CLINICAL AND LABORATORY ASPECTS OF SALICYLATE THERAPY.

THESIS SUBMITTED FOR THE DEGREE OF M.D. OF THE UNIVERSITY OF GLASGOW

by

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INTRODUCTION.

Although salicylates have been in common use in therapeutics for generations, it is only in comparatively recent times that precise laboratory methods have become available for the study of the complex changes produced in the organism during treatment with these drugs. Any consideration of the therapeutic applications of a drug must take into account its toxic manifestations, and these, for their proper assessment, should if possible be correlated with the concentration of the drug in the tissues and with detectable alterations in the composition of body fluids. A considerable literature on the toxicology of salicylates has accumulated, but less attention appears to have been given to the importance of the details of the laboratory investigation of the salicylate concentration of the blood than this aspect of the subject demands.

This thesis reviews previous work on the pharmacological action of salicylate in rheumatism, and describes a critical investigation of a widely used method of determining serum salicylate concentrations. The limitations of the method having been clearly defined and the experimental conditions necessary for its maximum usefulness determined, the technique has been applied to

a study of the factors influencing the blood salicylate concentration in one hundred individuals to whom the sodium salt was administered. Normal subjects and patients suffering from different conditions have been studied, and the investigation was carried out with particular reference to haemorrhagic manifestations and their relationship to ascorbic acid and vitamin K requirements.

It was apparent from the outset that the programme necessitated the co-operation of others, and due acknowledgement will be made to them.

CHAPTER I.

EFFECTS OF SALICYLATE ADMINISTRATION - REVIEW OF THE LITERATURE.

CHAPTER I.

Effects of Salicylate Administration - Review of the literature.

The action of a drug is dependent on an adequate concentration being maintained in the blood stream and tissues, and this level is in turn dependent on the manner and rate of absorption, distribution, and excretion.

The absorption and excretion of salicylates were studied by Scott, Thorburn, and Hanzlik (1917), and these investigators showed that most compounds of salicylic acid were absorbed equally well. They stated also that excretion was rapid, no appreciable amount of the drug being detected in the blood in man fortyeight hours after its administration had been stopped. The same workers found that in individuals suffering from rheumatic fever, the total amount excreted was about fifteen per cent less than in normal subjects. Excretion was also reported to be reduced in alcoholism, morphinism, and tuberculosis, but the administration of sodium bicarbonate was not observed to produce any alteration in the amount of salicyl radical excreted in the urine.

Smull, Wegria, and Leland (1944) described a series

of estimations of serum salicylate on patients who were given, first, sodium salicylate alone, and then sodium salicylate and sodium bicarbonate together in equal amounts. They found that when the serum salicylate concentration was maintained at a fairly constant level with the patient receiving sodium salicylate alone orally, the concurrent administration of sodium bicarbonate caused the serum salicylate concentration to fall; also, they reported that when sodium salicylate and sodium bicarbonate were given together from the beginning in a series of patients, the concentration of salicylate attained in the serum was never as high as that obtained when the same dose of sodium salicylate was given alone. Huntingdon, Ryan, Butt, Griffith, Montgomery, Solley, and Leake (1946) carried out similar experiments on a smaller scale, but were unable to detect any significant difference in salicylate levels in the serum whether or not bicarbonate was administered concurrently. Earlier, Morris and Graham (1931) had expressed the view that sodium bicarbonate had the effect of increasing the concentration of salicylate in the serum, but later work by Parker (1948) seemed to confirm the views of Smull and his co-workers. Smith, Gleason, Stoll and Ogorzalek (1946) also studied the effect of sodium

bicarbonate on salicylate excretion, and demonstrated in their cases that administration of alkali was accompanied by an increased excretion of free salicylate in the urine, but that the total salicylate excretion was unaffected.

Quinke (1882) published an account of the symptoms of salicylate intoxication as they were recognised at that time. They consisted of headache, dizziness, tinnitus, deafness, nausea, vomiting, collapse, and sometimes albuminuria. Modern textbooks add little to this description. In his paper, Quinke described a disturbance of respiration which he had noted in subjects receiving salicylate therapy, to which he gave the name "salicyl dyspnoea." He stated that the depth and the rate of breathing might be increased independently or together, and he compared the condition with the hyperpnoea of diabetic acidosis. Quinke recommended the administration of alkali to combat the acidosis which he believed to be the underlying disorder.

Langmead (1906) described eight cases of salicylate poisoning in children, of which two were fatal. Hyperpnoea was a prominent sign, but as some of the cases showed starvation and acetonuria, ketonuria cannot be ruled out as the true cause of the hyperpnoea in these cases.

Scott and Hanzlik (1916) referred to the occurrence of albuminuria during therapy with sodium salicylate, stating that they found it in apyrexial as well as in febrile subjects, and in both non-rheumatic and rheumatic cases. The albuminuria was described as renal in origin, and in experimental animals renal haemorrhages were detected. In man suffering from intoxication with salicylates the damage to the kidneys did not appear to be permanent.

Hanzlik, Scott, and Reycroft (1917) described the occurrence of water retention in the tissues during sodium salicylate therapy. They demonstrated that while small doses of the drug were followed by a diuresis, large doses gave rise to partial suppression of water excretion by the kidney, due in part to diaphoresis, but also to some extent to a diminution in renal function. An increase in weight in normal controls receiving sodium salicylate in therapeutic doses was observed. Since no evidence of haemo-dilution was found they stated that the water retention was chiefly in the tissues. This water retention they called "salicyl oedema", and emphasised that it in no way resembled the pitting oedema commonly observed

in cases of cardiac failure or nephritis.

In contradistinction to the claim that haemodilution does not occur, Barbour (1921) showed that the exhibition of antipyretics in fever caused an increase in blood volume, and he expressed the view that as a result of this change the heat centre permitted increased heat loss, and also that haemodilution increased sweating.

Lyons (1944) studied the effect of sodium intake on plasma volume and body weight. He reported a significant increase in plasma volume in fourteen subjects after receiving forty grams sodium chloride or fifty grams sodium bicarbonate in fortyeight hours. The increase was accompanied by a rise in venous pressure and body weight, but there was no proportionate fall in the serum protein, haemoglobin, or red blood cell count.

The emetic action of salicylates was investigated over forty years ago by Waddell (1911), who found that intravenous and oral administration of sodium salicylate were equally active in causing vomiting. The vomitus after parenteral administration did not contain salicylate. Hanzlik (1913) was one of the first to subscribe to the view that the vomiting of salicylate

overdosage was central in origin, basing his views on the observation that emesis was just as likely to occur after the administration of salicylates in a form which passed through the stomach undissolved as after soluble preparations such as sodium salicylate.

Eggleston and Hatcher (1912, 1915) considered the problem in detail. They demonstrated that when the drug was administered intravenously, a smaller dose was required to produce vomiting than when the oral route was used, and the result was more prompt. Dogs from which the alimentary tract had been removed showed the typical phenomena of emetic response when salicylate was given intravenously. These results were claimed as experimental proof that the main emetic action of salicylates was not local but central in origin, but it was also suggested that a local irritant factor might be present in addition when the drug was taken by mouth.

Schredorf, Bradley, and Ivy (1936) made the observation that acetyl salicylic acid affected gastric mobility and gave rise to gastric irritation, even in individuals not hypersensitive to the drug. The total titratable acidity was increased by aspirin, and this, it was suggested, was another cause of gastric irritation. Sodium bicarbonate was said to diminish both effects, and Sable (1945) suggested that these actions of bicarbonate

supported its use along with salicylate. He described cases in which no ill effects of bicarbonate had been noted. Sodium bicarbonate, however, liberates carbon dioxide when it reacts with acid, and the gas released not only causes discomfort, but is recognized as bringing about a secondary secretion of gastric juice.

Much controversy has arisen over the question, does acidosis arise during salicylate therapy? Reference has already been made to Quinke's views, and he undoubtedly considered the hyperpnoea of salicylism as acidotic. Johnson (1930) administered salicylate to cats, and found that a fall in blood lactate and the alkali reserve resulted. He concluded that salicylate could cause an acidosis, but Scott (1916) and others had earlier carried out similar experiments and they found little change in alkali reserve after the administration of massive doses of salicylate.

Veil (1926), Gebert (1931), and Odin (1932) after separate experiments all subscribed to the view that there was a marked disparity between the degree of hyperpnoea and the change in the alkali reserve of the blood. They found that during salicylate therapy the urinary pH was generally increased, and they believed this indicated an irritative effect on the respiratory

centre by the drug, which in turn gave rise to hyperventilation and, therefore, to a decrease in the CO_2 tension in the alveolar air and in the blood. A reduction of the fixed base in the blood was brought about by the excretion of an alkaline urine. It is important to realise that if salicylate does cause alkalosis, the treatment of salicylate intoxication by administration of alkali is contra-indicated.

Bowen, Roufa, and Clinger (1936) described poisoning by salicylate in a child of eighteen months who had taken several tablets of aspirin and a sip of dilute oil of wintergreen. Hyperpnoea was very marked, but the carbon dioxide combining power of the blood was 50 volumes per 100 volumes. This supports the view that the hyperpnoea of salicylism is due, not to acidosis, but to some other effect, probably central.

Dodd (1937) reported a human case of poisoning in which he found a fixed type of acidosis with an increased CO_2 capacity and lowered blood pH. In experiments on dogs, however, he found the blood pH increased and no alteration in the acid/base equilibrium; some animals manifested an increased loss of CO_2 from the blood due to hyperpnoea, and an associated increase in blood pH which was followed by a compensatory loss of base and the excretion of an alkaline urine. Dodd believed

his results were further evidence in support of the view that the cause of hyperpnoea was central.

More recently Reid has investigated this problem. In his earlier studies Reid (1948) suggested that salicylate induced an acidosis, but later with Watson and Sproull (1950) he carried out detailed studies of plasma CO_2 concentrations in adults under treatment with salicylate for rheumatic fever, and produced convincing evidence that a respiratory alkalosis is caused by the drug. Graham and Parker (1948) had a short time before shown that the hyperventilation produced when the drug was given intravenously to animals could be abolished by cutting both vagi; this seemed to explain the hyperpnoea of salicysm in man on the basis of stimulation of the vagus nerves.

The haemorrhagic manifestations of salicylate poisoning have been studied more in recent years, although over a quarter of a century ago Wetzel and Nourse (1926) described the occurrence of purpura in methyl salicylate poisoning. They stated that the effects of this drug differed from those of sodium salicylate only in degree, but suggested that with the methyl compound vascular and haemorrhagic lesions were more prominent. They describe multiple subserous haemorrhages in the heart, lungs, brain,

and kidneys, and also extensive subdural extravasations of blood.

Madisson (1934) published a report on the histological appearances seen in salicylate poisoning in man and experimental animals. He stated that in the acute stage of salicylate poisoning examination revealed engorgement of blood vessels and haemorrhages in most organs. There was cloudy swelling in the heart, liver, and kidneys. At the same time Madisson discovered that the intensity of changes was lessened by the administration of glucose. Others found punctate haemorrhages on all serous surfaces, and in fatal human cases haemorrhages were occasionally found in the gastric and duodenal mucosa - substantiating the observations of Quinke (1884), when he found congestion with poor aeration of the lung bases and slight haemorrhagic pericardial effusions in human cases.

Hurst and Lintott (1939) reported a number of cases of haematemesis after the administration of aspirin tablets. The local effect was observed by gastroscopy, and the hyperaemia and haemorrhage which were seen were considered to be the result of an increased sensitivity to the effect of aspirin on the tissues.

Melaena is said also to occur in aspirin therapy, and Sable (1945) considers its aetology to be identical with that of haematemesis.

Link (1943) and his colleagues made a study of the haemorrhagic tendencies observed during therapy with salicylates and dicoumarol. In 1931 Roderick had shown that the haemorrhagic diathesis in cattle known as "sweet clover disease" was associated with a lowered concentration of prothrombin in the blood, but it was not until 1941 that Link, Campbell and others succeeded in isolating the responsible agent from improperly cured hay made from the sweet clovers Melilotus alba and M. officinalis. The first compound isolated had the composition $C_{19}H_{12}O_6$, and was described as 3,3' methylene-bis (4-hydroxycoumarin). The naturally occurring substance was shown to have the same effects as the synthetic compounds.

Alkaline degradation of 3,3' methylene-bis (4-hydroxycoumarin) was carried out, and cleavage of the C = C linkage of the intermediate diketone resulted in the quantitative formation of salicylic acid.

Link found that dicoumarol in vitro had no effect on the clotting properties of blood, and also that in vivo there was no effect until several hours after oral or intravenous administration. He showed that when a single dose was given, the drug disappeared from the blood stream before hyperprothrombinaemia could be detected; from this he deduced that dicoumarol was altered in the body before prothrombin or its synthesis was affected.

A further series of chemical degradations was carried out, and the products subjected to physiological and chemical tests. Link found that only these compounds which might theoretically yield salicylic acid or ortho-hydroxy-benzoic acid on degradation possessed anti-coagulant activity. He showed also that when salicylic acid was given to rabbits or rats which had been fed on a diet poor in vitamin K, the blood prothrombin content was lowered, and when the drug was administered for periods of over twenty days, haemorrhage which was frequently fatal ensued. It was also shown that the maximum effect of a single dose of salicylate occurred twelve hours after administration, and that the blood prothrombin concentration had returned to normal after twenty hours. When vitamin K was administered along with the salicylate, no haemorrhages were observed.

Ashworth and McKemie (1944) stated that in salicylate poisoning, death could occur as a result of a haemorrhagic encephalitis, and they described two fatal cases.

Shapiro, Redish, and Campbell (1943) confirmed Link's observation that salicylate could cause a depression of the prothrombin concentration in the blood, and showed that the administration of six grams of aspirin daily along with 20 mg. vitamin K by mouth and 6 mg. intravenously did not cause hypo-prothrombinaemia. Shapiro referred to the observation of Davidson and

McDonald (1943) that vitamin K in clinical doses did not reverse or prevent the effects of dicoumarol on prothrombin concentration or the coagulation of the blood, and concluded that there was little reason to expect a different response when the increase in the prothrombin time was the result of salicylate therapy.

Rhoads (1943) alleged also that vitamin K was ineffective in controlling prolonged prothrombin times, but later Davidson (1943) showed that dicoumarol in a dose of 0.5 to 1 gram was effectively "neutralised" by 180 - 450 mg. vitamin K, and that there were no toxic effects.

Shapiro (1943) found some evidence that dicoumarol and salicylates were cumulative in their effect with regard to the depression of prothrombin concentration.

Meyer and Howard (1943) described the effect of daily administration of 5.3 grams aspirin to thirteen cases. They found that the prothrombin level fell to 50 per cent of normal in 3-4 days, and that there was a relative prolongation of the blood coagulation time. Normal values were obtained again 2-4 days after the aspirin therapy was stopped. Similar results were obtained when sodium salicylate was used. 6 mg. vitamin K per day did not correct the depression of the prothrombin concentration.

Rapoport and Guest (1943) confirmed Meyer and Howard's findings. When children aged six to fourteen years received from 1.5 to 8 grams sodium salicylate or aspirin daily, the prothrombin concentration in the blood was observed to fall to 50 per cent of normal in those individuals receiving six to eight grams salicylate per day. Fashena and Walker (1944) carried out similar experiments, and obtained similar results.

Shapiro (1944) studied the effect of vitamin K administration in preventing hypoprothrombinaemia in patients receiving salicylate therapy over long periods. Thirteen cases were observed. The prothrombin times were prolonged by the third to the fifth day after the salicylate therapy had been begun. One case had acute rheumatic carditis; the prothrombin time became prolonged on the second day of salicylate therapy, and the drug was stopped on the fifth day. The prothrombin time, however, remained abnormally long even after 6.5 mg. vitamin K had been given for three days. Nevertheless, salicylate was recommenced two days later, along with 9 mg. vitamin K. For the first six days thereafter there was complete protection against hypoprothrombinaemia, then a gradual increase in the prothrombin time was found in spite of the vitamin K administration. On the seventeenth day, 400 mg. ascorbic acid were given intravenously and repeated on the next three days. The

prothrombin time fell almost to normal. When the ascorbic acid treatment was stopped, the prothrombin time again rose.

Shapiro summarised the lessons from these results thus: The prothrombin time should be determined regularly throughout salicylate therapy. 1 mg. vitamin K will protect the body against the prothrombinoaemic effects of 1 gram of aspirin. If other factors are present which tend to lower the vitamin C content of the blood (such as fever, starvation) ascorbic acid should be administered in addition to vitamin K.

Richards and Cortell (1942) observed that when dicoumarol was administered to scorbutic and healthy guinea pigs, the scorbutic animals were the first to die, and histological examination of their livers showed fatty degeneration.

Overman, Stahman, and Link (1942) showed that feeding alalfa hay to rabbits protected them against the anticoagulant effect of dicoumarol. Vitamin C in high dosage given along with vitamin K reduced or protected completely against the effects of dicoumarol, and Vitamin C alone was able to prevent an increase in the prothrombin time in some but not all their experimental animals. They commented that dicoumarol may upset vitamin C and vitamin K metabolism, and compared the pathological lesions in deficiency diseases associated with a lack of vitamin C and vitamin K with the lesions found in cases treated

with excessive amounts of dicoumarol. The small amount of dicoumarol which often causes a rise in prothrombin time suggested that its action is due to a blocking of an enzyme system, probably in the liver. The inactivation of enzymes by substances structurally related to their substrates, the structural similarity of dicoumarol to substances which possess an activity similar to that of vitamin K, and the antagonistic action of vitamin K to dicoumarol lend support to the theory that vitamin K and dicoumarol act through a common system.

Tomaszewski (1939) and Campaira (1941) reported an apparent physiological relationship between vitamin K and ascorbic acid.

Since an interference with certain enzymes has been connected with the action of dicoumarol, it would not be surprising if salicylates played a similar role. The antagonism between the sulphonamides and p-aminobenzoic acid is now generally accepted. It is believed that the combination of an enzyme with p-aminobenzoic acid is blocked by sulphonamide. Ivanowics (1942) pointed out a similar antagonism between salicylate and pantothenate, and showed that the growth of staphylococcus aureus and B. coli which synthesize pantothenate was prevented by M/1000 salicylate. This action could be counteracted by adding pantothenate to the medium. Euler and Ahlstrom (1943) made further observations on the effect

of sodium salicylate on enzyme systems, and Lutwak-mann (1942) demonstrated the profound effect of salicylate on the enzyme system of living animals. He showed that liver glycogen disappeared after the intravenous administration of salicylate, and that in twentyfour hours normal conditions had been restored. The oxidation of dihydroxyacetone by liver slices from rats suffering from vitamin B_1 deficiency was diminished by the presence of salicylate, although the dismutation of hexose-phosphate was not affected. These observations suggest that the salicylate ion is a bacterial inhibitor in a non specific manner, and that it is also an enzyme inhibitor. This effect on the enzyme system may be associated with the fact that an adverse effect on the bone marrow has been ascribed to salicylates. Hawkinson and Kerr (1943) reported a case of agranulocytic anaemia and staphylococcal septicaemia after 2.7 to 4.0 grams aspirin daily for several years. The case had received scarlet fever toxoid followed by a severe reaction shortly before death, but they considered that the prolonged administration of salicylate was the cause of death.

Since Coburn (1943) suggested that a high concentration of salicylate in the blood - about 370 micrograms per millitre - was important in rheumatic fever to avoid cardiac complications, much work has been carried out to assess the value of this treatment, and a careful study of the toxic

signs has been made.

Keith (1945) found that nausea, vomiting, and tinnitus were more frequent at the beginning of therapy. He seldom found it necessary to stop treatment although his dosage ranged around 150 to 200 grains daily with an average blood level of 270/310 micrograms per millilitre. These levels were less than those advocated by Coburn in 1943, and the salicylate dosage was also lower. Keith stated that no renal damage was evident, but three cases developed severe hyperpnoea, one became comatose, and one developed acne. All these symptoms subsided on cessation of therapy. Although sodium bicarbonate was also given in these cases, he found that the same or higher blood salicylate levels could be attained on a dosage of 5 to 10 grams sodium salicylate alone as were reached when 10 to 13 grams salicylate were given along with equal amounts of sodium bicarbonate. The reduction in blood salicylate level brought about by the addition of sodium bicarbonate to the therapeutic regime did not compare with the 50 per cent reduction described by Smull (1944).

Studies of the effect of treatment with sodium bicarbonate and sodium salicylate in rheumatic fever were reported also by Butt (1945). This worker reported that there was a fall in the prothrombin level, but he observed no haemorrhages, no effect on liver function,

nor any evidence of renal injury. Prolonged high dosage caused the haemoglobin and red cell count to fall, but the total and differential white cell counts remained unaltered.

Coombs (1945) and others treated eightyfour cases of rheumatic fever with ten to sixteen grams of sodium salicylate daily, and found that when blood salicylate levels were maintained at over 300 micrograms per millilitre, the incidence of toxic manifestations was increased. They observed also a tendency for the blood pH and blood chloride to rise, while at the same time there was an increased loss of sodium in the urine, and a retention of water in the body. They concluded that salicylate had a direct irritative effect on the respiratory centre and that the signs of alkalosis were secondary to this, and suggested also that the administration of sodium bicarbonate along with salicylate tended to lower the concentration of the latter in the blood. They denied that the prothrombin concentration was affected even with serum salicylate levels exceeding 350 micrograms per millilitre.

In an attempt to correlate the divergent opinions expressed, Sable (1945) reviewed the literature, and from his studies concluded that more controlled investigations

were required by those aware of the many aspects of the problem, and that there was need for a better understanding of these commonly used drugs.

Review of Cases of Poisoning by Salicylate from 1882.

Quinke (1882) described a case in a girl aged 17 years suffering from rheumatism of long standing who died after receiving 34 grams of sodium salicylate in three days.

Langmead (1906) reported a case in a five year old boy suffering from rheumatic endocarditis who was given 100 grams in eleven weeks.

Balazs (1930) reviewed all the cases of attempted suicide with aspirin in Budapest. Only four were fatal, and of these one had consumed 20 grams; the others had taken between 30 and 40 grams. In all the cases, the quantity taken ranged from 5 to 95 grams

Dyke (1935) and Paisseau (1934) and others described other cases, and in the Lancet, 1935 (Sept. 14th) there appeared an article on the dangers of uncontrolled therapy with aspirin. Dyke's case was that of a girl who swallowed 450 grains of aspirin in a single dose. She suffered from nausea and hiccough and later became stuperose with symptoms of cerebral irritation. She was treated with abundant fluids, glucose intravenously, and lumbar puncture, and made a complete recovery.

Neale (1936) reported four fatal cases in males. One case had received 750 grains, and two had taken 1,000 grains. Symptoms of intoxication were delayed for several hours, and began with evidence of gastro-intestinal irritation, but signs of involvement of the central nervous system were more prominent and reached a maximum in twelve hours. A female case, aged 52, had received 500 grains of aspirin. The routine treatment of gastric lavage with dilute sodium bicarbonate solution was initiated, but no improvement set in until lumbar puncture was performed and 30 ml. fluid were withdrawn. Recovery set in almost at once, and was complete. Neale observed that without lumbar puncture recovery was unlikely in serious intoxication.

Andrews (1938) reported two cases, both of which recovered after ingesting 625 grains.

Evans (1938) also described a case with recovery after 1,500 grains. His patient suffered from emesis, and this may have contributed to his recovery.

Sell (1939) reported poisoning in a boy, aged 8, who had been given 10 grains sodium salicylate with 10 grains sodium bicarbonate four times a day. When 190 grains had been given, hyperpnoea was marked, there was tachycardia and profuse sweating, and frequent vomiting. Abdominal pain developed and the patient ultimately became delirious. Recovery set in after the administration of glucose and

sodium bicarbonate intravenously.

Oakley and Donnell (1942) reported a case which recovered after 65 grains.

Charters (1944) described salicylate poisoning in a woman who recovered after 750 grains. Lumbar puncture was not performed, but Charters gave insulin and glucose to abolish ketosis and counteract any toxic hepatitis.

Ashworth and McKemie (1944) described two deaths from encephalitis after large doses of salicylate.

Fatalities from salicylate poisoning do not constitute a large proportion of the total number of deaths registered annually in this country, but that the toll of life taken yearly by these compounds is not diminishing is shown by the following tables, compiled from information supplied by the Registrar-General for Scotland (1953) and the Registrar-General for England and Wales (1946, 1947, 1948, 1949, 1950.).

TABLE I

Deaths from Aspirin and Salicylate poisoning, 1944-1951, Scotland.

Year.	Total.				Accidental.	
	Aspirin.		Other Salicylates.		Both sexes, all salicylates.	Total Deaths
	Male.	Female.	Male.	Female.		
1944	2	1	-	-	1	3
1945	1	1	1	1	3	4
1946	2	3	-	1	4	6
1947	3	3	-	-	2	6
1948	4	3	1	-	4	8
1949	-	1	1	2	3	4
1950	4	2	5	1	6	12
1951	2	-	-	-	2	2

TABLE II

Deaths from Aspirin and Salicylates, 1944-1950, England & Wales.

Year.	Aspirin.		Other Salicylates.		Accidental. Both sexes, all salicylates.	Total Deaths.
	Male.	Female.	Male.	Female.		
1944	14	30	nil	nil	16	44
1945	20	33	nil	nil	25	53
1946	20	51	2	nil	20	73
1947	42	61	1	nil	22	104
1948	56	101	3	3	32	163
1949	51	82	1	1	27	135
	Aspirin and Salicylates.					
	Male.		Female.			
1950	56		92		31	148

Aspirin, being the salicylate compound most readily accessible to the lay public, was responsible for most deaths, and accidental poisoning was responsible for only a minority of the fatalities. Nevertheless the figures serve to remind us that the compounds are potential poisons

and that they should not be prescribed in a casual fashion, but used with discrimination and with due regard to their toxic properties.

CHAPTER II.

PHARMACOLOGY OF SODIUM SALICYLATE.

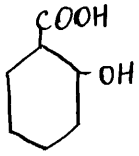
CHAPTER II

Pharmacology of Sodium Salicylate.

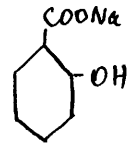
Historical.

The antipyretic action of a decoction of the bark of the willow tree has been known to the Hottentots for centuries, but it was not until 1874 that Maclagan introduced salicin to this country as a specific remedy for acute rheumatism. Leroux had discovered the presence of the glycoside salicin in willow bark in 1827, and showed that on hydrolysis it yielded an alcohol. Eleven years later, Piria prepared salicylic acid from salicin, and in 1844 Calcours made it from oil of gaultheria (wintergreen). All these methods, however, were relatively expensive, but when in 1860 the organic chemist Kolbe synthesized salicylic acid from phenol, the drug became much cheaper and its use as a febrifuge soon became popular. Its value in rheumatic fever was discovered in 1876 by Stricker, shortly after Maclagan's introduction of salicin. In 1899 Dremer synthesized the acetyl derivitive of salicylic acid, which rapidly became popular as an analgesic and antipyretic under the name "aspirin".

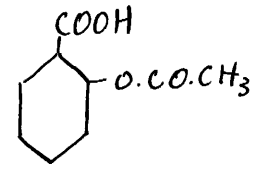
Chemical constitution of the Salicylates.



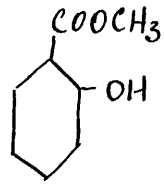
Salicylic acid (o-hydroxy benzoic acid)



Sodium Salicylate



Acetyl salicylic acid



Methyl salicylate.

The structural formulae of the compounds commonly used in therapeutics show their relationship to salicylic acid and to one another. The sodium salt and the methyl ester are formed by replacement of the "acidic" hydrogen of the carboxylic group; aspirin is the compound formed by substitution in the hydroxyl group.

Pharmacological and Physiological Actions.

Barbour and Wing (1913) applied sodium salicylate to the corpus striatium in the experimental animal, and observed that a fall in body temperature resulted. The value of their observations is now open to doubt, since the presence of a heat regulating centre has been demonstrated in the hypothalamus. Later, Barbour (1919)

showed that although aspirin was effective in lowering the temperature of fevered individuals, it had little or no effect on heat loss in normal subjects. He suggested that the action of the drug was a direct one on the heat regulating centre, causing the temperature control level to be set at a lower level in the pyrexial patient. Two years later, (Barbour, 1921) he showed that the administration of antipyretics caused an increase in the blood volume of the fevered subject. This, he thought, might facilitate sweating and so increase heat loss.

Miller (1914) demonstrated that after absorption, salicylic acid appeared in the tissues as salicylate, usually in the form of the ammonium salt. In vitro, ammonium salts of salicylic acid possessed no germicidal activity: for such an effect, the free acid had to be present. These findings led Miller to advance the view that sodium salicylate owed its therapeutic value to an increased carbon dioxide tension in the inflamed joint tissues liberating free salicylic acid from the salt. Miller claimed that cases of rheumatic fever experienced more rapid relief from joint pain when treated with the free acid instead of the sodium salt, but he believed that they relapsed more rapidly. The total period of incapacity was no shorter and cardiac complications were no less.

Hanzlik, Scott, and Thorburn (1917) determined the concentration of salicylate in the blood and joint fluid in a number of cases receiving the drug, and found values which were substantially the same. In contrast to Miller's results, they found no free acid in the joint fluid.

Swift (1925) believed that by relieving pain, salicylates were beneficial in themselves, but he pointed out that pain was a protective mechanism and that relief from pain might mask activity of a pathological process. For this reason he valued the erythrocyte sedimentation rate and the white blood cell count as a guide to the physician.

Hitch (1930) and Dyke (1935) showed the presence of salicylate in the cerebro spinal fluid after oral therapy.

Much controversy has arisen over the question whether sodium salicylate does have a curative action in rheumatic fever, and the question still remains unsettled. Goodman and Gilman (1941) state that the course of the disease is not changed, but since then the work of Coburn (1942) has received much attention. He suggested that the older belief that sodium salicylate not only relieved symptoms but influenced rheumatic inflammatory reactions was correct. He studied the prophylactic action of sodium salicylate in 186 cases of rheumatism with

streptococcal throat infections, and claimed that in the part of the group which received the drug for four weeks after the respiratory infection only one case developed a rheumatic recurrence, while 41 per cent of the untreated control part of the group suffered a recognizable recurrence of rheumatic symptoms. Later, (Cockburn, 1943) he used sodium salicylate in 101 cases of acute rheumatism. One part of the group were given small doses, and 21 out of 61 developed signs of heart disease. The 38 in the other part were treated with relatively massive dosage, and none showed any evidence of a cardiac lesion. The level of salicylate in the blood was measured throughout as a control, and Coburn claimed to have proved the value of maintaining high blood salicylate levels throughout in the treatment of rheumatic fever. He laid it down that the blood level should never be allowed to fall below a concentration of 36 mg. per 100 ml., that to ensure this frequent administration in high dosage - using the intravenous route if necessary - should be practised, and that the patients' complaints of unpleasant side effects should be ignored. He alleged that a serum concentration of less than 20 mg. per 100 ml. was dangerous in that while certainly relieving symptoms it masked the activity of the disease process.

Wegria and Smull (1945) made further observations on

cases of acute rheumatism treated on the lines suggested by Coburn. They maintained the blood salicylate concentration between 35 and 50 mg. per 100 ml. until the erythrocyte sedimentation rate was less than 20 mm. in one hour and remained so for two weeks. They found the intravenous route of administration no more effective than the oral.

Keith and Ross (1945) maintained a blood salicylate level of 27 - 31 mg. per 100 ml. in a small number of cases, but they were unable to confirm Coburn's views that the evidence of heart lesions was much reduced.

Jequier-Doge (1945) compared the efficiency of salicylate with sulphonamides in rheumatic fever, and found that the former was of much greater value.

Clark (1940) described the fate of salicylate in the body thus: "when a single dose is given by mouth, excretion begins in the urine in fifteen minutes and continues for 40 hours. Thereafter only small amounts are excreted. About 50 per cent is excreted in the first 24 hours, and 20 per cent in the next 24 hours. In the normal individual, 80 per cent of the dose is excreted, the febrile case excreting about 60 per cent. These figures agree reasonably well with the findings of Hanzlik (1917). Others have shown that the drug is excreted chiefly as salicyluric acid, formed in the detoxification process

by union of salicylic acid with glycine, thus:

$$C_6H_4OH.COOH. + H_2N.CH_2.COOH \quad C_6H_4OH.CO.NH.CH_2.COOH + H_2O$$

The principal toxic manifestations of salicylates have been listed by Graham and Parker (1948), and these have already been discussed (see Chapter I).

CHAPTER III.

THE DETERMINATION OF THE SALICYLATE CONTENT OF PLASMA OR SERUM.

CHAPTER III

The Determination of the Salicylate Content of Plasma or Serum.

A variety of methods have been developed for the chemical estimation of the salicylate ion in body fluids, the most popular being based on the colour reaction with iron salts first discovered in 1798 by White in the course of his investigations into the chemical nature of the active substance in willow bark. Brodie (1944) published details of a method which has been widely adopted on account of its relative simplicity, and which was used by Smull (1944), Keith (1945), and Sable (1945) in their investigations.

Smull stated that there was little difference between the results of salicylate estimation in serum and plasma obtained from the blood of patients receiving salicylate therapy, plasma giving slightly lower results than serum. Storage of the specimens for 36 hours at room temperature did not affect this difference.

In the work described in this thesis, serum was used throughout for the routine determination of salicylate levels.

Method. Apparatus: 3 100 ml. round-bottomed flasks with ground glass stoppers ("Quickfit and Quartz")

3 100 ml. conical flasks

1 5 ml. microburette

4 1 ml. pipettes

Spekker H 760 Absorptiometer

Cambridge "Spot" galvanometer

Reagents: Ethylene dichloride

6 N. Hydrochloric acid (Analar)

1 per cent solution of ferric chloride in 0.07 N. nitric acid.

Distilled water.

Procedure: 10 ml. venous blood are collected and centrifuged for five minutes at 3000 revolutions per minute. 1 ml. of the serum is then placed in a round-bottomed flask with 1 ml. salicylate-free serum, followed by 0.5 ml. 6 N.HCl to precipitate the protein. Finally, 30 ml. ethylene dichloride are introduced and the stoppered flask shaken vigorously for five minutes. The precipitated protein is filtered off and 20 ml. of the ethylene dichloride layer is measured from the burette into a conical flask. To this are added 10 ml. distilled water and 0.25 ml. ferric chloride reagent, and the mixture shaken vigorously for five minutes. The purple aqueous layer is then removed and its optical density measured in the absorptiometer, using Ilford spectrum blue-green 603 filters. By reference to a standard curve the concentration of salicylate in micrograms per ml. is determined.

The absorptiometer used throughout is shown in the photograph on the next page, and the general arrangement is represented diagrammatically.

Light from a centrally placed lamp is divided into two paths by apertures in the housing surrounding the light source. A spring loaded shutter, normally in the closed position, protects the sensitive photo-cells from light except when a reading is being taken.

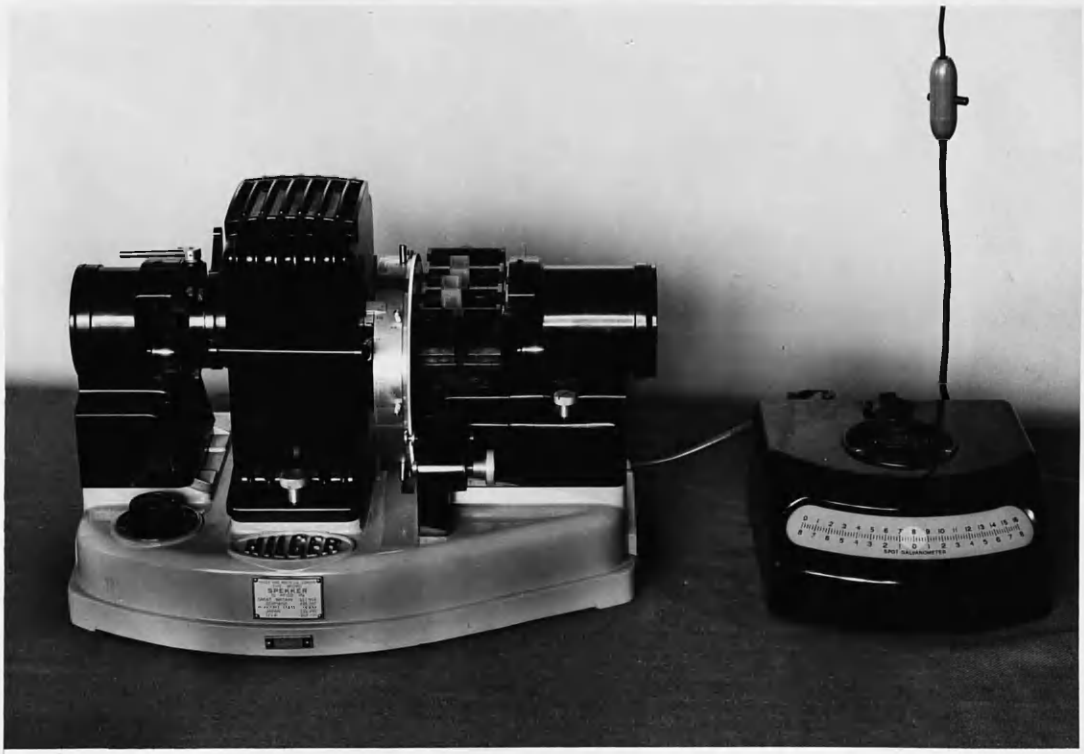
Light from the right hand aperture passes successively through a light filter, a variable aperture incorporated in a calibrated drum, a lens which converts it into a parallel beam, a glass cell containing the coloured solution, and finally is made to converge on a photo-cell.

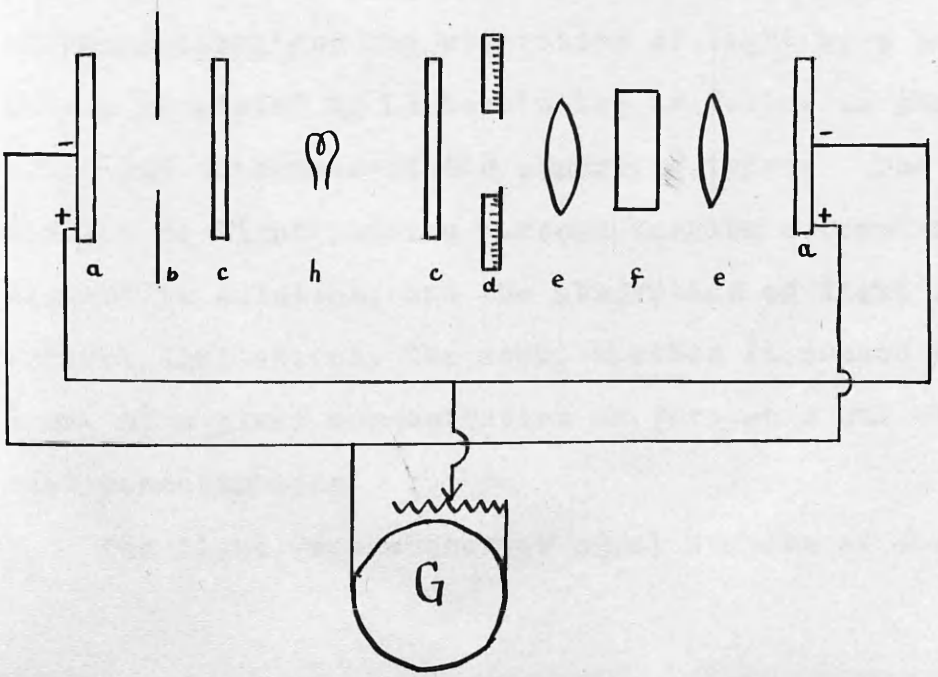
Light from the left-hand aperture passes through a light filter, a variable (but uncalibrated) opening on to a second photo-cell.

The two photo-cells are connected to a sensitive galvanometer.

Method of use:

The calibrated drum is set to read 1.0 and water is placed in the cell. The uncalibrated aperture on the left is closed, the galvanometer sensitivity set at its minimum, the lamphouse shutter opened, and the galvanometer switch turned until the maximum deflection is obtained. The compensating diaphragm is then slowly opened until the galvanometer deflection is again zero. The coloured





a PHOTOCELL.

b IRIS DIAPHRAGM.

c FILTER

d CALIBRATED DRUM. & IRIS DIAPHRAGM.

e LENS.

f COLOURED FLUID.

G GALVANOMETER.

h LAMP

solution whose concentration is to be determined is then brought into the path of the light in the position previously occupied by the water; less light now falls in the right hand photo-cell and the galvanometer shows a deflection. The calibrated aperture is opened until the galvanometer again reads zero, and the reading taken on the calibrated drum.

The process is repeated with a solution of known concentration.

By using a series of standard solutions, a standard curve can be constructed and the concentration of an unknown solution determined by direct reference to the graph.

Outline of the Theory of Absorptiometric Analysis.

All photometric measurements are based on the laws of absorption, and the absorption of light by a homogeneous medium is stated by Lambert's law to follow in geometric ratio the thickness of the absorbing layer. The same law applies to light passing through varying concentrations of pigment in solution, and the absorption of light is, with several limitations, the same, whether it passes through 1 cm. of a given concentration or through 2 cm. of half that concentration.

The light rays encounter equal numbers of molecules

in passing through layers of equal thickness provided that the liquid is homogeneous.

If the number of molecules in the path of the light is increased, a greater amount of light will be absorbed if other conditions remain equal. Closely packed or well separated molecules make no difference to the amount of light absorbed and therefore concentrations of the solution and thickness of the layer are interchangeable in a proportional manner.

These findings were proved by Beer (1852) and this law is known as Beer's law.

Preparation of Standard Curve for Sodium Salicylate Estimation.

The standard solutions were made with salicylate-free serum in order that the salicylate could be extracted under conditions identical with those prevailing when patients' sera were being analysed. Salicylate-free serum was prepared by pooling sera obtained from healthy blood donors for grouping purposes.

Procedure: 0.5 gram pure dry sodium salicylate was dissolved in serum and made up to 1 litre. Serial dilutions of this stock solution were made as shown in Table III.

TABLE III.

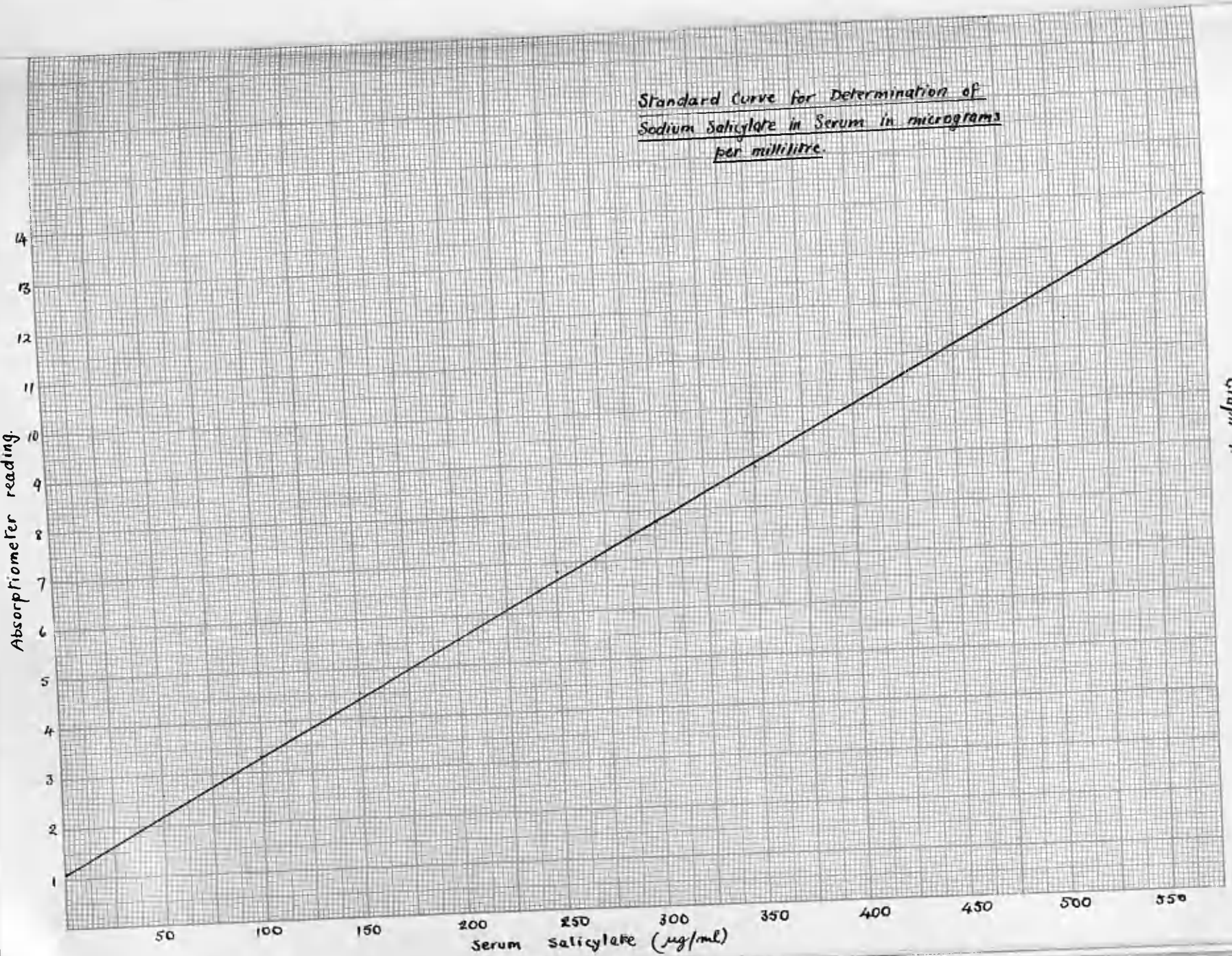
Stock Solution sodium salicylate (ml.)	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Salicylate-free serum (ml.)	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0
Standard solution of sodium salicylate (micrograms/ml.)	50	100	150	200	250	300	350	400	450	500

To eliminate experimental error as far as reasonably possible, five separate lots of standard solutions were made, the coloured salicylate-iron complexes prepared and the mean optical densities plotted against concentrations. The data are shown in tabular form and graphically.

TABLE IV.

Sodium salicylate soln. (µg./ml.)	50	100	150	200	250	300	350	400	450	500
Absorptiometer readings	2.3	3.1	4.6	5.4	6.8	7.5	8.8	10.1	11.2	12.4
	2.9	3.2	4.8	5.4	6.8	7.0	8.8	10.2	11.3	12.0
	2.6	3.2	4.1	5.6	6.8	7.9	8.6	9.6	11.1	12.3
	2.4	3.6	4.5	5.5	6.8	7.5	8.5	9.7	11.2	12.3
	2.8	3.4	4.5	5.6	6.7	7.6	9.3	10.4	11.1	12.4
Mean readings	2.6	3.3	4.5	5.5	6.8	7.7	8.8	10.2	11.2	12.3

Standard Curve for Determination of Sodium Salicylate in Serum in micrograms per millilitre.



Graph 1.

CHAPTER IV.

INVESTIGATION OF THE METHOD OF DETERMINING THE SALICYLATE CONTENT OF SERUM AND PLASMA.

CHAPTER IV.

Investigation of the Method of Determining the Salicylate Content of Serum and Plasma.

The concentrations of salicylate occurring in blood even under conditions of gross overdosage, are relatively low. In the study of the effects of this drug, therefore, it is essential to know the limits within which the method adopted for its estimation is sufficiently accurate. The following investigations were made in order to assess the reliability of Brodie's method, and the reproducibility of the results obtained by it.

1. Investigation of the percentage extraction of sodium salicylate from standard solutions of the salt in

   (a) distilled water
   (b) serum
   (c) plasma from heparinized blood
   (d) plasma from oxalated blood
   (e) plasma from citrated blood

2. The effect of time and temperature of storing the blood sample between collection and analysis.

3. Investigation of the amount of sodium salicylate retained in the precipitated protein.

4. The influence of pH on the final extract.

5. The stability of the salicylate-iron coloured complex.

1. (a). Investigation of the percentage extraction of sodium salicylate from distilled water.

Experimental:

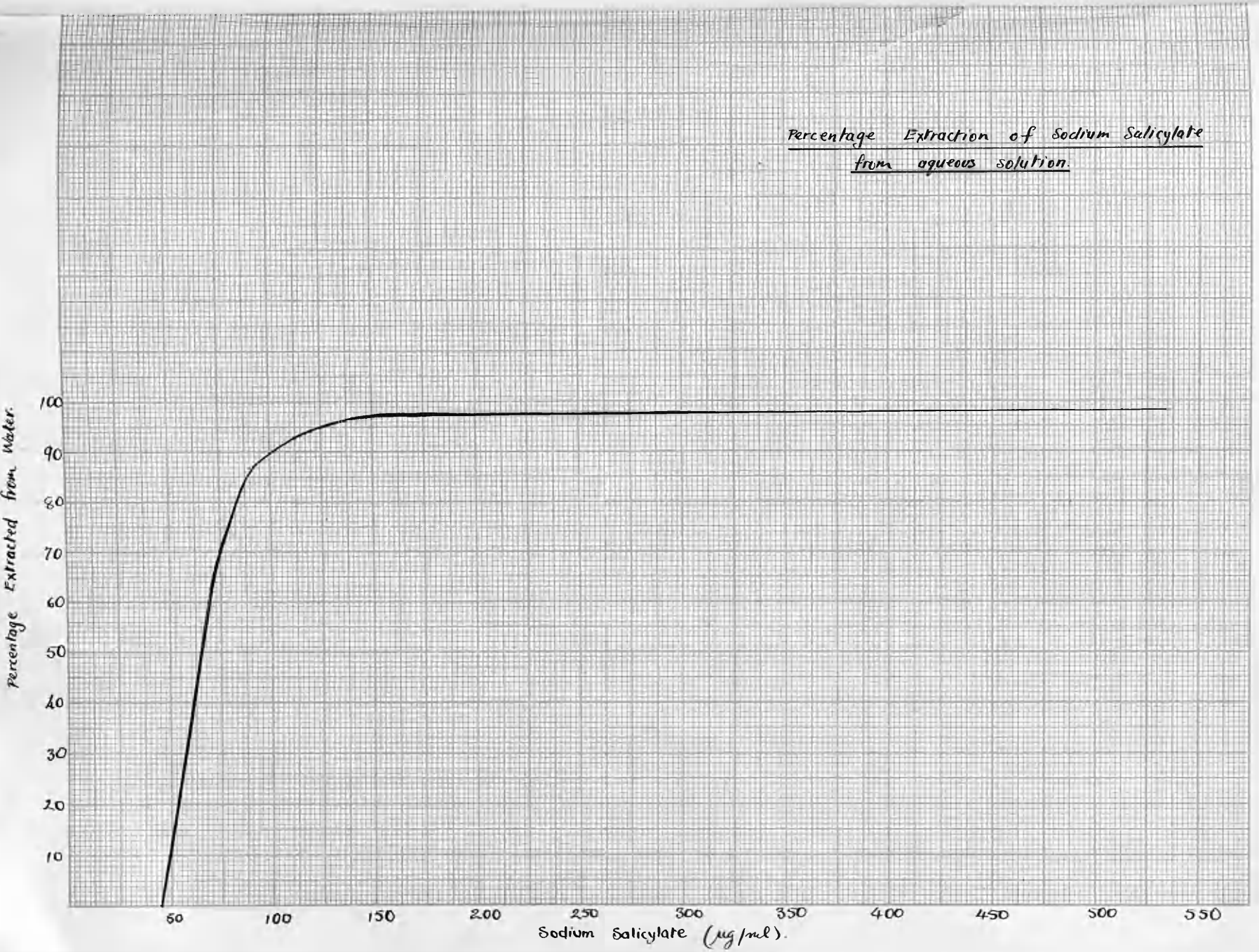
Ten solutions of sodium salicylate of different concentrations were prepared by dissolving accurately weighed amounts in measured volumes of distilled water. The extraction process was carried out, and the apparent concentration of each determined by reference to graph I. The results are shown in Table V and graph II.

Results:

TABLE V.

Sodium Salicylate solution: prepared concentration (µg./ml.)	Absorptiometer reading.	Concentration found.	Conc.found as % conc. taken.
48	-	-	-
72	2.6	46	64
90	2.9	78	87
120	3.6	115	94
168	4.8	165	97
246	6.4	240	97
298	7.3	280	97.5
354	8.8	345	97.5
420	9.9	410	98
512	12.2	500	98

Graph II.


Percentage Extraction of Sodium Salicylate from aqueous solution.
Percentage Extracted from Water.
100
90
80
70
60
50
40
30
20
10
50
100
150
200
250
300
350
400
450
500
550
Sodium Salicylate (µg/ml).

1. (b). Investigation of the percentage extraction of sodium salicylate from serum.

Experimental:

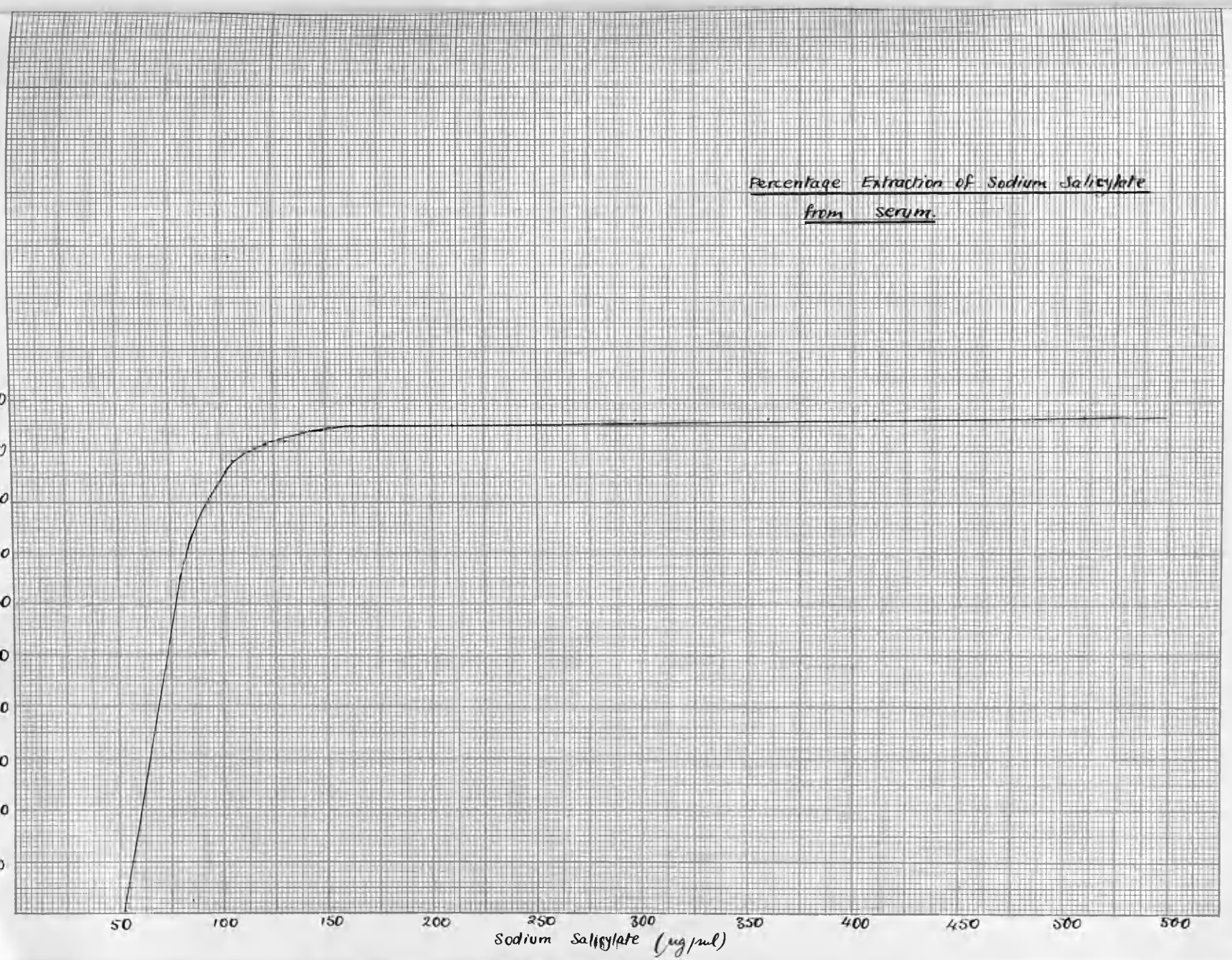
A series of ten solutions of sodium salicylate in serum was prepared covering approximately the same range as those made for the preceding investigation, the extraction carried out as before, and the apparent concentration of each solution found by reference to graph I.

Results:

TABLE VII.

Sodium salicylate solution: prepared concentration (µg./ml.)	Absorptiometer reading.	Concentration found. (µg/ml).	Conc. found as % conc. taken.
52	-	-	-
86	2.5	63	74
105	3.1	92	88
130	3.8	120	92
148	4.2	140	94
212	5.5	200	95
294	7.3	280	96
360	8.8	345	96
410	9.8	394	96
508	12.0	488	96

Graph III.


Percentage Extraction of Sodium Salicylate from serum.
Percentage Extracted from Serum.
100
90
60
70
60
50
40
30
20
10
50
100
150
200
250
300
350
400
450
500
Sodium Salicylate (µg/ml)

1. (c). Investigation of the percentage extraction of sodium salicylate from heparinized plasma.

Experimental:

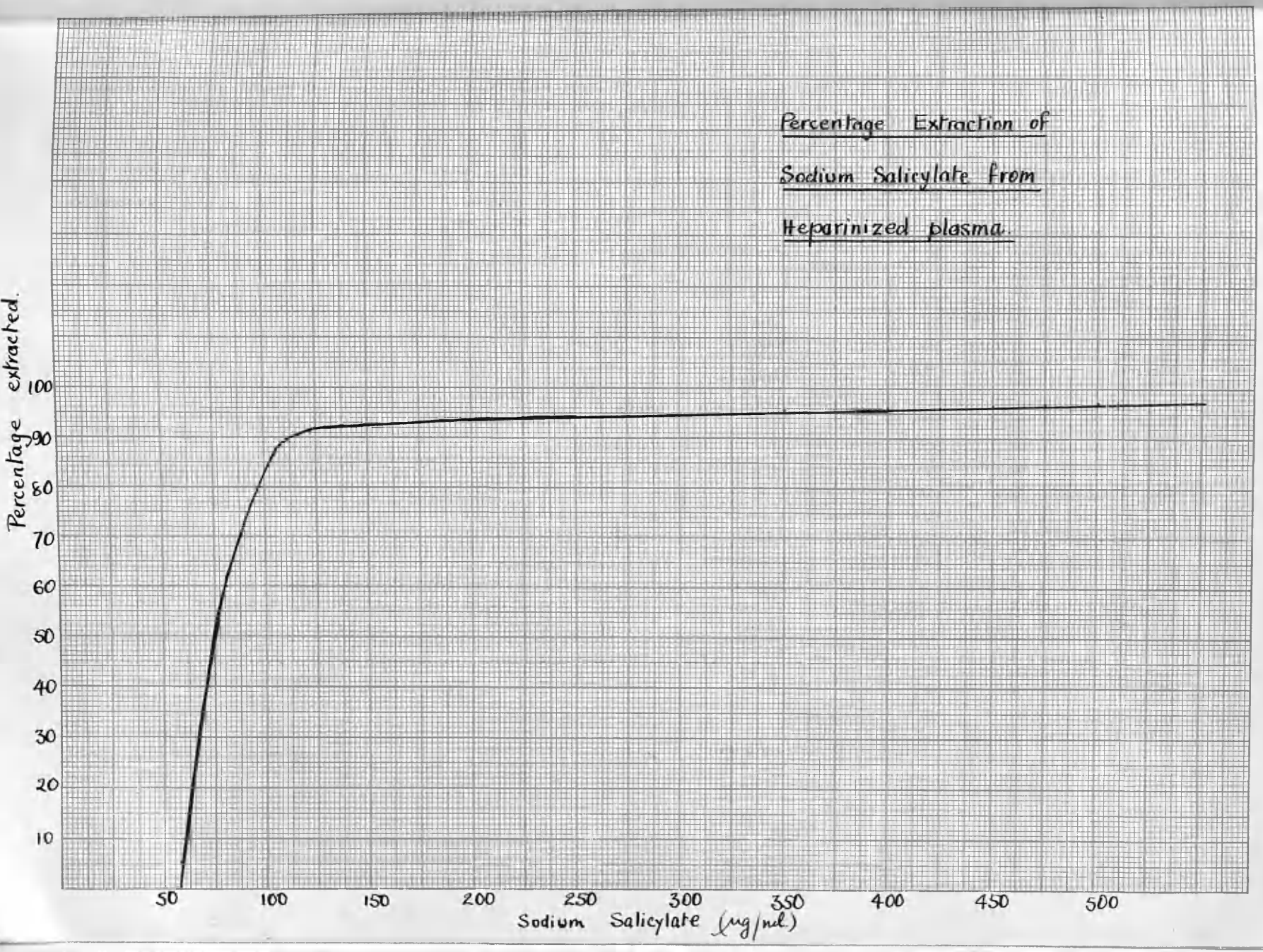
A series of solutions of sodium salicylate was prepared as in the preceding experiments, but plasma obtained from blood which had been collected in a vessel containing heparin as an anticoagulant was used as the solvent. The extraction was carried out as before, the apparent concentration of salicylate determined, and this result calculated as a percentage of the known concentration of the solution in each case. The results are recorded in tabular and graphical form.

Results:

TABLE VII.

Sodium salicylate soln: prepared concentration (μg./ml.)	Absorptiometer reading.	Concentration found (μg./ml.)	Conc. found as % of conc. taken.
55	-	-	-
90	2.5	64	71
100	2.9	82	82
115	3.4	105	92
180	4.3	165	93
230	5.8	216	94
300	7.4	285	95
375	9.0	360	96
420	10.0	405	96
500	11.9	485	97

Graph IV.


Percentage Extraction of
Sodium Salicylate from
Heparinized plasma.
Percentage extracted.
100
90
80
70
60
50
40
30
20
10
50
100
150
200
250
300
350
400
450
500
Sodium Salicylate (µg/ml)

1. (d). Investigation of the percentage extraction of sodium salicylate from oxalated blood.

Experimental:

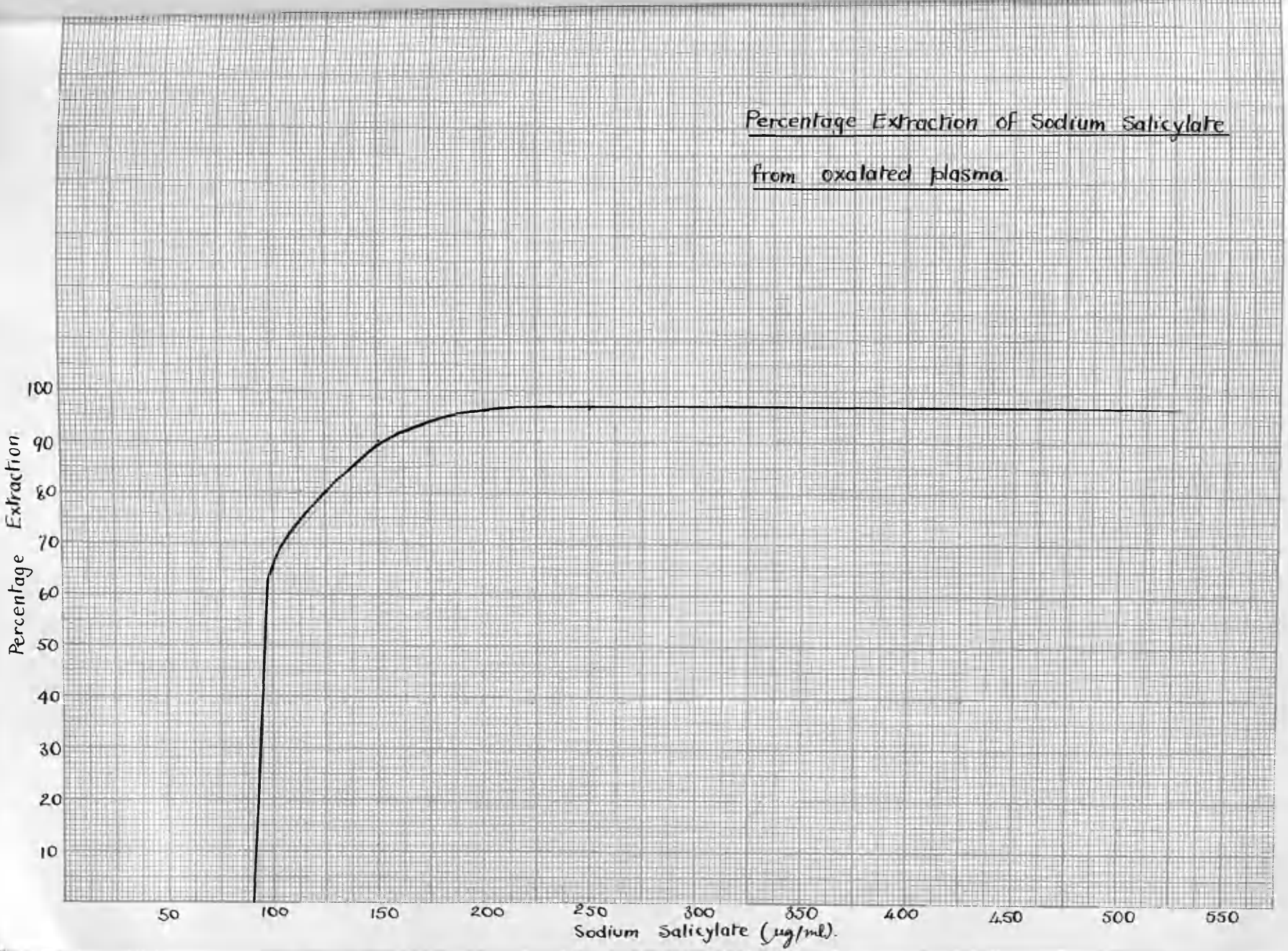
The procedure followed was the same as in the previous investigation, except that the solvent used was plasma obtained from blood collected in vessels containing sodium oxalate.

Results:

TABLE VIII.

Sodium Salicylate solution: prepared concentration (µg./ml.)	Absorptiometer reading.	Concentration found (µg./ml.)	Conc. found as % of conc. taken.
50	-	-	-
90	-	-	-
100	2.5	65	65
140	3.6	120	86
190	5.1	180	95
280	7.1	270	97
400	9.6	390	97
500	11.9	485	97

Graph V


Percentage Extraction of Sodium Salicylate
from oxalated plasma
Percentage Extraction
100
90
80
70
60
50
40
30
20
10
50
100
150
200
250
300
350
400
450
500
550
Sodium Salicylate (µg/ml).

1. (e). Investigation of the percentage extraction of sodium salicylate from citrated plasma.

Experimental:

A similar procedure was observed as in the previous investigations, but the solvent used for the preparation of the various concentrations of sodium salicylate was citrated plasma.

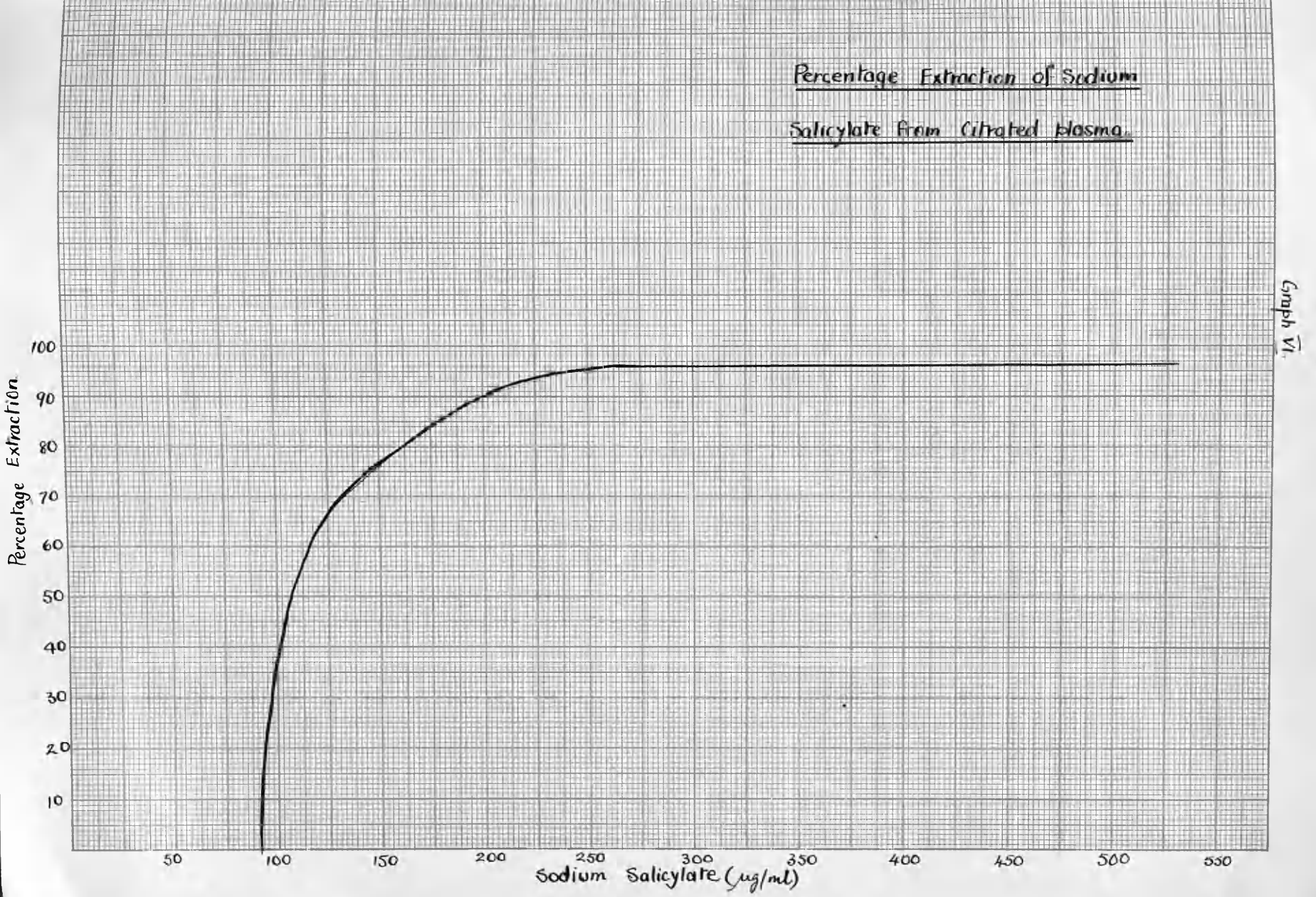
Results:

TABLE IX.

Sodium Salicylate solution: prepared concentration (µg./ml.)	Absorptiometer reading.	Concentration found (µg./ml.)	Conc. found as % of conc. taken.
50	-	-	-
90	-	-	-
100	1.9	35	35
125	3.0	83	66
150	3.6	115	76
200	5.1	180	90
250	6.4	238	95
300	7.5	290	96
400	8.5	385	96
500	11.8	480	96

Graph VI.

Percentage Extraction of Sodium Salicylate from Citrated Plasma.



The Effect of Time and Temperature of Storage on the accuracy of Serum Salicylate determination.

Experimental:

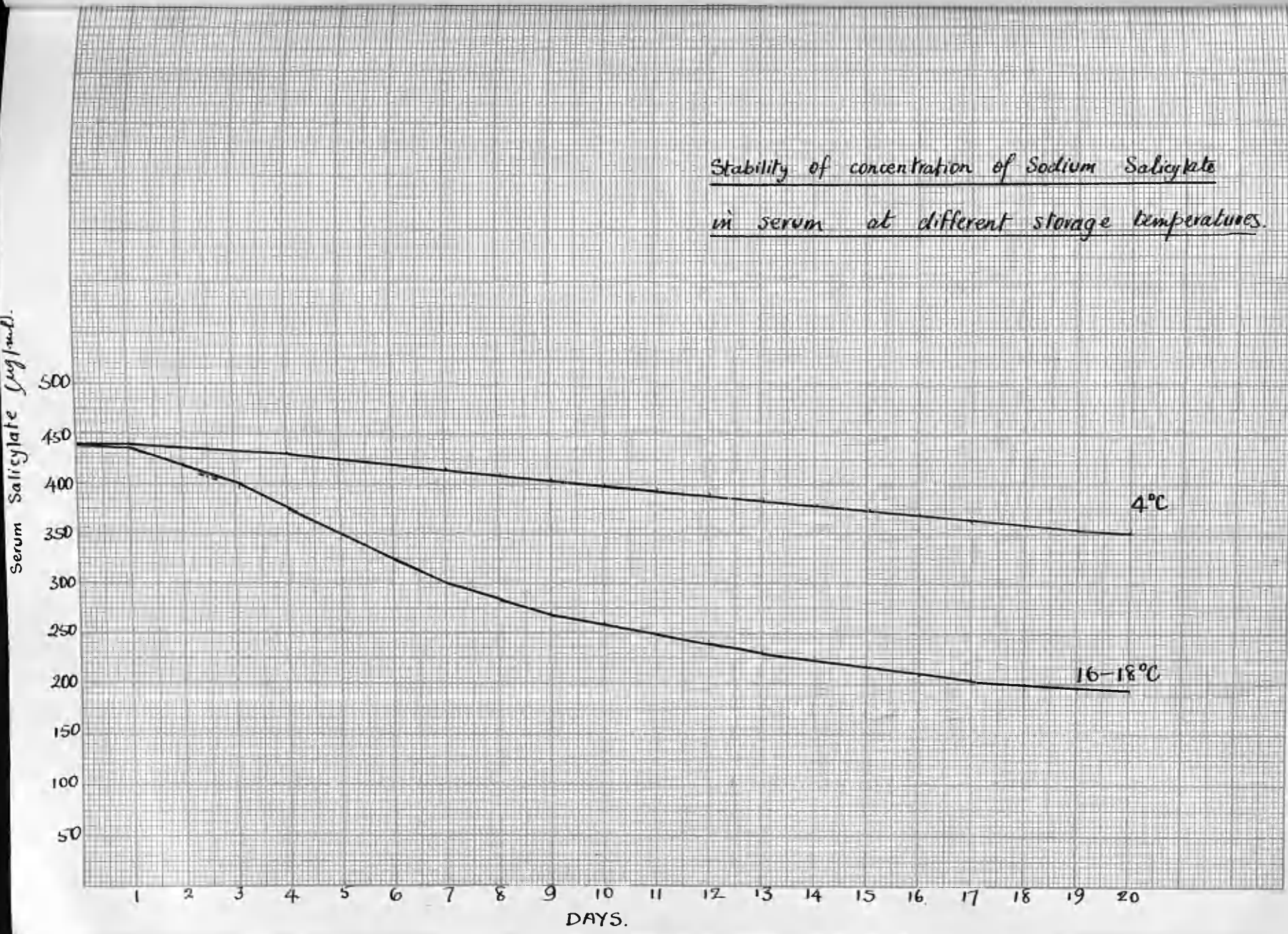
A solution of sodium salicylate in serum - 440 micrograms per millilitre - was prepared, and divided into a number of portions. One half of these was stored in the refrigerator at 4°C, the other in the laboratory at room temperature (16° - 18°C). The sodium salicylate content was estimated at intervals.

Results:

TABLE X.

Period of Storage. Days	Hours.	Salicylate concentration (μg./ml.) found after storage at 4°C.	16° - 18°C.
	½	438	440
	1	442	440
	2	436	435
	3	438	440
	4	432	435
	8	435	435
	12	435	435
	24	438	430
	36	442	425
	48	435	410
	96	430	375
7		417	300
8		410	285

Period of storage.		Salicylate concentration (μg./ml.) found after storage at	
Days	Hours.	4°C.	16° - 18°C.
9		407	272
10		400	260
11		395	250
12		390	240
13		385	232
14		380	225
15		375	217
16		370	210
17		360	205
18		355	200
19		352	195
20		350	190


Stability of concentration of Sodium Salicylate in serum at different storage temperatures.
Serum Salicylate µg/ml.
500
450
400
350
300
250
200
150
100
50
4°C
16–18°C
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS.

Graph VII.

Effect of pH of Aqueous extract on the Result of Serum Salicylate determination.

Experimental:

A solution of sodium salicylate in serum was prepared - 400 µg/ml. - and the final aqueous extract obtained in the usual manner. The pH of this was varied over a wide range by the addition of measured amounts of hydrochloric acid and sodium hydroxide solutions. Each sample prepared was subjected to the salicylate extraction process and the salicylate concentration found was compared with the known concentration. Results are recorded in table XI and in graphical form.

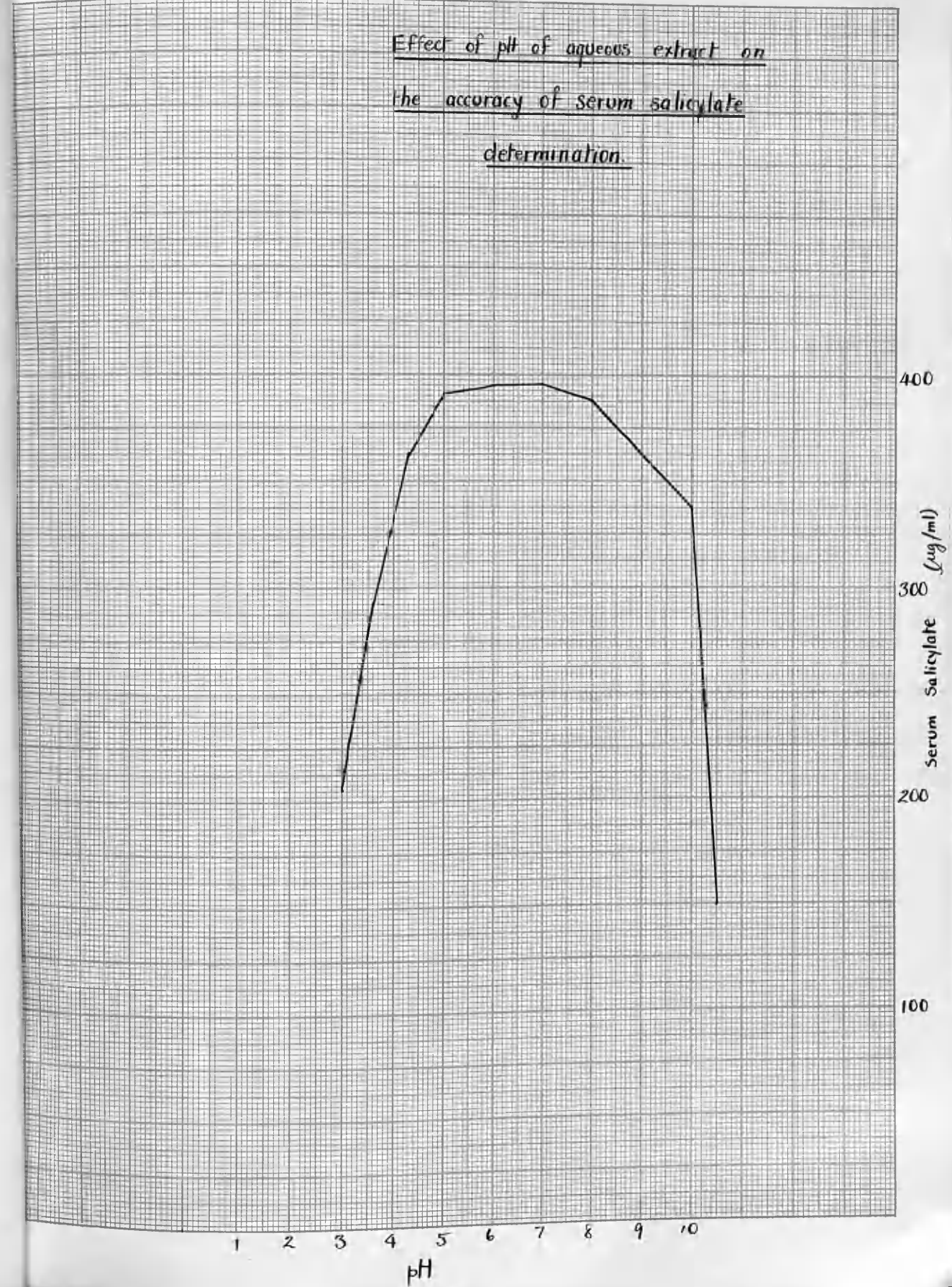
Results:

TABLE XI.

pH of aqueous extract.	3	3.6	4.3	5.0	5.3	6.1	7.0	8.0	10.1	10.5
Concentration of salicylate found (µg./ml.)	205	295	367	390	393	395	395	385	333	150

Graph VIII.

Effect of pH of aqueous extract on the accuracy of serum salicylate determination.



The Stability of the Salicylate-iron coloured complex when exposed to daylight.

The procedure for the estimation of sodium salicylate was carried out with three solutions of the salt in serum having concentrations of 150 µg/ml., 300 µg/ml., and 450 µg/ml. respectively, and the optical densities measured after standing in daylight for varying intervals.

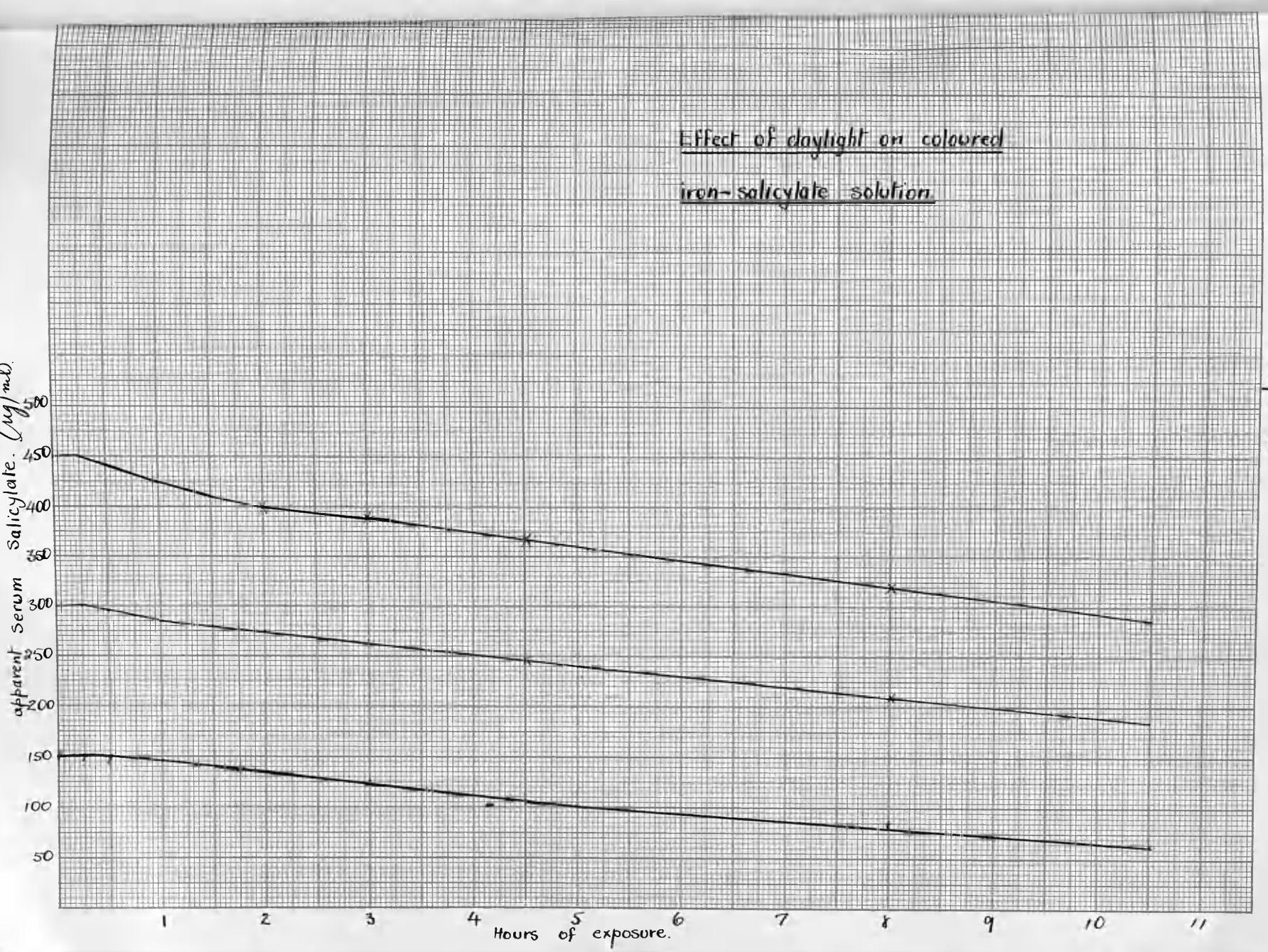
Results:

TABLE XII.

Apparent concentration (µg/ml.) - hours after preparation of complex.

0			¼		½	¾	1	1½	2	3	4½	8	10
450	450	450	450	450	450	445	440	410	400	390	375	320	285
300	300	300	300	300	290	285	285	280	275	260	245	210	185
150	145	150	150	145	145	140	140	135	132	122	105	80	65

Graph IX.


Effect of daylight on coloured iron-salicylate solution
apparent serum Salicylate. (mg/ml).
500
450
400
350
300
250
200
150
100
50
1
2
3
4
5
6
7
8
9
10
11
Hours of exposure.

Investigation of the amount of Sodium Salicylate retained in the Protein precipitated during the estimation procedure.

Experimental:

The percentage of sodium salicylate extracted from solutions of various concentrations in distilled water and serum has already been reported. The difference between the percentage extracted from serum and from distilled water at any given concentration of sodium salicylate was taken to be a measure of the amount of the solute retained by the protein precipitated from the serum. The percentages extracted from serum and from distilled water for ten different concentrations of the salt are recorded as taken from graphs II and III.

Results:

TABLE XIII

Sodium Salicylate concentration (µg/ml.)	%age extracted from water (from graph II)	%age extracted from serum (from graph III)	Difference
500	98	97	1
450	98	96	2
400	98	96	2
350	98	96	2
300	97.5	95.5	2
250	97	95	2
200	97	95	2
150	97	94.5	2.5
100	90	84	6
50	15	0	15

Comment on results of the investigations into the method of Brodie (1944) for determining serum salicylate concentration:

The method is, on the whole, satisfactory for general clinical use, but certain factors not specifically mentioned by Brodie require to be controlled.

Plasma, whether obtained from citrated, oxalated, or heparinized blood, gives values lower than those obtained when serum is used, when the salicylate concentration is low, and the results from serum approach more nearly the true figure.

The pH of the aqueous extract has a marked effect on the final result obtained, but not within the range of hydrogen ion concentration found in the blood in life. The most extreme conditions of alkalosis or acidosis, therefore, will not appreciably affect the accuracy of the result of blood salicylate determinations. Gross deviations of the pH of the extract, however, will give seriously misleading results, and accordingly in all succeeding serum salicylate determinations the pH of the extract was checked, using pH paper strips (Messrs. Johnston).

Optical densities require to be measured within fifteen minutes to avoid the effect of fading which becomes appreciable after this time. The rate of fading is much the same whether the concentration be high or low.

The concentration of sodium salicylate in blood falls

off appreciably when the sample is stored at room temperature for more than twentyfour hours. Refrigeration at 4°C permits a delay between collection of blood and analysis which should not exceed one day. As a routine, all subsequent analyses reported in this thesis were carried out within thirty minutes of collection of the specimen.

An appreciable amount of salicylate appears to be retained in the precipitated protein; above serum concentrations of 200 µg./ml. the inaccuracy thus introduced is unlikely to have any material significance when the analyses are carried out for routine control of salicylate therapy in clinical practice.

CHAPTER V.

SOME GENERAL FACTORS AFFECTING SERUM SALICYLATE LEVELS.

CHAPTER V.

Some General Factors affecting Serum Salicylate Levels.

The toxic manifestations which frequently result following the administration of salicylates appear to be closely related to the level of the drug in the blood: some investigation, therefore, seemed to be indicated regarding certain general factors which could influence these levels.

The following were the general factors investigated:

1. The dose of salicylate, and the frequency of administration.
2. The route of administration
   - (a) Orally
   - (b) By rectum
   - (c) Intravenously.
3. The Rate of Absorption and Excretion.
4. The fluid intake and the urine output.

The Dose of Sodium Salicylate and its frequency of Administration.

Experimental:

For this investigation a series of patients was selected who had been admitted to hospital for treatment of some local condition, generally of an orthopaedic nature, or eye disease, and in whom no evidence of serious organic disease could be detected by clinical examination. Their ages ranged from 25 to 50 years, and all were of average height and weight. Fluid intake was strictly controlled throughout the investigation, and was limited to three pints in 24 hours. Sodium salicylate was administered in 1 ounce of water at regular intervals day and night. Blood was collected periodically for determination of the serum salicylate concentration.

TABLE XIV.

Results: Serum salicylate levels when varying doses of sodium salicylate are given at varying intervals.

Case.	Dosage. (grains)	Frequency. (hours)		Serum salicylate (µg/ml.) - after time (in hours) from beginning of dosage.							
				8	12	16	20	24	30	36	40
1	5	4		-	-	110	130	138	150	170	174
2	5	4		-	-	98	122	132	142	158	164
3	5	4		-	-	96	126	130	144	174	180
4	5	4		-	-	108	120	140	152	162	166
5	5	4		-	-	98	122	142	146	160	162
			Mean:	-	-	102	124	136	147	165	169
1	5	2		-	98	130	160	194	240	270	276
2	5	2		-	92	126	150	202	246	272	278
3	5	2		-	90	120	148	186	220	250	258
4	5	2		-	102	134	168	188	228	258	263
5	5	2		-	108	140	194	205	266	285	280
			Mean:	-	98	130	164	195	240	265	271

TABLE XV.

Serum salicylate levels when different doses of sodium salicylate are given at different intervals.

Case.	Dosage. (grains)	Frequency. (hours)		Serum salicylate (µg/ml.) after time (in hours) from beginning of dosage.								
				4	8	12	16	20	24	30	36	40
1	10	4		-	-	84	124	160	190	235	260	264
2	10	4		-	-	88	130	166	194	238	266	270
3	10	4		-	-	92	136	172	198	242	272	278
4	10	4		-	-	80	120	145	180	220	248	252
5	10	4		-	-	86	130	152	183	240	279	286
			Mean:	-	-	86	128	159	189	235	265	270
1	10	2		-	76	118	170	222	290	350	408	430
2	10	2		-	80	120	175	230	295	355	416	435
3	10	2		-	82	128	180	235	295	350	390	415
4	10	2		-	70	110	155	210	280	360	392	412
5	10	2		-	72	114	175	238	280	345	415	444
			Mean:	-	76	118	170	227	288	352	405	427

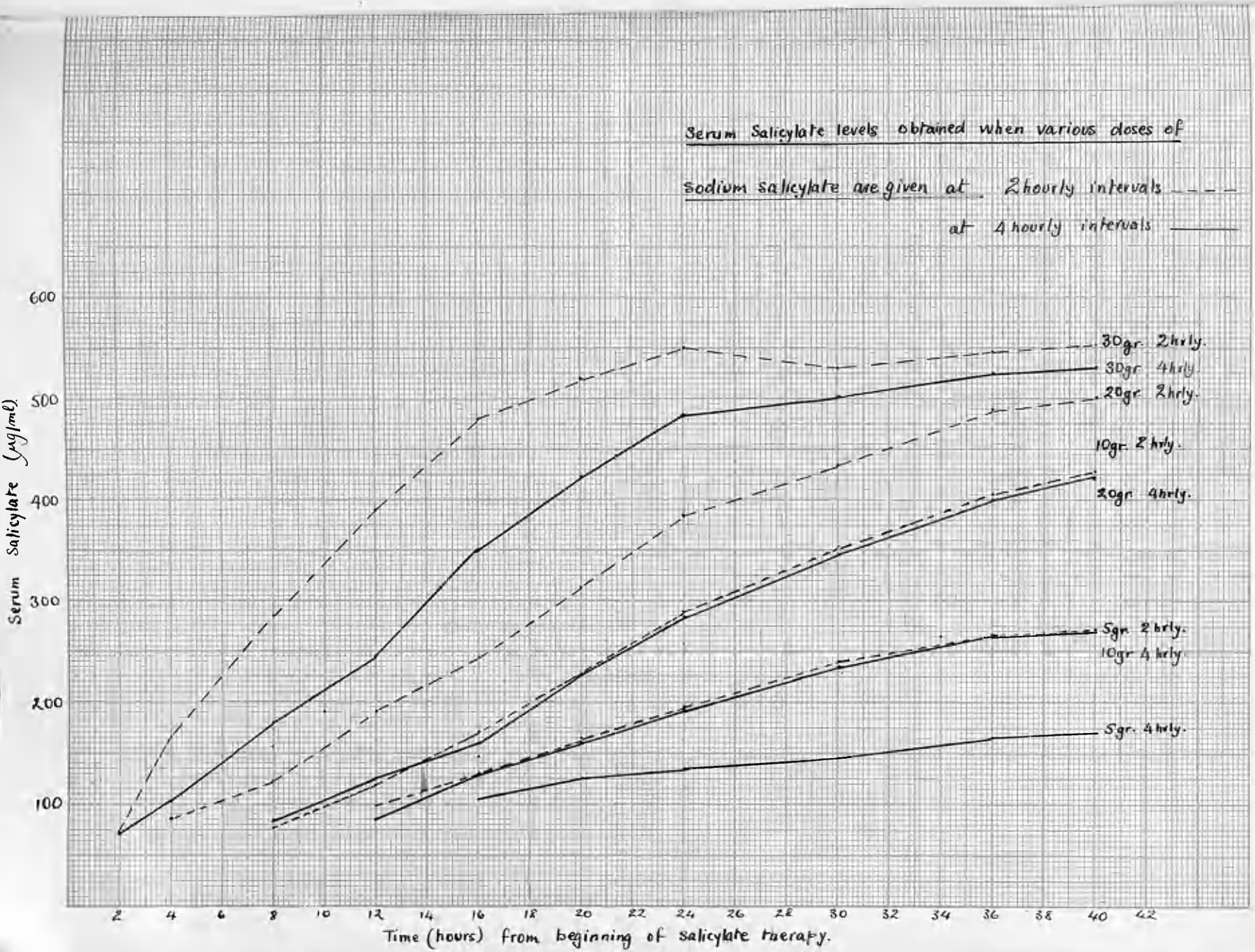
TABLE XVI.

Serum salicylate levels when varying doses of sodium salicylate are given at different intervals.

Case.	Dosage. (grains)	Frequency. (hours)		Serum salicylate (μg/ml.) after time (in hours) from beginning of dosage.								
				4	8	12	16	20	24	30	36	40
1	20	4		-	85	120	160	232	273	345	410	430
2	20	4		-	90	130	170	240	285	355	416	440
3	20	4		-	92	135	175	240	280	355	408	442
4	20	4		-	80	128	145	216	260	328	378	402
5	20	4		-	78	112	150	202	322	347	398	386
			Mean:	-	85	125	160	226	284	346	400	420
1	20	2		80	120	190	252	312	385	430	501	506
2	20	2		88	132	212	255	310	380	430	492	500
3	20	2		92	135	207	230	302	370	422	476	491
4	20	2		78	130	192	234	316	396	440	481	490
5	20	2		72	93	150	249	320	389	438	500	513
			Mean:	82	122	190	244	312	384	433	490	500

TABLE XVII.

Case.	Dosage. (grains)	Frequency. (hours)		Serum salicylate (μg./ml.) after time in hours from beginning of dosage.									
				2	4	8	12	16	20	24	30	36	40
1	30	4		70	104	182	245	360	420	496	498	502	531
2	30	4		72	102	162	235	342	380	436	478	536	540
3	30	4		60	100	168	229	346	391	440	533	540	520
4	30	4		82	109	200	260	358	440	518	500	502	528
5	30	4		66	95	193	250	344	480	530	496	545	536
			Mean:	70	102	181	244	350	422	284	501	525	531
1	30	2		68	160	292	400	490	525	550	505	530	555
2	30	2		81	190	310	405	465	490	560	510	555	540
3	30	2		72	155	260	370	462	538	542	538	512	560
4	30	2		70	160	272	385	498	518	520	512	518	565
5	30	2		70	165	291	380	490	530	578	575	615	530
			Mean:	72	166	285	388	481	520	550	528	546	552


Serum Salicylate levels obtained when various doses of
Sodium salicylate are given at 2 hourly intervals - - - -
at 4 hourly intervals ———
30gr. 2hrly.
30gr. 4hrly.
20gr. 2hrly.
10gr. 2 hrly.
20gr. 4hrly.
5gr. 2 hrly.
10gr 4 hrly.
5gr. 4 hrly.
Serum Salicylate (µg/ml)
600
500
400
300
200
100
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42
Time (hours) from beginning of salicylate therapy.

Graph X.

The Effect of Route of Administration, and Rate of Absorption and Excretion on Serum Salicylate levels.

(a) Oral administration.

Experimental:

Two subjects were selected and placed on fluid intakes of 2½ pints and 3 pints in 24 hours respectively. Each subject was given 4 grams sodium salicylate in two ounces of water at 8a.m., and the serum salicylate levels were determined at periodic intervals. The subjects had previously been found to exhibit no idiosyncrasy when the drug had been given in a dosage of 20 grains four hourly for five days.

Results:

see next page.

TABLE XVIII.

Results:

Time after administration of 4 grams sodium salicylate orally. (hours)	Serum salicylate levels (µg/ml.) Case 1.	Case 2.
¼	-	-
½	75	82
¾	-	-
1	160	180
2	316	340
2½	400	422
3	300	420
4	-	400
6	255	270
8	222	200
10	210	180
12	200	172
16	190	160
20	180	153
24	175	146
28	170	140
32	168	130
36	160	112
40	160	-
44	-	-
46	-	-
48	-	-
50	-	-

(b) Rectal Administration.

Experimental:

One case was available for this investigation. Four grams of sodium salicylate was dissolved in 220 ml. saline at 37°C and slowly injected per rectum. Blood was collected at intervals and the salicylate level in the serum determined. As before, the fluid intake was restricted to three pints in 24 hours.

Results:

TABLE XIX.

Time after rectal administration of 4 grams sodium salicylate (hours)	Serum salicylate level (μg/ml.)
¼	250
½	326
¾	392
1	422
1½	354
2	200
2½	184
3	170
4	162
8	148
10	136
12	128
16	122
20	112
24	110

Time after rectal administration of 4 grams sodium salicylate (hours)	Serum salicylate level (µg/ml.)
28	104
32	100
36	90
40	82

(c) Intravenous.

Experimental:

4 grams sodium salicylate was dissolved in isotonic sodium chloride solution (0.85%), sterilized in the autoclave, and administered by slow intravenous drip to one case. The infusion was given at a uniform rate over a period of 3 hours 40 minutes and no untoward reactions were observed. Blood was withdrawn from a vein in the other arm at intervals and analysed as before.

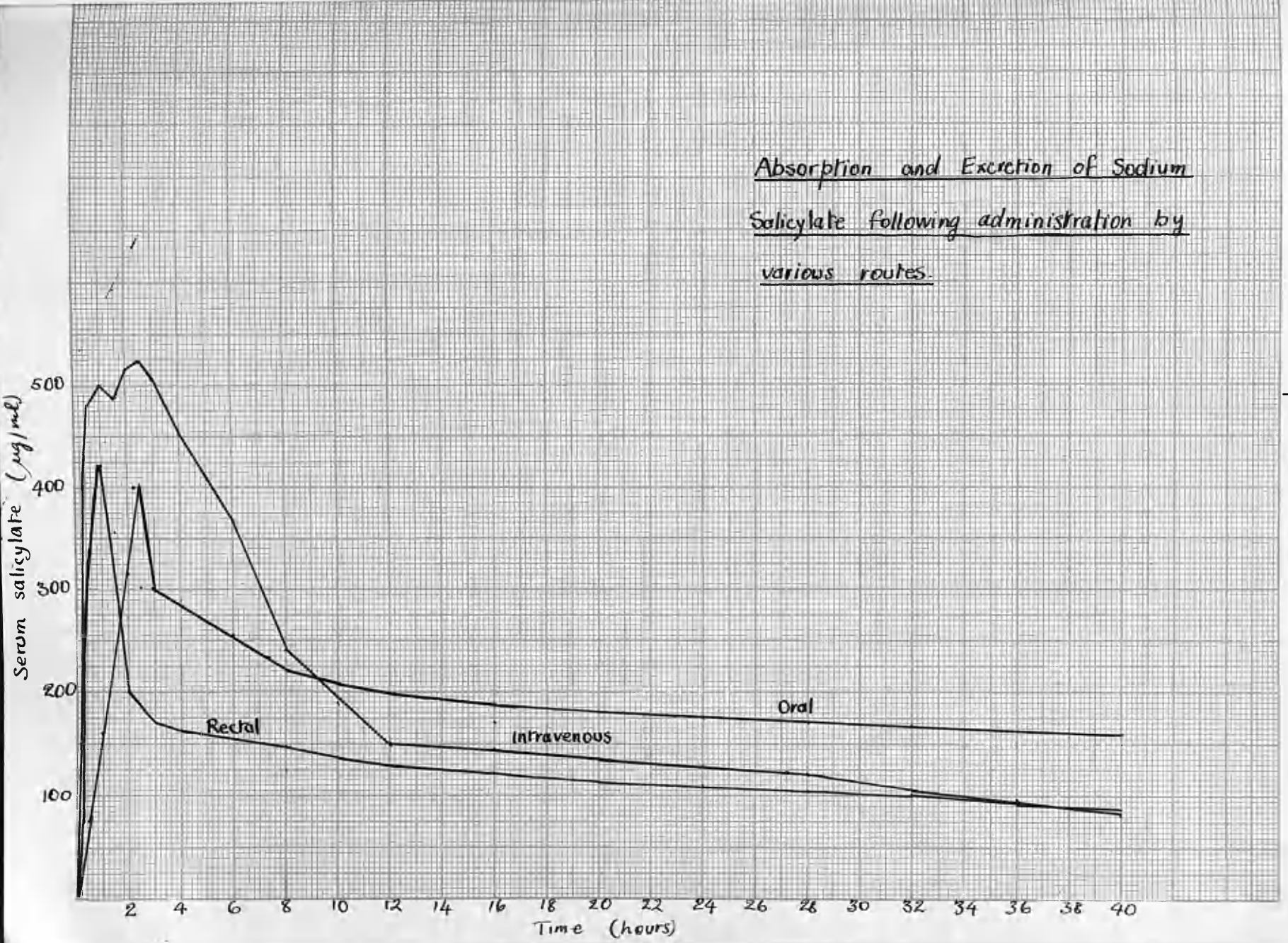
Results:

TABLE XX.

Time in hours after beginning infusion	Serum salicylate levels (µg./ml.)
¼	400
½	480
¾	490
1	500
1½	486
2	516
2½	522
3	502
4	450
6	366
8	240
10	188

Time in hours after beginning infusion	Serum salicylate levels (µg./ml.)
12	150
16	146
20	136
24	128
28	120
32	112
36	96
40	82

Graph XI.


Absorption and Excretion of Sodium Salicylate following administration by various routes.
Serum salicylate (µg/ml)
500
400
300
200
100
Rectal
Intravenous
Oral
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Time (hours)

Comment:

A standard dose - 4 grams - was administered orally to two subjects, by rectum to a third, and intravenously to a fourth. Blood levels were determined at intervals for a period of over 40 hours.

Oral administration was followed by a rapid rise in the serum salicylate content to a maximum of 400 µg./ml. in two and a half hours, followed by a gradual fall. After eight hours had elapsed, the concentration was 222 µg/ml., and appreciable amounts could still be detected after forty hours. These results suggest that when the drug is administered by the oral route, the rate of absorption is greater than the rate of excretion.

Rectal administration resulted in more rapid absorption of the drug than occurred after oral administration, for a peak of 422 µg/ml. was reached in one hour. Serum concentrations fell rapidly, however, and after four hours the level was only 162 µg/ml. - when the level after oral therapy had just passed its maximum.

When the same dose of the salt was administered by the intravenous route, a high concentration - 400 µg/ml. - was found in the blood within 15 minutes. The maximum concentration of 600 µg/ml. was attained in 2½ hours; thereafter blood levels fell.

Investigation of the Effects of Alterations in Fluid Intake and Output on the Serum salicylate level.

Experimental:

Three subjects free from serious organic disease were selected. Each was given sodium salicylate four hourly in a dosage of 15 grains in one ounce of water, day and night, and this dosage was maintained throughout the investigation. Serum salicylate levels were determined at the same time daily, and when a fairly constant level had been maintained for three days, the experiment proper began. The fluid intake was kept constant for a period, and the 24 hour output of urine carefully collected and measured. Daily estimations of the serum salicylate were continued. Later, the daily intake of fluid was increased to a measured amount, and the urine output and serum salicylate levels recorded as before.

Finally, the fluid intake was returned to its original level and the observations continued for a further period.

Results:

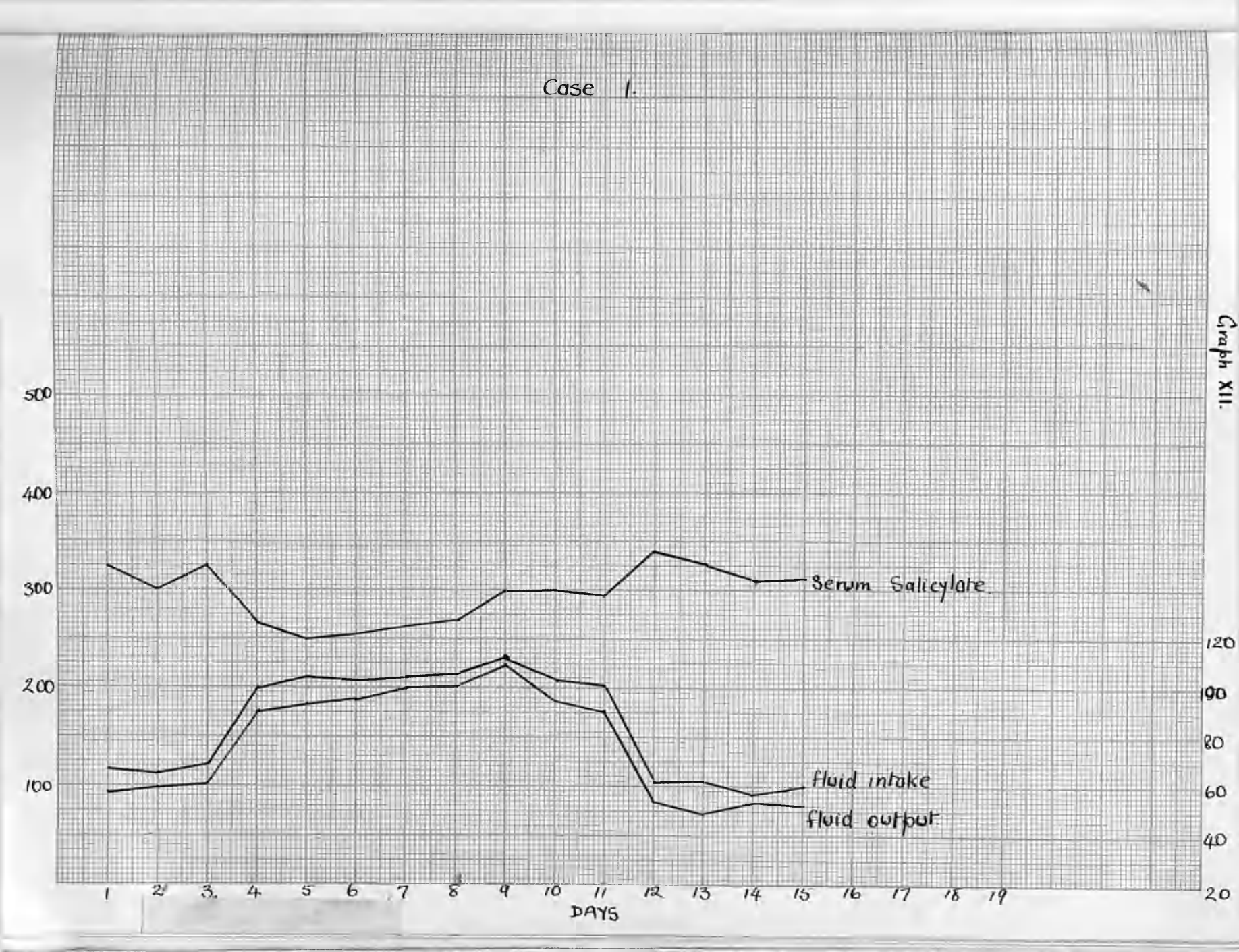
see next page.

Results:

Case 1.

TABLE XXI.

Time (days).	Fluid intake (fluid ounces).	Fluid output (fluid ounces).	Serum salicylate (micrograms/ml.).
1	66	57	325
2	65	60	300
3	68	61	325
4	100	90	265
5	104	93	250
6	102	96	255
7	104	100	265
8	106	100	270
9	112	110	300
10	103	95	300
11	100	90	295
12	62	54	340
13	62	48	325
14	56	53	310
15	60	52	312

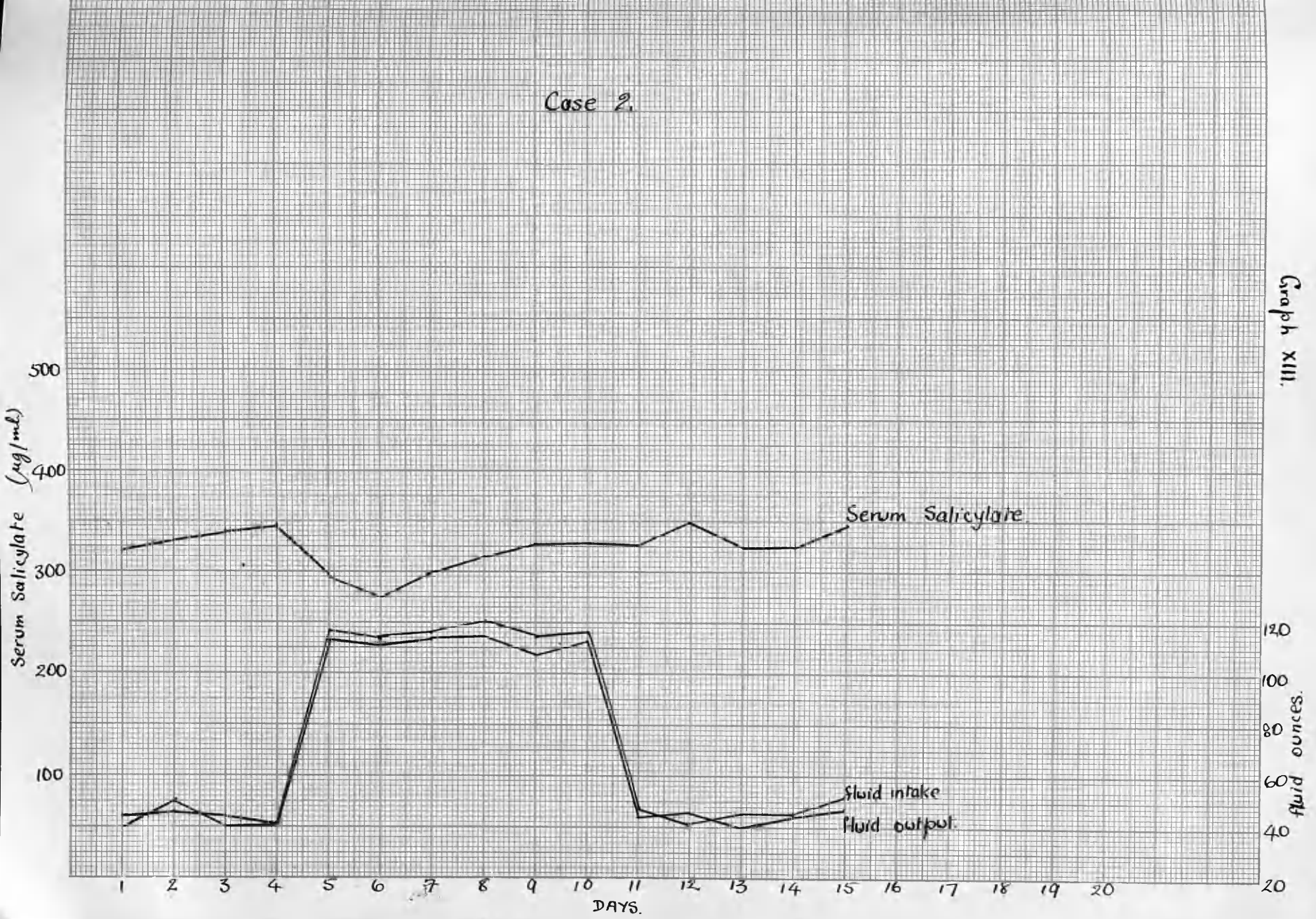

Case 1.
Graph XII.
500
400
300
200
100
120
100
80
60
40
20
Serum Salicylate
fluid intake
fluid output
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
DAYS

Case 2.

TABLE XXII.

Time (days).	Fluid intake (fluid ounces).	Fluid output (fluid ounces).	Serum salicylate (micrograms/ml.).
1	44	40	320
2	45	50	330
3	44	40	340
4	40	40	345
5	116	112	295
6	114	110	275
7	116	114	300
8	120	114	315
9	114	108	325
10	116	112	327
11	46	44	325
12	42	46	350
13	46	40	325
14	44	44	325
15	52	46	345

Case 2.


Graph XIII.
Serum Salicylate (μg/ml)
500
400
300
200
100
Serum Salicylate
Fluid intake
Fluid output.
120
100
80
60
40
20
fluid ounces.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS.

Case 3.

TABLE XXIII.

Time (days).	Fluid intake (fluid ounces).	Fluid output (fluid ounces).	Serum salicylate (micrograms/ml.).
1	64	60	375
2	68	54	360
3	63	57	375
4	112	106	275
5	106	104	260
6	98	102	260
7	106	108	310
8	102	100	325
9	100	102	330
10	105	100	325
11	100	97	355
12	56	60	330
13	60	62	325
14	64	64	320
15	62	64	325
16	55	60	337

Comment:

Three subjects, free from serious organic disease, were given standard doses of 15 grains of sodium salicylate in one


Case 3.
Graph XIV.
Serum Salicylate (mg/ml).
500
400
300
200
100
Serum Salicylate
Fluid intake
Fluid output
120
100
80
60
40
20
Fluid ounces.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS.

ounce of water every four hours, and the effect of varying their fluid intake on the level of salicylate in the serum was observed.

The total volume of urine excreted daily paralleled closely the intake in 24 hours.

In case 1, an increase in the fluid intake from 65 to 104 fluid ounces with a corresponding increase in urine volume from 60 to 93 fluid ounces gave rise to a fall in serum salicylate from 325 µg/ml. to 265 on the second day of increased diuresis. Later, the serum salicylate level rose gradually to its original level, but this rise began before the fluid intake was cut down to the former value of 62 fluid ounces.

Cases 2 and 3 showed similar changes throughout the investigation.

These results suggest that a rise in the volume of urine excreted will cause a depression in serum salicylate level initially because of an increase in the rate of excretion of the drug. Since these serum levels return to their original levels while the increased fluid output is being maintained, it is concluded that equilibrium is once more reached between the amount of salicylate absorbed and the amount distributed between the blood and the intercellular fluid.

Undesirable fluctuations in the serum salicylate level

when the drug is prescribed for therapeutic purposes may result if the fluid intake is not maintained at a fairly constant level.

CHAPTER VI.

SPECIAL FACTORS INFLUENCING BLOOD SALICYLATE LEVELS.

CHAPTER VI.

Special Factors Influencing Blood Salicylate Levels.

(a). The Effect of Sodium Bicarbonate administration on Serum Salicylate levels.

Experimental.

The investigation was carried out on patients and healthy controls. Serum salicylate levels were determined regularly while the subjects were receiving sodium salicylate in fairly uniform dosage at regular intervals day and night. The investigation was divided into three periods. In the first, sodium salicylate was given alone. In the second, combined treatment with equal doses of sodium bicarbonate and sodium salicylate was carried out. The salicylate was again the only drug exhibited in the third period.

In some cases, the periods were interchanged. Some subjects were allowed an unrestricted fluid intake, and in others the volume of fluid taken was limited.

The 24 hour output of urine was measured daily and each subject weighed at intervals in order to assess whether any increase in the extracellular fluid might be occurring which would tend to cause variations in the serum salicylate levels by increasing the concentration of salicylate in the tissues.

Case 1. Disease: Rheumatic fever, of three days' duration.

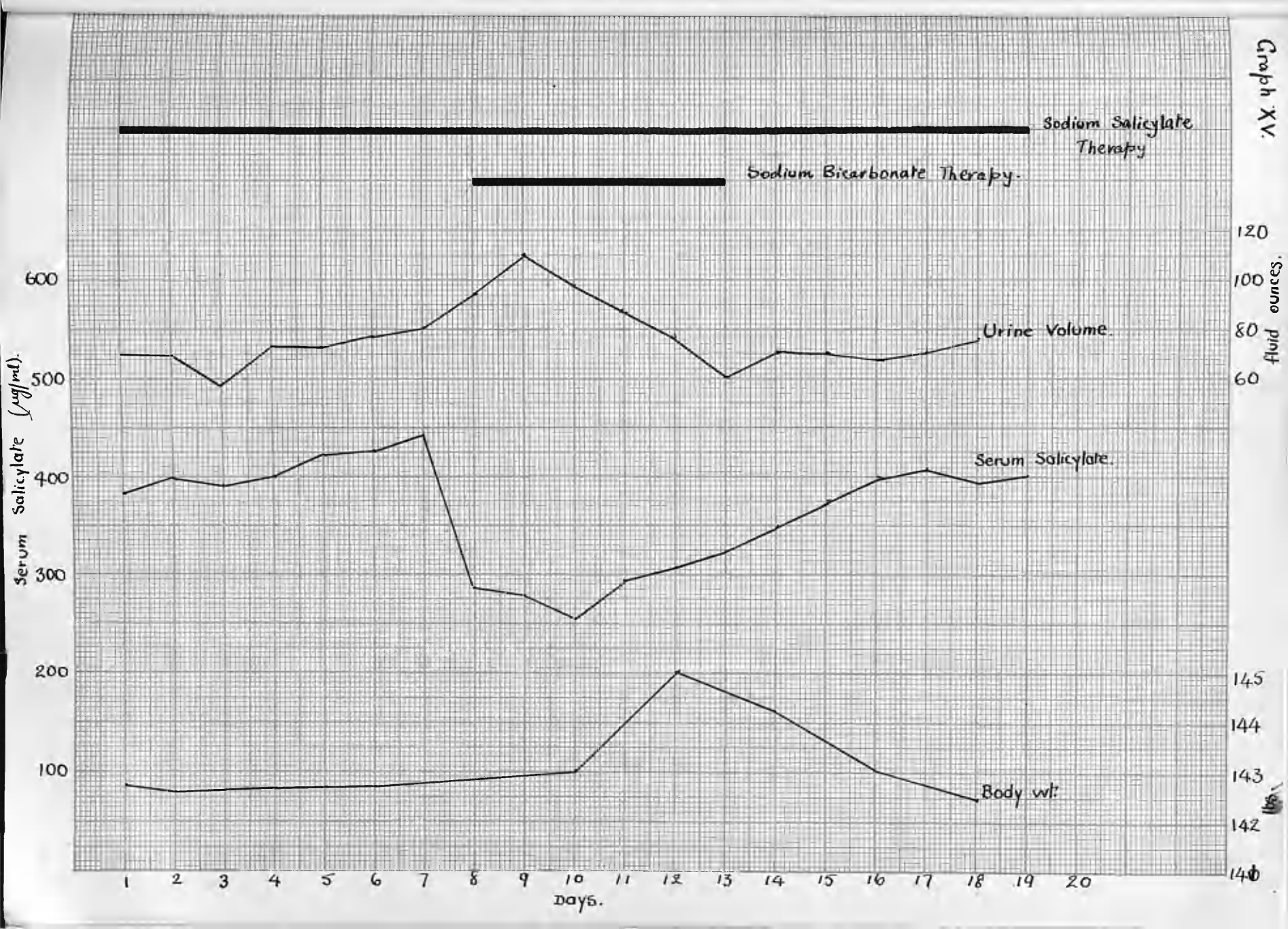
Treatment: Sodium salicylate, 20 grains every four hours.

Fluids unrestricted.

Results:

TABLE XXIV.

Time (days).	Weight (lb.)	Urine volume (fl.oz.).	Serum salicylate (µg./ml.).
x 1	142.7	70	380
2	142.6	70	400
3	-	58	390
4	-	74	400
5	142.7	74	420
6	-	79	425
7	142.8	80	440
xx 8	-	93	285
9	-	110	280
10	143	97	255
11	-	87	295
12	145	78	305
xxx13	-	60	325
14	144.2	70	348
15	-	70	374
16	143.1	68	398
17	-	70	407


Graph XV.
Sodium Salicylate Therapy
Sodium Bicarbonate Therapy.
Urine Volume.
Serum Salicylate.
Body wt.
Serum Salicylate (µg/ml).
Fluid ounces.
lbs.
600
500
400
300
200
100
120
100
80
60
145
144
143
142
140
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.

Time (days).	Weight (lb.).	Urine volume (fl.oz.).	Serum salicylate (µg./ml.).
18	142.4	76	393
19	-	-	400

x sodium salicylate administration.
xx combined salicylate and bicarbonate therapy begun.
xxx bicarbonate discontinued.

Comment: When the administration of sodium bicarbonate along with sodium salicylate was begun on the eighth day, the concentration of salicylate in the serum fell, but rose again after the bicarbonate was discontinued on the thirteenth day.

The urine volume rose on the first day of bicarbonate administration, reaching a maximum on the ninth day. Over the succeeding three days, however, the urine output fell to its original level, and it showed no alteration when bicarbonate treatment was stopped.

The body weight rose during the time bicarbonate was administered reaching a maximum at the end of this period. Thereafter it declined slowly to its original value.

The following explanation of these changes is suggested: the increased urine output following combined sodium salicylate and sodium bicarbonate therapy is probably due to the diuretic action of the alkali. This effect is most marked at first and tends to decline later. The tendency of

salicylates to cause partial suppression of water excretion by the kidney which Hanzlik (1917) described may be diminished by the bicarbonate.

The increase in body weight which was considered due to an increase in the extracellular tissue fluid may appear at first a contradictory observation, but graph XV shows that the maximum body weight was recorded after the urine volume had returned to normal at the end of the period of bicarbonate treatment: at this stage it may be that an increase in extracellular fluid was the result of increased sodium ingestion. The sodium of the salicylate may be partially responsible for the so-called "salicyl oedema" (Hanzlik 1917), and the administration of extra sodium as bicarbonate would tend to enhance the effect.

The marked fall in serum salicylate on the seventh day can be explained by the increased excretion in the urine. After cessation of bicarbonate therapy, a gradual rise in serum salicylate takes place as the volume of extra cellular fluid returns to normal.

Case 2. Disease: Rheumatic fever of 9 days' duration.

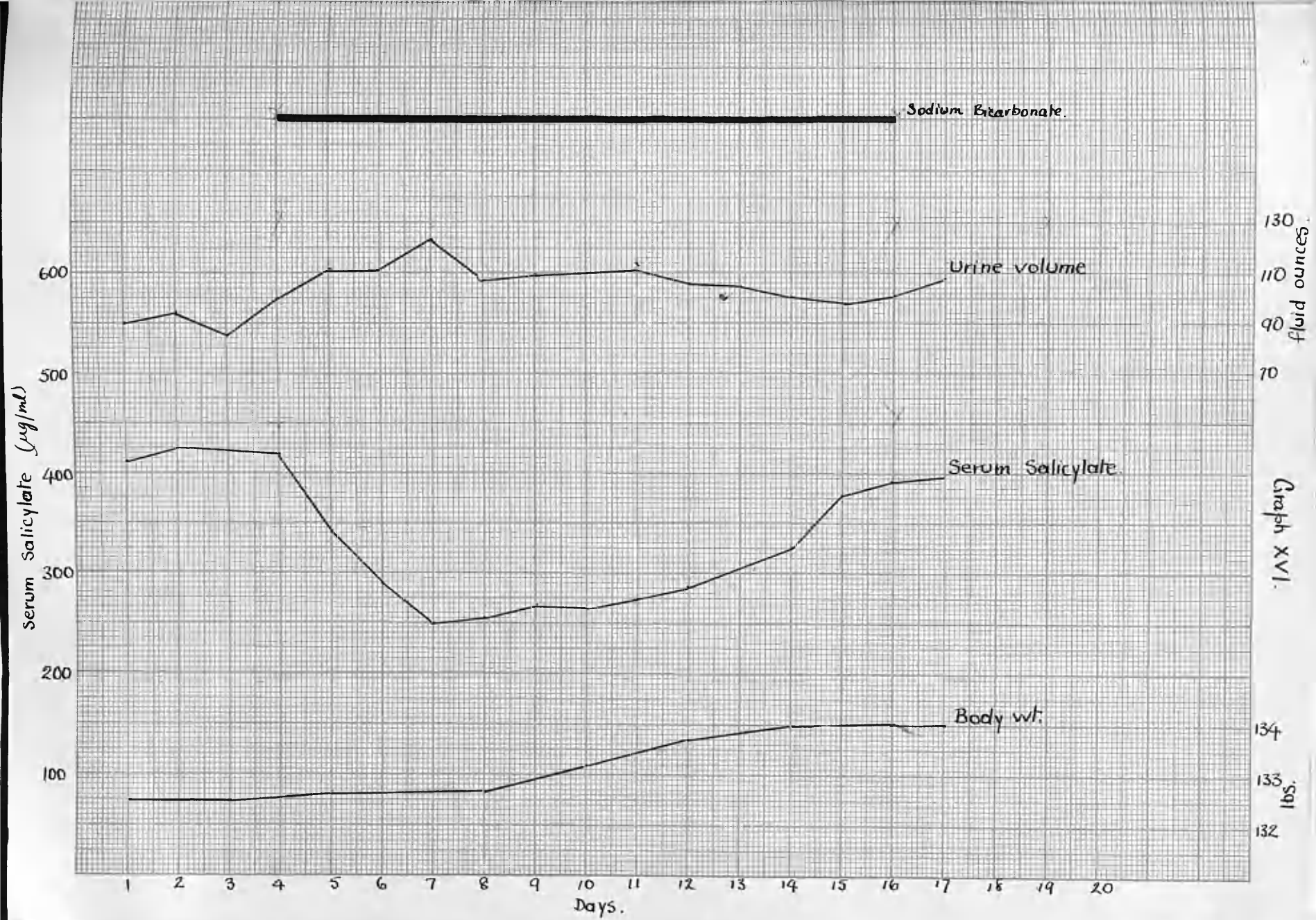
Treatment: Sodium salicylate, 20 gr. every four hours.

Fluids were not restricted.

Results:

TABLE XXV.

Time (days).	Weight (lb.).	Urine volume (fl.oz.).	Serum salicylate (µg./ml.).
1	132.5	90	410
2	-	94	425
3	132.5	85	-
4	-	100	420
5	132.6	110	340
6	-	110	290
7	-	123	250
8	132.7	107	255
9	-	109	265
10	133.2	-	265
11	-	110	-
12	133.7	106	285
13	-	105	-
14	134	100	325
15	-	97	380
16	-	100	390
17	134	107	395


Sodium Bicarbonate.
Urine volume
Serum Salicylate.
Body wt.
Serum Salicylate (μg/ml)
600
500
400
300
200
100
130
110
90
70
fluid ounces
134
133
lbs.
132
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.
Graph XVI.

Comment: In this investigation, the period of combined bicarbonate and salicylate therapy was twelve days. The following observations were made:

1. On the first day of combined treatment there was and increase in the urine output, and this rose to a maximum on the third day. Over the remaining twelve days slightly lower volumes were recorded. When bicarbonate was stopped, the urine output returned to around its initial volume in three days.

2. As in case 1 the body weight rose, the maximum being recorded at the end of period II.

3. The serum salicylate levels were lowered following the institution of combined bicarbonate and salicylate therapy, reaching 250 μg/ml. on the seventh day of the investigation. From this time to the end of the period of combined treatment the salicylate levels rose gradually and reached their original level when the bicarbonate was stopped.

The results in this case are similar to those obtained in case 1 and lead to similar conclusions.

Case 3. Rheumatic fever of 4 days' duration.

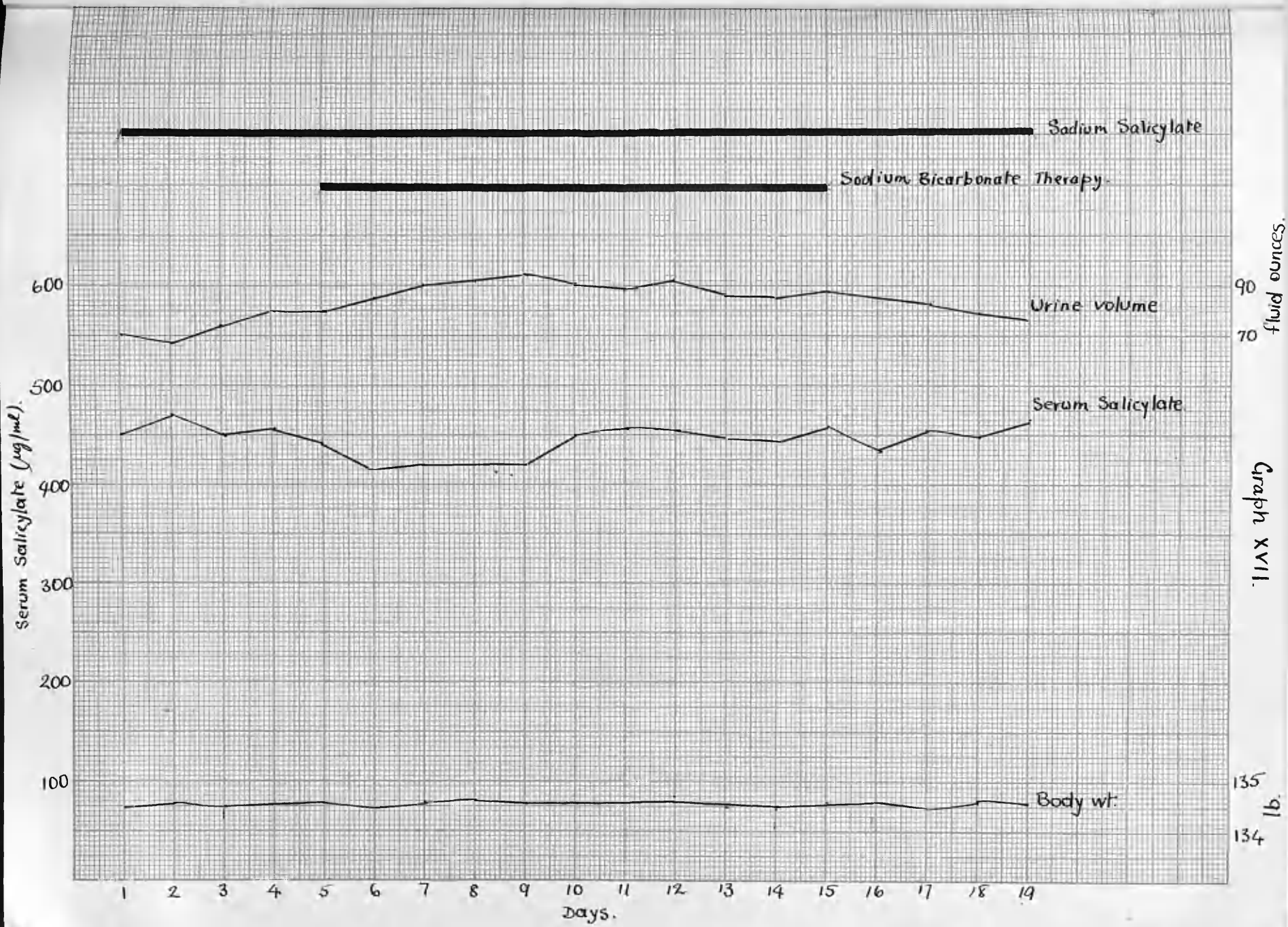
Treatment: Sodium salicylate 25 gr. every four hours.

Fluids were restricted to a daily volume of 60 fluid ounces in this case.

Results:

TABLE XXVI.

Time (days).	Weight (lb.).	Urine volume. (fl. oz.).	Serum salicylate (µg./ml.).
1	134.5	70	450
2	134.6	68	472
3	134.5	74	450
4	134.6	80	455
xx 5	134.6	80	442
6	134.5	84	415
7	134.6	90	420
8	134.6	92	422
9	-	94	420
10	134.6	90	450
11	-	88	456
12	134.5	92	455
13	-	87	448
14	-	86	440
xxx15	134.5	87	455
16	134.5	-	432


Sodium Salicylate
Sodium Bicarbonate Therapy.
Urine volume
Serum Salicylate
Body wt.
Serum Salicylate (µg/ml).
600
500
400
300
200
100
90
70
fluid ounces.
135
134
lb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Days.

Graph XVII.

Time (days).	Weight (lb.).	Urine volume (fl. oz.).	Serum salicylate (µg./ml.).
17	-	83	452
18	13.6	78	450
19	-	-	465

xx combined salicylate and bicarbonate therapy begun.
xxx bicarbonate discontinued.

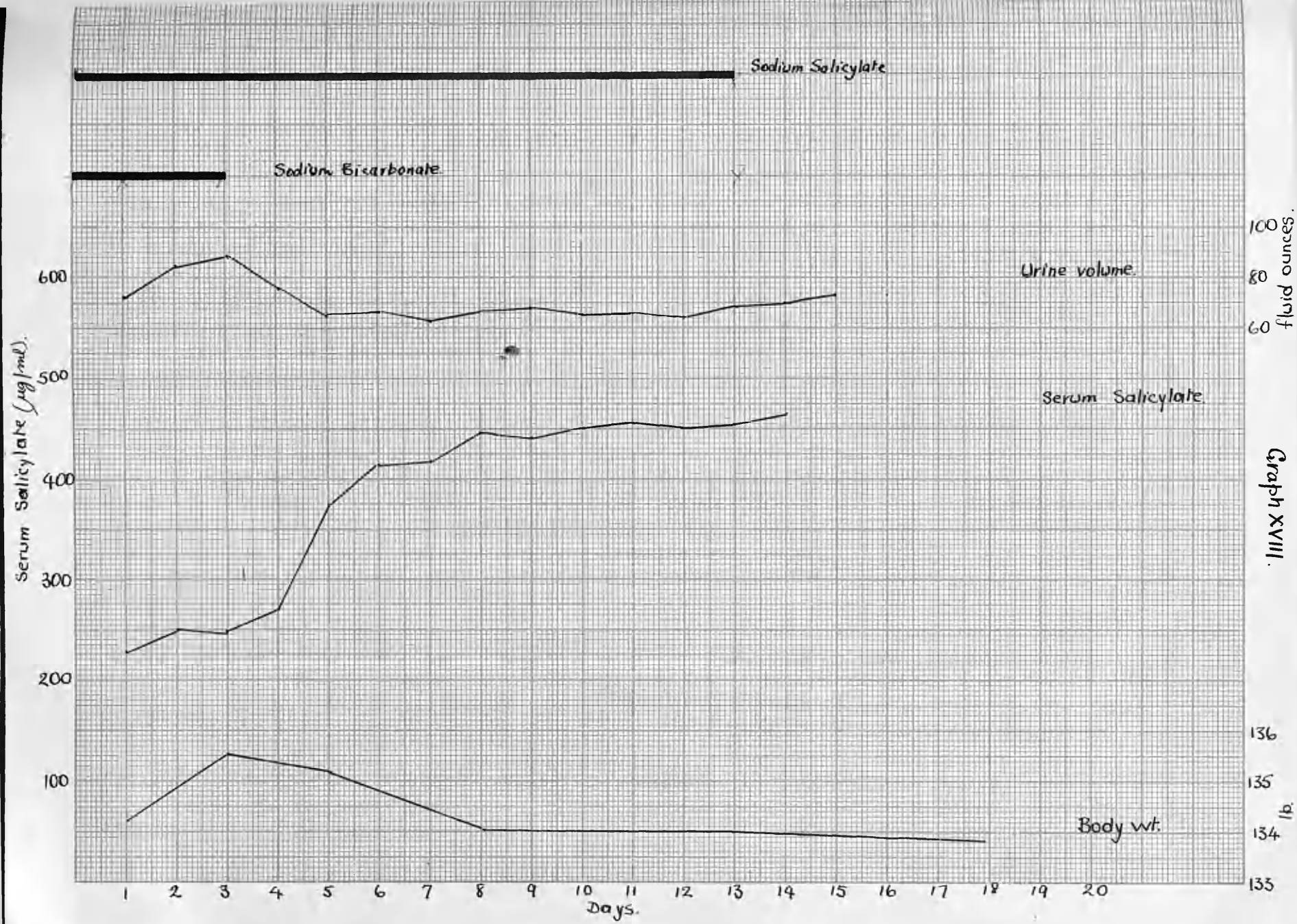
Comment: In this case, urine volume, serum salicylate concentration, and body weight all remained reasonably constant throughout. This suggests that when the fluid intake is restricted, increased diuresis changes in the extracellular fluid are much less marked, and that several of the factors which affect the serum salicylate level are thereby eliminated.

Case 4: This subject was a normal healthy male aged 26 years. He received 20 gr. sodium salicylate and 20 gr. sodium bicarbonate every four hours for three days, then sodium salicylate alone for ten days. After an interval of six days, sodium salicylate and sodium bicarbonate were given every four hours, in the same dosage as before, for 13 days, then sodium salicylate alone for a further 7 days. A second period of six days was allowed to elapse, and the subject was given sodium salicylate for 5 days followed by combined salicylate and bicarbonate therapy for 8 days, and lastly sodium salicylate alone.

Results:

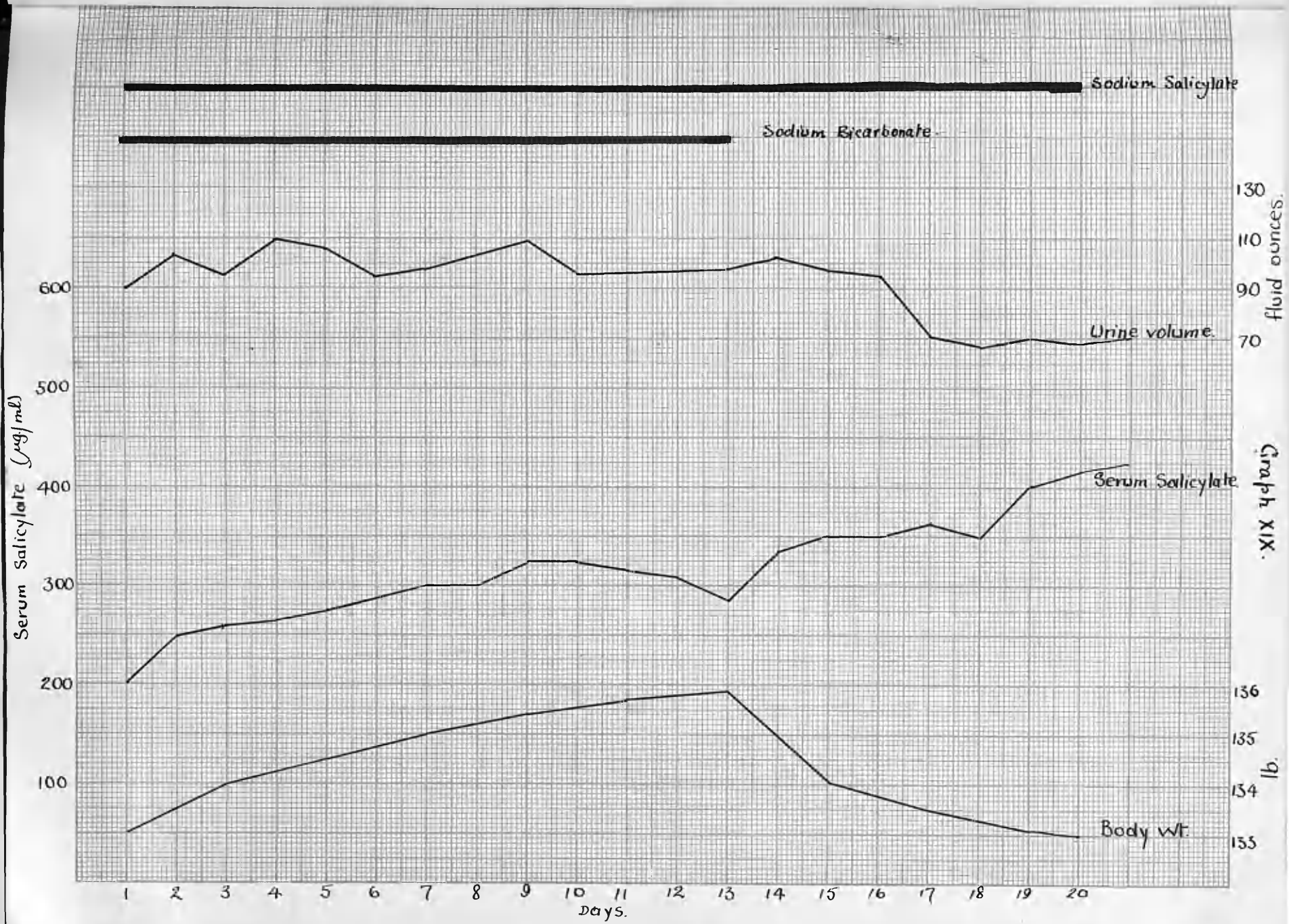
TABLE XXVII.

Time (days.)	Body weight (lb.).	Urine volume (fl. oz.).	Serum salicylate (µg./ml.).
1	134.3	74	226
2	-	84	250
3	135.5	88	245
4	-	75	270
5	135.2	65	374
6	-	66	414
7	-	63	418
8	134	66	445
9	-	68	440


Sodium Salicylate
Sodium Bicarbonate
Urine volume.
Serum Salicylate.
Body wt.
Serum Salicylate (µg/ml).
600
500
400
300
200
100
100
80
60
fluid ounces.
136
135
134
133
lb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.

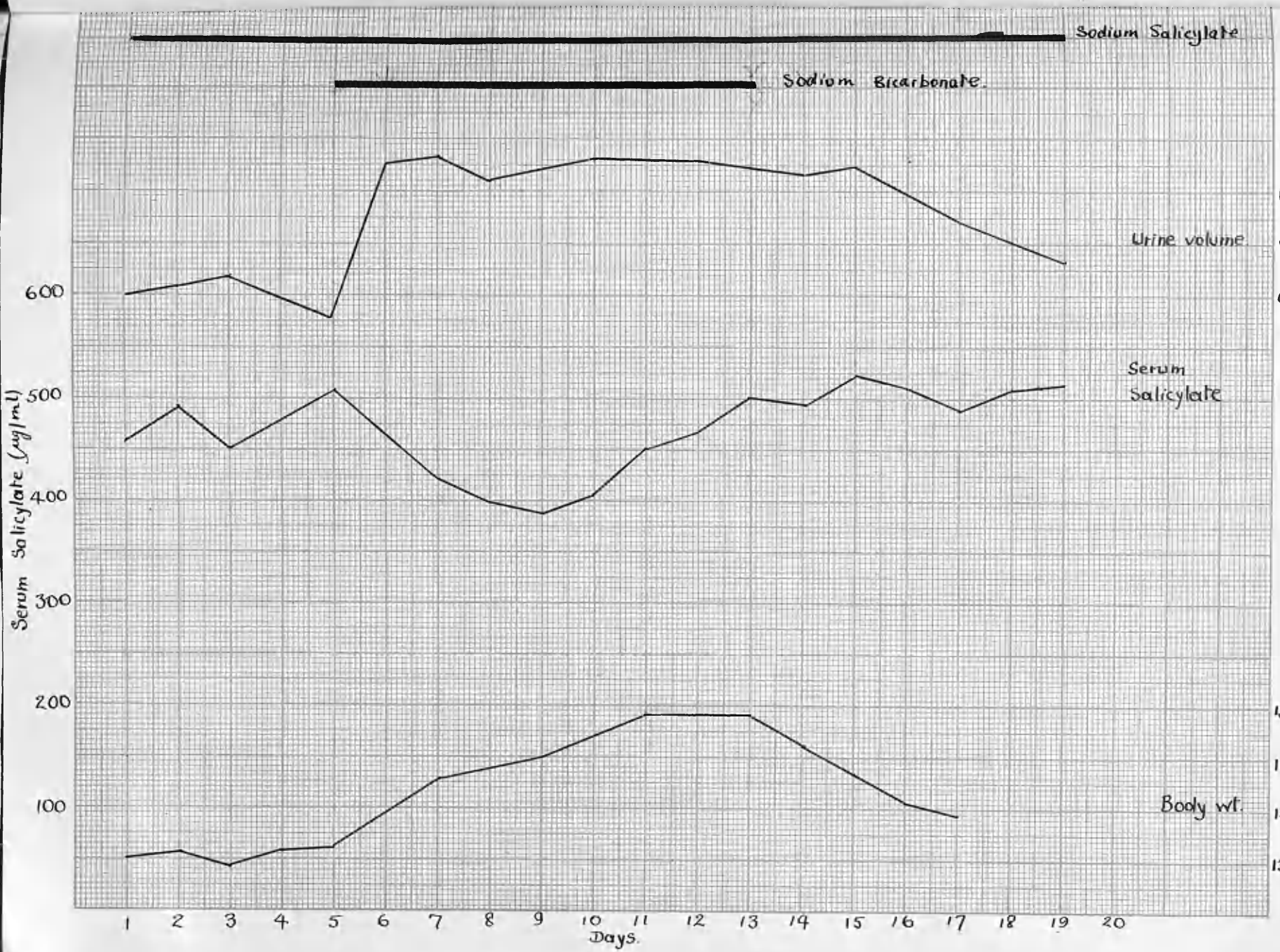
Graph XVIII.

Time (days.).	Body weight (lb.).	Urine volume (fl. oz.).	Serum salicylate (µg./ml.).
10	-	65	450
11	134	66	456
12	134	64	450
13	134	68	455
.....	Six days' interval	.....	
1	133	90	200
2	-	103	250
3	134	95	258
4	-	110	264
5	134.5	106	275
6	-	95	285
7	135	98	300
8	-	-	300
9	135.4	108	325
10	-	96	325
11	135.7	-	314
12	-	-	310
13	135.8	97	285
14	-	102	335
15	134	97	350
16	-	95	350
17	133.5	70	364
18	-	66	350


Sodium Salicylate
Sodium Bicarbonate
Urine volume.
Serum Salicylate
Body wt.
Serum Salicylate (μg/ml)
600
500
400
300
200
100
130
110
90
70
fluid ounces.
136
135
134
133
lb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.
Graph XIX.

Time (days.).	Body weight (lb.).	Urine volume (fl. oz.).	Serum salicylate (µg./ml.).
19	-	70	400
20	133	68	415
.....	Six days' interval	.....	
1	133	60	457
2	133.1	63	490
3	132.8	67	450
4	133.3	-	480
5	133.3	52	512
6	-	110	465
7	134.6	113	420
8	-	104	398
9	135	-	387
10	-	112	406
11	135.8	-	450
12	-	112	468
13	135.8	-	500
14	135.2	106	495
15	134.7	110	520
16	134.1	-	510
17	133.8	98	490
18	-	-	510

Comment: First period: During the three days of combined


Sodium Salicylate
Sodium Bicarbonate.
Urine volume
Serum Salicylate
Body wt.
Serum Salicylate (µg/ml)
600
500
400
300
200
100
120
100
80
60
ounces fluid
136
135
134
133
lb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.

Graph XX.

treatment at the beginning of the investigation, the body weight rose sharply, while the serum salicylate level remained approximately constant, and the urine output rose slightly. When the alkali was stopped, the body weight fell back to its original value over a period of five days, and remained constant thereafter. Meanwhile the serum salicylate rose rapidly for five days, then, like the body weight, remained constant. When the bicarbonate administration was stopped the urine volume began to fall until it reached the original figure on the fifth day. Thereafter it fluctuated only slightly.

Second period: The body weight showed an early and gradual rise until the alkali was stopped on the thirteenth day: after that, there was a return to normal in a relatively short time.

The urine output fluctuated slightly during the thirteen days of combined treatment, and when bicarbonate was stopped, fell to a lower level in two days, remaining fairly constant thereafter.

The serum salicylate concentration rose during the first nine days of combined treatment from 200 micrograms per ml. to 350 micrograms per ml. During the following four days, the concentration was fairly constant. On cessation of bicarbonate administration on the thirteenth day, the value of the serum salicylate began to rise, and by the twentieth day was 415 µg/ml.

Third period: When salicylate was administered alone, urine output, serum salicylate concentration, and body weight remained fairly constant. When combined salicylate and bicarbonate was begun on the fifth day, there was an immediate rise in the urine output, but this soon became constant at a new higher level. Serum salicylate concentration fell steadily and the body weight rose. By the ninth day of the investigation the serum concentrations began to rise again and reached 500 µg/ml. by the thirteenth day, when the alkali was stopped. Thereafter levels remained constant, but with cessation of bicarbonate the body weight and urine output again began to fall.

These results again suggest that there is a tendency for serum salicylate levels to rise from relatively low concentration if a sufficient period is allowed for equilibrium to be reached between the amount of drug excreted in the urine and the amount retained in the tissues and circulating in the blood. The administration of sodium bicarbonate with the sodium salicylate appears to delay the attainment of optimum salicylate concentration of over 350 µg./ml., since such concentrations are found 24-48 hours after the administration of sodium salicylate alone in adequate doses.

Case 5. This subject was a healthy male aged 40 years. Sodium salicylate and sodium bicarbonate were administered together for nine days in a dosage of 20 gr. every four hours. The bicarbonate was then stopped, and salicylate given alone for a further seven days.

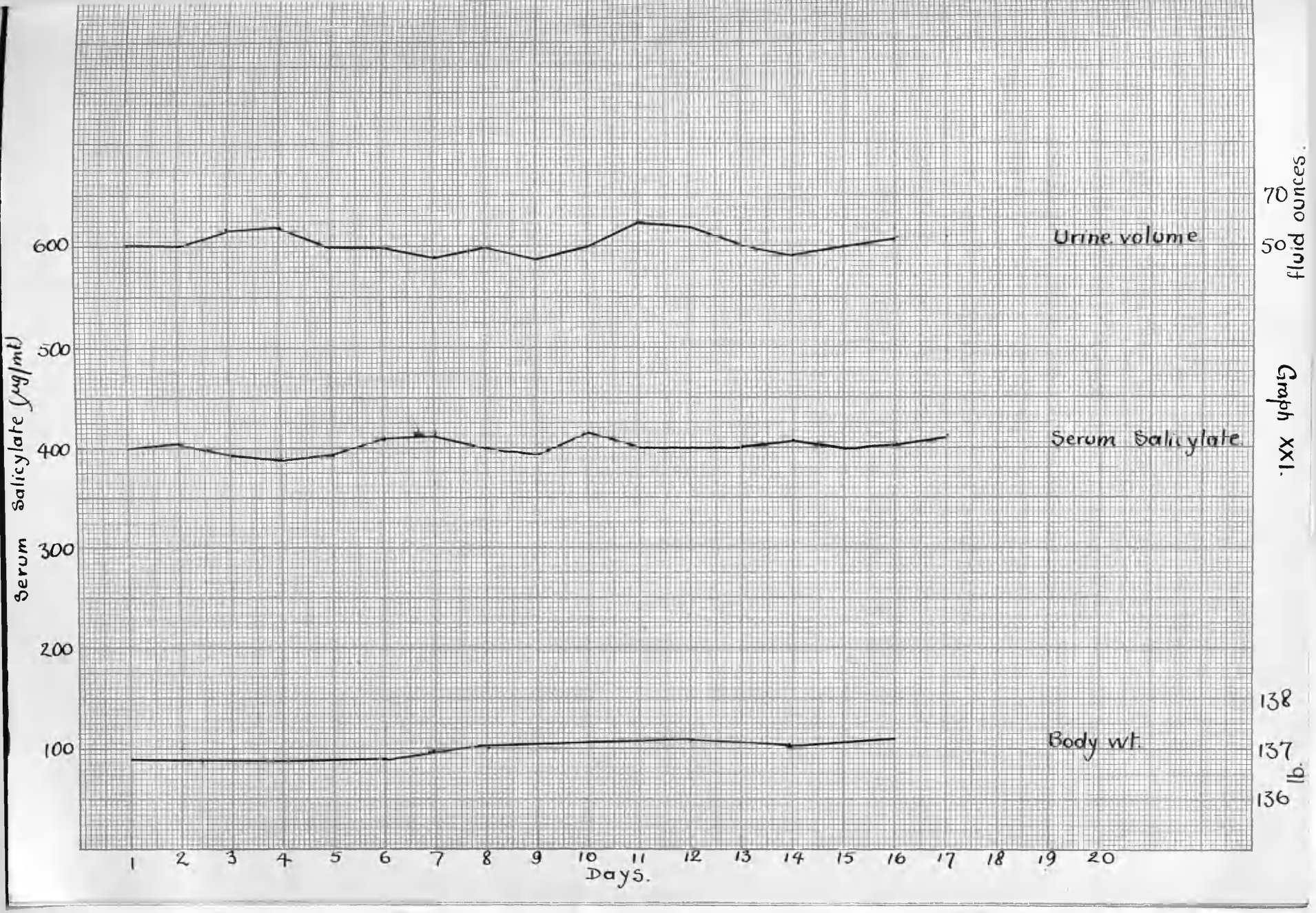
The fluid intake was restricted to 55 ounces per day.

Results:

TABLE XXVIII.

Time (days).	Body weight (lb.).	Urine output (fl.oz.)	Serum salicylate (µg./ml.)
1	136.8	50	400
2	-	50	405
3	136.6	55	393
4	-	57	390
5	-	50	395
6	136.8	50	410
7	-	46	410
8	137	50	400
9	-	45	392
x10	-	50	415
11	137.2	60	400
12	137.2	58	400
13	-	50	400
14	137	46	405
15	-	-	400
16	137.2	53	405

x bicarbonate discontinued.


Graph XXI.
Serum Salicylate (μg/ml)
600
500
400
300
200
100
Urine volume
Serum Salicylate
Body wt.
70
50
fluid ounces.
138
137
136
lb.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Days.

Comment: Serum salicylate concentrations and the body weight remained constant throughout this investigation, and the urine output showed only a slight rise on the third and fourth days, and again on the eleventh and twelfth days.

These result suggest that the restriction of fluid intake decreases the tendency for sodium bicarbonate administration to lower the serum salicylate concentration.

(b). <u>The Effect on the Serum Salicylate concentration of certain clinical states</u>.

At this juncture, it was decided to compare the serum salicylate levels obtained when sodium salicylate in standard dosage was administered to normal subjects with the serum concentration found when the drug was given to patients suffering from certain specified diseases. The standard dose selected was 0.13 gram per kilo body weight, and the drug was administered orally every four hours. The fluid intake was unrestricted, and the serum salicylate was determined daily.

The cases investigated were as follows:

1. Normal subjects: four cases from the surgical wards free from serious general disease.

2. Acute rheumatism: four cases.

3. Pernicious anaemia: three cases of differing degrees of severity.

4. Chronic myeloid leukaemia: three cases.

5. Chronic ulcerative colitis: two cases.

6. Subacute rheumatism: one case. This subject had had recurrent acute attacks of rheumatism

over a period of eight years, and was admitted to hospital in one of these. She was found to have a marked hypochromic anaemia, mitral stenosis, and auricular fibrillation. After sixteen days' treatment with salicylate she developed acute heart failure, oedema, and oliguria.

The results of each group are shown in detail in tabular form, and the mean levels are also recorded graphically.

Results: Normal subjects.

TABLE XXIX.

Serum salicylate (μg./ml.)

Day.	1	2	3	4	5	6	7	8	9	10
Case 1.	350	377	370	382	390	379	382	380	394	408
Case 2.	402	422	404	410	398	399	388	406	420	400
Case 3.	374	381	396	398	408	388	404	406	368	400
Case 4.	362	377	386	356	340	386	366	372	362	360
Mean.	372	386	389	384	384	388	385	391	386	392

Acute Rheumatism.

TABLE XXX.

Serum salicylate (μg./ml.)

Day.	1	2	3	4	5	6	7	8	9	10
Case 1.	180	232	308	316	328	337	338	326	333	358
Case 2.	252	290	360	352	364	356	360	371	362	348
Case 3.	236	332	342	339	321	332	328	316	330	312
Case 4.	212	276	376	385	347	367	378	371	335	358
Mean.	220	282	344	348	340	348	351	346	340	342

Pernicious anaemia.

TABLE XXXI.

Serum salicylate (μg./ml.)

Day.	1	2	3	4	5	6	7	8	9	10
Case 1.	386	394	402	396	382	370	408	400	386	386
Case 2.	370	386	378	384	378	388	398	366	374	372
Case 3.	390	390	396	408	414	439	400	386	398	406
Mean.	382	390	392	296	388	399	402	384	386	388

Chronic myeloid leukaemia.

TABLE XXXII.

Serum salicylate (μg./ml.)

Day.	1	2	3	4	5	6	7	8	9	10
Case 1.	440	436	452	444	438	450	464	442	452	460
Case 2.	430	430	436	442	456	440	444	448	444	450
Case 3.	444	460	450	422	456	431	472	436	460	464
Mean.	438	442	446	436	450	440	460	442	452	458

Chronic ulcerative colitis.

TABLE XXXIII.

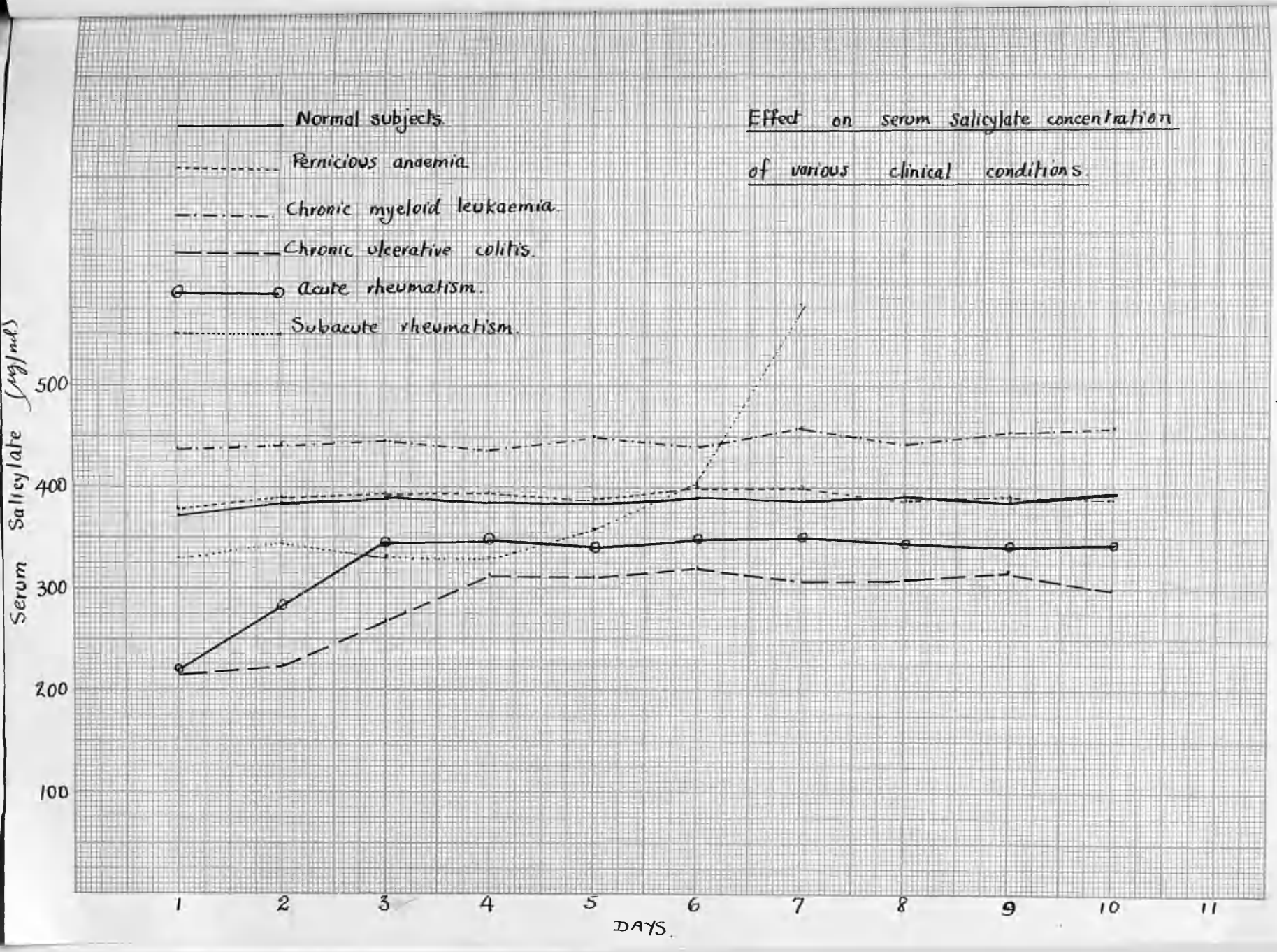
Serum salicylate (μg./ml.)

Day.	1	2	3	4	5	6	7	8	9	10
Case 1.	212	202	260	300	312	324	328	300	316	292
Case 2.	220	246	276	332	312	316	290	324	320	308
Mean.	216	224	268	316	312	320	309	312	318	300

Subacute rheumatism.

TABLE XXXIV.

Day.	1	2	3	4	5	6	7
Serum salicylate (μg./ml.)	328	344	331	330	382	404	580


Normal subjects.
Pernicious anaemia.
Chronic myeloid leukaemia.
Chronic ulcerative colitis.
Acute rheumatism.
Subacute rheumatism.
Effect on serum salicylate concentration of various clinical conditions.
Serum Salicylate (mg/ml)
500
400
300
200
100
1
2
3
4
5
6
7
8
9
10
11
DAYS.

Graph XXII.

Comments: The serum salicylate concentrations obtained in normal individuals receiving a dose of 0.13 g. per kilogram body weight remained reasonably constant from the second day after administration of the drug was begun. Results in cases of pernicious anaemia were substantially the same as in normal subjects.

In acute rheumatism, the mean level on the first day was lower than in normal subjects, and a constant level of the drug in the serum was not attained until after three days' therapy.

In the chronic myeloid leukaemia group, maximum concentrations of the drug were attained on the second day, and the values found were appreciably higher than in the other groups. These higher concentrations, it is suggested, might have been the result of diminished excretion by kidneys whose function was impaired because of chronic anaemia or leukaemic infiltration. Reduced rate of destruction of salicylate by the leukaemic liver might also be advanced as a cause of the high blood levels.

In the chronic ulcerative colitis cases, mean serum salicylate levels of less than 320 μg/ml. were obtained by the fourth day. These figures suggest that in this condition, absorption of salicylate from the intestinal tract is impaired: or the explanation might be that the increased loss of water in the fluid stools increased the rate of excretion of salicylate and thus kept the concentration in the serum at a lower level.

Subacute rheumatism with cardiac failure: This subject was given the standard dose of 0.13 g. sodium salicylate per kilogram body weight for 12 days before the serum levels reported in table XXXIV were begun. On the fourth day of salicylate therapy, auricular fibrillation developed, the patient became oedematous, and the urinary output diminished. By the seventh day, vomiting was severe and it was deemed inadvisable to maintain treatment with salicylate. No further blood specimens were obtained.

The rise in serum salicylate concentration after the onset of cardiac failure appeared to be a direct result of the almost complete suppression of urine and, therefore, consequent reduction in elimination of salicylate. Some degree of hepatic failure was probably present in addition, and this factor might also be concerned with the tendency of blood levels to rise as destruction of the drug in the liver decreased.

The suggestion has been made earlier that an increase in extracellular fluids might depress serum levels, and it seems reasonable to believe that in this case, oedema might have had a similar effect. If there had been no oedema, it is likely that the serum concentrations would have been even higher, provided the other factor - renal failure - had been present in the same degree.

CHAPTER VII.

THE INCIDENCE OF THE COMMONER GENERAL MANIFESTATIONS OF SALICYLISM.

CHAPTER VII.

The Incidence of the Commoner General Manifestations of Salicylism.

The cases described in this section include those in whom toxic manifestations were observed at some stage during therapy with sodium salicylate while the investigations reported in this thesis were being carried out. Signs of intoxication associated with an increased tendency to haemorrhage are described later, the object at this juncture being to describe the incidence of the commoner signs and symptoms of salicylism, and to determine whether these have any relationship to the concentration of the drug in the blood.

The total number of subjects studied in the course of the work described in this thesis was 94: of these, 88 developed one or more symptoms ascribed to salicylate administration. These symptoms can be classified under the following headings:

1. Epigastric discomfort.
2. Nausea.
3. Vomiting.
4. Tinnitus.
5. Deafness.

6. Vertigo.

7. Headache.

8. Others.

In the following paragraphs, each of these is described with reference to incidence, degree of severity, and duration, associated with each of the following serum salicylate concentrations.

(a) less than 100 micrograms per ml.
(b) 100 to 200 " " "
(c) 200 to 300 " " "
(d) 300 to 450 " " "

1. Epigastric discomfort:

This effect of salicylate therapy being purely subjective, complete reliance had to be placed on the patient's description. Loss of appetite accompanied by a vague feeling of gastric distention was the commonest complaint. Heartburn was occasionally mentioned, and when serum levels were over 200 μg/ml., this usually preceded vomiting. With concentrations below 200 μg/ml., discomfort affected the greatest percentage of subjects in the first few days and became negligible later, whereas when the serum concentration was greater than 200 μg/ml. this symptom generally persisted throughout the period of administration of the drug.

Results:

TABLE XXXV.

(a) Serum salicylate below 100 µg/ml. 12 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age with symptoms.	25	40	40	25	17	17	9	0	0	0	0

(b) Serum salicylate 100-200 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age with symptoms.	31	38	38	38	31	25	13	13	13	6	0

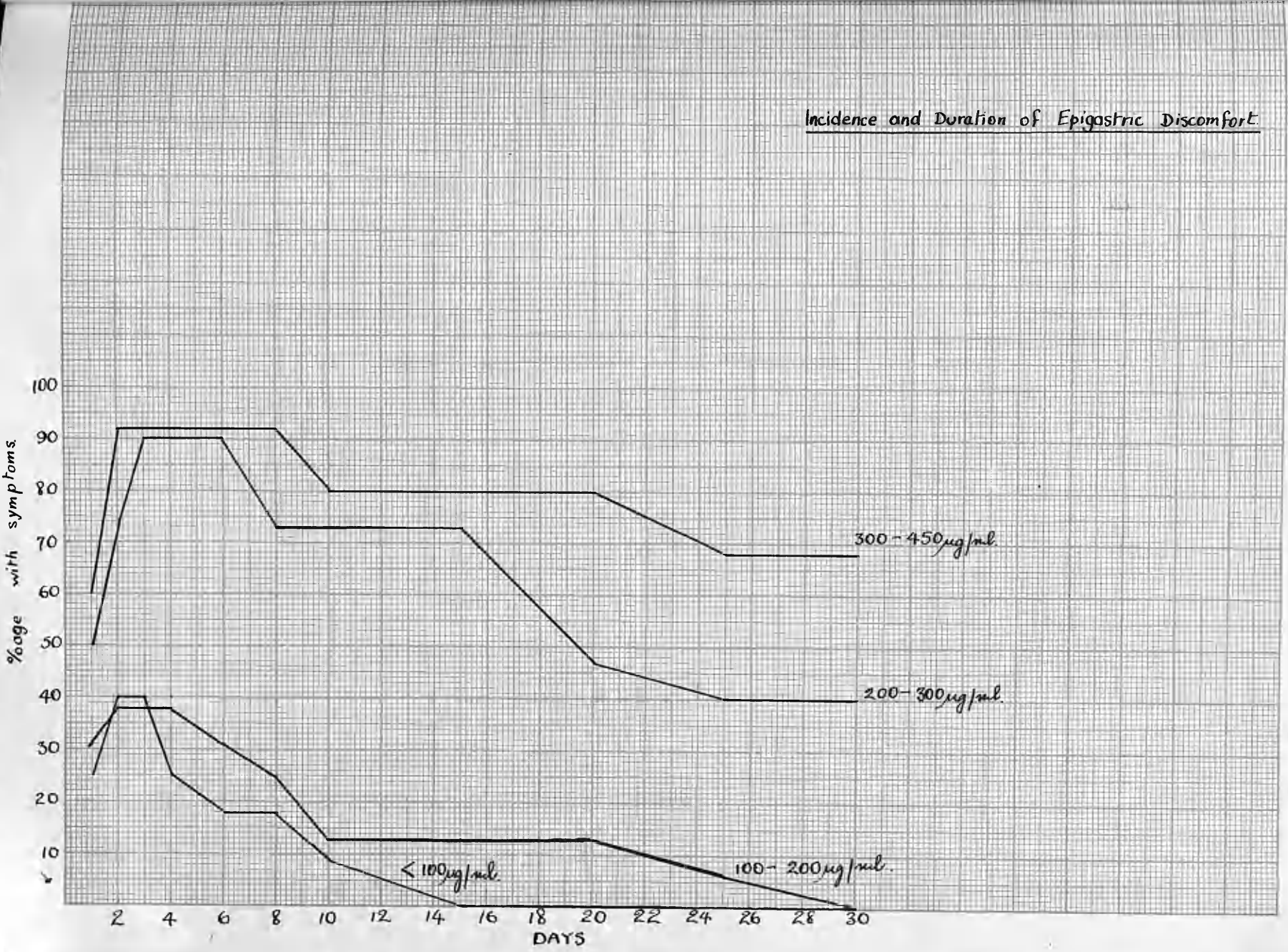
(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age with symptoms.	50	73	90	90	90	73	73	73	47	40	40

(d) Serum salicylate 300-450 µg/ml. 25 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age with symptoms.	60	92	92	92	92	92	80	80	80	68	68

Graph XXIII.


Incidence and Duration of Epigastric Discomfort
%age with symptoms
100
90
80
70
60
50
40
30
20
10
300 - 450 µg/ml
200 - 300 µg/ml
< 100 µg/ml
100 - 200 µg/ml
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS

2. Nausea:

Epigastric discomfort and nausea frequently occurred together, but the incidence of the latter was not so great. When the higher concentrations of the drug were being maintained, it frequently preceded vomiting, and it was most in evidence when headache and tinnitus were most severe.

As the administration was continued fewer cases were affected. The second and third days of treatment were the times when nausea was most troublesome.

Results:

TABLE XXXVI.

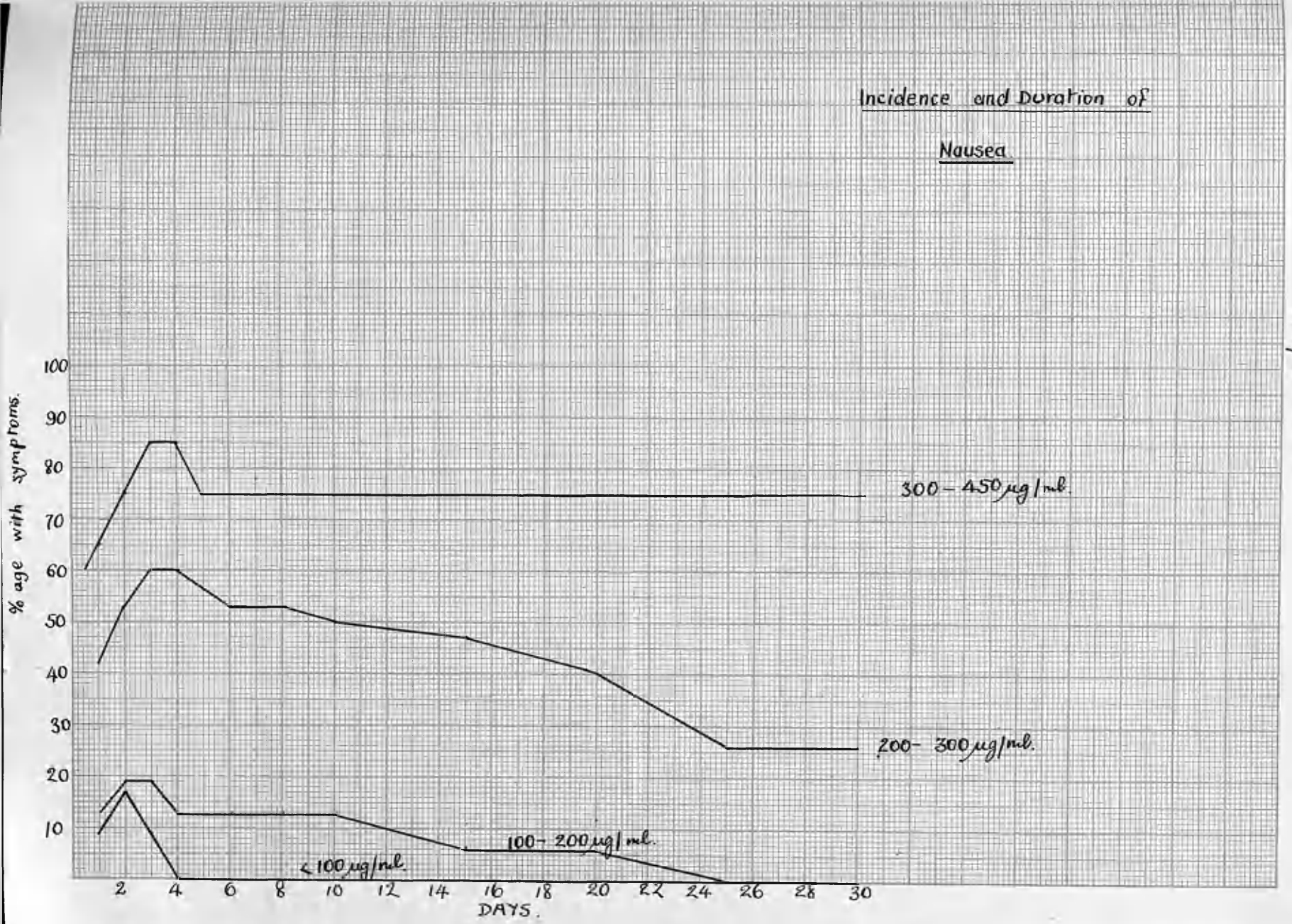
(a) Serum salicylate below 100 µg./ml. 12 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	9	17	9	0	0	0	0	0	0	0	0

(b) Serum salicylate 100-200 µg/ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	13	19	19	13	13	13	13	6	6	0	0

Graph XXIV.


Incidence and Duration of
Nausea
% age with symptoms.
100
90
80
70
60
50
40
30
20
10
300 – 450 µg/ml.
200 – 300 µg/ml.
100 – 200 µg/ml.
< 100 µg/ml.
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS.

(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	42	53	60	60	53	53	50	47	40	26	26

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	65	75	85	85	75	75	75	75	75	75	75

3. Vomiting:

This symptom of intoxication was not observed in any case when the serum salicylate was below 100 µg/ml., and with values between 100 µg/ml and 300 g/ml. it tended to pass off after the second day.

With concentrations above 300 µg/ml., vomiting persisted in approximately 50 per cent of cases. In three cases it was so severe on the second and third days that administration

was stopped for 24 hours. The concentration of salicylate in the serum was found to be over 520 µg./ml. When therapy was resumed the serum levels were not allowed to rise above 400 µg./ml. and vomiting did not recur.

Results:

TABLE XXXVII.

(a) Serum salicylate below 100 µg./ml. No cases.

(b) Serum salicylate 100-200 µg./ml.

Time (days)	1	2	3	4
%age affected.	6	12	6	0

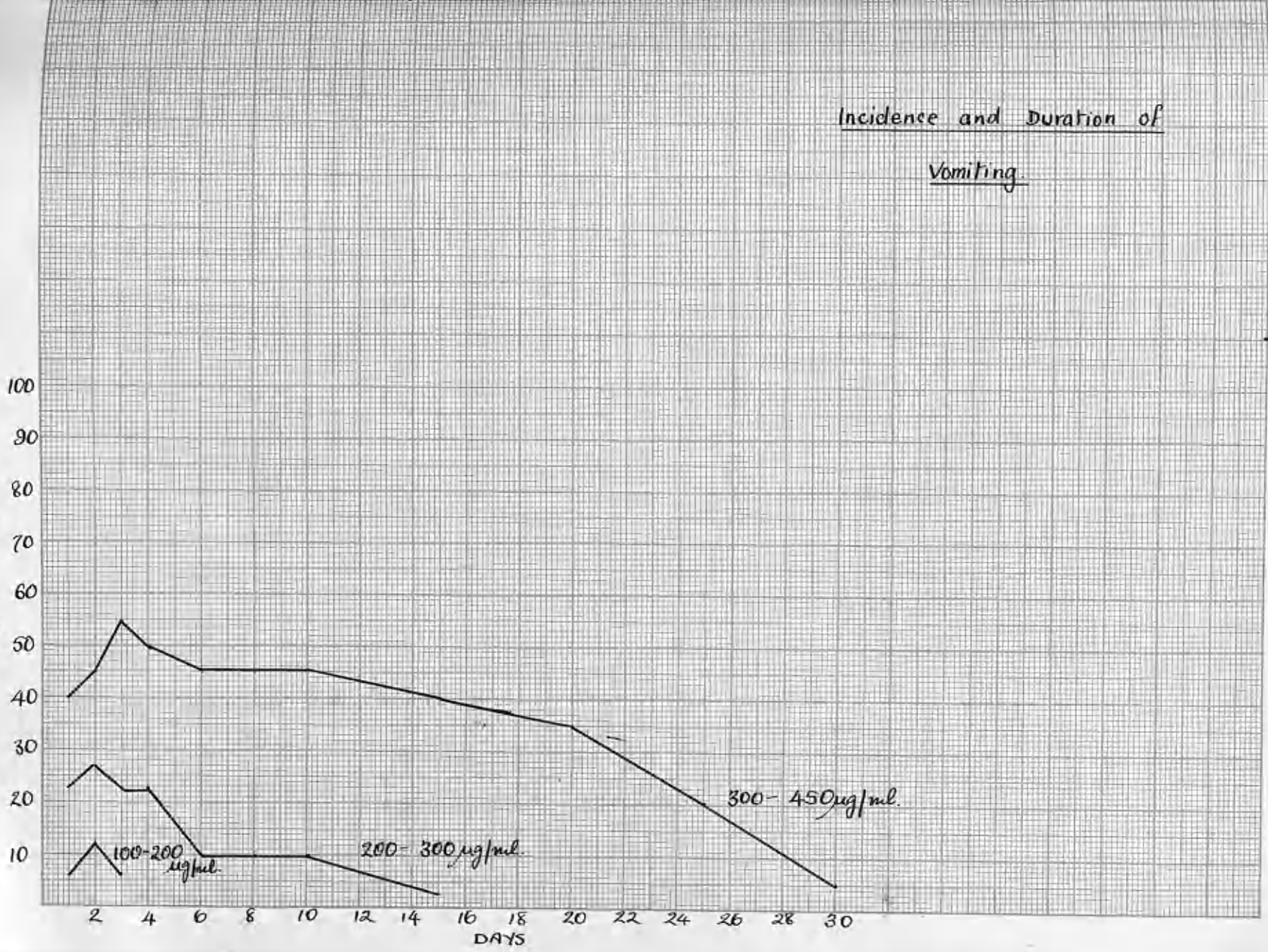
(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15
%age affected.	23	27	23	23	10	10	10	3

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	40	45	55	50	45	45	45	40	35	20	5

Graph XXV.


Incidence and Duration of
Vomiting.
% age with symptoms
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS
100-200 µg/ml.
200- 300 µg/ml.
300- 450 µg/ml.

4. Tinnitus:

Tinnitus occurred in a percentage of cases irrespective of the serum salicylate concentration. Its incidence and duration were similar to those of nausea, although both symptoms were not always complained of at the same time. Although it was severe in the three cases who suffered from excessive vomiting, it was never considered necessary to cease administration of the drug on account of this complaint alone.

Results:

TABLE XXXVIII.

(a) Serum salicylate below 100 µg./ml. 12 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	-	8	16	16	8	8	8	-	-	-	-

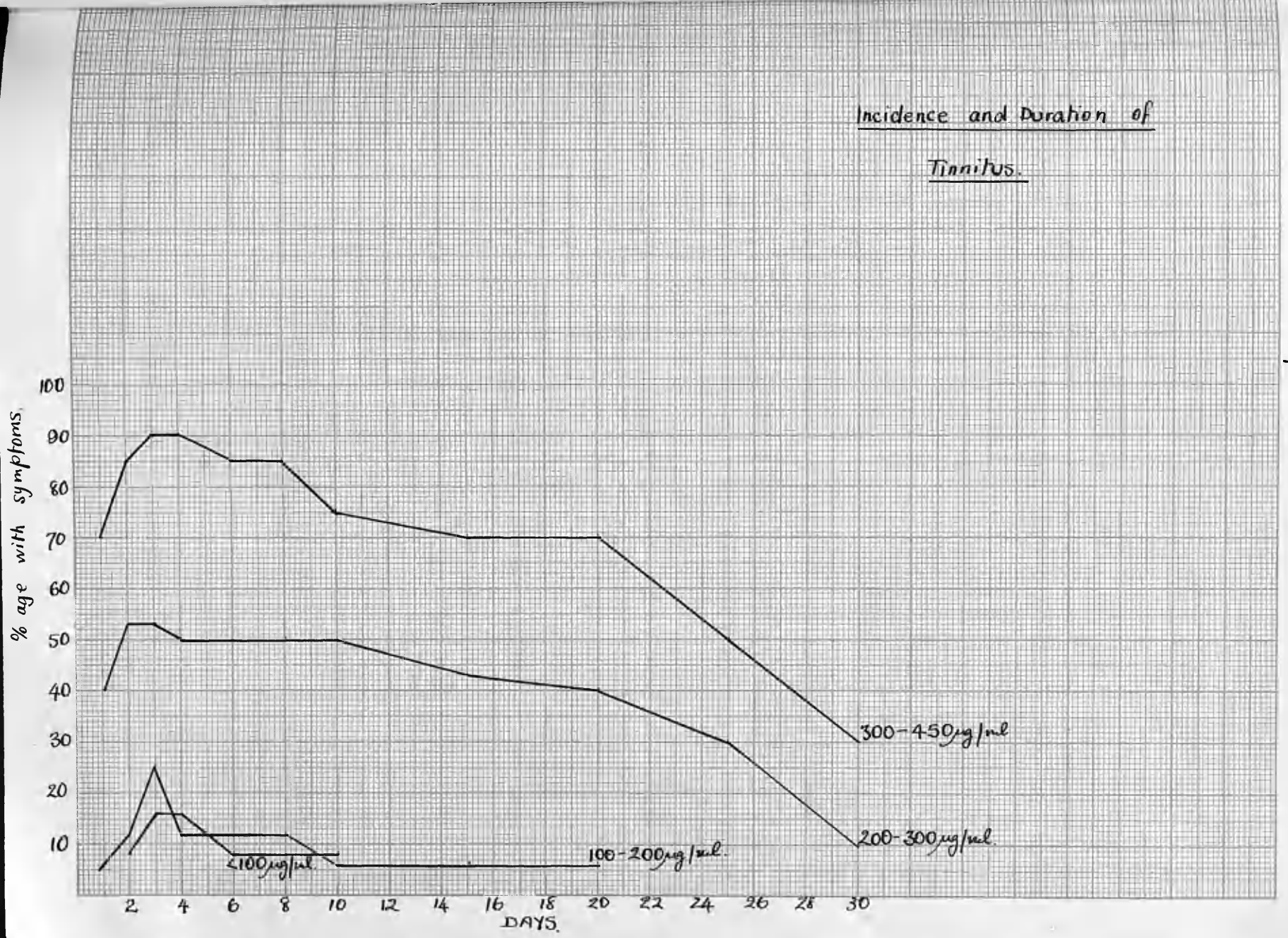
(b) Serum salicylate 100-200 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	6	12	25	12	12	12	6	6	6	-	-

(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	40	53	53	50	50	50	50	43	40	30	10

Graph XXVI.


Incidence and Duration of
Tinnitus.
% age with symptoms
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS.
300-450 µg/ml
200-300 µg/ml
100-200 µg/ml.
<100 µg/ml.

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	70	85	90	90	85	85	75	70	70	50	30

5. Deafness:

This manifestation of salicylism was commoner than tinnitus. In those cases where deafness and tinnitus were both present it is probable that severe tinnitus accentuated the degree of impairment of hearing.

Results:

TABLE XXXIX.

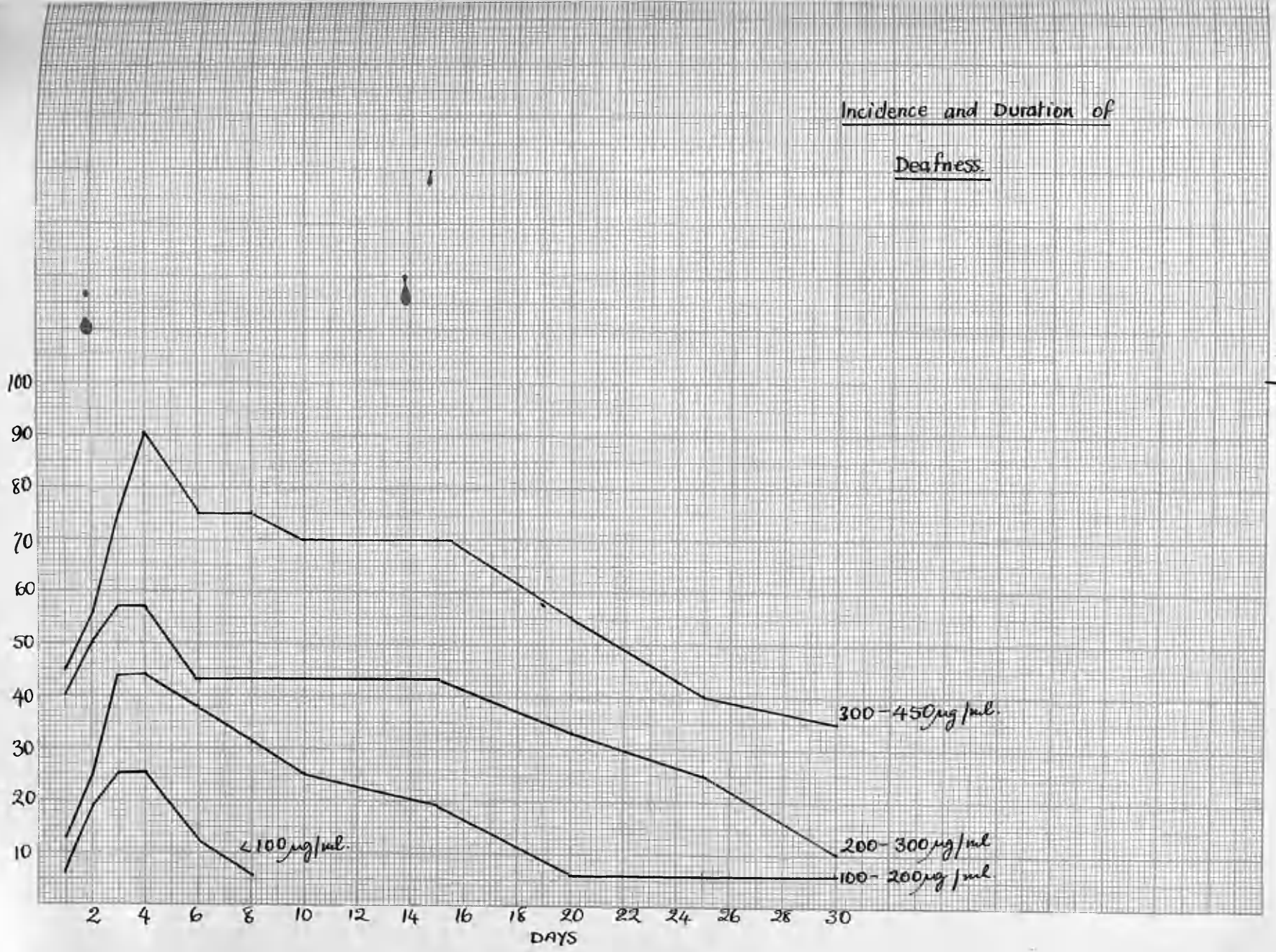
(a) Serum salicylate less than 100 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	6	19	25	25	12	6	-	-	-	-	-

(b) Serum salicylate 100-200 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	13	25	44	44	38	31	25	19	6	6	6

Graph XXVII.


Incidence and Duration of
Deafness
% age with symptoms
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
DAYS
300 - 450 µg/ml.
200 - 300 µg/ml
100 - 200 µg/ml.
< 100 µg/ml.

(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	40	50	57	57	43	43	43	43	33	25	10

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	45	55	75	90	75	75	70	70	55	40	35

6. Vertigo:

Subjective sensations of vertigo appeared to be related to the occurrence of tinnitus and deafness when the higher concentrations of salicylate were being maintained. Vertigo was seldom present in the absence of the other two symptoms.

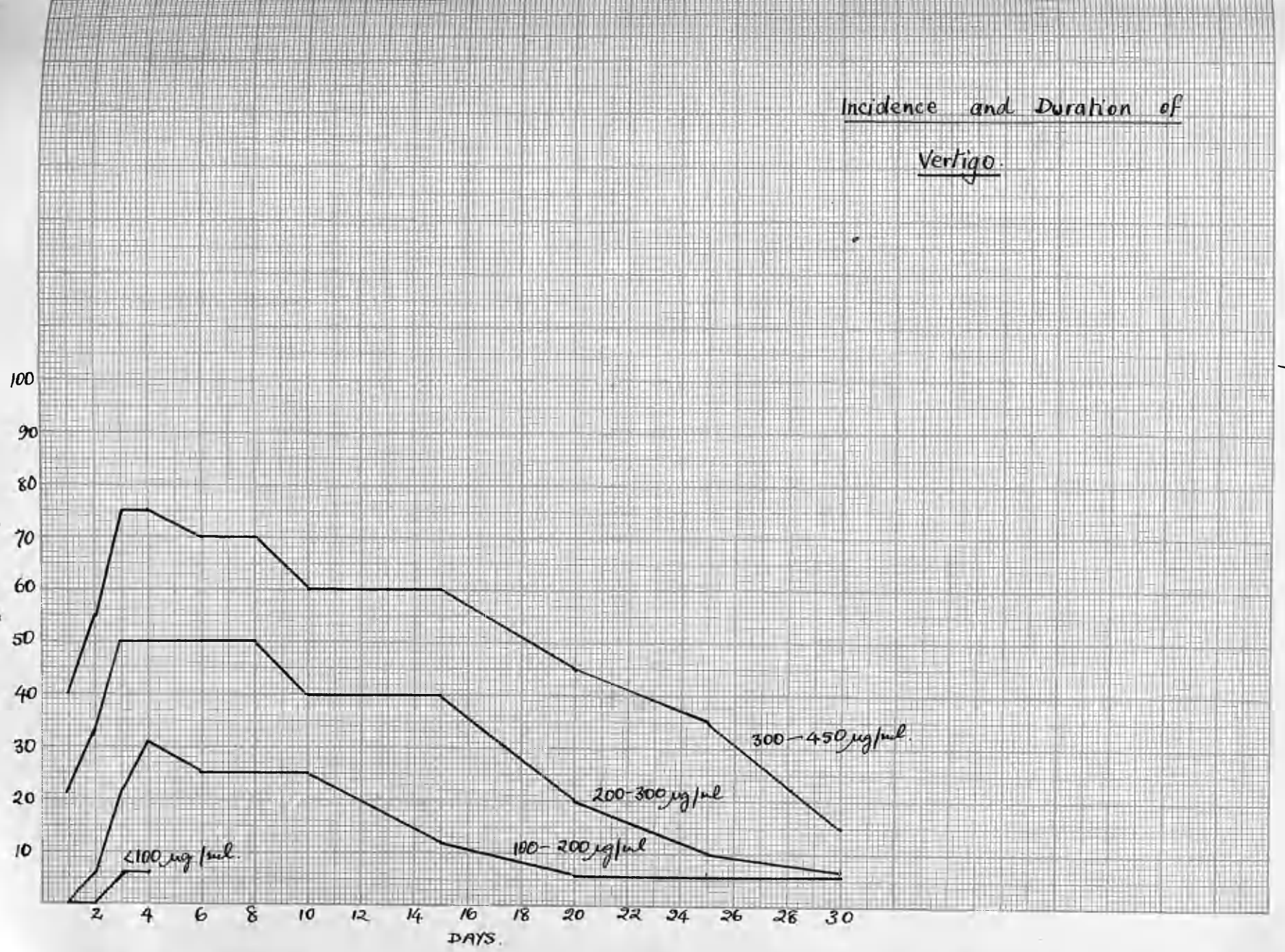
Results:

TABLE XL.

(a) Serum salicylate less than 100 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	-	0	6	6	-	-	-	-	-	-	-

Graph XXVIII.



(b) Serum salicylate 100-200 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	-	6	22	31	25	25	25	12	6	6	6

(c) Serum salicylate 200-300 µg./ml. 30 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	22	33	50	50	50	50	40	40	20	10	7

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	40	55	75	75	70	70	60	60	45	35	15

7. Headache:

Headache was present in varying degree at all concentrations of salicylate. It did not appear to have any direct relation to the other symptoms of salicylism, but as a rule when it was severe other evidence of intoxication was also present.

Results:

TABLE XLI.

(a) Serum salicylate less than 100 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	6	12	12	6	-	-	-	-	-	-	-

(b) Serum salicylate 100-200 µg./ml. 16 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	19	25	25	25	19	19	19	12	12	12	6

(c) Serum salicylate 200-300 µg./ml. 30 cases.

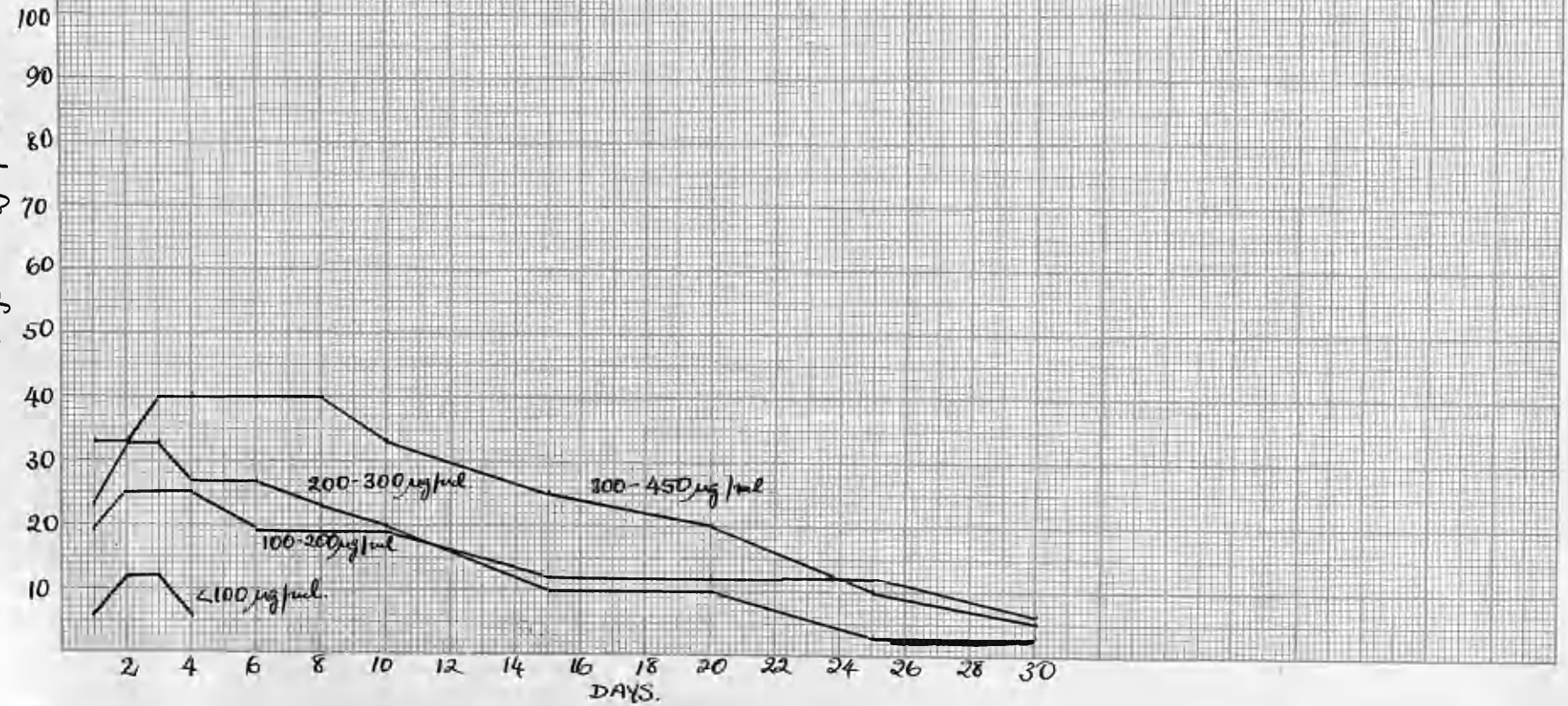
Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	23	33	33	27	27	23	20	10	10	3	3

(d) Serum salicylate 300-450 µg./ml. 20 cases.

Time (days)	1	2	3	4	6	8	10	15	20	25	30
%age affected.	33	33	40	40	40	40	33	25	20	10	5

Graph XXIX.

Incidence and Duration of Headache



Other signs and symptoms.

During the routine investigations, other well known accompaniments of salicylate therapy were observed, but the incidence of these was much lower than those recorded above. They are for that reason recorded briefly.

Proteinuria was detected in four cases whose urine was normal before therapy was begun. Within a week of cessation of salicylate administration the protein had disappeared.

Hypernoea was common in a slight degree but only in three cases was it so severe that further treatment with salicylate was regarded as inadvisable.

Mental confusion: Two subjects became confused after the serum salicylate had been maintained at a level of approximately 400 µg./ml. for three days. Eighteen hours after cessation of treatment, both subjects were perfectly clear headed.

CHAPTER VIII.

BLOOD COAGULATION AND PROTHROMBIN.

CHAPTER VIII.

Blood Coagulation and Prothrombin.

A vast literature on the subject of blood coagulation has accumulated, but the processes involved are still not fully understood, and understanding of the various theories advanced to account for the phenomenon has not been made easier by the multiplicity of terms used for what are probably identical factors. In a recent review, Milstone (1952) described the theory as being in a state of upheaval. Despite the complexity of the reactions which take place the simplified scheme proposed by Eagle (1937) based on the theory of Morawitz has been generally accepted as a working hypothesis. According to this view, when blood clots it does so because fibrinogen, a protein in the plasma, is converted to a network of insoluble fibrin in which the blood cells are enmeshed. The substance which interacts with the fibrinogen, converting it to fibrin, is thrombin. It is not generally agreed whether or not this conversion is a catalytic reaction, and the nature of the alteration which takes place in the fibrinogen molecule is still obscure. The circulating precursor of thrombin is called prothrombin, and is a protein occurring in the globulin fraction and containing 4 per cent carbohydrate. Thrombin itself does

not exist in the free state in circulating blood, since its presence would cause coagulation.

In the classical theory, the conversion of prothrombin to thrombin involves at least two other factors: thromboplastin, and Calcium ions. Thromboplastin is generally distributed throughout the tissues, and when blood is shed, disintegrating platelets liberate thromboplastin and the clotting mechanism is set in motion.

This simplified scheme can be represented thus:

$$\text{Prothrombin} + Ca^{++} + \text{thromboplastin} \longrightarrow \text{Thrombin}$$

$$\text{Thrombin} + \text{fibrinogen} \longrightarrow \text{Fibrin (clot)}$$

In parenthesis, it may be noted here that Lovelock and Burch (1951) reported experiments which they had carried out in vitro, the results of which suggested that the element calcium was not essential for clotting, but that the anticoagulant activity of oxalate, citrate, and fluoride ions was due to their ionic charge and not to their incidental property of removing calcium ions from solution.

In the present study, the effect of salicylate on the prothrombin level of blood has been investigated; it is pertinent, therefore, at this stage to refer to some characteristics of this constituent of the blood.

Brinkhous (1940) and Edsall (1944) state that prothrombin is associated with the pseudoglobulin fraction of the plasma

protein, and has a minimum solubility at pH 5.3 It can be salted out at between one third and half saturation with ammonium sulphate, and can be precipitated by the addition of acetone to plasma. It is non-dialysable through collodion and cellophane. A number of colloidal substances, such a magnesium and aluminium hydroxides, can be used to adsorb prothrombin. It is thermolabile, being destroyed by maintaining at 36°C for thirty minutes, and somewhat more slowly at room temperature. In frozen plasma it can be preserved for weeks.

Crude, but biologically active, samples of prothrombin were extracted by Mellanby (1930) and Seegers (1938).

The Source of Prothrombin.

The suggestion that the bone marrow was the source of prothrombin was made by Wohlisch (1929), but Barnes (1941) reported that the depression of bone marrow activity in animals by large doses of X-ray did not cause any alteration in the plasma prothrombin concentration.

Smith (1937) and his associates produced a hepatitis by administering chloroform to experimental animals, and observed that this effect was accompanied by a fall in the plasma prothrombin level within 24 hours, with a return to normal in about six days.

Quick (1938) made similar observations on dogs which

had been subjected to the deleterious effects of chloroform on liver function. These findings seemed to point to the liver as the site of prothrombin formation.

Stewart (1939) and Wilson (1940) working independently, showed that the administration of vitamin K in normal therapeutic dosage had little effect on the hypo-prothrombinaemia associated with obstructive jaundice, and Pohle and Stewart (1941) described a number of similar cases with extensive destruction of liver tissue in which they considered the plasma prothrombin concentration to be a useful index of liver function.

Kark (1941), however, investigated a series of subjects with obstructive jaundice, and he was unable to report any relation between the depression of prothrombin concentration and the results of the hippuric acid excretion test of hepatic function.

Lucia and Aggeler (1941) made a similar study, and reached the same conclusions.

Bay (1940) and Kohler (1940) suggested that a more accurate assessment of hepatic function might be achieved by observing the response of the prothrombin level to vitamin K administration rather than by simply making observations on the original prothrombin concentration. The same suggestion was put forward by Lord and Andrus (1941) but they did not claim that the test could be more than a crude index of hepatic efficiency.

The Estimation of the Prothrombin Content of Plasma.

Historical: Nearly forty years ago, Howell (1914) described a method of estimating the amount of prothrombin in the blood which consisted essentially of measuring the time required for coagulation after recalcification of plasma from which calcium ions had previously been removed. An improved method which was suggested by Quick (1935) depended on the addition of an excess of thromboplastin to oxalated plasma, followed by recalcification. At the same time, Quick showed that the concentration of fibrinogen in normal blood varied from 0.3-0.75 per cent, but this variation had no effect on the clotting time.

Ravidin, Riegel, and Morrison (1940) found that the normal physiological variations in the plasma of healthy individuals had also no effect on the clotting power, and Quick concluded that with a fixed concentration in optimal amount of Calcium and an excess of thromboplastin, the only variable in the factors concerned in coagulation could be the prothrombin.

The "prothrombin time" as determined by Quick's (1935) method is entirely different from that found by Howell's method. This latter figure is now known as the Coagulation Time, and normal values may be obtained when Quick's prothrombin time is only 20 per cent of normal. It is for this reason that normal coagulation time is frequently found

in subjects exhibiting haemorrhagic signs, for the prothrombin time has to be at a low level for a considerable period before any significant alteration in the coagulation time becomes apparent.

A more intricate method was developed by Smith, Warner, and Brinkhous (1937), in which the estimation was carried out in two stages. They first converted the prothrombin in defibrinated plasma to thrombin, then added fibrinogen to serial dilutions of the thrombin solution. The coagulation times observed were considered to be indicative of the prothrombin content of the original plasma.

In 1937 Quick and Leu (1937) announced that the rate of blood coagulation was a function of the concentration of thrombin, and that the production of thrombin in oxalated plasma was proportional to concentration of prothrombin, provided that excess of thromboplastin was present and that an optimum amount of calcium was added to the solution of the reactants.

Quick's method was adopted by Page (1941) but he substituted a 1:1000 solution of viper venom for the thromboplastin extract of rabbit's brain. This modification was investigated by Kleinmann (1945), who found that the use of viper venom allowed more flexibility in technique in so far as small variations in the amount of calcium ions, oxalate, or volume of blood used did not affect the final

result observed to the same extent as when rabbit's brain extract was used as the source of thromboplastin. In the same year, however, Hobson and Wills (1941) demonstrated that when Russell viper venom was used alone as the thromboplastin, the prothrombin time was affected by haemolysis, lipaemia, the platelet count in vivo, and in some cases by the speed and duration at which the blood was centrifuged. They showed further that by the addition of 5 mg. lecithin to each millilitre of serum solution, these inconsistencies were eliminated. In practice, brain tissue has been found much more satisfactory than snake venom as a source of thromboplastin, and the use of venom has been abandoned by most workers.

Method: The technique followed in this investigation for the measurement of the prothrombin content of plasma was arranged to include those refinements which appear to be essential to give consistent results, and at the same time to be as simple and practical as possible.

Apparatus and Reagents.

1. A series of uniform serological test tubes was carefully selected. These were cleaned in a bichromate-sulphuric acid mixture, rinsed overnight in running tap water, given a final rinse in distilled water, and dried in a hot air oven.

2. 0.1 Molar Sodium oxalate (Analar).

1.34 g. sodium oxalate in 100 ml. water.

3. 0.02 Molar Calcium chloride (Analar).

0.25 g. anhydrous calcium chloride was dissolved in 100 ml. water, standardised against silver nitrate solution, and adjusted by dilution to contain 0.222 g. calcium chloride per 100 ml.

4. Thromboplastin Extract.

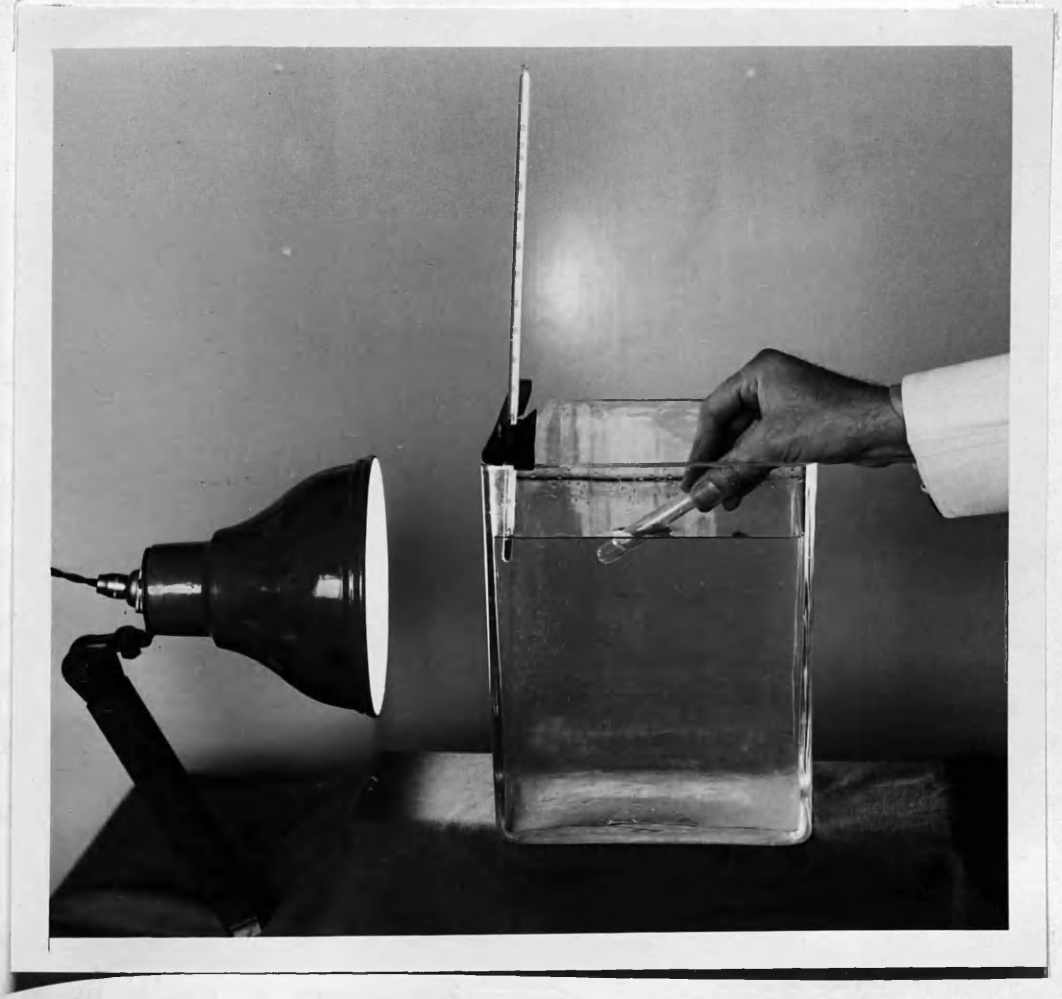
Bacto Thromboplastin (Difco) was used, and prepared for use daily. The contents of one ampoule were dissolved in 4 ml. 0.85 per cent sodium chloride solution and incubated at 45°-50°C for ten minutes with occasional gentle shaking.

Procedure: 45 ml. of venous blood were mixed immediately after collection with 0.5 ml. sodium oxalate solution, and centrifuged at 1500 revolutions per minute for five minutes. The plasma thus obtained was inspected for haemolysis; if this was evident, the specimen was discarded.

A clean, dry serological tube was placed in the 37°C water bath to allow the glass to attain the same temperature. Tubes containing the thromboplastin extract and the calcium chloride solution were treated similarly.

0.1 ml. plasma was then placed in the serological tube, followed by 0.1 ml. thromboplastin extract, and the contents mixed by rotating the tube. 0.1 ml. calcium chloride

solution was then added by forcibly blowing it in from the pipette and the stop watch started. This was done most conveniently with the aid of an assistant. The tube was then rotated gently while held in a sloping position, and closely observed for the formation of delicate fibres. The appearance of these was taken as the end point. It was found by experience that the end point could be seen more easily if the special water bath shown in the photograph was used. This consists of a pathological specimen jar



filled with water, standing on a black tile. The vessel is filled with water at 37°C, and the "Anglepoise" lamp containing a 100 watt lamp is placed so that it shines horizontally through the jar with the bulb about one inch from the glass. With this arrangement the temperature of the water is kept at a temperature of 37°-38°C, and the first appearance of the fibrin threads is easily observed.

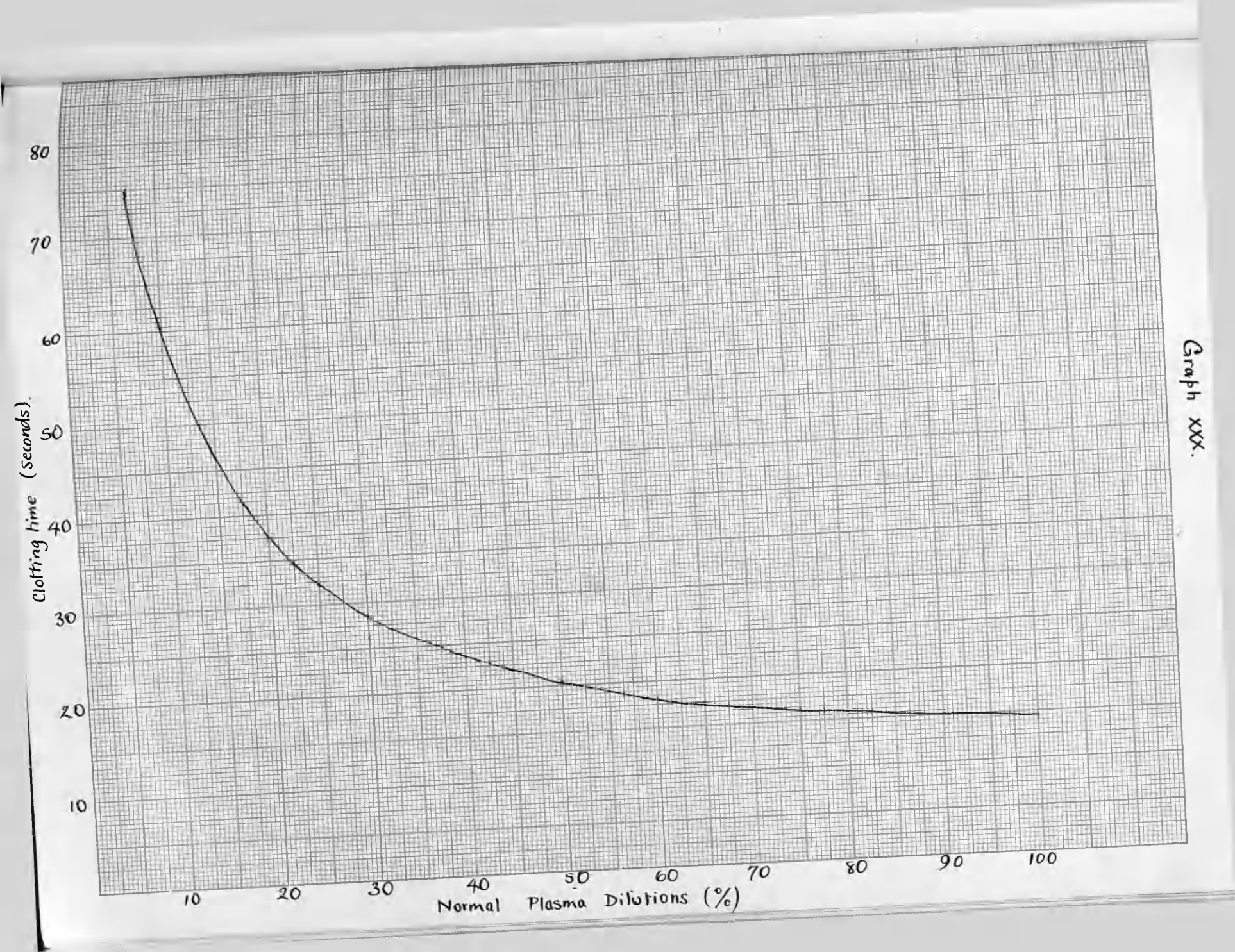
Expression of Results:

The results of prothrombin determination are expressed by some workers as a Prothrombin Index, using the formula

$$\frac{\text{Prothrombin Time of Normal Plasma} \times 100}{\text{Prothrombin Time of Test Plasma}}$$

This has been shown to give misleading results, however, (Whitby and Britton: 1950) and a more satisfactory measure of the comparative amount of prothrombin in plasma is obtained by expressing the results as Prothrombin Activity. The prothrombin time is determined as described above, using a normal plasma undiluted and at dilutions (with 0.85 per cent saline) containing 50, 30, 20, and 10 per cent plasma. From the results obtained a calibration curve is constructed. The Prothrombin Activity of the test plasma, expressed as per cent of normal, is determined by reference to the graph.

A fresh graph was made daily throughout the investigations on Prothrombin Activity reported in this work, and a typical one is shown in graph XXX.


Graph XXX.
Clotting time (seconds).
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100
Normal Plasma Dilutions (%)

CHAPTER IX.

THE EFFECT OF SODIUM SALICYLATE ADMINISTRATION ON PROTHROMBIN ACTIVITY.

CHAPTER IX.

The Effect of Sodium Salicylate administration on Prothrombin Activity.

The prothrombin activity was determined in 36 subjects who were receiving sodium salicylate orally. They were divided into three groups.

1. Twelve normal subjects.
2. Seventeen patients sufffering from various conditions.
3. Seven cases selected from patients found to be suffering from vitamin C deficiency.

Further studies with vitamin C and vitamin K were carried out on some of these subjects. These investigations are described and the results reported in later chapters.

Group 1. The Effect of the administration of Sodium Salicylate on the Prothrombin Activity of Normal subjects.

Results:

Case 1. Sodium salicylate dosage: 5 grains (0.33 g.) every four hours.

TABLE XLII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity. (%)
1	126	100
2	140	100
3	138	100
4	140	100
5	126	100
6	128	74
7	132	74
8	130	55
9	136	48
10	138	48
11	132	48
12	134	48

Total amount of sodium salicylate administered in twelve days: 24 grams.

Comment: The administration of 0.33 g. sodium salicylate

every four hours to a normal subject caused the prothrombin activity to fall to 74 on the sixth day and to 48 on the twelfth day after 24 g. had been administered.

Case 2. Prothrombin activity before administration of sodium salicylate: 112 per cent.

Sodium salicylate dosage: 5 gr. (0.33 g.) every four hours.

TABLE XLIII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity (%)
1	180	112
2	193	112
3	191	112
4	202	112
5	206	100
6	200	67
7	204	67
8	198	53
9	198	45
10	194	41
11	212	41
12	206	41

Total amount of sodium salicylate administered in twelve days: 24 g.

Comment: The administration of 0.33g. sodium salicylate every four hours to a normal subject produced a fall in prothrombin activity from 112 per cent to 100 per cent on the fifth day, and a final value of 41 per cent was obtained on the twelfth day, after 24 g. had been given.

Case 3. Prothrombin activity before sodium salicylate administration: 100%

Sodium salicylate dosage: 5 gr. (0.35 g.) every four hours.

TABLE XLIV.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity (%)
1	146	100
2	171	100
3	170	100
4	174	100
5	170	100
6	166	74
7	170	67
8	170	67
9	176	53
10	173	50
11	175	50
12	171	50
13	171	50
14	173	50

Total amount of sodium salicylate administered in fourteen days: 28 g.

Comment: In this case, an initial prothrombin activity of 100 per cent fell to 74 per cent after sodium salicylate had been given in a dose of 0.33 g. every four hours for six days,

and after fourteen days of similar treatment the prothrombin activity was reduced to 50 per cent when a total of 28 g. of the drug had been given.

Case 4. Sodium salicylate dosage: 10 gr. (0.66 g.) every four hours.

TABLE XLV.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	205	73
2	290	73
3	282	73
4	286	73
5	292	60
6	284	60
7	286	51
8	290	39
9	284	30
10	286	27
11	285	30
12	280	27

Total amount of sodium salicylate administered in 12 days: 48 g.

Comment: In this subject, four hourly sodium salicylate in a dosage of 0.66 g. produced a depression in prothrombin activity from 73 to 60 on the fifth day, and after twelve days the level was further reduced to 27 - when a total of 48 g. of the drug had been given.

Case 5. Sodium salicylate dosage: 10 grains (0.66 g.) every four hours.

TABLE XLVI.

Time (days)	Serum salicylate (µg./ml.).	Prothrombin activity
1	172	100
2	262	100
3	268	100
4	261	100
5	258	57
6	268	57
7	270	43
8	262	40
9	266	38
10	264	37
11	270	34
12	262	34
13	265	32

Total amount of sodium salicylave given in thirteen days: 52 g.

Comment: This subject showed a drop in prothrombin activity from 100 to 57 on the fifth day of medication with sodium salicylate in a dose of 10 g. four hourly: on the thirteenth day, the activity had fallen to 32 - after a total of 52 g. of salicylate had been administered.

Case 6. Prothrombin activity before sodium salicylate administration: 158%

Sodium salicylate dosage: 10 gr. (0.66 g.) every four hours.

TABLE XLVII

Time (days).	Serum salicylate (μg./ml.)	Prothrombin activity (%)
1	194	158
2	272	122
3	274	80
4	268	52
5	268	33
6	276	25
7	272	22
8	266	21
9	274	21
10	270	20
11	276	19
12	278	18
13	269	18

Total amount of sodium salicylate administered in thirteen days: 52 g.

Comment: When sodium salicylate was given four hourly in a dose of 0.66 g. to this subject, a fall in prothrombin activity from 158 to 25 took place in six days: by the thirteenth day, the prothrombin activity was 18.

Case 7. Sodium salicylate dosage: 15 grains (1.0 g.) every four hours.

TABLE XLVIII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity
1	210	100
2	332	77
3	336	68
4	330	60
5	328	52
6	337	48
7	330	41
8	335	38
9	329	34
10	330	29
11	337	24
12	332	21

Total quantity of salicylate given: 72 grams.

Comment: When given sodium salicylate in a dosage of 15 grains (1.0 g.) every four hours, this subject showed a fall in the prothrombin activity of the blood to 77 on the second day, 48 on the sixth day, and 21 on the twelfth day. When this level had been reached, the total amount of sodium salicylate administered was 72 grams.

Case 8. Prothrombin activity before administration of sodium salicylate: 100%

Sodium salicylate dosage: 15 gr. (1.0 g.) four hourly.

TABLE XLIX.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity
1	200	100
2	315	91
3	320	85
4	312	78
5	308	67
6	316	60
7	316	53
8	324	47
9	320	39
10	326	31
11	318	26
12	312	20

Total quantity of sodium salicylate given in twelve days: 72 g.

Comment: This normal case showed a fall in prothrombin activity from the initial level of 100 to 67 after five days, and a figure of 20 was reached on the twelfth day when 72 g. in all had been administered.

Case 9. Sodium salicylate dosage: 20 grains (1.33 g.) every four hours.

TABLE L.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity
1	304	100
2	450	90
3	454	81
4	446	63
5	448	45
6	451	40
7	454	38
8	444	36
9	448	34
10	449	29
11	456	22
12	453	19

Total quantity of sodium salicylate given: 96 g.

Comment: 20 grains of sodium salicylate given four hourly produced a fall in prothrombin activity from 100 to 63 in four days: by the twelfth day the prothrombin activity had dropped to 7, when 96 g. of sodium salicylate had been administered.

Case 10. Prothrombin activity before administration of sodium salicylate: 100%

Sodium salicylate dosage: 20 gr. (1.33 g.) every four hours.

TABLE LI.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity (%)
1	395	100
2	432	83
3	430	70
4	436	56
5	428	49
6	430	46
7	424	42
8	431	36
9	426	32
10	424	29
11	430	24
12	434	20

Total amount of sodium salicylate administered in twelve days: 96 g.

Comment: Sodium salicylate, when given in a dose of 1.33 g. four hourly to this normal subject, caused the prothrombin activity to fall from 100% to 49% by the fifth day of therapy, and by the twelfth day, when 96 g. had been administered, the prothrombin activity was 20%.

Case 11. Prothrombin activity before commencement of sodium salicylate therapy: 100%
Sodium salicylate dosage: 25 gr. (1.66 g.) every four hours.

TABLE LII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity (%)
1	380	100
2	460	100
3	462	78
4	458	67
5	456	59
6	464	51
7	468	47
8	460	38
9	466	31
10	462	25
11	470	20
12	466	17

Total quantity of sodium salicylate administered in twelve days: 120 g.

Comment: The prothrombin activity in this subject was 100% beofre sodium salicylate therapy was begun in a dosage of 1.66 g. four hourly. After three days it was 78%, and by the twelfth day it had dropped to 17%.

Case 12. Prothrombin activity before salicylate administration: 100%.

Sodium salicylate dosage: 20 gr. every four hours.

TABLE LIII.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity (%)
1	344	100
2	400	89
3	402	82
4	398	77
5	407	70
6	409	66
7	409	50
8	400	40
9	410	33
10	397	27
11	402	23
12	404	19

Total amount of sodium salicylate administered: 96 g.

Comment: In this subject, a fall in prothrombin activity from 100% to 77% after four days' treatment with 20 gr. sodium salicylate every four hours was observed. After twelve days, the activity was 19%.

These observations on the prothrombin activity following the administration of sodium salicylate to healthy individuals show that the depression of the activity becomes evident earlier in the investigation as higher doses of salicylate are given. Further, the effect of salicylate is cumulative to some extent since the lowest levels are produced after several days' treatment. As higher doses of the drug are given, the greatest depression of prothrombin activity appears to occur after a shorter time, just as the first effects are similarly observed earlier and the rate of depression is more rapid.

Group 1.

The Effect of Discontinuing Sodium Salicylate Administration on the lowered Prothrombin Activity.

Results:

Case 3: The total amount of sodium salicylate administered before the drug was stopped on the fourteenth day was 28 g., and the prothrombin activity then was 50.

TABLE LIV.

Time (days from commencement of sodium salicylate administration)	Serum salicylate (μg./ml.)	Prothrombin activity.
14	170	50
15	80	50
16	-	64
17	-	100
18	-	
19	-	100
20	-	100
21	-	100
22	-	100

Comment: In this case, on the fourth day after administration of salicylate had been discontinued, the prothrombin activity

had returned to its original level.

The time taken for the plasma prothrombin activity to return to normal following the discontinuance of the drug is much shorter than the time required for the salicylate to bring about the maximum depression of prothrombin activity in a normal subject.

Case 6. Total amount of sodium salicylate administered before cessation of salicylate therapy on the thirteenth day was 52 g., and the prothrombin activity had fallen to 18 by that time.

TABLE LV.

Time (days from start of salicylate administration).	Serum salicylate (µg./ml.)	Prothrombin activity.
11	274	18
12	120	39
13	-	72
14	-	158
15	-	158
16	-	158
17	-	158
18	-	158
		158

Comment: The prothrombin activity returned to its initial level on the fourth day after stopping the administration of sodium salicylate.

The time taken for the prothrombin activity to return to normal was the same in this case as in case 3, although in case 6 the dose of the drug which was administered every four hours was twice the amount given at the same intervals to case 3.

Case 11. Total amount of sodium salicylate administered when the drug was stopped on the twelfth day: 120 g. Prothrombin activity on the twelfth day: 17%

TABLE LVI.

Time (days from start of salicylate administration).	Serum salicylate. (µg./ml.).	Prothrombin activity.
12	466	17
13	310	17
14	163	27
15	-	51
16	-	78
17	-	100
18	-	100
19	-	100
20	-	100
		0
		00

Comment: In this case 120 g. sodium salicylate had been given over a period of twelve days before discontinuing medication, compared with 28 g. and 52 g. respectively which had been given to cases 3 and 6. It was observed that the prothrombin activity returned to its original level six days after cessation of the administration of salicylate, although the maximum depression of the prothrombin activity was obtained only after twelve days treatment.

Group 2.

The Effect of the Administration of Sodium Salicylate on the Prothrombin Activity of Subjects suffering from various diseases.

This group of subjects consisted of 20 cases undergoing investigation in hospital for various conditions, as follows:

1. Pernicious anaemia - 4 cases.
2. Hyperthyroidism - 3 cases.
3. Chronic myeloid leukaemia - 3 cases.
4. Rheumatoid arthritis - 3 cases.
5. Ulcerative colitis - 2 cases.
6. Acute and subacute rheumatism - 5 cases.

Sodium Salicylate Medication.

oup in a range of dosage similar to that adopted in the investigations carried out on the normal subjects.

No restriction was placed on the intake of fluid throughout the investigation.

The Effect of the Administration of Sodium Salicylate on the Prothrombin Activity of Subjects suffering from various diseases.

Pernicious anaemia.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per Kilo body weight every four hours.

Prothrombin activity before administration of sodium salicylate: 33%.

Blood picture:

Total red cell count	Hb (% Haldane)	W.B.C.
1,8000,000	44	3,100

TABLE LVII

Time (days)	Serum salicylate	Prothrombin activity
2	370	21
3	365	17
4	352	14
5	218	13
6	83	15

Comment: Before beginning the administration of sodium salicylate, the prothrombin activity was 27%. On the first day of the investigation, the activity fell to 21%

and by the fifth day a level of 13 had been reached. Salicylate medication was stopped on the fourth day because of troublesome vomiting, and on the sixth day there was some melaena. Occult blood in the faeces was detected for the next few days. No other haemorrhagic manifestations were observed. Two days after stopping salicylate administration the vomiting ceased and did not recur.

Pernicious anaemia.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Blood picture:

Total red cell count.	Hb (% Haldane)	W.B.C.
2,720,000	70	5,300

TABLE LVIII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	390	53
2	410	35
3	422	24
4	412	20
5	404	20
6	416	21
[illegible]	[illegible]	[illegible]

Comment: Before beginning salicylate therapy the prothrombin activity was 53%, and this figure fell to 35% on the second day. On the fourth day the activity had reached the low level of 20 and remained at this value for the remainder of the investigation. No toxic manifestations that could be ascribed to the salicylate were noted.

Pernicious anaemia.

Results: Case 3. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Blood picture:

Total red cell count.	Hb (% Haldane)	W.B.C.
3,610,000	86	6,500

TABLE LIX.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity (%)
1	302	100
2	318	78
3	312	52
4	324	35
5	322	22

Comment: No toxic manifestations were observed. The prothrombin activity was 100% before salicylate administration was started, but it fell to 78% on the second day of treatment, and to 20% on the sixth day.

Pernicious anaemia.

Results: Case 4. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Blood picture:

Total red cell count.	Hb (% Haldane)	W.B.C.
4,420,000	103	8.200

TABLE LX.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity (%)
1	408	112
2	428	60
3	424	35
4	430	26
5	410	21
6	416	20

Comment: No toxic manifestations were noted. The Prothrombin activity which was initially 112% fell to 20% on the sixth day and closely simulated the findings in case 3.

From the results obtained in all four cases, it appears that the greater the severity of the anaemia, the lower is the individual's prothrombin activity, and that administration of sodium salicylate to the patient will further reduce the activity.

Hyperthyroidism.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight.

Basal Metabolic Rate: +46%.

TABLE LXI.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	322	30
2	364	25
3	362	19
4	366	17
5	360	16
6	376	15
7	360	16
[illegible]	[illegible]	[illegible]

Comment: On the ninth day, the patient suffered from severe epistaxis. This may have been related to the low prothrombin activity of 16. No previous history of bleeding was obtained, and twenty four hours after cessation of medication, the condition had passed off completely.

The initial prothrombin activity of 30% fell to 15 on the sixth day.

Hyperthyroidism.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight.

Basal Metabolic Rate: +60%.

TABLE LXII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	412	53
2	443	43
3	446	29
4	442	20
5	440	19
6	438	19
7	445	19
8	[illegible]	19

Comment: This case showed no toxic manifestations. The prothrombin activity fell from its initial value of 53 to 20 after four days.

Hyperthyroidism.

Results: Case 3. Sodium salicylate dosage: 0.13 g. per kilo body weight.

Basal Metabolic Rate: +44%.

TABLE LXIII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	368	40
2	410	32
3	412	23
4	406	23
5	406	24
6	412	23
7	416	23
8	[illegible]	[illegible]

Summary. The patient's prothrombin activity fell from 46% to 23% after two days treatment with salicylate, and remained at that level until the administration of the drug was stopped on the eighth day.

Hyperthyroidism is frequently associated with disturbed liver function, and this impairment might be the cause of the initial low prothrombin activity. The administration of salicylate to such cases might depress the prothrombin activity to a dangerously low level.

Chronic myeloid leukaemia.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Blood picture.

R.B.C.	Hb (% Haldane)	W.B.C.
3,350,000	48	250,000

TABLE LXIV.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	466	24
2	475	21
3	466	18
4	480	15
5	472	13
	474	13

Comment: The prothrombin activity was 28 before therapy was begun, and fell to 24 on the first day. On the sixth day epistaxis began, and there was a purpuric rash on the arms, legs, and upper part of the chest. The blood platelet count was 19,800 - a level above that at which haemorrhage usually occurs. It was concluded that the condition was primarily the result of a lowered prothrombin level, and administration of salicylate was at once discontinued.

For the next two days there was intermittent epistaxis, then this symptom ceased, but during the following two weeks fresh crops of petechiae continued to appear. Throughout this period vitamin K was administered in a dosage of 20 mg. daily.

The gradual return to normal of the prothrombin activity is described later.

Chronic myeloid leukaemia.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight, every four hours.

Blood picture:

R.B.C.	Hb (% Haldane)	W.B.C.
4,430,000	64	87,000

TABLE LXV

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	420	29
2	424	23
3	422	19
4	428	16
5	422	15
7	426	14
8	430	15
9	430	15

Comment: The prothrombin activity was 29 on the first day of the investigation, and by the sixth day it had fallen to 15. No haemorrhagic manifestations developed in this case.

Chronic myeloid leukaemia.

Results: Case 3. Sodium salicylate dosage: 0.13 g. per body weight every four hours.

Blood picture:

R.B.C.	Hb (% Haldane)	W.B.C.
3,860,000	60	102,000

TABLE LXVI.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	436	59
2	432	55
3	438	55
4	438	43
5	430	37
6	432	30
8	436	22
9	430	18
10	432	18
11	428	18
12	436	18
13	436	18

Comment: Before salicylate therapy was begun the prothrombin activity was 59 - considerably higher than in cases 1 and 2. The maximum reduction in activity was not reached until after

nine days, when the prothrombin activity was 18. These results suggest that the time required for the haemorrhagic tendency to be brought about by salicylate medication is dependent on the individual's original prothrombin concentration.

Rheumatoid arthritis.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXVII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	298	63
2	332	63
3	336	54
4	332	54
5	338	39
6	345	29
7	340	25
8	343	23
9	336	25
[illegible]	[illegible]	22
12	336	23

Comment: The original prothrombin activity of 63 was reduced to 22 on the tenth day: the time required for maximum reduction in activity in this case compares closely with the time observed in the investigations in normal subjects.

No untoward reactions were seen.

Rheumatoid arthritis.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXVIII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	310	48
2	342	48
3	338	48
4	344	42
5	346	33
6	340	30
7	340	27
8	342	25
9	340	27

hose in case 1: the maximum depression in activity was reached after eight days, compared with ten days in case 1: but the original level was lower in this individual.

Rheumatoid arthritis.

Results: Case 3. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXIX.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	230	100
2	286	100
3	282	65
4	288	65
5	281	45
6	280	31
7	286	31
8	286	28
9	290	28

ase remained lower throughout the investigation than in cases 1 and 2, values of 290 micrograms per ml. never being exceeded. The original activity of 100 fell to 28 after eight days, whereas similar depressions occurred in the two previous cases on the sixth and seventh days respectively when higher serum salicylate levels were maintained and the initial prothrombin concentrations were lower than normal.

There were no haemorrhagic manifestations.

Chronic Ulcerative Colitis.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXX.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	232	38
2	252	35
3	290	35
4	310	29
5	312	27
6	308	23
7	312	20
8	310	17
11	308	16
12	306	15
13	310	15

Comment: The serum salicylate level did not rise much above the mean value of 300 micrograms per ml. throughout, and reasonably constant levels were not maintained until after the third day of treatment.

The prothrombin activity of 38 at the beginning of the investigation fell to 15 on the twelfth day and appeared to

have attained its maximum depression at this stage.

Despite a low initial prothrombin level which was further depressed by salicylate administration, no clinical manifestations of an increased haemorrhagic tendency were observed.

Chronic Ulcerative Colitis.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXI.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	273	38
2	294	33
3	318	27
4	322	21
5	316	21
6	320	19
7	320	18
8	324	17

Comment: The results obtained in this investigation corresponded closely to those in case 1, although the greatest depression of the activity (from the original level of 38 to 15) was observed on the ninth instead of the twelfth day.

This patient had a tooth extracted five days before the start of the investigation: there was no abnormal bleeding at the time, but the removal of a second tooth on the seventh day of the investigation - when the prothrombin

activity was 18 - was followed by profuse haemorrhage from the wound. The condition was considered to be the result of a lowered prothrombin concentration brought about by salicylate.

Acute and sub-acute Rheumatism.

Results: Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXII.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	208	100
2	268	100
3	344	70
4	340	70
5	342	54
6	339	33
7	336	23
8	338	19
		16
		17

Comment: By the third day of salicylate medication the prothrombin activity had fallen from the original level of 100 to 70, and a maximum depression to 16 was reached by the ninth day. No haemorrhagic manifestations were observed. The complaint of joint pains passed off on the third day when the prothrombin activity first began to fall, and the serum salicylate level first reached a concentration of 340-350 micrograms per ml.

Acute and Sub-acute Rheumatism.

Results: Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXIII.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity
1	246	76
2	316	76
3	376	57
4	370	37
5	362	26
6	368	22
7	375	20
8	368	17

Comment: The initial activity of 76 was reduced to 57 on the third day of the investigation, and the lowest recorded level of 16 was observed after the salicylate had been administered for nine days.

At no time were any haemorrhagic symptoms detected.

Acute and Subacute Rheumatism.

Results: Case 3. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXIV.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	190	74
2	250	74
3	288	54
4	304	54
5	312	38
6	312	35
7	318	25
8	310	20
9	314	20

Comment: Observations on this case were similar to those obtained in the preceeding investigation in cases 1 and 2. The original prothrombin activity of 74 first became reduced on the third day when the value was 54. The lowest value of 20 was reached on the eighth day. No haemorrhagic manifestations were seen throughout the entire period.

Acute and Subacute Rheumatism:

Results: Case 4. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXV.

Time (days).	Serum salicylate (μg./ml.)	Prothrombin activity
1	256	137
2	324	137
3	372	100
4	376	54
5	370	59
6	368	50
7	372	36
8	364	27
9	368	27

Comment: The prothrombin activity in this subject was 137 before salicylate medication was instituted, and this fell to 100 after the drug had been given for two days. Throughout the remaining period of the investigation the activity fell to 27 by the eighth day, remaining stationary until treatment with salicylate was stopped on the eleventh day.

It appears that a high initial prothrombin level may be

an indication of a satisfactory intake of Vitamin K in the diet, and of a normal synthesis of prothrombin in the body - both factors which would protect the body against the prothrombinopaenic action of salicylate.

Acute and Subacute Rheumatism.

Results: Case 5. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

TABLE LXXVI.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity.
1	216	100
2	280	74
3	332	100
4	342	56
5	338	42
6	330	37
7	338	28
8	342	22
9	336	20
--	344	20

Comment: From an initial level of 100 the prothrombin activity fell to 74 in the second day, but on the third day it returned to 100. On the fourth day a fall to 56 was observed, and by the ninth day the lowest level of 20 was reached.

No haemorrhagic manifestations were observed at any time.

The effect of discontinuing the Salicylate administration on the lowered prothrombin activity in certain of the cases suffering from various diseases.

Pernicious Anaemia.

Case 1. (Already described on page 147).

TABLE LXXVII.

Time (days)	Serum salicylate (μg./ml.)	Prothrombin activity
x 4	352	14
5	218	13
6	83	15
7		20
9	-	26
10	-	28
11	-	28
12	-	28

x Salicylate stopped.

Comment: Sodium salicylate administration was stopped on the fourth day because of vomiting (see page 148). The serum salicylate fell from 352 micrograms per ml. to 218

micrograms per ml. on the following day, and to 83 micrograms per ml. on the sixth day. Thereafter there was no detectable salicylate in the blood.

On the sixth day the prothrombin activity showed a slight increase from 13 to 15, and during the next four days this rise became much more marked until a final level of 28 was reached on the tenth day.

From these observations it was concluded that the prothrombinopoenic action of sodium salicylate soon passed off when medication was stopped. The prothrombin activity of this subject did not rise to the normal value of 100, but to a figure close to that determined before the investigation was begun.

Chronic Myeloid Leukaemia.

Case 3. (Already described on page 158).

TABLE LXXVIII.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity
x 7	474	13
8	312	13
9	182	13
10	74	13
11	-	14
12	-	16
13	-	18
14	-	18

x Salicylate stopped.

tration, a

in this case,

values of 18 being found on the thirteenth and fourteenth days compared with 13 when the drug was stopped. These results, compared with the activity of 24 when the investigation was begun, suggest that the synthesis of prothrombin was below normal, possibly as a result of impaired liver function caused by leukaemic infiltration of that organ.

Rheumatoid arthritis.

Case 1. (Already described on page 160).

TABLE LXXIX.

	Time (days)	Serum salicylate (μg./ml.)	Prothrombin activity.
	11	334	22
x	12	336	23
	13	220	23
	14	102	26
	15	-	30
	16	-	43
	17	-	39

x Salicylate administration stopped.

Comment: Sodium salicylate was stopped on the twelfth day

[illegible]ourteenth day

[illegible]ed on the

sixteenth day. The activity before salicylate treatment was begun was 63, and despite the administration of Vitamin K (to be described later), this figure was not reached again.

Acute and Subacute Rheumatism.

Case 2. (Already described on page 168).

TABLE LXXX.

Time (days)	Serum salicylate (µg./ml.)	Prothrombin activity.
x 8	368	17
9	210	16
10	115	16
11	-	18
12	-	24
13	-	27
14	-	50
15	-	64
16	-	64
17	-	57
18	-	76
19	-	100
20	-	76
21	-	76

x Salicylate discontinued.

Comment: Sodium salicylate was discontinued on the eighth day of the investigation, and on the eleventh day the activity began to rise. On the nineteenth day a figure of 100 was determined. This fell to 76 on the following day. As values of over 50 were found from the fourteenth day it was concluded that in this subject, synthesis of prothrombin was satisfactory.

Group 3. The effect of Sodium Salicylate administration on the Prothrombin activity of Ascorbic acid-deficient subjects.

This group consisted of seven cases selected from subjects who had been found to have varying degrees of ascorbic acid deficiency when examined in hospital, and in whom no other serious organic lesion had been discovered.

Sodium salicylate was administered orally in dosage similar to that given in the preceding groups, and before ascorbic acid had been given therapeutically.

The ascorbic acid determinations were carried out by the colorimetric method of Roe and Kuether (1943), using the Spekker absorptiometer already described.

The Effect of Sodium Salicylate administration on the Prothrombin activity of Ascorbic acid-deficient subjects.

Case 1. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.34 mg./100 ml. Plasma: 0.3 mg./100 ml.

Normal control: ascorbic acid level:

Whole blood: 1.35 mg./100 ml. Plasma: 1.1 mg./100 ml.

TABLE LXXXI.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity
1	302	67
2	398	67
3	404	53
4	410	48
5	408	38
6	402	26
7	412	21
8	416	17
9	416	17
10	418	15
11	420	17

Comment: This subject gave a history of bruising of the arms, legs, thighs, and thorax of three weeks' duration. During the investigation no increase in the severity of the condition

was observed. Before the administration of salicylate was begun, the prothrombin activity was 67. This figure fell to 53 after three days' medication with sodium salicylate. The lowest prothrombin activity was observed on the tenth day of the investigation when a figure of 15 was obtained.

The degree of depression of the plasma prothrombin activity and the rate of its fall were similar to that observed in the normal subjects previously described when serum salicylate levels of over 400 micrograms per ml. were maintained for similar periods.

Case 2. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.20 mg./100 ml. Plasma: 0.14 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 1.22 mg./100 ml. Plasma: 1.0 mg./100 ml.

TABLE LXXXII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
1	248	100
2	290	100
3	280	78
4	286	45
5	286	33
6	296	24
7	302	19
8	292	17
9	294	17
10	290	18
11	294	17

Comment: This subject gave a history of three weeks' recurrent epistaxis, and had been admitted to hospital for further investigation and treatment of anaemia. His prothrombin activity was 100 before beginning salicylate medication, but it fell to 78 on the third day of the investigation and by the eighth day was 17.

No epistaxis occurred during the investigation.

Case 3. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.24 mg./100 ml. Plasma: 0.21 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 0.98 mg./100 ml. Plasma: 0.92 mg./100 ml.

TABLE LXXXIII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
1	280	68
2	342	53
3	392	35
4	392	40
5	400	38
6	398	33
7	410	24
8	402	21
9	406	18
10	398	18
11	398	18
12	407	18
13	392	18

Comment: No history of haemorrhage was obtained in this case. The initial prothrombin activity of 68 fell to 53 on the second day and to 18 on the ninth. Similar observations were made daily until salicylate treatment was stopped on the thirteenth

day. No clinical signs of a haemorrhagic tendency were noted during the investigation.

Case 4. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.31 mg./100 ml. Plasma: 0.28 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 1.16 mg./100 ml. Plasma: 0.92 mg./100 ml.

TABLE LXXXIV.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
1	310	100
2	346	100
3	382	55
4	378	34
5	386	26
6	380	18
7	378	17
8	376	16
9	378	17
10	382	17
11	376	17

Comment: This subject complained of persistent bleeding from the gums after brushing her teeth: the condition had been present for six months. Bleeding and clotting times were normal. On the third day after beginning salicylate administration, the prothrombin activity fell from the original figure of 100 to 55, and on the eighth day to 16.

Although oral bleeding continuted it did not increase in severity, and no other haemorrhagic manifestations were seen.

Case 5. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.18 mg./100 ml. Plasma: 0.15 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 0.97 mg./100 ml. Plasma: 0.92 mg./100 ml.

TABLE LXXXV.

Time (days).	Serum salicylate (µg./ml.)	Prothrombin activity.
1	330	74
2	418	63
3	454	63
4	452	48
5	458	33
6	444	24
7	452	18
8	440	15
9	450	13
10	452	12
11	448	13
12	452	12

Comment: This patient was admitted suffering from a purpuric rash over the anterior surface of both legs, of two weeks' duration.

His initial prothrombin activity was 74, and on the second day of salicylate treatment a drop to 63 was observed. By the

tenth day the activity had fallen to 12 and on the twelfth day severe epistaxis occurred which did not subside until six days later.

Case 6. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid levels on admission:

Whole blood: 0.15 mg./100 ml. Plasma: 0.12 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 1.12 mg./100 ml. Plasma: 1.04 mg./100 ml.

TABLE LXXXVI.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
1	210	100
2	240	100
3	246	60
4	271	53
5	268	38
6	274	24
7	276	20
8	268	17
9	268	16
10	272	17
11	266	16

Comment: This patient was admitted with a nine weeks' history of intermittent haematuria. Apart from low blood ascorbic acid levels, no abnormal condition was found. The initial prothrombin activity of 100 fell to 60 on the third day following the institution of salicylate medication, and on the ninth day a value of 16 was observed. Although the prothrombin activity was considerably depressed, the haematuria ceased on the fifth

day and there was no recurrence.

Case 7. Sodium salicylate dosage: 0.13 g. per kilo body weight every four hours.

Ascorbic acid blood levels on admission:

Whole blood: 0.38 mg./100 ml. Plasma: 0.31 mg./100 ml.

Normal control ascorbic acid levels:

Whole blood: 1.0 mg./100 ml. Plasma: 0.92 mg./100 ml.

TABLE LXXXVII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
1	214	65
2	230	65
3	238	51
4	246	36
5	238	33
6	244	27
7	258	22
8	260	20
9	249	19
10	252	18

Comment: This subject's complaint was of extensive bruising of arms, face, neck, and buttocks which first appeared three weeks before admission.

The initial prothrombin activity of 65 fell to 51 after three days treatment with salicylate, and on the tenth day the activity was 18.

No further bruising or other haemorrhagic manifestations were observed.

The Effect of Discontinuance of Sodium Salicylate administration on the lowered Prothrombin Activity of certain scorbutic subjects.

Case 3. (already described on page 183).

Prothrombin activity before commencement of salicylate medication: 68

Prothrombin activity when salicylate medication was stopped: 18

TABLE LXXXVIII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
12	407	18
x 13	392	18
14	291	19
15	116	19
16	-	21
17	-	24
18	-	38
19	-	38
20	-	38
21	-	38

x Salicylate administration stopped.

Comment: Sodium salicylate treatment was stopped on the thirteenth day when the prothrombin activity was 18, and the

serum salicylate level 392 micrograms per ml. On the sixteenth day no salicylate could be detected in the serum, and the activity was then 21. Two days later the prothrombin activity had risen to 38, and it remained at this level - compared with a value of 68 before the investigation began.

Case 7. (already described on page 191).

Prothrombin activity before commencement of salicylate medication: 65

Prothrombin activity when salicylate medication was stopped: 18

TABLE LXXXIX.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
9	249	19
x 10	252	18
11	102	18
12	-	20
13	-	26
14	-	36
15	-	51
16	-	47
17	-	51

x Salicylate administration stopped.

Comment: Sodium salicylate was stopped on the tenth day, when the prothrombin activity was 18. By the twelfth day no salicylate was detectable in the blood, although the prothrombin activity had risen only to 20. On the seventeenth day an activity of 51 was observed. In this case no greater tendency was observed for the activity to return to its original value of 65 when salicylate was stopped than in case 3.

CHAPTER X.

VITAMIN K IN SALICYLATE THERAPY.

CHAPTER X.

Vitamin K in Salicylate therapy.

Vitamin K - Historical.

The discovery of vitamin K followed the work of Dam (1929) of Copenhagen, who described a haemorrhagic syndrome in chicks fed on a diet poor in fats. This haemorrhagic condition was often fatal, but later work by Dam (1935 & 1936) and others demonstrated that the administration of non-saponifiable, non-sterol fractions of hog liver fat controlled the bleeding. The active principle of the fat he called vitamin K (Koagulation-vitamin) a name that has been generally adopted.

Almquist and Stokstad (1935) confirmed Dam's observations, and Schonheyder (1936) suggested that a deficiency of prothrombin in the plasma was responsible for the haemorrhage. When vitamin K was restored to the diet of chicks which had developed haemorrhagic symptoms on a fat poor diet, the plasma prothrombin level returned to normal and the tendency to bleed was abolished.

Quick (1935) carried out investigations on patients suffering from Jaundice, and found that the prothrombin level of the plasma was reduced. Hawkins (1936) reported similar findings in dogs with biliary fistulae. From these results, Quick (1937) suggested that a lack of bile in the intestine of

man might bring about faulty absorption of the fat-soluble vitamin K and give rise to a reduction in prothrombin production. Greaves and Schmidt (1937) carried out animal experiments and their results supported Quick's hypothesis.

Following the publication of these observations, numerous reports appeared which demonstrated the value of vitamin K administration in cases of jaundice to reduce the tendency to haemorrhage, particularly before undertaking operative treatment. The first series treated in this manner was described by Warner (1938), and in the same year Butt (1938) and others at the Mayo Clinic published a similar report.

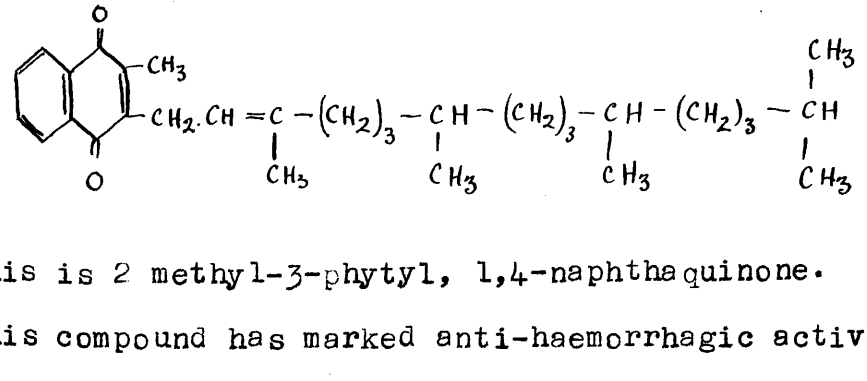
Dam (1938), Olsen (1939), and Rhoads (1939) and others made further studies and vitamin K therapy was soon placed on a sound clinical and experimental basis.

Vitamin K has a wide natural distribution. Green plants are the richest sources, although appreciable quantities occur in the animal body. Alfalfa, spinach, carrot tops, tomatoes, cabbage, and seaweed all have a fairly high content. It is present in some vegetable oils such as those obtained from soya bean and pine needles, but it is not found in any appreciable concentration in fish liver oils. The vitamin can be prepared by bacterial action on fish meal, bran, and rice. Its presence in faeces has been proved,

and is believed to be brought about by intestinal micro-organisms, especially of the coli group. The vitamin is released on putrefactive disintegration of the bacterial cells.

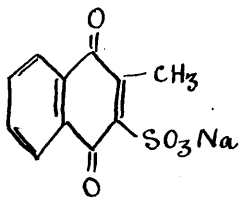
Early attempts to isolate vitamin K and determine its chemical structure were made by Almquist (1936, 1937), and he succeeded in obtaining it in a reasonably pure form, although the crystals melted to an oil at room temperature. MacCorquodale (1939) and McKee (1939) isolated two different substances from alafalfa meal and putrifying fish meal respectively: the first they named vitamin K_1, the second vitamin K_2.

Further work has shown that many substances have an antihaemorrhagic action, and Almquist and Klose (1939) showed that phthiocol, a compound isolated from the tubercle bacillus, had physical and chemical properties similar to vitamin K, and possessed some anti-haemorrhagic properties. In 1939, Buckley (1939) claimed that the chemical structure of vitamin K was represented by the formula

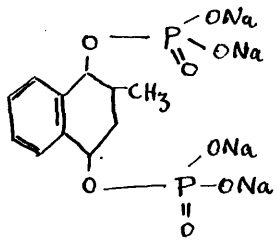


This is 2 methyl-3-phytyl, 1,4-naphthaquinone.
This compound has marked anti-haemorrhagic activity, but is only sparingly soluble in water. The water soluble sodium

salt of 2-methyl-1:4 naphthohydroquinone-3-sulphonic acid is rather less active, but is of much greater practical use in clinical medicine.



In the investigations described in this thesis, the synthetic water soluble analogue of vitamin K - the tetra sodium salt of 2-methyl-1:4 naphthohydroquinone diphosphoric acid - was used. Its structural formula is



Wilkinson (1940), in an account of the treatment of haemorrhagic disease in man, claimed that the synthetic substitutes of vitamin K were equally effective when the oral, intramuscular, or subcutaneous routes were used, provided the dosage was adequate.

Palladin (1945) suggested that treatment with vitamin K may also be effective in conditions where the blood prothrombin was normal, and he studied its effect in epistaxis, uterine haemorrhage, and trauma. He stated that in scurvy the capillary haemorrhage was reduced and that healing of wounds was stimulated.

The Effect of vitamin K on hypoprothrombinaemia following salicylate administration.

A number of cases were selected from the three groups of subjects described in chapter IX to whom sodium salicylate had been or was still being administered.

Shapiro (1944) asserted that the lowered prothrombin activity found in patients treated with aspirin could be counteracted by vitamin K, but Meyer and Howard (1943) and Clausen and Jager (1946) denied that the hypoprothrombinaemia following salicylate administration was in any way affected.

A report is given in the following pages of an investigation of the effect of vitamin K on the lowered prothrombin activity noted at different serum salicylate concentrations. The synthetic water-soluble 'Synkavit' (Roche) was used - the tetra sodium salt of 2-methyl 1:4 naphthoquinone diphosphoric acid. In the majority of cases the vitamin was given by mouth in a dosage of 20 mg. daily.

The Effect of vitamin K therapy on the depressed prothrombin activity of normal individuals receiving sodium salicylate orally.

In the first three cases described in this section, the sodium salicylate administration was stopped on the day on which vitamin K treatment was instituted.

Case 2 (already described on page 129).

Prothrombin activity before administration of salicylate: 112

Prothrombin activity when salicylate was stopped and vitamin K therapy was begun on the 12th day: 41

TABLE XL.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	206	41
	13	87	67
	14	-	112
	15	-	112
xx	16	-	112
	17	-	112
	18	-	110
	19	-	112
	20	-	110
	21	-	112

x Salicylate stopped and vitamin K begun.

xx vitamin K treatment stopped.

Comment: After stopping salicylate administration and giving 20 mg. vitamin K daily, the prothrombin activity rose from 41 on the twelfth day to 112 on the fourteenth.

It is not possible, from these observations, to conclude whether the vitamin K therapy acutally produced any significant reduction in the period required for the prothrombin activity to return to its normal value, for only 2 to 3 days were necessary for this to occur when no vitamin K was given to normal subjects in whom similar salicylate levels had been maintained.

Case 7 (already described on page 135).

Prothrombin activity before administration of salicylate: 100

Prothrombin activity when salicylate was stopped and vitamin K therapy was begun on 12th day: 21

TABLE XLI.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	332	21
	13	195	30
	14	72	57
	15	-	100
	16	-	100
xx	17	-	100
	18	-	65
	19	-	80
	20	-	100
	21	-	100

x Salicylate stopped and vitamin K begun.

xx vitamin K stopped.

Comment: In this case, the prothrombin activity rose from 21 on the twelfth day to 100 on the fifteenth day. As in case 2, there was no significant acceleration in the return to normal of the prothrombin activity although vitamin K was given: cessation of salicylate therapy alone appeared to give similar results.

It might be concluded that, in a normal individual with a normal intake and absorption of vitamin K, the return to normal of a prothrombin activity depressed by salicylate therapy is not hastened by oral administration of vitamin K.

Case 10 (already described on page 138).

Prothrombin activity before administration of salicylate: 100

Prothrombin activity when salicylate was stopped and vitamin K therapy was begun on 12th day: 20

TABLE XLII.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	434	20
	13	227	25
	14	106	42
	15	-	62
	16	-	100
xx	17	-	100
	18	-	100
	19	-	96
	20	-	100
	21	-	100

x Salicylate stopped and vitamin K begun.

xx vitamin K stopped.

Comment: The prothrombin activity of 20 on the twelfth day had risen to 100 on the sixteenth, and again it did not appear that the rate of the return to normal of the prothrombin level had been in any way influenced by vitamin therapy.

In the following three cases, similar observations were made, but the salicylate administration was continued without interruption along with vitamin K. The dosage of the latter was 20 mg. per day.

Case 1. (already described on page 127).

Prothrombin activity before administration of sodium salicylate: 100

Prothrombin activity when vitamin K therapy was begun on 12th day: 48

TABLE XLIII.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	134	48
	13	137	48
	14	127	67
	15	134	100
xx	16	135	100
	17	130	74
	18	130	55
	19	132	55
	20	136	48
	21	130	48

x vitamin K begun.

xx vitamin K stopped.

Comment: In this case the administration of vitamin K produced a rise in activity from 48 to 100 after three days although reasonably constant serum salicylate levels were

maintained.

Cessation of vitamin K treatment was followed by a fall in prothrombin activity to 48 again in four days.

Case 4 (already described on page 132).

Prothrombin activity before administration of sodium salicylate: 73

Prothrombin activity when vitamin K therapy was begun on 12th day: 27

TABLE XLIV.

Time (days).		Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	280	27
	13	280	33
	14	280	52
	15	286	61
	16	285	100
	17	282	100
xx	18	280	100
	19	286	52
	20	282	36
	21	280	30
	22	285	30
	23	286	30

x vitamin K begun.

xx vitamin K stopped.

Comment: The level of serum salicylate in this case was about 280 micrograms per ml. throughout the investigation, compared with the relatively low values of 130 micrograms per ml. in case 1.

The prothrombin activity rose from 27 on the twelfth day

to 100 on the seventeenth while vitamin K was being given. After cessation of vitamin K treatment the activity dropped back to a value of 30 within four days.

Case 12. (already described on page 140).

Prothrombin activity before administration of sodium salicylate: 100

Prothrombin activity when vitamin K therapy was begun on 12th day: 19

TABLE XLV.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	404	19
	13	400	26
	14	409	36
	15	405	58
	16	410	100
	17	406	100
xx	18	402	100
	19	408	65
	20	400	34
	21	404	21
	22	407	18
	23	410	18

x vitamin K begun.

xx vitamin K stopped.

Comment: The serum salicylate was maintained at a much higher level in this case, with a dosage of 20 gr. four hourly. The activity rose from 19 to 100 after four days treatment with vitamin K - a period similar to that observed in case 4, and slightly longer than in case 1 where the serum salicylate levels were low.

Cessation of vitamin K therapy was immediately followed by a sharp fall in the prothrombin activity to 34 in two days and to 18 after a further two days.

The Effect of Vitamin K therapy on the depressed prothrombin levels of individuals suffering from various diseases and receiving sodium salicylate orally.

Pernicious anaemia.

Case 3. (already described on page 150).

Prothrombin activity before administration of salicylate: 100

Prothrombin activity when salicylate was stopped and vitamin K therapy was begun on 7th day: 21

TABLE XLVI.

	Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
x	7	318	21
	8	260	21
	9	110	23
	10	-	29
	11	-	42
	12	-	74
	13	-	100
	14	-	100

x Salicylate stopped, and vitamin K therapy begun.

Pernicious anaemia.

Case 4 (already described on page 151).

Prothrombin activity before salicylate administration: 112

Prothrombin activity when vitamin K was begun on the 7th day (the salicylate treatment being continued) : 20

TABLE XLVII.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	7	420	20
	8	418	20
	9	420	24
	10	424	37
	11	416	60
	12	420	100
	13	422	88
	14	420	100

x vitamin K treatment begun.

Comment: In cases 3 and 4 of pernicious anaemia the prothrombin activity was about 20 after seven days' salicylate administration.

Treatment with 20 mg. vitamin K per day from the seventh day gave rise, in case 3, to an increase in the activity to 100 after six days; in case 4, the prothrombin activity reached 100 after five days even although high salicylate levels were maintained, in contrast to case 3.

These results seem to indicate that vitamin K was just as effective in counteracting the depressing effect of salicylate on prothrombin activity when administration of the drug was continued. It is possible, nevertheless, that a smaller dose of the vitamin might have been ineffective in case 4, the relatively high dose of 20 mg. per day giving similar results in both cases because it might have been in excess in case 3 and near the optimal dose in case 4.

Hyperthyroidism.

Case 1. (already described on page 152).

Basal Metabolic Rate +46%

Prothrombin activity before salicylate treatment was commenced: 30

Prothrombin activity when vitamin K treatment was commenced on the 9th day : 16

Sodium salicylate medication was continued throughout this investigation.

TABLE XLVIII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 9	366	16
10	366	17
11	361	22
12	357	28
13	363	48
14	360	63
15	366	63

x vitamin K therapy commenced.

Comment: Treatment with 20 mg. vitamin K per day was begun on the ninth day when the activity was 16. Within five days, the prothrombin activity had risen to 63 although the serum salicylate level had been maintained around a concentration of 360 micrograms per ml.

In this case, not only did the vitamin K therapy correct

the hypoprothrombinaemic effect of salicylate, but it reduced to a considerable extent the original prothrombin deficiency.

Chronic myeloid leukaemia.

Case 1 (already described on page 155).

Prothrombin activity before salicylate administration was begun: 24

Prothrombin activity when salicylate was stopped on the 7th day: 13

Treatment with vitamin K was not begun until the fourteenth day, when the prothrombin activity was: 18

TABLE C.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
14	-	18
15	-	19
16	-	25
17	-	38
18	-	53
19	-	42
20	-	53
21	-	53

Chronic myeloid leukaemia.

Case 2. (already described on page 157).

Prothrombin activity before salicylate treatment was begun:29

Prothrombin activity when vitamin K therapy was begun on the 9th day: 15

TABLE XLIX.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 9	430	15
10	428	15
11	435	19
12	439	20
13	430	20
14	433	23
15	434	24
16	436	24
17	430	22
18	432	24
19	434	26

x vitamin K therapy begun.

Comments: In case 1, there was an interval of seven days between the cessation of salicylate administration and the beginning of vitamin K therapy. Prothrombin activity rose from 18 on the fourteenth day to 53 on the eighteenth, 20 mg. of vitamin K being administered daily during this period. Over the succeeding three days the prothrombin level showed

no tendency to reach higher values. The response noted was considered fairly satisfactory in view of the low activity of 24 which was found at the beginning of the investigation before any salicylate had been given, and which had been attributed to defective function of the leukaemic liver.

In case 2, the concentration of serum salicylate was maintained a round 430 micrograms per ml. Vitamin K treatment, commenced on the ninth day when the activity was 15 (compared with an initial value of 29) brought about a rise to only 24 after six days, and no appreciable increase occurred thereafter.

From these observations, it was concluded that in case 2 the continued administration of salicylate depressed the prothrombin activity to a level which could not be counteracted by the administration of vitamin K because the leukaemic liver was unable to synthesis effective amounts of prothrombin. In case 1, although liver function was impaired, cessation of salicylate threw less strain on the leukaemic liver and aided by the vitamin K, the liver was able to synthesis sufficient prothrombin to raise the activity to 53.

Chronic Rheumatoid Arthritis.

Case 1 (already described on page 160).

Vitamin K treatment was instituted on the seventeenth day, five days after salicylate medication was discontinued.

Prothrombin activity before salicylate was begun: 63

Prothrombin activity when salicylate was stopped on the 12th day : 23

Prothrombin activity on seventeenth day: 39

TABLE CI.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 17	-	39
18	-	39
19	-	39
20	-	41
21	-	39

x vitamin K therapy begun.

Chronic Rheumatoid Arthritis.

Case 3. (already described on page 162).

Sodium salicylate medication was continued throughout this investigation without interuption.

Vitamin K therapy was begun on the ninth day.

Prothrombin activity before salicylate administration was begun: 100

Prothrombin activity when vitamin K therapy was begun on the 9th day : 28

TABLE CII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
x 9	290	28
10	296	28
11	291	33
12	304	33
13	300	35
14	296	35
15	292	35
16	301	42
17	297	45
18	290	65

x vitamin K begun.

Comment: In case 1, salicylate medication was stopped on the twelfth day and the serum was free of salicylate on the

seventeenth day of the investigation, when treatment with vitamin K was begun in a dose of 20 mg. per day. No appreciable alteration in prothrombin activity occurred from the seventeenth to the twentyfirst day while vitamin K was being administered, despite the diminution of the effect of the salicylate which must have been taking place during this period. It was considered that either the vitamin K was not being absorbed, or that in this subject there was some unusual interference with prothrombin synthesis. Before salicylate administration there was apparently a moderate impairment of prothrombin synthesis, for the activity at the start of the investigation was 63.

In case 3, a rise in prothrombin activity from 28 on the ninth day to 65 on the eighteenth was observed, despite the maintenance of a serum salicylate concentration around 300 micrograms per ml. These figures seem to indicate that absorption of vitamin K was satisfactory and that prothrombin synthesis was able to proceed normally when sufficient vitamin K was present to counteract the effect of salicylate.

Chronic Ulcerative Colitis.

Case 1. (already described on page 163).

Sodium salicylate was administered throughout this investigation.

Prothrombin activity before salicylate administration was begun: 38

Prothrombin activity when vitamin K therapy was begun on the 14th day: 15

TABLE CIII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
14	312	15
15	318	15
16	326	17
17	322	24
18	310	33
19	307	44
20	316	67
21	324	53
22	309	59
23	302	68
24	306	68
25	317	68
26	309	68

Chronic Ulcerative Colitis.

Case 2 (already described on page 165).

Sodium salicylate administration was continued throughout.

Prothrombin activity before salicylate was begun: 38

Prothrombin activity when vitamin K was begun on tenth day: 15

TABLE CIV.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
10	316	15
11	316	15
12	320	16
13	324	18
14	318	24
15	312	29
16	320	27
17	326	27
18	319	29

Comment: Both cases of ulcerative colitis showed an increase in prothrombin activity when vitamin K therapy was instituted.

In case 1, the activity rose from 15 to 67 after seven days - although there was a slight drop next day - and the activity showed little alteration thereafter.

In case 2, there was a rise in prothrombin activity from 15 on the tenth day to 29 on the fifteenth, the figure

remaining about this value during the remaining three days of the investigation.

These results seemed to indicate that in case 1, vitamin K was effective in counteracting the effect of the salicylate: in case 2, however, vitamin K had less effect, either because absorption from the damaged intestine was impaired, or synthesis of prothrombin was less efficient than normal.

Acute and Subacute Rheumatism.

Case 1. (already described on page 167).

Sodium salicylate treatment was continued throughout this investigation.

Prothrombin activity before salicylate administration was begun: 100

Prothrombin activity when vitamin K treatment was begun on the 11th day: 17

TABLE CV.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
11	334	17
12	338	17
13	330	22
14	339	28
15	342	43
16	331	74
17	334	120
18	336	100
19	330	100
20	338	70
21	340	100

Acute and Subacute Rheumatism.

Case 3. (already described on page 169).

Sodium salicylate was administered throughout in this case.

Prothrombin activity before salicylate treatment was begun: 74

Prothrombin activity when vitamin K treatment was begun on the 11th day: 20

TABLE CVI.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
11	318	20
12	316	20
13	314	25
14	318	25
15	312	38
16	320	38
17	317	54
18	323	42
19	316	62
20	310	74
21	318	74
22	312	62
23	321	62
24	326	74
25	314	74
26	314	74

Comment: In case 1, a rise in prothrombin activity from 17 on the 11th day (when vitamin K therapy was instituted) to 120 on the seventeenth day was observed, despite a concentration of serum salicylate of 330-340 micrograms per ml.

In case 3, the experimental conditions were similar, but the rise in prothrombin activity was slower, a figure of 20 rising to 42 after seven days vitamin K treatment, to 74 after nine days, and remaining around this figure thereafter.

These results suggest that considerable individual variations in the response to vitamin K treatment occur.

CHAPTER XI.

VITAMIN C IN SALICYLATE THERAPY.

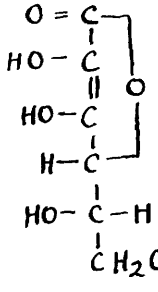
CHAPTER XI.

Vitamin C in Salicylate Therapy.

Vitamin C - Source and Distribution.

In 1924, Zilva (1924) made one of the earliest attempts to isolate vitamin C, and he succeeded in effecting a 300 fold concentration. Four years later, Szent-Gyorgi isolated from adrenal cortex and cabbage hexuronic acid.

In 1932, Waugh and King (1932) isolated the vitamin from lemon juice and identified it with hexuronic acid. The chemical constitution of the vitamin was established by the work of Haworth, Hirst, and a number of other workers in 1932, and a year later Haworth and Hirst (Ault et al) (1933) synthesized ascorbic acid. Its formula was shown to be



Ascorbic acid contains an asymmetric carbon atom, and consequently displays optical activity. The biologically active form is laevo-ascorbic acid. The dextro-form is inactive. Ascorbic acid is readily oxidized to dehydroascorbic acid, a substance which is still biologically active. The products of further oxidation possess no activity.

Vitamin C is found to the greatest extent in black currants, rose hips, citrus fruits, and many fresh green vegetables. Most animal foods are poor sources, and little is stored in the body. Here it is found in greatest concentration in the adrenal cortex.

Physiological Action.

Ascorbic acid appears to be intimately concerned with the formation of intercellular collagenous material. The manner in which the vitamin acts in this process is unknown. This function of the vitamin offers an explanation of the observations of generations of seafarers, who described the failure of wounds to heal and the weakening of ligaments in sailors the victims of scurvy.

It also appears that vitamin C is concerned with carbohydrate metabolism, but the exact relationship has not yet been made clear. It seems also to be concerned with several enzymatic processes, but the part played by ascorbic acid remains obscure.

Scurvy.

The essential pathological lesion in severe vitamin C deficiency in man appears to be a weakening of the capillary endothelial wall, giving rise to haemorrhagic manifestations at the sites of stress or trauma.

Haemorrhages may occur in the muscles and extend into

the skin causing extensive discolouration or simply a petechial rash. Epistaxis, subperiosteal haemorrhage, retinal and cerebral haemorrhage, and bleeding from the gastro-intestinal tract may be seen, with the result that a severe anaemia can soon develop, which if not promptly treated may have a fatal result.

Definite interference with the clotting mechanism of blood itself, however, has not been described in this condition.

Studies on the Effect of Ascorbic Acid on lowered Prothrombin activity caused by Salicylate Administration:

In the following series of investigations, the effect of ascorbic acid was studied when given

(a) alone, either before or after vitamin K therapy, and

(b) along with vitamin K.

Group 1: Normal subjects receiving sodium salicylate throughout the investigation, to whom 450 mg. ascorbic acid was administered daily, at first without vitamin K, and later along with 20 mg. vitamin K daily.

Case 5 (previously described on page 133).

Ascorbic acid therapy began on the thirteenth day and was continued throughout the investigation, supplemented by vitamin K (20 mg. daily) from the eighteenth day.

Prothrombin activity before salicylate administration: 100

Prothrombin activity when ascorbic acid therapy was begun on 13th day: 32

TABLE CVII.

Time (days).	Serum salicylate (μg./ml.)	Prothrombin activity.
x 13	265	32
14	260	32
15	266	38
16	263	32
17	263	34
xx 18	260	34
19	265	44
20	266	60
21	263	78
22	260	100

x ascorbic acid therapy begun.

xx combined vitamin C and K therapy begun.

Normal subjects.

Case 8. (previously described on page 136).

Ascorbic acid was administered from the twelfth day, and was supplemented with vitamin K orally from the seventeenth day.

Prothrombin activity before salicylate administration: 100

Prothrombin activity when ascorbic acid therapy was begun: 20

TABLE CVIII.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	312	20
	13	318	20
	14	324	19
	15	320	19
	16	316	20
xx	17	322	20
	18	319	24
	19	319	31
	20	320	60
	21	322	100

x vitamin C therapy begun.

xx combined vitamin C and K therapy begun.

Normal subjects.

Case 9 (Previously described on page 137).

Ascorbic acid therapy was started on the twelfth day, and vitamin K was added on the 17th day.

Prothrombin activity before salicylate administration: 100

Prothrombin activity when ascorbic acid therapy was begun: 19

TABLE CIX.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 12	453	19
13	456	19
14	448	19
15	444	19
16	456	20
xx 17	450	19
18	454	25
19	448	39
20	456	54
21	453	74
22	453	100

x vitamin C begun.

xx combined vitamin C and K therapy begun.

Comment: In each of these three cases, administration of ascorbic acid alone caused no appreciable alteration in prothrombin activity. When vitamin K was given along with

vitamin C, however, a rise in prothrombin activity at once began, and normal values were attained in the next 4 or 5 days - a period similar to that which had been already observed in other normal subjects when vitamin K had been administered alone.

These results suggest that the administration of ascorbic acid had no effect on the prothrombinopaemic action of salicylates in normal subjects.

Group 2. The Effect of Ascorbic Acid treatment on the lowered prothrombin activity due to salicylate administration in subjects suffering from various diseases.

Ascorbic acid was given daily in doses of 450-500 mg. to all cases in this group, and vitamin K in doses of 20 mg. daily unless otherwise indicated. Sodium salicylate was given uninteruptedly throughout.

Pernicious anaemia.

Case 2. (Previously described on page 149).

Prothrombin activity before beginning salicylate administration: 53

Prothrombin activity when ascorbic acid therapy began on 7th day: 20

TABLE CX.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 7	410	20
8	412	20
9	412	20
10	412	20
xx 11	414	20
12	416	20
13	420	22
14	420	31
15	414	41
16	412	44
17	406	41
18	416	41

x vitamin C begun.

xx vitamin C and K begun.

Comment: This subject received ascorbic acid 450 mg. orally per day from the seventh day when the activity was 20, but no

change in this figure was observed after four days.

From the eleventh day, vitamin K was given along with the vitamin C in a dose of 20 mg. daily, and the prothrombin activity was then observed to rise to 44 in five days - a figure which approached the initial value of 53.

These observations were similar to those noted previously in the normal cases, and again it appeared that ascorbic acid did not pay an essential part in counteracting the depressing effect of salicylate on prothrombin activity.

Hyperthryoidism.

Case 1 (previously described on pages 152 and 215).

20 mg. vitamin K had been given daily for six days before ascorbic acid (500 mg. daily) was given along with the vitamin K.

Prothrombin activity before salicylate treatment: 30

Prothrombin activity when ascorbic acid was started on 15th day: 63

TABLE CXI.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
x 15	366	63
16	360	63
17	368	55
18	364	74
19	358	63
20	360	63

x combined vitamin C and K therapy begun.

Comment: The activity was 63 when ascorbic acid treatment was commenced, and vitamin K had already been given for six days.

No appreciable elevation in the prothrombin activity took place during the period of combined vitamin C and vitamin K therapy, from which it appeared that in hyperthyroidism also, vitamin C had no apparent action in counteracting salicylate's effect on the prothrombin activity.

Hyperthryoidism.

Case 2 (previously described on page 153).

Ascorbic acid was given alone at first, in daily doses of 500 mg., then later it was combined with a dose of 20 mg. vitamin K.

Prothrombin activity before beginning salicylate: 53

Prothrombin activity when ascorbic acid therapy began on 7th day: 19

TABLE CXII.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	7	445	19
	8	440	19
	9	443	19
	10	448	21
	11	444	19
	12	440	19
xx	13	448	19
	14	448	19
	15	446	24
	16	442	33
	17	445	48
	18	445	68
	19	449	100
	20	446	100

x vitamin C therapy commenced.

xx combined vitamin C and vitamin K begun.

Comments: After six days treatment with ascorbic acid no significant change in prothrombin activity was observed; when vitamin K was administered along with the ascorbic acid from the thirteenth day, the activity at once began to rise, a figure of 100 being attained over a further period of six days.

These observations further substantiate the conclusion reached in case 1, namely, that ascorbic acid had no significant effect in counteracting the hypoprothrombinaemia of salicylate therapy.

Chronic myeloid leukaemia.

Case 1. (previously described on pages 155 and 217).

Vitamin K had already been given for seven days before combined vitamin C and vitamin K treatment was started on the twenty-first day. It will be recalled that this subject developed haemorrhagic manifestations following salicylate administration, and the vitamin K was given to counteract these effects.

Prothrombin activity before beginning salicylate administration: 24

Prothrombin activity when combined vitamin therapy was begun on the 21st day: 53

TABLE CXIII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 21	-	53
22	-	53
23	-	53
24	-	53
25	-	47

x combined vitamin C and vitamin K treatment begun.

Chronic myeloid leukaemia.

Case 2. (previously described on pages 157 and 218).

This subject received sodium salicylate throughout the investigation. Vitamin K had already been administered for ten days before this investigation with combined vitamin C and vitamin K treatment was begun. (see page 218).

Prothrombin activity before salicylate treatment was begun: 29

Prothrombin activity when combined therapy was begun on the 19th day: 26

TABLE CXIV.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
x 19	434	26
20	432	24
21	436	26
22	430	31
23	439	45
24	435	66
25	438	58
26	430	58

x combined vitamin C and vitamin K therapy begun.

Chronic myeloid leukaemia.

Case 3. (previously described on page 158).

This subject was given ascorbic acid daily (500 mg.) for seven days before combined vitamin therapy was instituted, the sodium salicylate being administered uninterruptedly throughout.

Prothrombin activity before beginning salicylate administration: 59

Prothrombin activity when vitamin C therapy was begun on the 13th day: 18

TABLE CXV.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 13	436	18
14	436	18
15	440	18
16	435	18
17	432	18
18	434	19
19	430	19
xx 20	437	18
21	435	18
22	435	20
23	433	24
24	439	28
25	441	31
26	440	38
27	433	43
28	438	41

x vitamin C begun.
xx combined vitamin therapy begun.

Comment: When ascorbic acid therapy was begun on the twenty-first day of the investigation on case 1, the prothrombin activity had already increased to 53 over the seven day period of vitamin K treatment. No further increase in activity was detected during the succeeding four days of combined therapy.

Case 2: This subject had already been given vitamin K for ten days before the combined vitamin treatment was started, and the activity had risen slowly from 15 to 26 during this period (see page 218). On the twenty-fourth day, five days after ascorbic acid treatment had been begun, the activity was 66.

Case 3: This case began treatment with ascorbic acid alone on the thirteenth day, seven days before vitamin K treatment began, and during the period of vitamin C administration the prothrombin activity remained at 18. When vitamin K was given along with ascorbic acid, however, a rise in prothrombin activity immediately set in, and after another seven days a value of 43 was obtained.

From these results it is seen that in cases 1 and 3, ascorbic acid alone produced no alteration in prothrombin activity. In case 2, however, the slow rise in activity observed when vitamin K was given alone appeared to be accelerated when vitamin C was given along with the vitamin K. This seems to suggest that ascorbic acid plays a part in

the synthesis of prothrombin, and that in cases of hypoprothrombinaemia with asymptomatic ascorbic acid deficiency, vitamin K therapy will not by itself be effective in restoring the prothrombin activity to normal.

The effect of ascorbic acid on the depressed prothrombin activity of subjects with definite vitamin C deficiency is described later.

Chronic Rheumatoid Arthritis.

Case 2. (already described on page 161).

This case received ascorbic acid, 500 mg. daily, for five days before combined treatment was begun, and sodium salicylate was given throughout the investigation.

Prothrombin activity before salicylate was begun: 48

Prothrombin activity when ascorbic acid was begun on the 8th day: 25

TABLE CXVI.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 8	342	25
9	340	27
10	344	27
11	344	27
12	338	25
xx 13	342	27
14	348	27
15	348	30
16	346	38
17	338	42
18	342	46
19	347	57
20	340	66
21	340	76
22	348	66
23	339	100

x ascorbic acid begun.
xx combined treatment begun.

Chronic Rheumatoid Arthritis.

Case 3. (already described on pages 162 and 221).

This subject received sodium salicylate throughout. Vitamin K (20 mg. per day) had already been given from the ninth day of the investigation (page 221) and combined vitamin C and K therapy was started on the eighteenth day.

Prothrombin activity before beginning salicylate: 100

Prothrombin activity when combined therapy was begun on the 18th day: 65

TABLE CXVII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 18	290	65
19	286	65
20	286	65
21	293	76
22	290	65
23	288	65

x combined vitamin C and K therapy begun.

Comment: When 500 mg. ascorbic acid was given daily to case 2, from the 8th to the 13th day, the prothrombin activity remained unaltered. Following the institution of vitamin K therapy in addition to ascorbic acid, the activity began to rise and a figure of 100 was obtained after ten days combined therapy. In this case it appeared that ascorbic acid by itself lacked

any effect on the hypoprothrombinaemia.

Daily administration of 2C mg. of vitamin K to case 3 for nine days had raised the prothrombin activity from 28 to 65. When ascorbic acid was given, no further increase was observed. Again it was concluded that the vitamin C was without effect on hypoprothrombinaemia.

Chronic Ulcerative Colitis.

Case 2. (previously described on pages 165 and 224).

The serum salicylate concentration was maintained at a constant level throughout the investigation in this subject. 20 mg. vitamin K had been given orally for eight days before the combined vitamin C and K therapy was begun on the eighteenth day.

Prothrombin activity before salicylate treatment was begun: 38

Prothrombin activity when combined vitamin therapy was begun on 18th day: 29

TABLE CXVIII.

Time (days).	Serum salicylate (µg./ml.).	Prothrombin activity.
x 18	319	29
19	322	26
20	322	29
21	329	39
22	331	52
23	326	78
24	328	66
25	322	66

x combined vitamin C and K therapy begun.

Comment: 20 mg. vitamin K daily produced a rise in prothrombin activity in this case from 15 to 29 over a period of eight days.

Three days after combined therapy with vitamins C and K, the prothrombin activity was 39, and after a further two days treatment this figure had risen to 78.

In this patient suffering from chronic ulcerative colitis, the administration of ascorbic acid appeared to play an important part in hastening the return to normal of the prothrombin activity: a return which apparently therapy with vitamin K alone could not achieve.

Acute and Subacute Rheumatism.

Case 5 (previously described on page 172).

Sodium salicylate was administered to this subject throughout the investigation.

Ascorbic acid therapy was begun on the eleventh day, and combined vitamin C and K treatment on the fifteenth day.

Prothrombin activity before beginning salicylate administration: 100

Prothrombin activity when ascorbic acid was begun on the 11th day 20

TABLE CXIX.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	11	344	20
	12	348	21
	13	342	19
	14	338	20
xx	15	346	20
	16	346	20
	17	339	22
	18	342	30
	19	346	51
	20	345	66
	21	341	56
	22	338	74
	23	342	100
	24	342	111
	25	345	100

x vitamin C therapy begun.
xx combined vitamin C and K therapy begun.

Comment: This case of acute rheumatism showed no alteration in prothrombin activity during the five days she received 450 mg. of ascorbic acid daily. When, however, vitamin K was added (20 mg. per day), the prothrombin activity rose to 66 in five days and after a further four days a value of 111 was obtained.

From these results it was concluded that this patient's prothrombin synthesis was unaffected by ascorbic acid administration.

The Effect of treatment with Ascorbic Acid on the lowered prothrombin activity produced in Scorbutic subjects by the administration of Sodium Salicylate.

Case 1. (previously described on page 180).
The administration of Sodium salicylate was continued throughout this investigation.

Ascorbic acid treatment was begun on the twelfth day (450 mg. per day), and contained vitamin C and K therapy on the sixteenth.

Prothrombin activity before beginning salicylate administration: 67

Prothrombin activity when ascorbic acid treatment begun on the 12th day: 16

TABLE CXX.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 12	417	16
13	412	16
14	418	15
15	411	16
xx 16	412	16
17	420	16
18	422	20
19	416	24
20	410	31
21	418	44
22	421	78
23	417	100
24	420	100

x ascorbic acid begun. xx combined vitamin C and K begun.

Comment: During the four days of treatment with ascorbic acid alone, the prothrombin activity remained stable at a figure around 16. Seven days after combined therapy was begun, however, the activity had risen to 100.

Again it was concluded that ascorbic acid alone was unable in this case to influence the prothrombin activity.

Case 2. (previously described on page 182).

This case also received sodium salicylate without interruption.

Vitamin K had been given for seven days before combined vitamin C and K therapy was begun.

Prothrombin activity before beginning salicylate: 100

Prothrombin activity when combined vitamin C and K therapy begun on the 18th day: 33

TABLE CXXI.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 18	308	33
19	312	33
20	306	45
21	300	62
22	302	78
23	308	111
24	300	100

x combined vitamin C and K therapy began.

Comment: The prothrombin activity rose from 17 to 33 during the seven days treatment with vitamin K alone. The administration of ascorbic acid together with vitamin K was followed by a rise in prothrombin activity from 33 to 111 in five days.

It was concluded that the more rapid rise in activity

during the latter part of this investigation might be attributed to the ascorbic acid potentiating the effect of the vitamin K, since vitamin K alone had failed to produce such a marked rise in activity.

Case 3. (already described on page 183).

Salicylate administration had been stopped on the thirteenth day, when the prothrombin activity was 18. Ascorbic acid therapy was begun on the twenty-first, when the prothrombin activity had risen to 41. The figure had been constant at this level for three days. Combined vitamin C and K therapy was started on the twentyfifth day.

Prothrombin activity before salicylate administration: 68

Prothrombin activity when ascorbic acid therapy began on the 21st day: 41

TABLE CXXII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 21	-	41
22	-	38
23	-	38
24	-	38
xx 25	-	44
26	-	41
27	-	41
28	-	44
29	-	44
30	-	44

x ascorbic acid begun.

xx combined vitamin therapy begun.

Comment: During the period of treatment with ascorbic acid alone, the prothrombin activity showed no tendency to rise. After five days combined vitamin treatment, a slight rise to 44 was observed: the original figure at the beginning of the investigation was 68.

From these observations it was concluded that combined vitamin therapy in doses similar to that given to the cases already described failed to produce a marked increase in prothrombin concentration in the plasma: this may have been due to some impairment of absorption or to some obscure disturbance of synthesis of prothrombin in this particular patient.

Case 4. (previously described on page 185).

The concentration of serum salicylate was kept at about 370-380 micrograms per ml. throughout this investigation.

Combined vitamin C and K treatment began on the eleventh day. Seven days later the ascorbic acid was discontinued, but vitamin K was given throughout. On the twenty-fourth day, combined therapy was resumed.

Prothrombin activity before beginning salicylate treatment: 100

Prothrombin activity when combined vitamin therapy was first started on the 11th day: 17

TABLE CXXIII.

overleaf

TABLE CXXIII.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 11	376	17
12	370	22
13	378	34
14	372	50
15	380	76
16	374	100
xx 17	374	100
18	379	100
19	381	66
20	372	76
21	376	66
22	375	56
23	383	50
xxx 24	371	44
25	374	43
26	370	44
27	378	64
28	382	100
29	374	100

x combined vitamin therapy begun.
xx ascorbic acid discontinued.
xxx combined therapy resumed.

Comment: Combined vitamin C and K therapy produced a rise in prothrombin activity from 17 on the eleventh day to 100

on the sixteenth. Two days later the bleeding from the gums was considerably reduced and after another seven days there was no evidence of haemorrhage anywhere in the body.

From the eighteenth to the twenty-fourth day no ascorbic acid was given, and the prothrombin activity fell to 44 over this period.

Treatment with ascorbic acid as well as vitamin K was resumed on the twenty-fourth day, and in four days a rise in prothrombin activity to 100 had taken place.

From these observations it appeared that ascorbic acid played an important part in the mechanism associated with the return to normal of the plasma prothrombin concentration in this patient, since cessation of vitamin C administration resulted in a drop in prothrombin activity to subnormal values, despite the maintenance of vitamin K therapy.

Case 5. (previously described on page 187).

The concentration of serum salicylate was maintained throughout at a level of 430-450 micrograms per ml. Vitamin K (20 mg. daily) was given from the twelfth to the twenty-first day. On the twenty-first day, vitamin C (500 mg. daily) was begun and the vitamin K stopped. On the twenty-sixth day, combined therapy with both vitamins was resumed.

Prothrombin activity before salicylate administration: 74

Prothrombin activity when vitamin K treatment began on 12th day: 12

TABLE CXXIV.

overleaf

TABLE CXXIV.

	Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x	12	452	12
	13	448	12
	14	450	13
	15	446	15
	16	440	21
	17	442	26
	18	449	38
	19	440	48
	20	447	43
xx	21	441	43
	22	444	39
	23	444	31
	24	448	23
	25	452	17
xxx	26	443	15
	27	447	15
	28	448	22
	29	440	26
	30	441	33
	31	440	48
	32	446	100
	33	449	100

x Vitamin K therapy begun.
xx Vitamin K stopped, vitamin C begun.
xxx Combined vitamin C and K therapy begun.

Comment: When vitamin K was given to this subject, there was an increase in prothrombin activity from 12 to 48 in seven days.

Replacement of vitamin K therapy by ascorbic acid (500 mg. daily) on the twenty-first day resulted in a drop in activity to 15 in five days. The resumption of ascorbic acid and continuation of vitamin K administration brought the activity back to 100 after six days.

These results suggested that although ascorbic acid by itself did not have any apparent beneficial effect on the depressed prothrombin activity, it increased the effect of vitamin K in restoring the plasma prothrombin concentration to normal.

Case 6. (previously described on page 189).

Sodium salicylate was administered to this patient throughout the investigation.

Ascorbic acid (500 mg. daily) was given from the eleventh to the seventeenth day, and vitamin K alone (20 mg. daily) from the seventeenth to the twenty-fourth day. From this time to the end of the investigation on the twenty-eighth day, both vitamins were given.

Prothrombin activity before beginning salicylate administration: 100

Prothrombin acitivity when ascorbic acid therapy began on the 11th day: 16

TABLE CXXV.

overleaf

TABLE CXXV.

Time (days).		Serum salicylate (μg./ml.).	Prothrombin activity.
x	11	266	16
	12	264	17
	13	260	17
	14	260	17
	15	267	16
	16	272	16
xx	17	262	16
	18	266	17
	19	274	20
	20	263	27
	21	260	44
	22	272	50
	23	270	49
xxx	24	266	49
	25	260	60
	26	270	100
	27	272	112
	28	264	100

x ascorbic acid begun.
xx ascorbic acid stopped, vitamin K begun.
xxx combined vitamin therapy begun.

Comment: During the period (11th to 17th day) that this patient received only the vitamin C, the prothrombin activity did not alter appreciably, but when ascorbic acid therapy

was replaced by vitamin K treatment, the activity rose from 16 to 49 in six days. Combined vitamin C and K therapy restored the activity to the original figure of 100 in three days.

These results seemed further to support the conclusion that a deficiency of ascorbic acid tended to reduce the beneficial effect of vitamin K treatment on the hypoprothrombinaemia of salicylate therapy.

Case 7. (previously described on page 191).

The administration of sodium salicylate to this subject had been stopped on the tenth day. On the seventeenth day, ascorbic acid (500 mg. per day) administration was started, and continued to the end of the investigation, supplemented by vitamin K in a dose of 20 mg. per day from the twenty-second day.

Prothrombin activity before beginning salicylate administration: 65

Prothrombin activity when ascorbic acid therapy was started on the 17th day: 51

TABLE CXXVI.

Time (days).	Serum salicylate (μg./ml.).	Prothrombin activity.
x 17	-	51
18	-	51
19	-	46
20	-	42
21	-	51
xx 22	-	51
23	-	51
24	-	51
25	-	51
26	-	51
27	-	51

x ascorbic acid begun.
xx combined therapy begun.

Comment: This subject showed no appreciable change in prothrombin activity during the whole period from the seventeenth to the twenty-seventh day, irrespective of whether ascorbic acid was given alone or in conjunction with vitamin K.

As the prothrombin activity had risen from 18 to 51 during the period between the cessation of salicylate administration and the beginning of ascorbic acid treatment, it appeared that there was no gross defect in absorption of vitamin or synthesis of prothrombin in this case to explain the failure, later in the investigation of vitamin C and K therapy to restore the prothrombin activity to its original value.

CONCLUSIONS.

Conclusions.

The present study has embraced a consideration of several factors which it was thought might be related to the concentration of salicylate in the serum of individuals receiving the drug, and the investigations of the author are reported. As a first step, the reliability of an accepted method of measuring serum salicylate was critically investigated, as appreciable inaccuracies would lead to false interpretations of the results of chemical analyses.

It appears from the findings of the experiments described in Chapter IV that a number of factors to which Brodie makes no reference in his original account have a marked effect on the accuracy of the analytical results. If the volume of serum taken for analysis is that suggested by Brodie and adopted by Coburn, and the concentration of salicylate is low - as may be the case when dosage is inadequate - the method is not precise enough to give accurate results. With serum levels above 150 μg/ml., however, the method is satisfactory, provided that strict attention is paid to a number of details.

The optical density of the coloured iron-salicylate complex was found to be markedly affected by the pH of the final solution. The apparent lower percentage of salicylate found - particularly when the actual concentration is low - when citrate or oxalate was used as an anticoagulant compared with the results found when serum was analysed may be caused

by the anticoagulants altering the pH of the solution.

Brodie does not refer to the effect on the result of delay between collection of the serum and its analysis, or to the stability of the colour of the final solution. These two factors have been investigated, and the results indicate that refrigeration at 4°C permits a delay of thirty-six hours to elapse without marked error arising; when the blood cannot be stored under these conditions, the absolute maximum period which should elapse between collection and analysis is twenty-four hours. This last point is of some importance in districts where the laboratory is some distance from the hospital. The optical density of the final coloured solution should be measured with the minimum delay, if possible within thirty minutes of preparation of the complex.

In studying some of the general factors which might influence serum concentrations, it was considered of value to investigate the effect of administering different doses at different time intervals. To obtain serum levels of 350 micrograms per millilitre - the optimum figure given by Coburn (1943) - a dose of 0.66 grams every two hours, or 1.33 grams every four hours, was found necessary: and with this dosage, satisfactory levels were not obtained until the second day of therapy. To obtain similar levels within 24 hours of beginning treatment, higher dosage was required. These findings are similar to those reported by Coburn, and lead to the conclusion that to obtain optimum serum concentrations

in the shortest time, high dosage must be given initially and continued at frequent intervals.

The results of the investigations of the relative rates of absorption and excretion of sodium salicylate when administered by various routes show that when the drug is given per rectum, absorption is more rapid than when the oral route is used: the rate of excretion, however, is also more rapid, so that of the two methods of administration, the conventional oral route is to be preferred. Coburn (1943) advised that in acute rheumatism, salicylate should be given intravenously: the results reported in Chapter V show that when this route is adopted excretion is rapid and the high concentrations obtained within a short time of injection cannot easily be maintained by this method alone. Except in special circumstances, therefore, administration by mouth is the method of choice, particularly when it is borne in mind that many cases requiring salicylate therapy are treated at home without special nursing facilities, and under conditions where repeated intravenous medication is not without its hazards.

The importance of the relationship between the volume of fluid intake and output and the maintenance of optimal serum concentrations of the drug has been investigated. The results lead to the conclusion that, in the absence of contraindications such as hyperpyrexia, excessive sweating, or impairment of renal function, the restriction of fluid

intake to about 3 pints in 24 hours might with advantage be adopted.

When sodium bicarbonate was administered concurrently with sodium salicylate, it was observed that the urine volume increased and at the same time serum salicylate values fell. When, however, fluid intake was restricted, sodium bicarbonate administration did not produce a significant drop in serum salicylate levels. Smull (1944) had observed a depression of serum salicylate following sodium bicarbonate administration, but she does not appear to have pursued the subject further. The present writer subscribes to the view that the fall in serum salicylate following administration of alkali may be due to the diuretic action of the latter causing an increased rate of excretion of the salicylate.

The concentration of salicylate obtainable in the blood in normal subjects has been compared with the levels in patients suffering from a variety of diseases who were given similar doses of the drug. In cases of acute rheumatism the serum concentration was found to be lower than in normal subjects, suggesting either an impairment in the rate of absorption from the alimentary tract of an increased rate of destruction: or perhaps increased retention in the tissues.

In addisonian or pernicious anaemia the blood levels were similar to those found in the normal controls. Chronic myeloid leukaemia was associated with relatively higher levels, and it is suggested that in this condition the

reason may be either that kidney function is impaired and excretion therefore diminished, or leukaemic infiltration of the liver interferes with the rate of destruction of the salicylate.

Chronic ulcerative colitis was associated with blood concentrations lower than normal. The explanation advanced is that in this condition absorption is impaired and abnormal amounts are lost in the frequent fluid stools.

In the cases studied in this work, the common signs noted were similar to those which Quincke (1882) described - namely, epigastric discomfort, nausea, vomiting, tinnitus, deafness, vertigo, and headache. These were studied in relation to their incidence and duration when various concentrations of salicylate were maintained in the blood. It was noticed that, contrary to what is usually observed in any intoxication as distinct from idiosyncrasy, the toxic effects were most in evidence during the early stages of therapy, tending to lessen in severity as treatment was continued. The clinician, therefore, should not regard these signs, when present to a moderate degree, as indications for modifying treatment, but should rather accept them as evidence of satisfactory absorption.

Epigastric discomfort was a common complaint, and was present even when the serum salicylate was low. Nausea,

on the other hand, was most in evidence when higher concentrations were being maintained. With levels below 100 micrograms per millilitre vomiting was absent. Coburn's (1943) assertion that salicylate medication should never be stopped unless manifestations of intoxication were severe appears to have been verified by the results reported here, for only in three cases was it deemed advisable to discontinue its administration. Tinnitus, like nausea, was uncommon when blood salicylate was low. The incidence and duration of this symptom was similar to that of nausea and vertigo, although these manifestations were not always present at the same time in the same case. Deafness was commoner than tinnitus at all serum concentrations, and was often persistent after tinnitus had passed off.

The incidence of headache was very variable, and seemed to be quite unrelated in its occurrence to the other toxic effects, and was almost as common at low serum levels as when the concentration of the drug in the blood was high.

The prothrombin activity of the plasma was studied in subjects receiving sodium salicylate, and the results reported in detail confirm the observations of Shapiro (1943) and Link (1943) that salicylate medication is frequently accompanied by a drop in blood prothrombin. The effect of the salicylate varies in different individuals, and that the

process is reversible is clear from the tabulated results. Generally, when treatment was stopped the prothrombin activity returned to normal; but occasionally some degree of hypoprothrombinaemia persisted. Low activities after cessation of salicylate treatment were usually found in those subjects in whom the activity had been subnormal before the drug had been given.

Serious bleeding did not occur in any of the cases investigated, but melaena was observed in one case of pernicious anaemia, a purpuric rash appeared in leukaemia, and one subject suffering from hyperthyroidism developed epistaxis. All these symptoms were associated with a lowered prothrombin activity occurring during treatment with sodium salicylate, and it seems established that the drug was the cause.

The efficiency of vitamin K in counteracting the hypoprothrombinaemia produced by salicylate therapy which Shapiro (1944) described has been confirmed. In some cases however, the response to vitamin K was less than was expected, and this suggested that some other factor might be lacking in these subjects. Shapiro had found in one of his cases that the prothrombin activity could not be restored to normal until treatment with ascorbic acid in addition to vitamin K was instituted; and this led to the decision in the present study to investigate the value of ascorbic acid in counteracting hypoprothrombinaemia of salicylate therapy. The

results reported in this thesis show that ascorbic acid, when administered concurrently with vitamin K, can produce a satisfactory anti-prothrombinopaenic effect. The investigations carried out on the scorbutic subjects demonstrate this more markedly.

The observations made seem to suggest that some relationship may exist between vitamin K and ascorbic acid which is associated with prothrombin production in the liver, and the action of salicylate in lowering the prothrombin content of the blood may be a result of interference by the drug with certain hepatic enzyme systems.

The general conclusion reached from these studies is that there is some evidence for administering both vitamin C and vitamin K to patients suffering from acute rheumatism who are being treated intensively with salicylate, if a tendency to haemorrhage is to be avoided.

SUMMARY.

1. The literature on the pharmacology of sodium salicylate in rheumatism is reviewed.

2. An investigation of Brodie's (1944) method of measuring the salicylate content of serum is reported, and certain practical precautions described.

3. A number of general factors affecting serum salicylate concentrations were investigated, and the effect of administering different amounts of sodium salicylate at different frequencies and by different routes have been studied.

4. The effect of sodium bicarbonate administration on serum salicylate concentrations under different conditions of fluid intake has been studied.

5. The level of serum salicylate attained in certain diseases under standard conditions of dosage has been investigated.

6. The duration and incidence of certain manifestations of salicylism in subjects in whom various serum salicylate concentrations were being maintained is described.

7. The effect of sodium salicylate on the prothrombin activity of the plasma in vivo was studied in normal subjects, a series of individuals under treatment for a variety of conditions, and a series of patient suffering from ascorbic

acid deficiency in varying degrees.

8. The effect of vitamin K administration, with and without ascorbic acid administration, on the hypoprothrombinaemia caused by salicylate therapy is reported.

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