Recoveries at all concentrations above 5 mg/100 ml were high (average 91%) and the standard errors were small.

Table 45. Optical densities by concentrations of salicyluric acid from 0.5 - 100 mg/100 ml in water, using procedure M7.1.

Concentration Salicyluric Acid mg/100 ml	Log. Concentration	Optical Densities				Standard Error
		1	2	3	Mean	
0.5	- 0.3010	2.8	3.2	2.8	2.93	± 0.13
1.0	0.0000	3.6	3.4	3.3	3.43	± 0.09
2.5	0.3979	4.2	4.2	4.2	4.20	± 0.00
5.0	0.6999	5.7	5.7	5.7	5.70	± 0.00
7.5	0.8751	7.7	7.6	7.5	7.60	± 0.06
10.0	1.0000	8.4	8.6	8.9	8.63	± 0.15
25.0	1.3979	14.9	16.9	16.5	16.10	± 0.61
50.0	1.6999	34.5	34.7	-	34.60	± 0.10
75.0	1.8751	47.5	49.0	47.7	48.70	± 0.47
100.0	2.0000	67.7	66.0	65.3	66.33	± 0.71

Figure 55. Calibration graph showing the relationship between log. aqueous concentrations of salicyluric acid between 0.5 and 100 mg/100 ml after treatment by the method described in Fig. 43 using dichloroethane in place of carbon tetrachloride (standard errors shown).

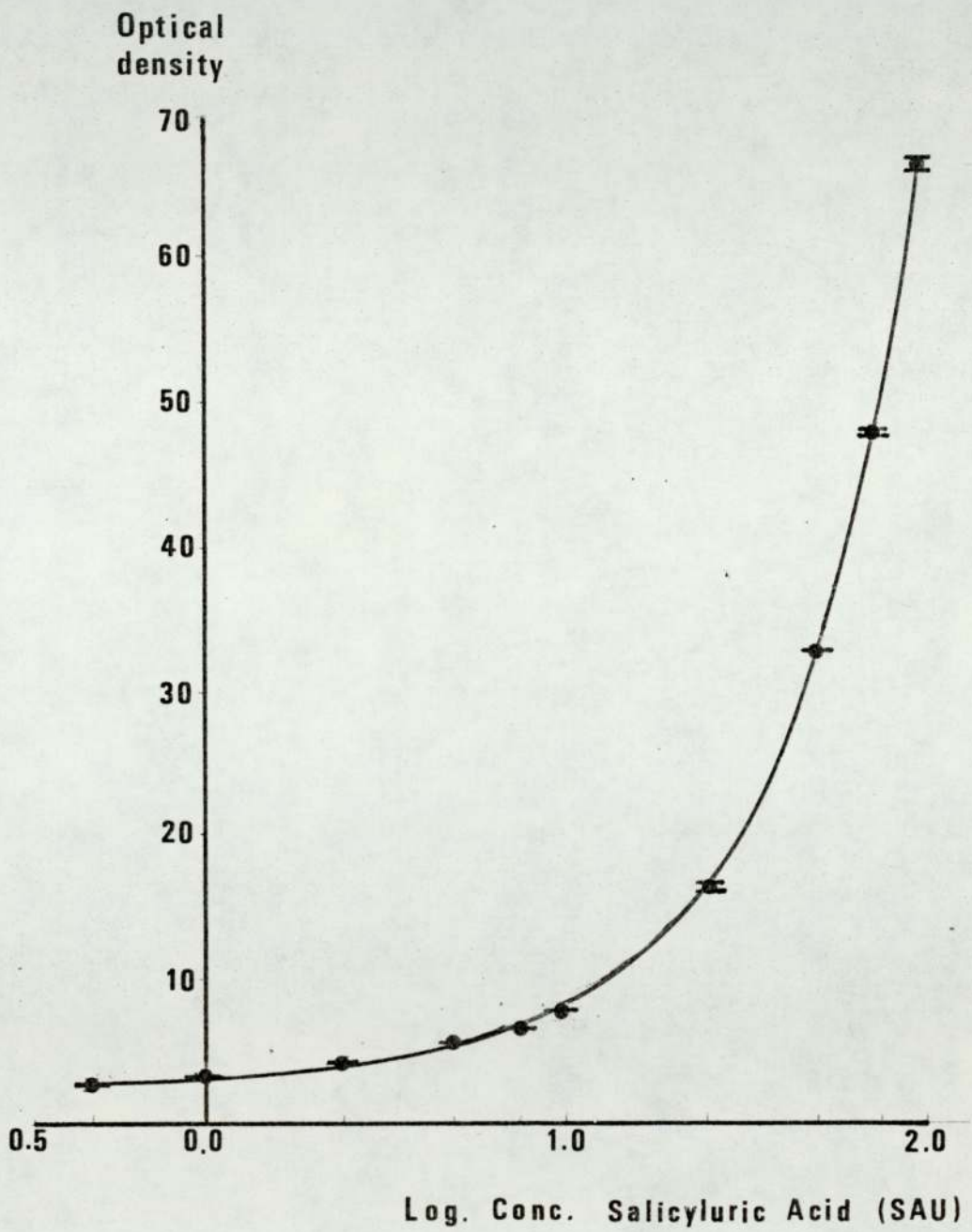
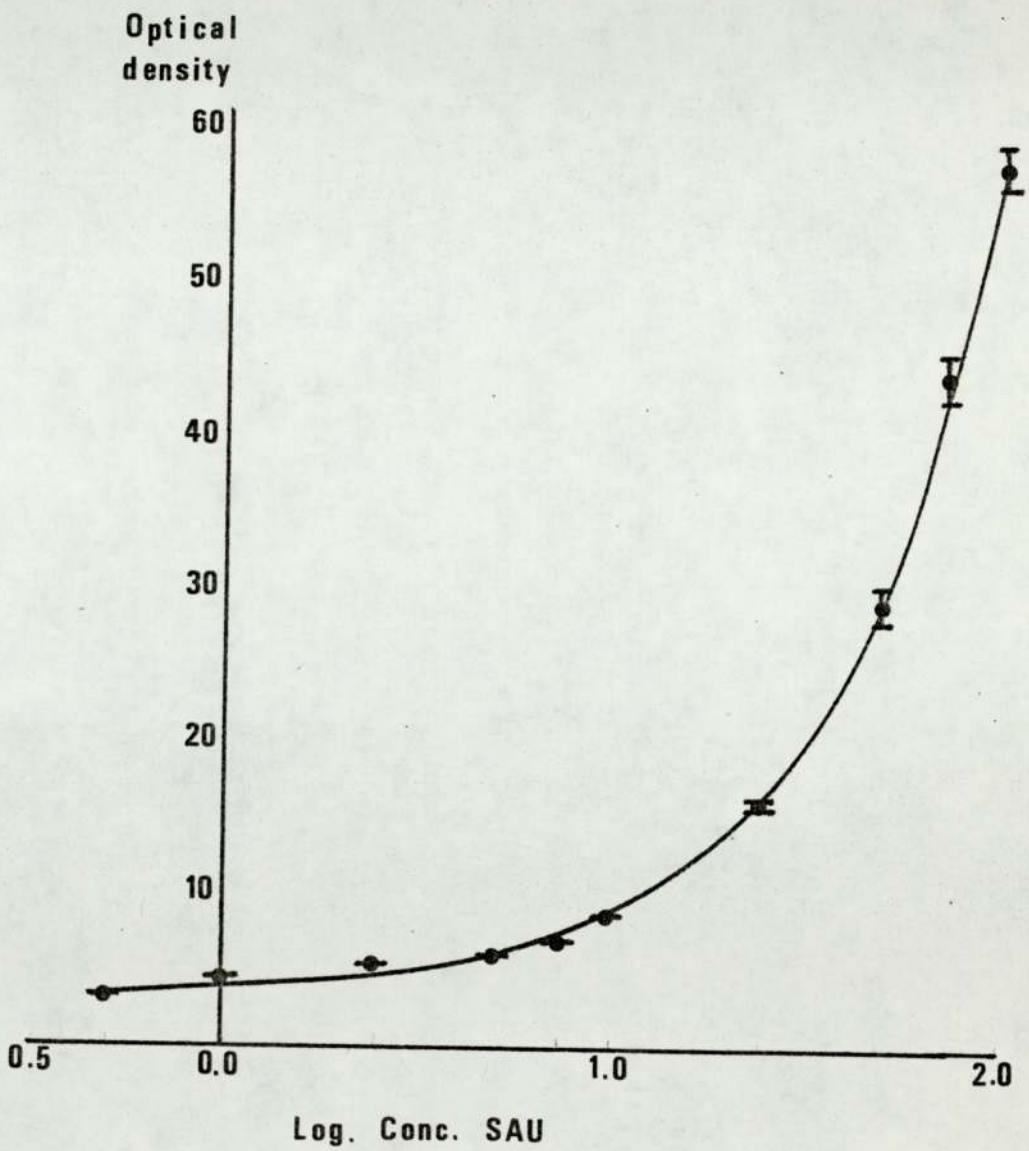


Table 46. Optical densities given by concentrations of salicyluric acid from 0.5 - 100 mg/100 ml in plasma, using procedure M7.2.

Concentration Salicyluric Acid mg/100 ml.	Optical Densities			Mean O.D.	Standard Error	Salicyluric acid found mg/100 ml.	% Recovery
	1	2	3				
0.5	-	3.0	2.5	2.75	± 0.25	> 0.50	-
1.0	4.2	4.1	3.7	4.00	± 0.15	2.11	211
2.5	5.1	-	5.0	5.05	± 0.05	3.80	152
5.0	5.8	5.6	5.2	5.53	± 0.18	4.68	93
7.5	7.1	6.1	6.9	6.70	± 0.31	6.46	86
10.0	9.0	8.1	8.0	8.37	± 0.32	9.40	94
25.0	17.0	16.5	14.0	15.83	± 0.93	23.71	94
50.0	30.7	27.0	-	28.85	± 1.85	46.99	93
75.0	49.0	40.0	42.0	43.67	± 2.73	69.50	92
100.0	62.5	53.7	57.2	57.80	± 2.56	88.51	88

Figure 56. Calibration graph showing the relationship between log. plasma levels of salicyluric acid between 0.5 and 100 mg/100 ml after treatment by the method described in Fig. 43 using dichloroethane in place of carbon tetrachloride (standard errors shown).



Discussion: The use of dichloroethane for extraction of salicyluric acid from body fluids is complicated by the fact that it also extracts salicylic acid at the same time. This was, in fact, the basis for a method of estimating salicylic acid (Brodie, Udenfriend and Coburn, 1944). This difficulty could be overcome if two provisos could be satisfied.

a) That the extraction of salicyluric acid from body fluids was quantitative.

b) The contribution of any simultaneously extracted salicylic acid to the resultant optical density could be quantified.

M7.2 has shown that extraction of salicyluric acid from plasma was quantitative and consistent.

M7.3

Aim: To quantify the extraction of salicylic acid from plasma with dichloroethane.

Equipment and Materials: As M7.2 except
Salicylic acid/ether stock solution
in place of salicyluric acid/methanol.

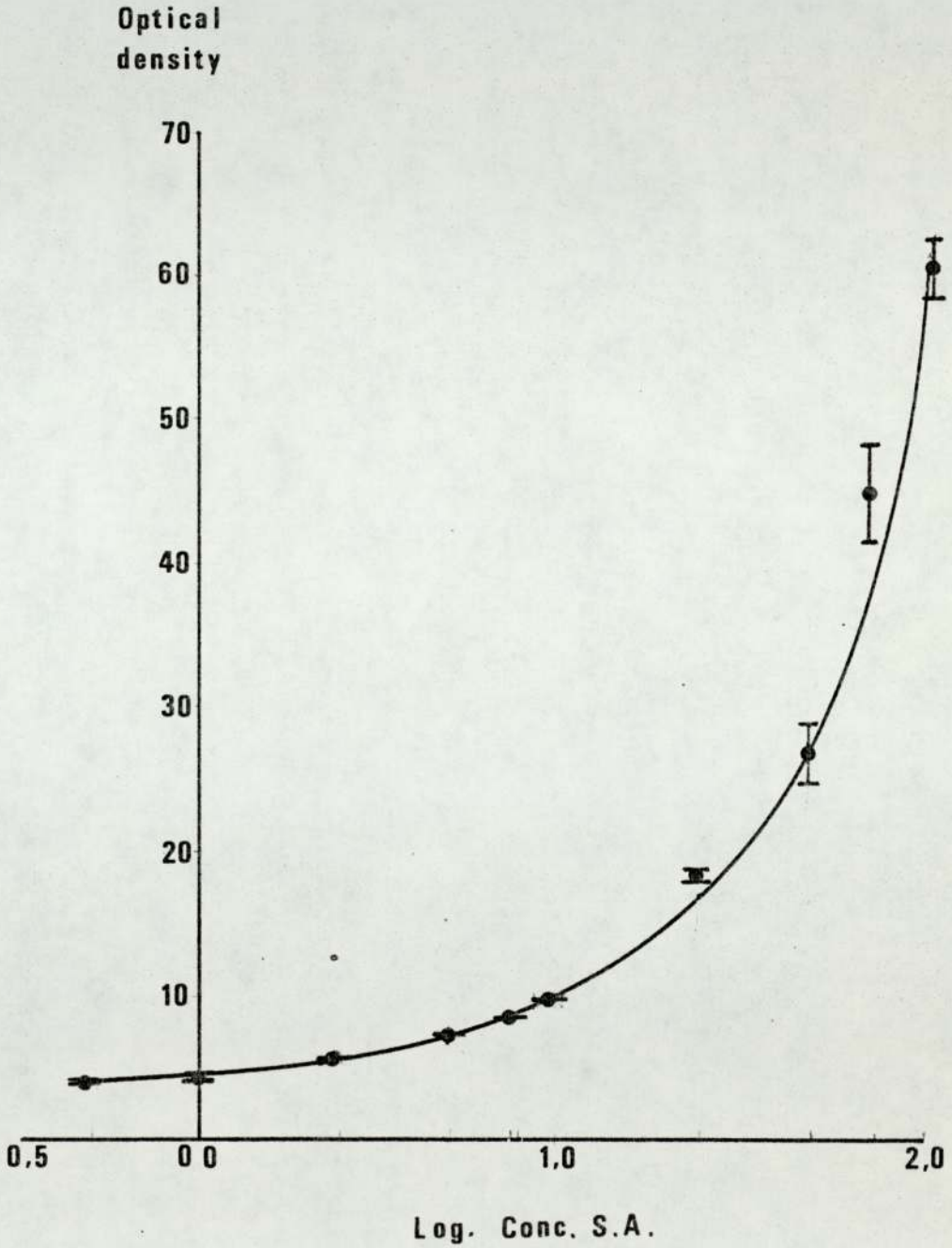
Method: Aliquots of the stock salicylic acid solution were evaporated to dryness and the residue taken up in plasma to give concentrations ranging from 0.5 - 100 mg/100 ml. These were extracted as in Fig.43 using dichloroethane in place of carbon tetrachloride.

Results and Discussion: The resulting optical densities are given in Table 47 and these plotted against log.-concentrations in Fig.57. Optical density increased with concentration and the standard

Table 47. Optical densities resulting from extraction of added salicylic acid from plasma with dichloroethane, using procedure M7.3

Concentration Salicylic acid mg/100 ml.	Optical Densities				Standard error
	1	2	3	Mean	
0.5	3.5	3.8	3.7	3.67	± 0.088
1.0	4.2	4.1	4.5	4.27	± 0.120
2.5	5.5	5.5	6.0	5.67	± 0.166
5.0	7.2	6.8	7.7	7.23	± 0.260
7.5	7.6	8.5	8.6	8.23	± 0.049
10.0	8.9	10.1	10.4	9.80	± 0.149
25.0	18.8	19.0	17.1	18.30	± 0.775
50.0	27.8	28.0	23.8	26.53	± 2.099
75.0	39.1	44.5	51.0	44.87	± 3.249
100.0	56.2	64.2	60.5	60.30	± 1.849

Figure 57. Calibration graph showing the relationship between log. plasma concentrations of salicylic acid between 0.5 and 100 mg/100 ml after treatment by the method described in Fig. 43 using dichloroethane in place of carbon tetrachloride (standard errors shown).



errors were small. Levy and Procknall (1968) found that the difference in the proportion of salicylic acid extracted by carbon tetrachloride and dichloroethane was constant throughout the concentration range. Table 48 shows that it was not so in this case but reference to Fig.57 shows a definite relationship between concentration and optical density.

Conclusions: This method could be used to estimate accurately concentrations of salicyluric acid in body fluid between 5 and 100 mg/100 ml. Estimation of salicyluric acid would depend on an initial estimation of the amount of salicylic acid present so that the contribution of the latter to the optical density after extraction with dichloroethane would be known. Difficulty might be expected in the measurement of low concentrations of salicyluric acid in the presence of high concentrations of salicylic acid.

ii) Acyl and phenyl glucuronides

Although GLC of these metabolites would have been the most desirable method of estimation, difficulties were encountered, due to the lability of the compounds, in obtaining pure samples for standardisation. As a result, this method was not pursued. It was considered that use might be made of the lability of the compounds in a way similar to that used by Prescott (1971) to estimate glucuronides of paracetamol. This consisted of an estimation of "free" paracetamol by GLC and an enzymatic hydrolysis of "conjugated" paracetamol to "free" paracetamol by use of glucuronide enzymes. The increase in "free" paracetamol was proportional to the amount of "conjugated" paracetamol present.

Table 48. Differences in optical densities at 540 mμ of salicylic acid extracted from plasma with carbon tetrachloride and dichloroethane.

Concentration of added Salicylic Acid mg/100 ml.	Mean O.D. CCl_4 (a)	Mean O.D. Dichloro-ethane (b)	Percentage Differe $\frac{a - b}{a} \times 100$
0.5	3.80	3.67	+ 3.5
1.0	4.33	4.27	+ 1.4
2.5	5.60	5.67	- 1.2
5.0	7.43	7.23	+ 2.8
7.5	12.56	8.23	+ 34.5
10.0	14.13	9.80	+ 44.1
25.0	28.33	18.30	+ 54.8
50.0	55.25	26.50	+ 108.3
75.0	83.00	44.87	+ 85.0
100.0	134.00	60.30	+ 122.2

M8.1

Aim: To estimate the amount of "conjugated" salicylic acid present in a sample from a patient who had taken an overdose of aspirin.

Equipment: As M5.1

Materials: Urine sample

Sodium acetate buffer pH 5.0

Glucuronidase

OV17 stationary phase

CQ 80-100 mesh support

M - toluic acid

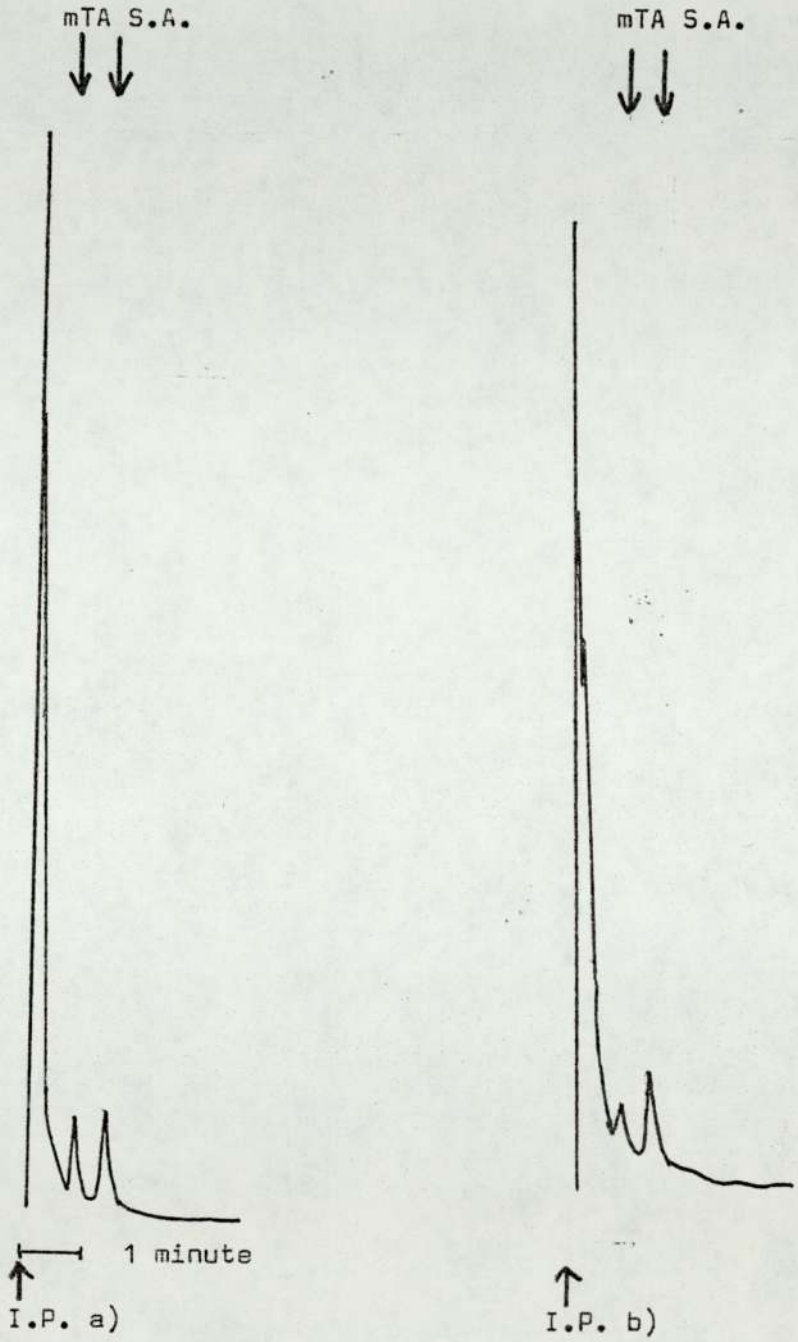
BSTFA

Anhydrous sodium sulphate

Method: A 1 ml sample of the urine was prepared as in M4.9 by extraction and derivatisation with BSTFA. Another 1 ml sample was incubated with 0.5 ml of buffer and 0.1 ml of glucuronidase at 37^{o}C for 22 hours after which time the incubate was treated as the first sample. 0.5 µl aliquots of the derivatised samples were placed onto the column.

Results and Discussion: The chromatograph obtained is shown in Fig.58. The peak height ratio (salicylic acid: m-toluic acid) increased from 1.26 to 1.86 after incubation, representing an initial salicylic acid of 10 mg/100 ml and a final concentration of 16 mg/100 ml. Thus it could be calculated that 6 mg/100 ml of salicylic acid had been conjugated with glucuronic acid. It is possible, therefore, to use this method for estimating the amount of salicylic acid which is conjugated with glucuronide in samples obtained from patients who had taken a dose of salicylate.

Figure 58. Chromatograms obtained from extraction and silylation of a sample of urine obtained from a patient after an overdose of aspirin a) before its treatment with glucuronidase and b) after its treatment with glucuronidase (M8.1).



iii) Other Metabolites

No suitable method was found in the literature for accurately estimating quantities of the minor hydroxymetabolites of aspirin. It may have been possible to assay them using GLC, but their range of molecular weights and polarities after derivatisation would have made this impractical without the advantage of temperature programming which was not available to the author.

5. Conclusions

. Salicylic acid concentrations in body fluids may be estimated by GLC methods and by an improved colourimetric method accurately enough for the purposes of this study.

. Salicyluric acid could be assayed by GLC if the problem of the unstable trisilylated derivative could be overcome. It may be assayed colourimetrically in conjunction with salicylic acid.

. Glucuronide-conjugated salicylic acid may be assayed by enzymatic hydrolysis and GLC.

. No suitable method was found for other metabolites. Some indication of their proportions may be obtained by subtracting the levels of salicylic acid, salicyluric acid and glucuronides from "total salicylates".

1. Volunteer Study

(i) Background

The aim of the volunteer study was to obtain a picture of the patterns of absorption, metabolism and excretion of a single therapeutic dose of aspirin. There were six subjects; four male and two female. All were healthy and had not taken any medication containing aspirin during the week prior to the study. All refrained from eating after 10 p.m. on the evening of the study and took only beverages on the morning of the study. After the dose of aspirin nothing was consumed for approximately three hours.

The dose taken was in the high therapeutic range and consisted of 4 x 300 mg Boots Soluble Aspirin in approximately 50 ml of water.

Sampling

Samples were taken of venous blood from the forearm obtained by means of a metal butterfly cannula. The blood was collected in 10 ml aliquots into plastic Lithium/ Heparin tubes which were stoppered and centrifuged as soon as convenient. The plasma was separated and frozen.

The total volume of each urine sample was measured and noted and approximately 30 mls of each was saved to be frozen.

Samples of both blood and urine were taken at zero time - immediately prior to the dose of aspirin. Urine

samples were collected every hour for six hours then there were 6-12 hour, 12-24 hour and 24-48 hour collections. Blood samples were taken at 5, 10 and 15 minutes then every 15 minutes until 2 hours had elapsed, every 30 minutes for a further 2 hours then every hour for a further two hours. It became impracticable to adhere strictly to this schedule and every sample was labelled with the actual time of collection.

Analysis

The samples were analysed for "total salicylates" using method M1. The calibration curve used is shown in Fig.62. Salicylic acid was estimated by g.l.c. (Method M4) the calibration curve used was Fig.38. An attempt was made to assay the samples for glucuronides using M8.1 but unfortunately the results were erratic in the extreme and have been omitted from the study.

(ii) "Total salicylates"

Results of the analysis of the plasma samples for "total salicylates" are given in Tables 49 to 54. They are plotted collectively in Fig.59. This figure shows that all volunteer subjects experienced a rapid rise in plasma salicylate levels between 0 and 40 minutes after dosing, followed by a further, more gradual rise to peak levels within approximately two hours of dosing. Except for one set of samples (J.A.S.) which showed a peak salicylate level at 195 minutes, levels then fell gradually, reaching 5 - 10 mg/100 ml at six hours after dosing. Peak salicylate

Table 49. Volunteer study: Levels of plasma "total salicylate" in samples taken from T.G.L.

Time (mins.)	Salicylate (mg/100 ml.)
0	0.00
5	2.00
10	6.75
15	5.00
60	6.75
75	6.50
90	7.00
105	7.50
120	8.50
145	9.25
180	7.75
210	8.00
252	6.50
300	5.00
360	6.00

Table 50. Volunteer study: Levels of plasma "total salicylate" in samples taken from A, B.

Time (mins.)	Salicylate (mg/100 ml.)
0.	0.00
4	0.00
10	1.50
15	6.00
30	9.00
45	11.75
60	12.00
80	12.25
90	14.75
105	13.90
120	13.50
165	13.50
205	12.25
245	11.75
300	8.00
360	9.00

Table 51. Volunteer study: Levels of plasma "total salicylate" and salicylic acid in samples taken from J.A.S.

Time (mins.)	Salicylate (mg/100 ml.)	Salicylic Acid (mg/100 ml.)
0	0.00	0.00
5	3.25	1.00
10	3.75	2.20
15	9.75	2.50
30	10.50	5.50
45	11.00	6.60
60	12.00	5.60
75	12.50	6.25
90	11.00	6.00
105	12.00	6.30
120	11.25	5.00
135	11.00	6.10
150	11.00	5.20
195	16.50	5.00
210	15.75	3.40
240	9.25	7.25
300	8.20	5.00
360	11.25	5.10

Table 52. Volunteer study: Levels of plasma "total salicylate" in samples taken from S.G.

Time (mins.)	Salicylate (mg/100 ml.)
0	0.00
6	0.20
10	4.50
15	6.00
30	10.00
52	12.00
63	10.30
75	10.00
90	10.30
105	8.50
125	8.50
141	8.00
150	9.00
195	9.50
210	7.20
240	6.50
304	6.00
360	5.55

Table 53. Volunteer study: Levels of plasma "total salicylate" and salicylic acid in samples from B.A.H.

Time (mins.)	Salicylate (mg/100 ml.)	Salicylic Acid (Mg/100 ml.)
0	0.00	0.00
5	2.00	0.85
10	5.00	1.50
16	5.50	3.50
30	7.75	6.10
46	6.75	6.00
62	7.75	7.30
75	8.50	7.10
90	8.25	6.10
107	8.25	7.20
120	12.00	10.00
135	6.00	5.05
150	6.75	7.40
182	5.50	-
215	5.50	5.10
240	5.50	5.25
270	5.00	4.85
300	5.25	-

Table 54. Volunteer study: Levels of plasma "total salicylate" and salicylic acid in samples taken from N.W.

Time (mins.)	Salicylate (mg/100 ml.)	Salicylic Acid (mg/100 ml)
0	0.00	0.00
5	1.75	1.40
11	5.00	1.95
16	11.00	5.00
30	9.50	7.45
45	12.00	8.00
60	9.50	-
75	12.25	11.50
91	10.50	5.80
105	7.25	-
120	9.00	5.80
135	7.75	8.50
150	9.25	9.50
165	7.25	7.20
180	9.50	6.75
210	7.50	6.50
245	7.75	5.25
268	6.75	5.65
305	7.00	6.85
330	10.00	6.35
368	6.50	6.25

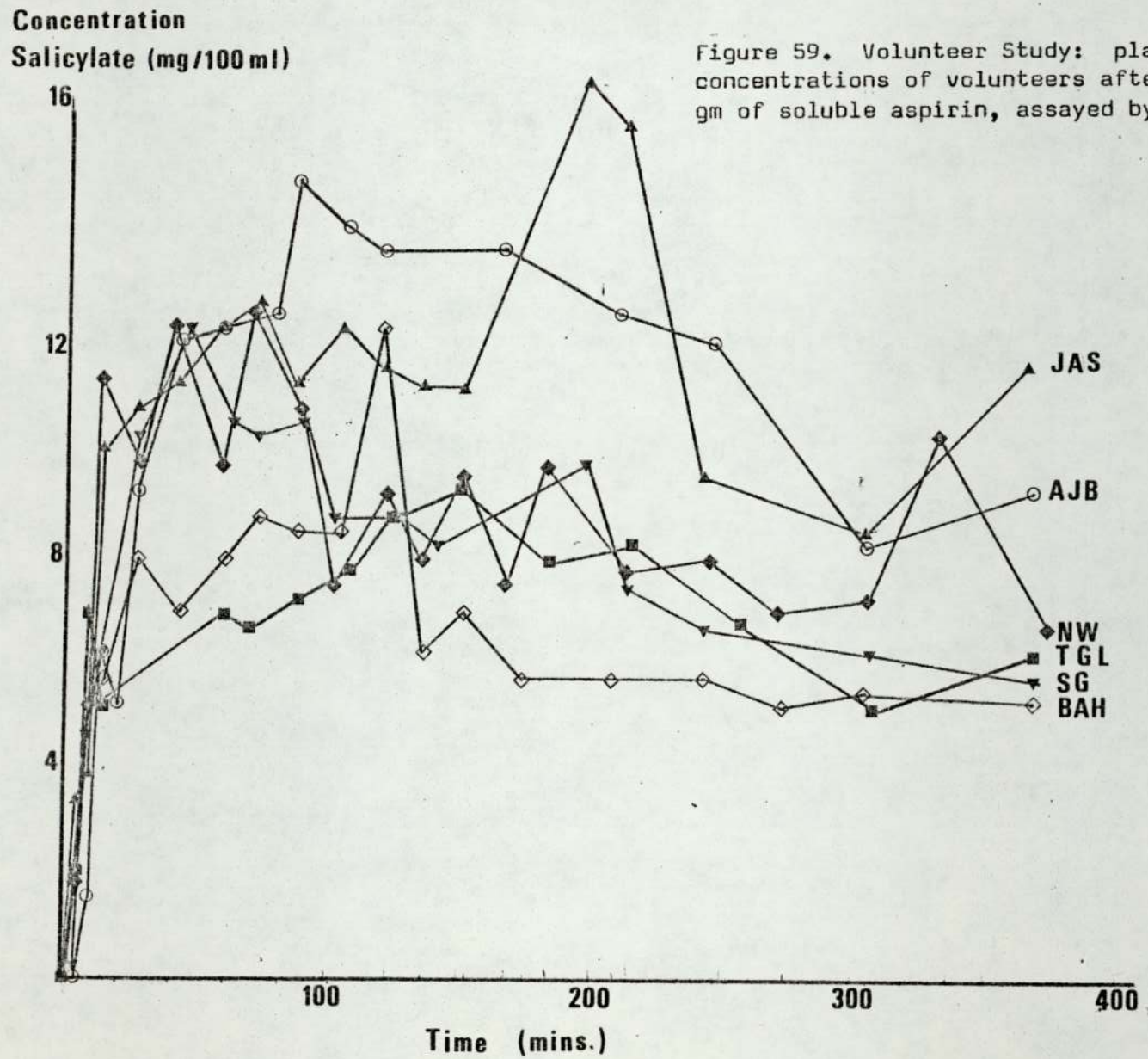


Figure 59. Volunteer Study: plasma "total salicylate" concentrations of volunteers after a single dose of 1.2 gm of soluble aspirin, assayed by procedure M1.

levels were between 9.25 and 16.5 mg/100 ml.

An interesting factor here is that total salicylate levels, far from showing a smooth increase then decline, were erratic in all subjects until the fourth hour after dosing, and there was no clear plateau level of salicylate in any one case. It is possible that, despite starving for twelve hours prior to the study, intermittent gastric emptying may have contributed to the erratic plasma levels. Normally, the irritant effect of aspirin would tend to delay gastric emptying and absorption would be primarily through the stomach wall. However if gastric emptying was affected by such factors as the mobility or positioning of subjects absorption rates would vary and this could account for the unstable plasma levels found. Attempts were made to reduce mobility of subjects during the first few hours of the study and no food or drink were consumed until after the fourth hour, but it is possible that gastric emptying may have contributed to the variable plasma levels seen.

Another possible explanation is that the distribution volume of salicylate within the body may consist of separate compartments which have different saturation levels. It could be that the dose level used here and the time period studied did not allow equilibrium between these theoretical compartments to be reached. Selective filling or emptying of these compartments could have contributed to the variability in plasma levels found.

In an attempt to ascertain if plasma salicylate levels

declined exponentially from peak levels, the logarithms of salicylate levels were plotted against times of samples (Fig.60). There was no clear pattern over the six hour period studied and when regression analysis was carried out on declining salicylate levels, in only one case (S.G.) was a log - linear relationship seen ($r = 0.92$). Cummings et al. (1966) suggest that linearity only occurs when salicylate levels fall below 4 mg/100 ml and, if that is correct, the lack of linearity found is not surprising. However, they also suggest that elimination of salicylate becomes more rapid as plasma levels fall. This may have been true over the period studied at that time but excretion rates (Table 55) obtained in the course of this volunteer study and expressed as cumulative percentages of the original dose excreted show a marked decrease after plasma levels had reached 5 - 10 mg/100 ml (Fig.61).

(iii) Salicylic acid

In three subjects (J.A.S., B.A.H. and N.W.) all plasma samples were also analysed for salicylic acid. The results of this analysis are shown in Tables, 51, 53 and 54. It was apparent in all three cases that changes in plasma levels of salicylic acid reflected changes in levels of total salicylates.

It was also noticeable that in the case of N.W. and B.A.H., that during the first two hours following ingestion, salicylic acid accounted for a lower proportion of the total salicylate than in the later stages. Between 0 and 107 minutes after ingestion the average proportion of

Figure 60. Volunteer study: log. plasma "total salicylate" concentrations of volunteers after a single 1.2 gm dose of soluble aspirin assayed by procedure M1.

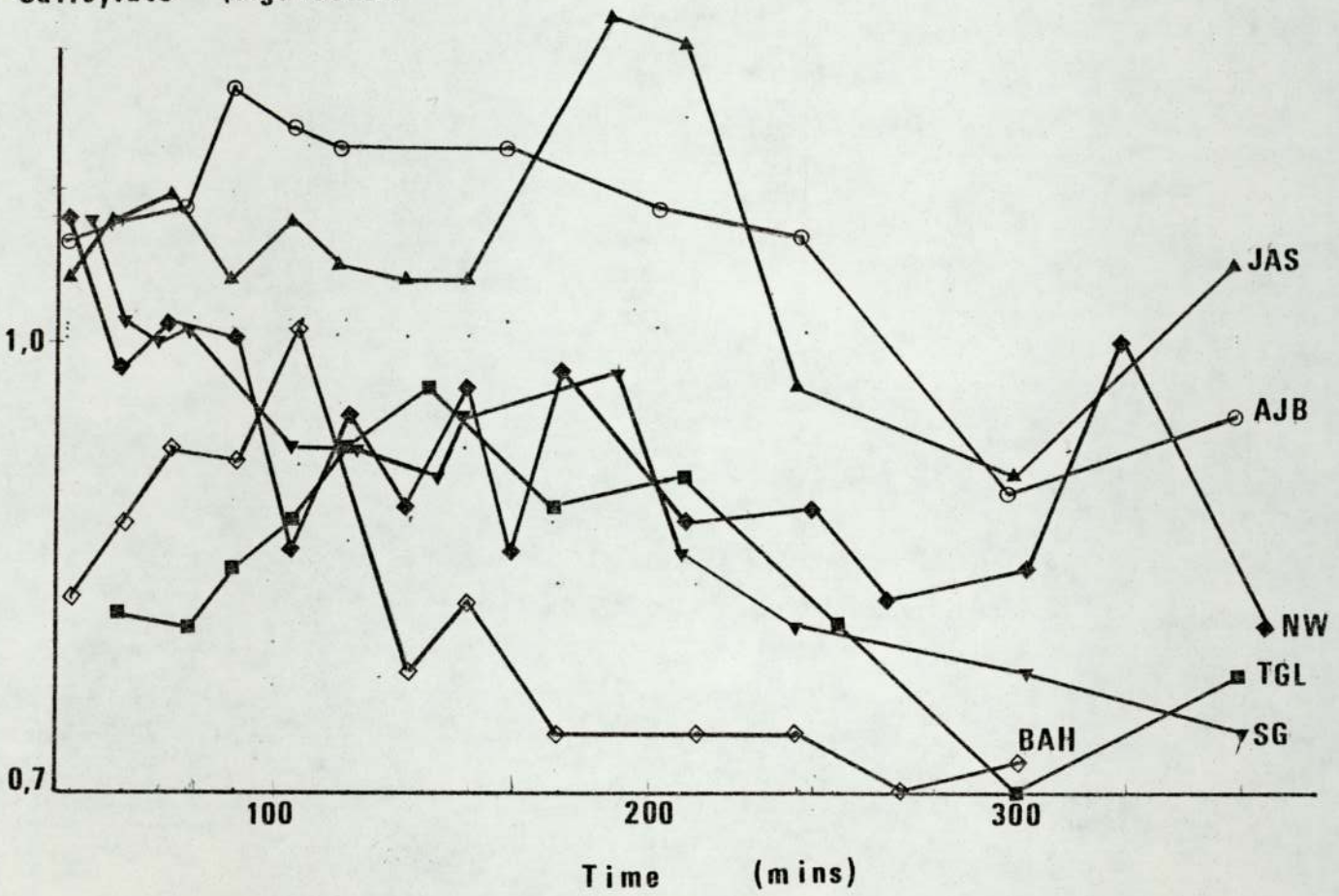


Figure 61. Volunteer Study: cumulative excretion of "total salicylates" of volunteers after a single dose of 1.2 gm of soluble aspirin, assayed by procedure M1.

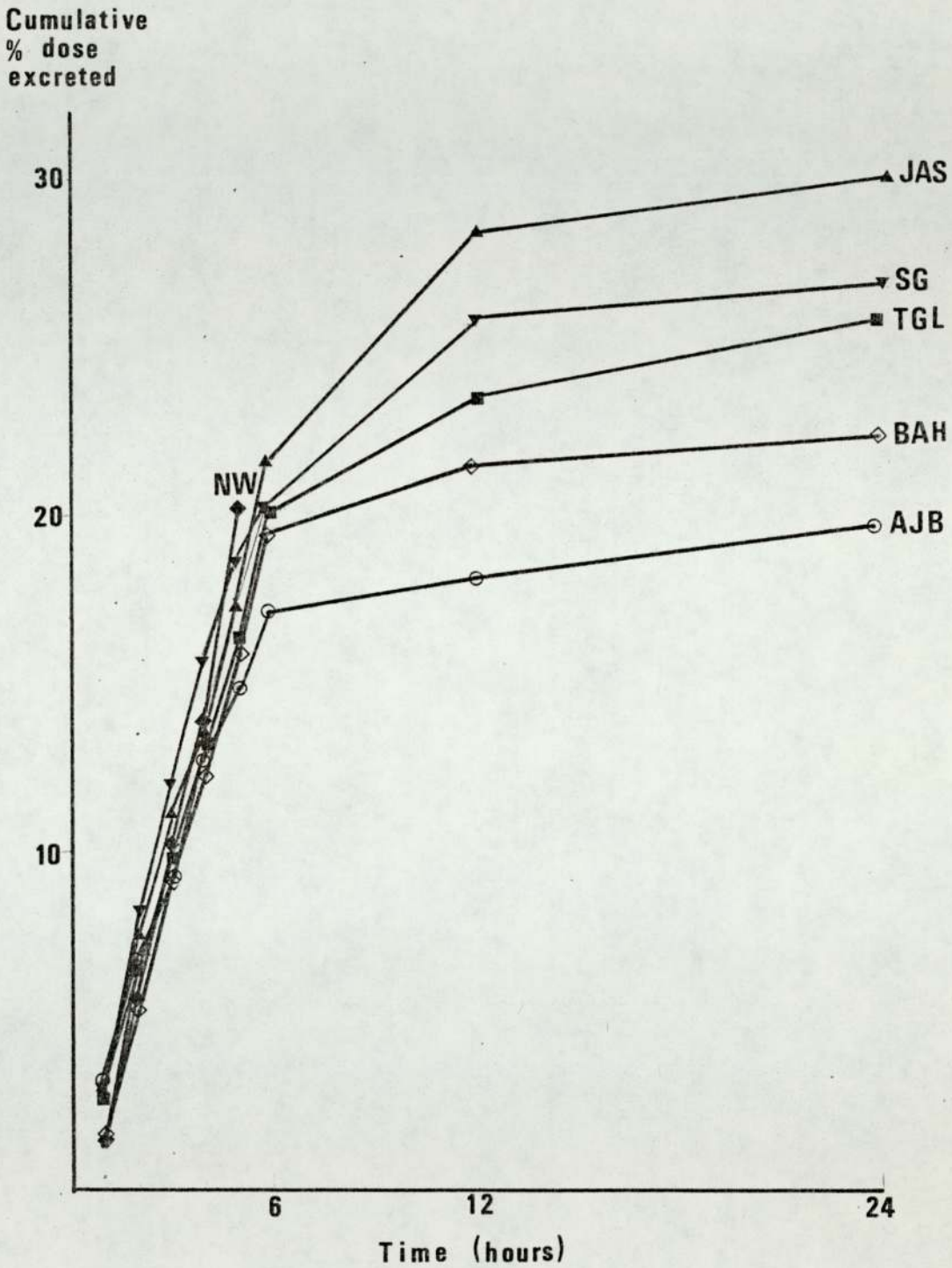


Table 55. Volunteer study: excretion rates (mg/hour) of total salicylate in six volunteers after a single 1.2 gm dose of aspirin.

Hour	A.B.	T.G.L.	B.A.H.	N.W.	J.A.S.	S.G.
1	38	33	19	17	34	36
2	43	46	44	48	55	63
3	31	37	48	57	43	46
4	40	42	37	44	29	44
5	26	37	43	77	48	36
6	27	47	42	-	51	30
12	12	41	25	-	81	55
24	19	28	11	-	21	13
36	-	-	7	-	-	-
48	-	-	-	-	12	4

total salicylates accounted for by salicylic acid in B.A.H's samples was 71.6% and over the rest of these samples this proportion rose to 90.4%. A similar phenomenon occurred in N.W's samples - 65.3% up to 120 minutes, then an average of 82.8%. This apparent increase in the proportion of salicylic acid occurred over a period when total salicylate levels had stabilised or were falling and it is difficult to explain in terms of saturation of other metabolic pathways. It is possible that when rates of production of all metabolites have been increased that some depletion of enzymes may occur and that this factor may be time - as well as dose - dependent. Cummings et al. (1966) suggest that the limitation of the rate of formation of one of the major metabolites of aspirin - salicyluric acid - could be considered in terms of the saturation of an enzyme or transport system. This explains the limitation of metabolite formation with increasing dose but does not explain the apparent limitation of metabolite formation with time seen here.

2. Samples from Overdose Patients

(i) Background

The results below represent the analyses of samples taken from patients admitted to the Regional Poisoning Treatment Centre, Dudley Road Hospital, Birmingham, after overdoses of aspirin or other salicylate containing preparations. The five patients were admitted over a period of several months and this made it difficult to

establish a rigid sampling schedule, however, I am indebted to the nursing staff of the R.P.T.C. for their goodwill which enabled samples to be collected, centrifuged where necessary and stored ready for analysis.

The small number of patients studied was partly due to the fact that, to enable cerebrospinal fluid (csf) to be collected, permission had to be obtained for a lumbar puncture to be performed.

Samples of blood were centrifuged when collected and the plasma separated (in some cases the blood was allowed to clot and, unfortunately, serum only could be obtained). The plasma, urine and csf samples were stored in a refrigerator at $4^{o}C$ until they could be transferred to Aston University for analysis (usually the next day).

Following the volunteer study, several factors indicated that a change of assay method for salicylic acid would be advantageous. Initially, it had been hoped that an estimation could be made of the metabolites of aspirin, especially salicyluric acid. This had not proved possible using gas-liquid chromatography. This factor and the apparently increasing unreliability of the instrument available for glc estimation made it necessary to estimate salicylic acid and its metabolites by procedures M5.6 and M7.3. Although more time consuming, this latter method gave the reliability required under the increasing pressure of time. Method M1 was still being used to assay "total salicylates", the calibration curve used being the same as that used for the volunteer study (Fig.62).

Table 56. Analysis of samples obtained from Patient A (C.M.P.)

	Time of sample (hours)	"Total salicylates" (mg/100 ml.)	Salicylic acid (mg/100 ml.)	Salicyluric acid (mg/100 ml.)	Other metabolites (mg/100 ml.)
Plasma	0	32.0	22.90	<0.50	8.60
	6	9.0	4.68	1.48	2.84
	13	4.3	1.05	<0.50	2.75
CSF.	0	7.5	3.85	<0.50	3.15
Urine	0-6	33.5	13.80	15.59	4.21
	6-13	57.0	44.67	20.18	0.00

Table 57. Analysis of samples obtained from Patient B (B.H.)

	Time of sample (hours)	"Total salicylates" (mg/100 ml.)	Salicylic acid (mg/100 ml.)	Salicyluric acid (mg/100 ml.)	Other metabolites (mg/100 ml.)
Plasma	0	82.3	76.56	<0.50	5.24
	2	62.0	53.22	<0.50	8.28
	4	50.0	40.93	<0.50	8.57
	6	41.8	33.50	<0.50	7.80
	8	39.3	29.92	<0.50	8.80
	12	26.2	24.43	-	-
	20	15.5	1.66	2.82	11.02
CSF	0	23.7	22.49	<0.50	0.71
Urine	0-7	237.6	164.40	4.30	68.90
	7-20	104.0	66.37	43.15	0.00

Table 58. Analysis of samples obtained from Patient C (S.S.)

Time of sample (hours)	"Total salicylates" (mg/100 ml.)	Salicylic acid (mg/100 ml.)	Salicyluric acid (mg/100 ml.)	Other metabolites (mg/100 ml.)
Plasma				
0	47.0	34.91	3.80	8.09
4	24.5	18.62	<0.50	5.38
6	18.0	15.74	<0.50	1.76
8	14.7	15.38	0.00	0.00
10	14.3	8.57	<0.50	5.23
12	11.0	6.31	<0.50	4.19
CSF				
0	12.0	8.77	<0.50	2.73
Urine				
0-1½	198.0	96.61	61.66	39.73
1½-2	67.0	54.70	6.76	5.54
2-4	148.0	99.54	19.72	28.74
4-5	116.0	38.46	8.61	68.93
5-7	75.0	45.19	11.75	18.06
7-10	71.0	41.88	15.49	13.63
10 -11½	33.5	15.03	5.37	13.10

Table 59. Anaylsis of samples obtained from Patient D (K.H.)

	Time of sample (hours)	"Total salicylates" (mg/100 ml.)	Salicylic acid (mg/100 ml.)	Salicyluric acid (mg/100 ml.)	Other metabolites (mg/100 ml.)
Plasma					
	0	51.0	33.50	<0.50	17.0
	½	50.1	29.51	"	20.1
	1	51.4	38.10	"	12.8
	1½	47.0	33.11	"	13.4
	2	43.0	29.99	"	12.5
	3	40.0	26.61	"	12.9
	4	37.8	25.41	"	11.9
	7	28.6	16.98	"	11.1
	9	23.4	10.50	"	12.4
	14	18.0	10.72	"	6.8
Urine					
	0-10	132.0	92.48	76.04	-
	10.22	110.0	59.44	111.26	-
	22.46	280.0	105.68	188.90	-

Table 60. Analysis of samples obtained from Patient E (E.O)

Time of sample (hours)	"Total salicylate" (mg/100 ml.)	Salicylic acid (mg/100 ml.)	Salicyluric acid (mg/100 ml.)	Other metabolites (mg/100 ml.)
Plasma				
3	56.2	39.81	-	-
5	42.5	22.39	-	-
7	27.5	13.49	-	-
10	17.5	9.12	6.46	1.92
14	10.0	5.75	1.03	3.22
18	8.5	3.16	<0.50	4.84
22	5.0	1.02	-	-
CSF				
0	7.0	5.37	0.00	1.63
Urine				
0	165.0	71.61	25.12	68.27
0-1½	160.0	59.57	8.91	91.52
1½-3	92.0	98.86	0.00	0.00
3-3¼	276.0	100.00	30.90	145.10
3¼-3½	110.0	33.88	<0.50	75.62
3½-4¼	122.0	83.18	0.00	38.82
4¼-5¼	100.0	84.53	0.00	15.47
5¼-5½	78.0	56.89	0.00	21.11
5½-6½	105.0	90.78	<0.50	13.72
6½-7½	67.0	59.57	0.00	7.43
7½-7¾	114.0	75.86	0.00	38.14
7¾-8½	76.0	-	-	-
8½-9½	73.0	-	-	-
9½-18	54.5	31.84	0.79	21.87

(ii) The patients

Patient A (C.M.P.) was a 24 year old female admitted about two hours after having taken approximately 100 Anadin (along with a small amount of Valium and Mogadon). She showed few symptoms of salicylate poisoning at this stage with no sweating or hyperventilating. Her stomach was washed out and she was given forced alkaline diuresis and made a satisfactory recovery.

Patient B (B.H.) was admitted about three hours after taking an unknown dose of aspirin. On admission he had vomited once and was sweating and hyperventilating. His stomach was washed out and he was given forced alkaline diuresis and recovered uneventfully.

Patient C (S.S.) was a 17 year old female admitted about 9 hours after having taken approximately 70 aspirin tablets. She had vomited several times and was sweating and hyperventilating. Forced alkaline diuresis was given and she recovered uneventfully.

Patient D (K.H.) was a 34 year old male admitted about 90 minutes after having taken 80 aspirin tablets and possibly some marijuana. On admission he was sweating and hyperventilating and complaining of tinnitus. His stomach was washed out revealing several undissolved tablets. He was given forced alkaline diuresis and recovered sufficiently to take his own discharge.

Patient E (E.O.) was a 24 year old female admitted approximately 12 hours after having taken about 70 aspirin

tablets. Before admission she had vomited, and on admission she was not sweating or hyperventilating but was complaining of tinnitus which persisted for some hours. Her stomach was washed out and forced alkaline diuresis commenced. She was readmitted a few days after her discharge with haematemesis.

(iii) Analysis of samples

Since the time since ingestion was not known exactly it was decided that the time of the first sample (usually plasma but occasionally urine) would be considered as time 0, and other samples labelled in hours accordingly.

Samples were analysed for "total salicylates", salicylic acid and salicyluric acid. "Other metabolites" were estimated by subtraction. Where salicyluric acid levels were measureable as only <0.50 mg/100 ml., for the purpose of the subtraction they were classed as equal to 0.50 mg/100 ml throughout. The results of the analyses are shown in Tables 56 to 60.

Other clinical information

Other clinical information was obtained from blood gas analysis carried out by the laboratory service at Dudley Road Hospital and is presented in Table 61. Amongst the analyses done were those for partial carbon dioxide levels in the blood. The $_pCO_2$ levels were depressed especially in patients C and E. This correlates with the degree of hyperventilation seen on admission of these two patients. Glucose levels were also analysed in the

Table 61. Additional information obtained from D.R.H. laboratory service.

Patient	Blood pH	CSF pH	mM CSF Glucose	mM Standard Bicarbonate
A	7.49	7.260	-	27.5
B	7.37	7.280	-	19.7
C	7.41	7.335	1.80	20.2
E	7.45	7.344	1.00	22.7

csf of two patients (C and E) and were found to be significantly low. Patient C had a csf glucose level of 1.8 mg/100 ml and Patient E had a level of 1.0 mg/100 ml. Normal csf glucose levels lie between 2.2 and 4.4. mg/100 ml. This depression of csf glucose is interesting in view of the suggestion of Thurston et al. (1970) that the uncoupling effect of salicylate on oxidative phosphorylation leads to a compensatory increase in cerebral glycolysis. They considered that brain glucose levels fell in the mice they studied because the rate of utilisation of glucose to maintain high energy phosphate levels in the brain exceeded the rate at which glucose could be supplied from the blood. They found that concurrent administration of glucose with salicylate restored brain glucose levels and improved the condition and survival rate of the animals. Prior to this study investigations of csf in human salicylate overdose have not included any estimations of glucose levels, and on the basis of the significant lowering of glucose seen here, this might be an area of future interest.

Results of analysis for salicylate and metabolites

Plasma levels of "total salicylates" on admission were all outside the normal therapeutic range and varied from 32 to 82 mg/100 ml. The difficulty of assessing the likely severity of poisoning is emphasised here as several variables may contribute to the clinical picture including the time since ingestion and whether the patient had vomited since taking them and, if so, whether any unabsorbed tablets were vomited or washed out during gastric lavage.

When the log. salicylate levels were plotted against time the decline in plasma salicylate concentrations was found to be log linear with high correlation coefficients - r = 1.00 (A); 0.99 (B); 0.98 (C); 0.99 (D); 0.99 (E) (see Fig.63). Similarly salicylic acid levels in plasma declined log linearly - r = 1.00 (A); 0.95 (B); 0.98 (C); 0.95 (D); 0.99 (E), (see Fig.64).

As the concentration of salicylates in plasma fell, there was a fall in the proportion of salicylic acid (Fig.65) and a concomitant rise in the proportion of other metabolites (including salicyluric acid). Clearly this is a reflection of the saturation of metabolic pathways known to exist even at therapeutic doses (Levy, 1965). It would be expected that this pattern would also exist in the elimination of salicylate in the urine, however the relationship was not so clear when salicylic acid levels in the urine were examined. This is surprising as other studies have confirmed the effect of rate limited metabolism using urine samples (Levy, 1965; Cummings et al., 1965).

When the urine samples from patients C, D and E were analysed for salicyluric acid, again no clear pattern was seen in the proportion of "total salicylates" excreted as salicyluric acid over the time period studied and the results of this analysis are therefore inconclusive.

Levels of salicylate in the central nervous system

The importance of the infiltration of salicylate into the central nervous system has been recognised for some

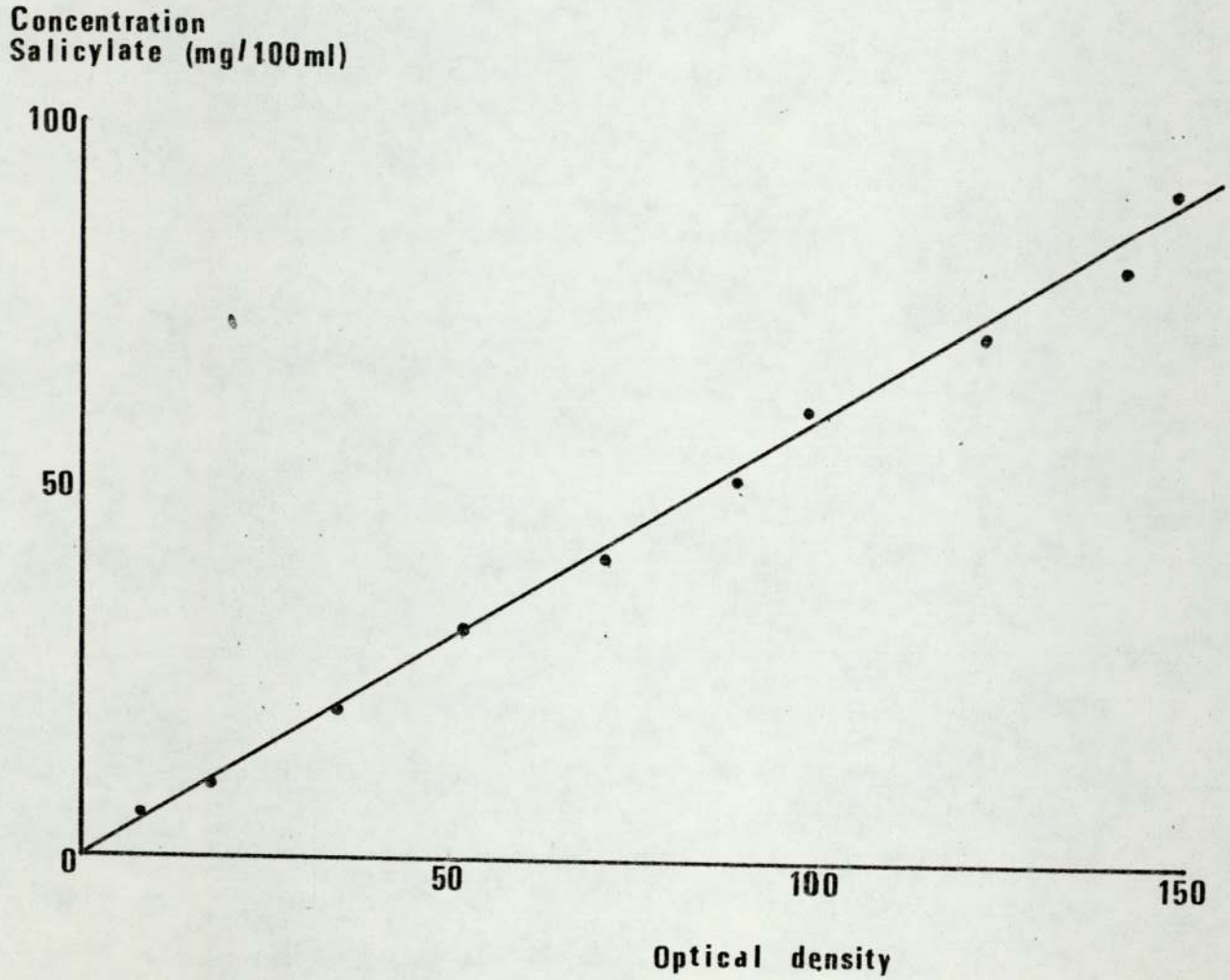


Figure 62. Calibration graph showing the relationship between salicylate concentrations of between 5 and 90 mg/100 ml and optical density at 540 mμ after treatment by procedure M1.

Figure 63. Log. plasma salicylate levels in five patients admitted to hospital after having taken an overdose of salicylate analgesics. Time 0 was the time of the first plasma or urine sample taken after admission.

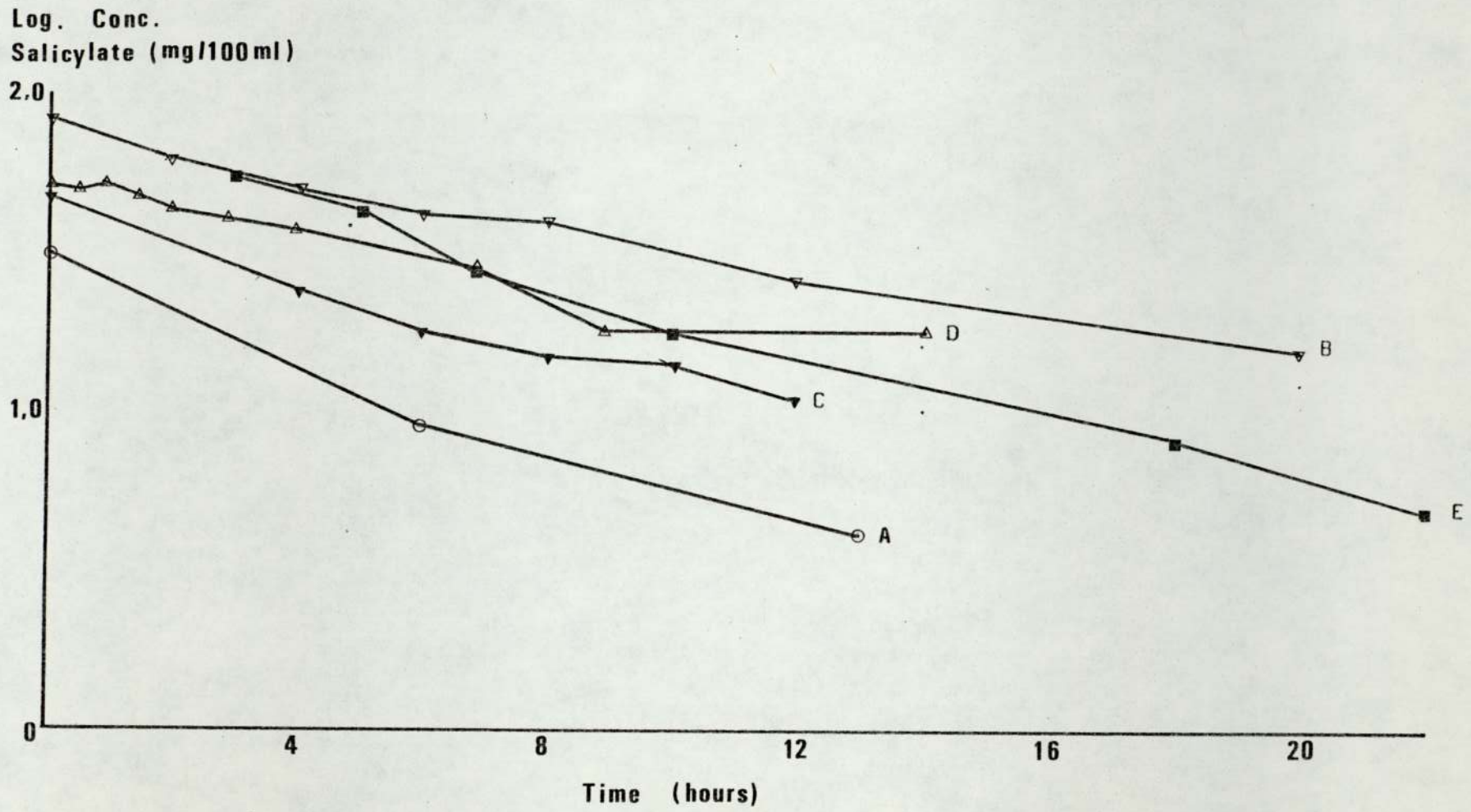


Figure 64. Log. plasma salicylic acid levels in five patients admitted to hospital after having taken an overdose of salicylate analgesics. Time 0 was the time of the first plasma or urine sample taken after admission.

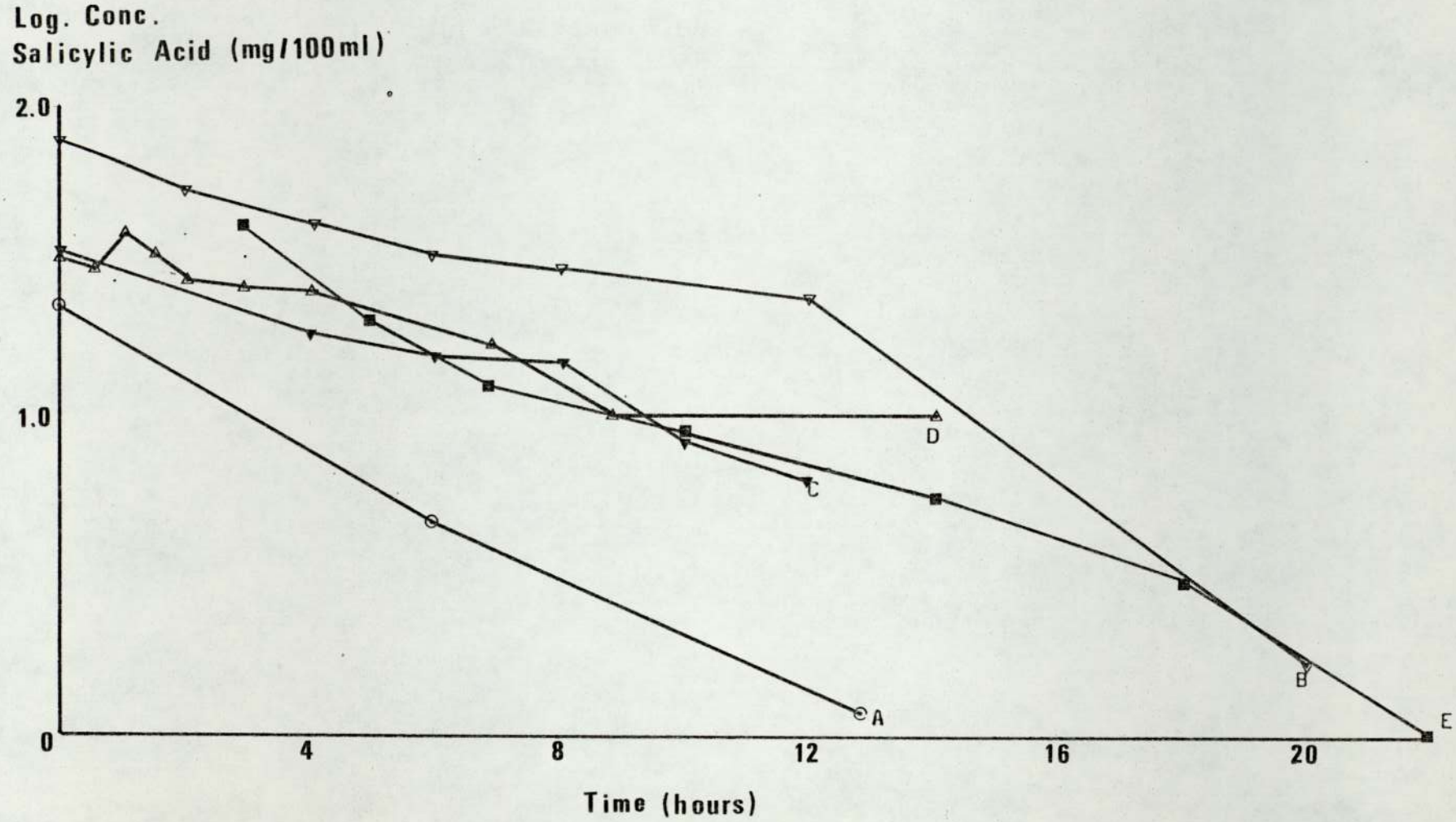
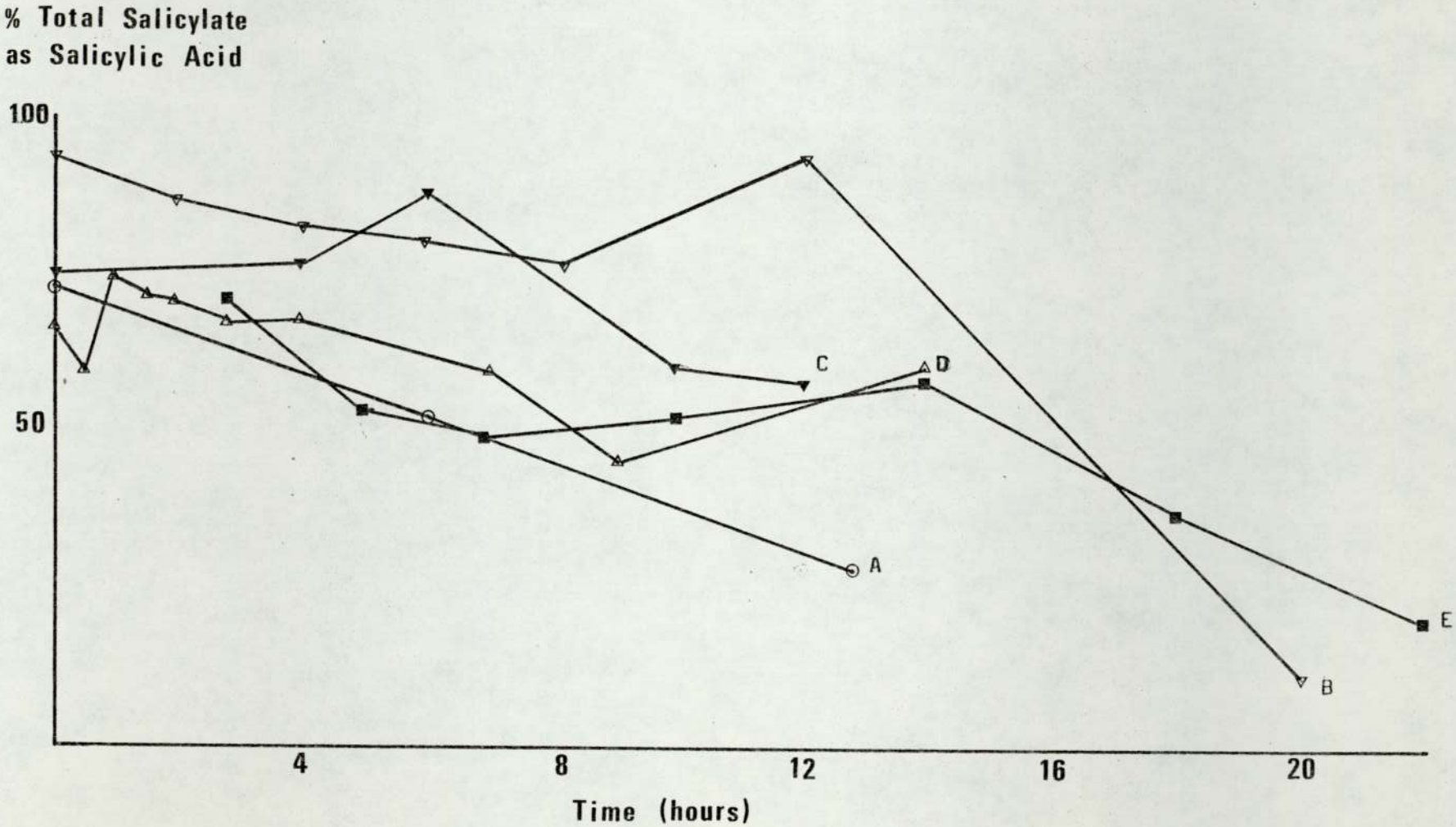


Figure 65. Salicylic acid as a proportion of "total salicylates" in the plasma of five patients admitted to hospital after having taken an overdose of salicylate analgesics. Time 0 was the time of the first plasma or urine sample taken after admission.



time (Tenney and Miller, 1955; Bron et al., 1973; Thurston et al., 1970). It is not only the direct toxic effect on brain glucose levels that is important here, but the possibility that salicylate in the vicinity of the respiratory neurones could stimulate respiration (Tenney and Miller, 1955; confirmed by Bron et al., 1973). Stimulation of respiratory neurones can lead to respiratory alkalosis which tends to decrease the amount of salicylate available to be absorbed into the central nervous system and can thus act as a "brake" on further absorption, and is a valuable feedback mechanism. However this respiratory alkalosis leads to several side effects including the reduction of the buffering capacity of blood and which lead to further disturbances in the acid/base balance of the patient.

Buchanan et al. (1975) suggested that salicylate was transported from the blood to the respiratory neurones in the medulla via the csf. Thus csf levels of salicylate could be important in diagnosis of salicylate intoxication Buchanan and Rabinowitz (1974) studied the ratio of serum and csf concentrations of salicylate and the bicarbonate level of plasma in ten infants and found a good correlation between actual bicarbonate and serum/csf salicylate ratio (Γ = 0.90). Standard bicarbonate levels were available for patients A, B, C and E and were compared with plasma/csf ratios of salicylate. No correlation was found (Γ = 0.17) however the fact that the bicarbonate levels were not depressed to such an extent in these adults as they were in the infants studied by Buchanan and Rabinowitz (1974) was probably a contributory factor here.

A reasonable amount of correlation ($r = 0.8$) was found between blood pH and plasma/csf levels of salicylate, confirming the principle that as the pH of plasma falls, more unionised salicylate is available to be transferred to the csf. The fact that pH of the blood can only be held 80% responsible for variation in the plasma/csf salicylate indicates that another factor must also influence this. It is possible that variations in plasma protein binding could be a contributory factor here as it could be expected that free salicylate would diffuse more easily into the csf than bound salicylate.

3. Comparison with volunteer study

It is unfortunate that, at the time the volunteer study was carried out, the methodology for assaying samples for salicyluric acid had not been perfected. It would have been interesting to compare the rate of elimination of salicylate with respect to the rate of formation of salicyluric acid in the volunteers with the rate of elimination found in the overose patients. However as there did not seem to be a consistent pattern in the rate of elimination of salicylate by the overdose patients, the comparison would, perhaps, have not yielded any concrete results.

The main points that arise from the comparison of plasma levels of salicylates in both cases are that, although no log - linearity was seen in the decline of plasma levels at therapeutic doses, there was a definite linear (i.e. first order) decline in the overdose patients. The possible reasons for the lack of linearity in the volunteer study have been discussed previously and it is difficult to exclude some criticism of the methodology with respect to the unexpectedly

high proportions of salicylic acid found in the "total salicylate" estimation. At the dose used in the volunteer study, it could be expected that the major metabolite would be salicyluric acid rather than salicylic acid itself. It is possible that the method used for assay of salicylic acid led to an overestimation of this portion.

The log - linearity of decline of salicylate levels in the plasma of overdose patients correlates well with the assumption that first order formation of metabolites is the major route of elimination of salicylate at high concentrations. Cumming, Duke and Widdowson (1964) have reported a log - linear decline in serum salicylate within the concentration range.100 to 30 mg/100ml and under conditions of forced alkaline diuresis.

4. Conclusions

. Variability in plasma levels seen in the volunteer study could be explained by reference to gastric emptying or the existence of compartments in the volume of distribution of salicylate which fill/empty at differential rates.

. As a result of these variations few conclusions can be drawn from comparison with levels in overdose patients.

. The log - linearity of decline of plasma levels of overdose patients is further evidence of the limitation of salicyluric acid formation at high doses of salicylate.

. Brain glucose may be depressed by high doses of salicylate in humans due to compensatory glycolysis.

. Adult overdose patients are not as susceptible to acid/base disturbances as infants who ingest a large dose of salicylate, even when the adults have a higher plasma level of salicylate. The ratio of salicylate in the csf to that in plasma does not reach such high levels as in infants, possibly due to the respiratory alkalosis of adult salicylate poisoning which tends to prevent salicylate entering the csf.

Appendix 1. Questionnaire used for pilot study.

Name: ....

Case No.: ..... Address: ....

....

Day and Date of Admission: ....

.....

Length of Stay: ..... Time of admission: .....

Age: .... D.O.B.: ..... Sex: ..... Pregnant: .....

Means of referral: .... Religion: ....

Civil State: ..... Social Class: .....

Type of admission: .....

Present History

Previous therapeutic drugs: .....

....

Previous misuse of Drugs: .....

....

Previous Self Poisoning: .....

.....

Other Suicide: ....

Previous Psychiatric therapy: I.P.: ..... Relatives: I.P.: .....
O.P.: ..... O.P.: .....

Alcohol - simultaneously: ..... Relatives: .....

clinic test: .....

Laboratory drug confirmation: ..... Blood: ..... Blood level:..

....

Emergency toxicology: ..... Emergency biochemistry: .....

.....

Coma Depth: ..... Length: .....

Ventilator/Airway: .....

Hypothermia: ..... Hypertension: ..... Blisters: .....

Treatment: .....

.....

Complications: .....

.....

Social Violence by Patient: .....

Social Violence against patient: .....

Time in present job: .....

Debts: ..... Criminal records: .....

....

Police investigation: .....

Psychiatric Diagnosis: .....

.....

Country Born: ..... How long here?: .....

Country Parents Born: ..... Father: .....
Mother: .....

How long here?: .....

Racial Stock: .....

Appendix 2. Questionnaire/Coding sheet used for overdose study.

OVERDOSE ADMISSIONS – CODING SHEET

PATIENT'S NAME .....

DATE OF ADMISSION .....

CARD NUMBER — S — 1 [1]

CASE NUMBER — S — 2 3 4 5 6 7

DATE — S — 8 9 10 11 12 13

DAY OF WEEK — S

N/AV	(0)	FRI	(5)
MON	(1)	SAT	(6)
TUES	(2)	SUN	(7)
WED	(3)		
THURS	(4)		

14

TIME OF ADMISSION — S

N/AV	(0)	14.00 - 17.59	(4)
02.00 - 05.59	(1)	18.00 - 21.59	(5)
06.00 - 09.59	(2)	22.00 - 02.00	(6)
10.00 - 13.59	(3)		

15

CIVIL STATUS — S

N/AV	(0)	SEPARATED	(4)
MARRIED	(1)	COHABIT	(5)
UNMARRIED	(2)	WIDOW	(6)
DIVORCED	(3)		

16

SEX — S

N/AV	(0)	FEMALE	(2)
MALE	(1)	PREGNANT	(3)

17

AGE — S — 18 19

AGE GROUP — S

N/AV	(0)	35 - 44	(5)
< 15	(1)	45 - 54	(6)
15 - 19	(2)	55 - 64	(7)
20 - 24	(3)	65 - 74	(8)
25 - 34	(4)	75 +	(9)

20

DATE OF BIRTH — S — 21 22 23 24 25 26

WHERE BORN — S

N/AV	(00)	PAKISTAN	(07)
ENGLAND	(01)	EUROPE	(08)
IRELAND	(02)	AFRICA	(09)
SCOTLAND	(03)	OTHER ASIAN	(10)
WALES	(04)	AMERICAN	(11)
W. INDIES	(05)	OTHER	(12)
INDIA	(06)		

27 28

RACIAL GROUP — N

N/AV	(0)	INDIAN	(4)
CAUCASIAN	(1)	PAKISTAN	(5)
W. INDIAN	(2)	OTHER ASIAN	(6)
AFRICAN	(3)	OTHER	(7)

29

RELIGION — S

N/AV	(0)	OTHER CHURCH	(5)
NIL	(1)	MUSLIM	(6)
C. of E.	(2)	SIKH	(7)
OTHER PROTESTANT	(3)	HINDU	(8)
R.C.	(4)	OTHER	(9)

30

SOCIAL CLASS - EITHER PATIENT OR NEXT OF KIN

N.B. If Wife - determined by Husband
If Child at home - determined by Father

N

N/AV	(0)	CLASS 5	(5)
CLASS 1	(1)	NO USUAL OCCUPATION	(6)
CLASS 2	(2)	STUDENT	(7)
CLASS 3	(3)		
CLASS 4	(4)		

31

ADDRESS — S — 32 33 34 35

REFERRED TO D.R.H. BY: — S

N/AV	(0)	POLICE	(4)	999	(8)
G.P.	(1)	SAMARITAN	(5)		
PSY. HOSP.	(2)	SELF	(6)		
GEN. HOSP.	(3)	OTHER	(7)		

36

REASON FOR ADMISSION — D

N/AV	(0)	SELF-INJURY ? TYPE	(6)
FOR KICKS	(1)	SELF-INJURY & SELF POIS.	(7)
INTENTIONAL SELF-POISONING	(2)	D.T.'s	(8)
ACCIDENTAL SELF-POISONING	(3)	COMPLICATED MISUSE	(9)
POISONING? TYPE	(4)		
SELF-INJURY INTENTIONAL	(5)		

37

LENGTH OF TIME BETWEEN INGESTION & ADMISSION (in hrs.) — N

N/AV	(0)	8 - 12	(5)
0 - 2	(1)	12 - 18	(6)
2 - 4	(2)	18 - 24	(7)
4 - 6	(3)	> 24	(8)
6 - 8	(4)		

38

EVIDENCE THAT POISON WAS TAKEN — D

N/AV	(0)
NIL	(1)
PATIENT'S HISTORY	(2)
OTHER HISTORY	(3)
CLINICAL SIGNS	(4)
TOXICOLOGY	(5)

MARK HIGHEST SCORE THAT IS APPLICABLE

39

PACKAGING
N

N/AV	(0)	BOTTLE	(3)
POP OUT	(1)	OTHER	(4)
SMALL QUANTITY CONTAINER	(2)		

40

NUMBER OF SUBSTANCES TAKEN OTHER THAN ALCOHOL
N

N/AV	(0)	5	(5)
1	(1)	6	(6)
2	(2)	6+	(7)
3	(3)	NIL	(8)
4	(4)		

41

ALCOHOL SIMULTANEOUSLY
N

N/AV	(0)	WINE	(3)
NIL	(1)	SPIRITS	(4)
BEER	(2)	HOME BREW	(5)
		SHERRY	(6)

42

PRINCIPLE POISON
D

000 = NOT APPLICABLE 43 44 45

SECONDARY POISON
D

000 = NOT APPLICABLE 46 47 48

TERTIARY POISON
D

000 = NOT APPLICABLE 49 50 51

PRIMARY REGULAR DRUG DURING PREVIOUS 2 WEEKS
D

000 = NOT APPLICABLE 52 53 54

SECONDARY REGULAR DRUG DURING PREVIOUS 2 WEEKS
D

000 = NOT APPLICABLE 55 56 57

ALCOHOL
D

N/AV	(0)
CHRONIC ALCOHOLIC	(1)
HEAVY SOCIAL DRINKER	(2)
SOCIAL DRINKER (CULTURAL NORM)	(3)
T.T.	(4)

58

SMOKER
N

N/AV	(0)	10 - 19	(3)
0	(1)	19 - 30	(4)
1 - 9	(2)	> 30	(5)

59

MISUSE OF DRUGS
D

N/AV	(0)
NO MISUSE	(1)
HABITUAL MISUSE - I.V.	(2)
HABITUAL MISUSE - ORAL	(3)
MARIJUANA	(4)

60

SOURCE OF DRUG TAKEN IN OVERDOSE
N

N/AV	(0)
NON-PRESC. – BOUGHT FOR O/D	(1)
NON-PRESC. – BOUGHT FROM CHEM. BY PATIENT/FRIEND	(2)
NON-PRESC. – OBTAINED ILLICITLY	(3)
PRESC. FOR SELF WITHIN 1 WEEK	(4)
PRESC. FOR SELF LONGER 1 WEEK	(5)
PRESC. FOR OTHER (FAMILY)	(6)
PRESC. DRUG OBTAINED ILLICITLY	(7)

61

DRUG LEVEL PRIMARY DRUG IN NG/LITRE 10^{-9}/LITRE
Max. 90×10^{-9}
D

N/AV = 00,000,000,000
Done but not codable 10,000,000,000,000

62 63 64 65 66 67 68 69 70 71 72

CARD NUMBER
S

1 2

CASE NUMBER
S

2 3 4 5 6 7

DATE OF ADMISSION
S

8 9 10 11 12 13

DRUG LEVEL SECONDARY DRUG
D
Max. 90×10^{-9}

14 15 16 17 18 19 20 21 22 23 24

DRUG DETECTED IN URINE
D

N/AV	(0)
YES	(1)
NO	(2)
EXCRETION RATE OBTAINED	(3)

25

ALCOHOL mg/100 ml
D

N/AV	(0)	100 - 149	(4)
Done - 0	(1)	150 - 199	(5)
1 - 49	(2)	200 - 300	(6)
50 - 99	(3)	>300	(7)

26

CARBON MONOXIDE
D

N/AV	(0)	20 - 40%	(4)
NONE	(1)	40 - 60%	(5)
>5%	(2)	>60%	(6)
5 - 19%	(3)		

27

LEVEL CONSCIOUSNESS (lowest)
D

0 1 2 3 4

N/AV 5

28

HYPOTHERMIA (lowest)
D

N/AV	(0)	33 - 34	(4)
NONE	(1)	32 - 33	(5)
35 - 36	(2)	< 32	(6)
34 - 35	(3)		

29

CLINICAL FEATURES – NEUROLOGY
D

N/AV	(0)
NORMAL EYE MOVEMENT	(1)
NYSTAGMUS	(2)
LOSS CONJUGATE GAZE	(3)
OTHER	(4)

30

CLINICAL FEATURES – REFLEXES
D

N/AV	(0)
NORMAL	(1)
DEPR.	(2)
INER.	(3)
ASYMETR.	(4)

31

CLINICAL FEATURES – PLANTARS
D

N/AV	(0)
NORMAL	(1)
↑	(2)
↓	(3)
ASYMETR.	(4)
OTHER	(5)

32

CLINICAL FEATURES – SPEECH
D

N/AV	(0)
NORMAL	(1)
PRESSURE	(2)
SLURRED	(3)

33

CLINICAL FEATURES – PUPILS
D

N/AV	(0)
NORMAL	(1)
CONSTR.	(2)
DILATED	(3)
UNEQUAL	(4)
OTHER	(5)

34

CLINICAL FEATURES – C.V.S.
D

N/AV	(0)
NORMAL	(1)
TACHYCARDIA	(2)
BRADYCARDIA	(3)
ARRYTHMIA	(4)
OTHER	(5)

35

CLINICAL FEATURES – RESPIRATORY
D

N/AV	(0)
NORMAL	(1)
NO GAG REFLEX	(2)
HYPOVENTILATION	(3)
OTHER	(4)

36

CLINICAL FEATURES – GASTRO-INTESTINAL
D

N/AV	(0)
NORMAL	(1)
ABNORMAL	(2)

37

CLINICAL FEATURES - LIVER
D

N/AV	(0)
NORMAL	(1)
ABNORMAL	(2)

38

CLINICAL FEATURES - GENITO-URINARY
D

N/AV	(0)
NORMAL	(1)
RETENTION	(2)
OTHER	(3)

39

COMPLICATIONS
D

N/AV	(0)	HEPATIC	(5)
NO	(1)	RENAL	(6)
CVS	(2)	NEUROLOGICAL	(7)
RESP.	(3)	MUSCULO/SKELETAL	(8)
ABDO.	(4)	DEATH	(9)

40

BLISTERS
D

N/AV	(0)
NO	(1)
YES	(2)

41

INVESTIGATION BIOCHEM
D

N/AV	(0)
NIL	(1)
EMERGENCY	(2)
RESEARCH	(3)
ROUTINE	(4)
BLOOD GASES	(5)

42

TOXICOLOGY

D

N/AV	(0)
NIL	(1)
EMERGENCY	(2)
RESEARCH	(3)
ROUTINE	(4)
BLOOD GASES	(5)

43

RADIOLOGY

D

N/AV	(0)
NIL	(1)
EMERGENCY	(2)
RESEARCH	(3)
ROUTINE	(4)

44

HAEMATOLOGY

D

N/AV	(0)
NIL	(1)
EMERGENCY	(2)
RESEARCH	(3)
ROUTINE	(4)

45

GASTRIC LAVAGE

D

N/AV	(0)
NOT DONE	(1)
YES WITHOUT ETT	(2)
YES WITH ETT	(3)
DONE BEFORE ADMISSION	(4)
IPECAC	(5)
OTHER	(6)

46

COMPLICATIONS GASTRIC LAVAGE

D

N/AV	(0)
NIL	(1)
ASPIRATION	(2)
LIPOID PNEU.	(3)
BLEEDING	(4)
OTHER	(5)

47

AIRWAY

D

N/AV	(0)
NORMAL	(1)
OROPHARYNGEAL	(2)
ETT	(3)
ETT AND VENTIL	(4)
TRACHY	(5)

48

MAINTENANCE FLUID

D

N/AV	(0)
NO	(1)
YES	(2)

49

HYPOTENSION

D

N/AV	(0)
NO	(1)
BED BLOCK	(2)
VASOPRESSOR	(3)
VOLUME EXPANSION	(4)

50

FORCED DIURESIS

D

N/AV	(0)
NO	(1)
SALICYLATE	(2)
BARBITURATE	(3)
AMPHETAMINE	(4)
OTHER	(5)

51

PERITONEAL DIALYSIS

D

N/AV	(0)
NO	(1)
YES– TO REMOVE POISON	(2)
YES– FOR RENAL FAILURE	(3)

52

HAEMODIALYSIS

D

N/AV	(0)
NO	(1)
YES– TO REMOVE POISON	(2)
YES– FOR RENAL FAILURE	(3)

53

CHARCOAL HAEMOPERFUSION

D

N/AV	(0)
NO	(1)
YES	(2)

54

MANNITOL INFUSION

D

N/AV	(0)
NO	(1)
YES FOR CEREBRAL OEDEMA	(2)
YES FOR DIURESIS	(3)
YES FOR RENAL FAILURE	(4)

55

SPECIFIC ANTAGONIST

D

N/AV	(0)
NONE	(1)
PARACETAMOL– CYSTEAMINE	(2)
– METHIONINE	(3)
OPIATE – NALOXONE	(4)
DESFERIOXAMINE	(5)
OTHER	(6)

36

PSYCHIATRIC INTERVIEW

P/SW

N/AV	(0)
SEEN BY ADULT PSYCHIATRIST	(1)
SEEN BY SOCIAL WORKER	(2)
SEEN BY ADULT PSY. & SOCIAL WORKER	(3)
SEEN BY CHILD PSYCHIATRIST	(4)
DISCHARGED BY PHYSICIAN WITH OP APPOINTMENT	(5)
DISCHARGED SELF– WITH OP APPT.	(6)
DISCHARGED SELF– NO OP APPT.	(7)
DISCHARGED BY PHYSICIAN	(8)
NOT SEEN PSY. – OTHER REASONS	(9)

57

PREVIOUS SELF-POISONING (Total)
(Admitted to Hospital)

P/SW		
	N/AV	(0)
	NONE	(1)
	ONE	(2)
	TWO	(3)
	THREE	(4)
	FOUR	(5)
	5 - 10	(6)
	>10	(7)

59

PREVIOUS SELF-POISONING
(Not Admitted to Hospital)

P/SW		
	N/AV	(0)
	NONE	(1)
	ONE	(2)
	TWO	(3)
	THREE	(4)
	>3	(5)

60

MOST RECENT ADMISSION TO HOSPITAL WITH AN OVERDOSE

P/SW				
	N/AV	(0)	WITHIN 6 MONTHS	(5)
	NONE	(1)	WITHIN 1 YEAR	(6)
	WITHIN 24 HOURS	(2)	1 - 5 YEARS	(7)
	WITHIN 1 WEEK	(3)	>5 YEARS	(8)
	WITHIN 1 MONTH	(4)		

61

PREVIOUS IN-PATIENT PSYCHIATRIC TREATMENT

P/SW		
	N/AV	(0)
	NONE	(1)
	IP AT TIME OF ADMISSION	(2)
	IP WITHIN 1 WEEK	(3)
	IP WITHIN 1 MONTH	(4)
	IP WITHIN 1 YEAR	(5)
	IP >1 YEAR	(6)

62

PREVIOUS OP PSYCHIATRIC TREATMENT

P/SW		
	N/AV	(0)
	NONE	(1)
	OP AT TIME OF ADMISSION	(2)
	OP WITHIN 1 WEEK	(3)
	OP WITHIN 1 MONTH	(4)
	OP WITHIN 1 YEAR	(5)

63

SEPARATION FROM MOTHER (Up to 15)

P/SW		
	N/AV	(0)
	NOT SEPARATED	(1)
	SEPARATED BEFORE AGE 10	(2)
	SEPARATED BETWEEN 10 - 15	(3)

64

SEPARATION FROM FATHER (Up to 15)

P/SW		
	N/AV	(0)
	NOT SEPARATED	(1)
	SEPARATED BEFORE AGE 10	(2)
	SEPARATED BETWEEN 10 - 15	(3)

65

PRESENT HOUSEHOLD LIVING WITH/IN
(List the Highest)

P/SW				
	N/AV	(0)	LODGINGS/HOSTEL	(5)
	SPOUSE	(1)	INSTITUTION	(6)
	PARENTS	(2)	ALONE	(7)
	SIBLINGS	(3)	OTHER	(8)
	OTHER RELATIVE/ FRIEND	(4)		

66

OVERCROWDING AT HOME

P/SW		
	N/AV	(0)
	YES	(1)
	NO	(2)

67

VIOLENCE WITHIN LAST YEAR

P/SW		
	N/AV	(0)
	NONE	(1)
	BY PATIENT	(2)
	AGAINST PATIENT	(3)
	BOTH	(4)

68

DEBTS

P/SW		
	N/AV	(0)
	NONE	(1)
	RENT ARREARS	(2)
	COURT ACTION THREATENED FOR RENT ARREARS	(3)
	H.P.	(4)
	COURT ACTION THREATENED FOR H.P.	(5)
	OTHER DEBT	(6)
	COURT ACTION THREATENED FOR OTHER DEBT	(7)

69

CRIMINAL RECORD

P/SW		
	N/AV	(0)
	NONE	(1)
	CONVICTION IN LAST YEAR	(2)
	CONVICTION IN MORE THAN A YEAR	(3)
	CURRENT POLICE PROCEEDINGS	(4)

70

PROBLEMS IN FAMILY

P/SW				
	N/AV	(0)	MARITAL DISCORD	(7)
	NONE	(1)	CULTURAL	(8)
	ALCOHOL	(2)		
	PSYCHIATRIC	(3)		
	ALCOHOL & PSYCHIATRIC	(4)		
	CRIMINAL	(5)		
	OTHER	(6)		

71

SELF-POISONING IN FAMILY

P/SW		
	N/AV	(0)
	NONE	(1)
	IMMEDIATE FAMILY	(2)
	EXTENDED FAMILY	(3)
	FRIEND	(4)

72

SUICIDE IN FAMILY

P/SW

N/AV	(0)
NONE	(1)
IMMEDIATE FAMILY	(2)
EXTENDED FAMILY	(3)
FRIEND	(4)

73

ILLNESS DIAGNOSIS

P/SW

N/AV	(0)
NO PSYCHIATRIC ILLNESS	(1)
DEPRESSIVE ILLNESS	(2)
DEPRESSIVE REACTION	(3)
ORGANIC PSYCHIATRIC DISORDER	(4)
SCHIZOPHRENIA	(5)
EPILEPSY	(6)
MANIA	(7)
OTHER (SPECIFY)	
.....	(8)

74

PERSONALITY DIAGNOSIS

P/SW

N/AV	(0)
NORMAL PERSONALITY	(1)
PERSONALITY DISORDER	(2)
SUBNORMALITY	(3)
DRUG ADDICTION	(4)
ALCOHOLISM	(5)
OTHER (SPECIFY)	
.....	(6)

75

AGREED PSYCHIATRIC DISPOSAL ON DISCHARGE

P/SW

N/AV	(0)
NONE	(1)
IP DETAINED	(2)
IP INFORMAL	(3)
DAY PATIENT	(4)
OP (DRH)	(5)
OP (ELSEWHERE)	(6)
GP	(7)
OTHER	(8)
DIED	(9)

76

AGREED PSYCHIATRIC DISPOSAL SOCIAL WORK

P/SW

N/AV	(0)
NONE	(1)
HOSPITAL SOCIAL WORKER	(2)
SOCIAL SERVICES DEPT.	(3)
HEALTH VISITOR	(4)
OTHER	(5)

77

LENGTH OF STAY

S

N/AV	(0)	5 - 7 DAYS	(5)
< 24 HRS	(1)	7 - 10 DAYS	(6)
24 - 48 HRS	(2)	10 - 14 DAYS	(7)
48 - 72 HRS	(3)	15 - 28 DAYS	(8)
3 - 5 DAYS	(4)	>28 DAYS	(9)

78

CONSULTANT PSYCHIATRIST WHO SAW PATIENT

S

.....

79

LENGTH OF TIME IN COUNTRY

P/SW

N/AV	(0)
LIFE	(1)
0-2 YEARS	(2)
2-5 YEARS	(3)
5-10 YEARS	(4)
10-15 YEARS	(5)
> 15 YEARS	(6)

80

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