"Chemical Studies of Synthetic Therapeutic Compounds"

Thesis

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Ronald David Watson, B.Sc.,

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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Contents.

Introduction.

Although the science of chemotherapy may truly be said to have been introduced by Ehrlich, it is with the synthesis of salicylic acid by Kolbe, and its application as an anti-rheumatic drug by Buss, that the first real union of synthetic organic chemistry with medical science is recorded. The work of Ehrlich established the important new concept of specific active groupings in each therapeutic agent, and his investigations were carried out with a breadth of vision sufficient to gather together the material foundations of a new science, but the work of Kolbe and Buss constituted a search for the active principle in an old remedy, the isolation and characterisation of the principle, and the confirmation of the formula by synthetic methods culminating in the replacement of the variable natural product by the pure synthetic product as a therapeutic agent. It is therefore not unfitting that "Chemical Studies of Therapeutic Compounds" should be concerned primarily with a search for further anti-rheumatic drugs with the valuation and assessment of the pharmacological and biochemical changes resulting from salicylate administration forming a basis for the project.

Such basic work is naturally predominantly clinical in nature. It is the physician who must be familiar with the natural course of the disease, so that he may distinguish the abnormalities which result from it, and as far as possible recognise clearly the characteristic

primary effects of the disease. When the disease process can be related to a well defined abnormality, speculation as to the mode of action of a drug is possible, and the search for new drugs can continue on a basis of hypothesis and trial. Fortuitous discoveries may still occur, but the ordered progress of chemotherapy, as of the other sciences, must be based on theoretical conception and practical examination. When the physician has succeeded in determining a characteristic effect of the disease, further progress is possible.

From the practical standpoint, it is also most helpful if the disease can be reproduced in animals. The same characteristic abnormality may then be followed by a pharmacologist working with laboratory animals such as mice and rats, so that initial trial of new drugs may be completed much more quickly than is possible with patients. At the same time, the pharmacologist determines the toxic dose and if possible the effective therapeutic dose of the drug in question, so that before healthy adults or patients are given the drug not only the limits of safety in dosage, but also some indication of the probable effect, may both have been determined.

With the formulation of a hypothesis and the need to test it by trial of new drugs, the organic chemist is required for synthetic work. When the initial basis of the project has been established it is possible to suggest drugs which may require to be synthesised, and this, of course, is the chief contribution of an organic chemist to such a project. But there is need and opportunity not only for the

preparation of new drugs by synthesis or extraction, but also for determination of methods of examination of the effects of the drug. Thus it is useful to know not only the dosage of a drug but also the plasma concentration resulting, and the variation in the plasma concentration from patient to patient when the same dosage is administered. Investigation of the mode of action of the drug invariably calls for a considerable number of analytical determinations in biological fluids, and the varying effects of the drugs frequently necessitate modification of existing methods to ensure accurate evaluations.

From the proportion of the drug dose excreted, from the time elapsing before excretion is complete, and from the nature of the degraded and conjugated drug products identified, much may be inferred. If the patients under examination are maintained on a known balanced diet throughout the course of the investigation, it is possible by analysis of the excreted material to determine to some extent the effect of the drug on the metabolic processes of the body.

With such information obtained for any one drug, and a knowledge of its chemical and physical properties, it may be possible to formulate a hypothesis which may be tested by the synthesis and clinical trial of a farther compound. Thus just as clinical observations constitute a specialised field for the physician, so the synthesis of new drugs forms a specialised chemical field, but between these two extremes of specialisation there remains a wide range of research in chemotherapy in which the greatest co-operation and mutual understanding is of great value.

Such a project is to some extent outlined in this synthesis. The treatment of rheumatic fever with sodium salicylate is widespread, but a great deal is as yet unknown as to the cause of the disease. It was hoped, therefore, that an initial investigation into the mode of action of the drug, with careful clinical and biochemical assessment of the remission of the disease under treatment, might give an indication of the mode of action of sodium salicylate and some insight into the disease process, and at the same time indicate some possible alternative drug. This work and its immediate consequences will be outlined in the following pages.

Sodium Salicylate.

Although in classical times Hippocrates has given a remarkably unmistakable account of the typical symptoms of rheumatic fever, in the latter days of the Roman Empire there arose ever increasing confusion of the disease with other joint conditions. This decadence of medical perceptions lasted throughout the middle ages, with medical writings of the period indicative of the mystical and philosophical approach of the alchemists, just as current medical writings indicate an essentially factual scientific approach. In this connection, it is interesting to note that work by Cullen in Edinburgh in 1769[1] was among the first to show a sufficiently analytical approach to the mass of confused and contradictory data available to differentiate acute from chronic rheumatism and to distinguish them from gout and other similar joint afflictions. Thus from the 18th century to the present day a steadily increasing insight into the symptoms of rheumatic fever has made possible its distinction from other similar diseases, and greatly facilitated its treatment.

The characteristic features of acute rheumatic fever are elevation of body temperature, abnormally high pulse rate, joint pain and swelling, and inflammation of the heart. Relapse is a frequent feature of the course of the disease, and recurrent attacks

are common. These essential manifestations of acute rheumatic fever are now clearly recognised and noted.

The efficiency of medical treatment of rheumatic fever has closely paralleled the accuracy of diagnosis throughout the years. Extracts of willow and poplar bark and leaves containing salicin, a glycoside of o-hydroxy benzyl alcohol, and of birch bark containing glycosides of methyl salicylate have been used in medicaments for over 2000 years. Hippocrates and Pliny alike refer to prescriptions the active principle of which is now known to be salicylic acid. However, interest in the active principle arose only when it was found towards the end of the 18th century that these willow bark extracts were an effective substitute for the relatively scarce "Peruvian bark" containing quinine. Thus the gradual dulling of diagnostic accumen which had concentrated attention almost exclusively on the more obvious pyrexia associated with rheumatic fever, with the consequence that treatment was predominently with antipyretics, was instrumental in effecting the introduction of salicylate treatment for the disease.

In 1838 Piria[2] prepared salicylic acid from salicin, but it was not until Kolbe in 1874[3] synthesised the acid by direct carbonation of sodium phenate that a plentiful, pure and moderately priced supply was available. Buss[4], testing Kolbe's acid for its antipyretic property, found that it was exceptionally effective in the treatment of acute rheumatism as compared with other fevers.

A year later, in 1876, Maclagan [5] published his own independent observations fully confirming the work of Buss, and from that time the drug has remained in general use in the treatment of rheumatic fever.

Even today, however, some controversy remains over the much debated question of whether sodium salicylate actually cures rheumatic fever or whether it merely alleviates symptoms. The use of aspirin (acetyl salicylic acid) as a treatment for headache is, of course, widespread, and there is no reasonable doubt that salicylate has some analgesic action, but a mild, deep seated action of this sort is not easily measured, and no accurate assessment has as yet been made. The antipyretic action, though obvious, is not easy to exclude in considering its action on the general course of the disease. Salicylate will undoubtedly reduce the body temperature of rabbits or men suffering from fever induced by different means, but the basic question as to whether sodium salicylate actually cures rheumatic fever remains. Moreover, in dealing with rheumatic fever an ever-present obstacle to securing truly significant data is spontaneous remission of the disease, liable to occur at any time, whether or not the patient is undergoing treatment.

However, in 1943 Coburn [6], considering the relationship between plasma salicylate concentrations and remission of symptoms, as opposed to salicylate dosage and clinical observations thereafter, determined the effective plasma concentration to be 35-40 mg.%.

He found that while a dosage of 2 g. four hourly making a total daily dose of 10-12 g. was in general satisfactory in attaining this concentration of sodium salicylate, many cases occurred in which patients receiving this dosage did not attain a sufficiently high blood concentration. Coburn also introduced to rheumatic fever the important concept of the high initial dose to build up an effective plasma concentration of drug as quickly as possible, rather than a gently increasing dose which may sometimes give less satisfactory results.

The work of Coburn was confirmed and considerably developed by Reid [7] who investigated the possibility of effective cure by sodium salicylate as opposed to mere relief of symptoms. He confirmed the importance of a blood concentration of 30-40 mg. % but stressed the necessity for frequent determinations, since not only is it impossible to be certain of reaching this effective concentration with any given dosage, but also it is impossible to be certain of maintaining this concentration with the same dose, even when it has been reached. Frequent observations established a distinct tendency for the concentration to fall after 6-8 days, so that in some patients a relapse might occur apparently in spite of adequate salicylate dosage, but actually due to an insufficiently high plasma salicylate concentration. It is probable that much previous conflicting evidence could be rationalised if this fact

is taken into consideration.

He also observed that the fluctuations in the rate of clinical improvement of the patients were closely paralleled by the variation in the erythrocyte sedimentation rate (E.S.R.), a measure of the rate of settling of erythrocytes in whole blood. While the patient was improving the sedimentation rate fell from the high initial values always found in patients suffering from rheumatic fever, but when a temporary relapse due to a fall in plasma concentration of salicylate occurred, the erythrocyte sedimentation rate again began to rise. Finally, when the patient was cured, and salicylate administration ceased, the E.S.R. returned to a more normal value. There is some evidence from experiments in vitro[89] that the alteration in sedimentation rates of erythrocytes may be due to direct physical action of sodium salicylate in the blood, rather than a secondary result of the breaking of the disease process, but Reid, established that a decrease in the E.S.R. does give a practical objective measure of the degree of improvement of the patient.

In the same paper, however, Reid also made certain preliminary observations on the relationship between these unexpected falls in plasma salicylate concentration and the urinary pH and plasma CO_2 combining power. Smull, Wegria and Leland [10] had found that when sodium bicarbonate was given together with sodium salicylate a lower plasma salicylate concentration was reached. Smith, *et al.*[11] suggested

that this was due to increased excretion in urine. Reid not only confirmed these observations, but also showed that when a relapse due to a fall in plasma salicylate concentration occurred in a patient, an increase in urinary pH and plasma CO_2 combining power was the invariable accompaniment, whether the patient was receiving treatment with sodium salicylate and sodium bicarbonate, or with sodium salicylate alone. When the plasma and urinary salicylate concentrations were rising, the sodium bicarbonate concentration of the plasma and urinary pH were falling. Moreover, when an effective plasma salicylate concentration was maintained, and regression of the disease as indicated by clinical observations and by a fall in the E.S.R. occurred, urine output always increased considerably.

With the importance of the plasma concentration of salicylate and its relationship to the E.S.R. thus established, the mode of action of the drug became of immediate interest, and these observations on the variations of the bicarbonate content of the plasma and urinary pH values were sufficient to indicate that further attention might well be paid to their fluctuations. Accordingly, with the co-operation of seven adult patients a detailed study was made of the progress of rheumatic fever under treatment with sodium salicylate, (Reid, Watson and Sproull, Quart. J. Med., 1950, 19, 1).

Some account of this work will now be given. It will be appreciated that the aim was to establish further the links between

plasma salicylate concentration, relief of disease symptoms and fall in E.S.R., and at the same time to trace as far as possible the biological changes accompanying each stage of the remission of the disease process.

The total water present within the body includes two main divisions: the intracellular water, which is the volume of water within the cells, and the extracellular fluid which comprises all fluid outside the cells. The extracellular fluid may be further subdivided into the plasma water, which is the volume of blood circulating in the body exclusive of the blood cells, and the interstitial water which is the remainder of the extracellular fluid. The changes in volume of these divisions of body water were followed by direct and indirect measurement.

Thus the variation in total body water was followed by constant measurements of body weight, urine volume and fluid intake throughout the period the patient was under observation. Losses due to sweating and respiration were not accounted for, so that although a reduction in total body water is certainly implied by a considerable fall in body weight accompanied by a urine volume much in excess of fluid intake, an increase in body weight and a fluid intake in excess of urine volume can less surely be ascribed to an increase in total body water.

The volume of the blood plasma was investigated directly by measurement of the degree of dilution of an injection of the dye T 1824[12].

Within the cells of the body, potassium constitutes about 95% of the total cation present, so that by following the potassium "balance" (potassium intake given by the diet less the urinary potassium excretion), an assessment was made of the variations in intracellular water. Again, all losses were not taken into consideration so that more importance must be attached to the decrease in intracellular volume implied by a urinary potassium excretion greater than potassium intake than to the opposite case of a positive potassium "balance".

Similarly, in extracellular water the dominant ions are sodium and chloride, so that by measuring the sodium and chloride intakes and urinary outputs a measure of the fluctuations of extracellular water was possible.

Plasma protein and non-protein nitrogen determinations, together with a measure of the nitrogen intake and urinary nitrogen output, gave some indication of the effect of sodium salicylate on protein metabolism, while the acid-base changes in the plasma were followed by measurement of the pH and CO_2 content. Five of the patients were given 1.5 g. sodium salicylate orally every four hours, and for comparison purposes two were given 2 g. sodium salicylate and

2 g. sodium bicarbonate every four hours. The relationship between plasma salicylate concentration, E.S.R. and clinical condition will first be discussed, followed by observations on the absorption, excretion and degradation of sodium salicylate and finally by the results of plasma acid-base investigations and the body water studies.

Plasma salicylate concentration, E.S.R. and clinical condition.

In all seven patients an effective plasma salicylate concentration of 35-40 mg.% was attained within five days, with the disappearance of acute rheumatic manifestations, and a noticeably increased depth and decreased rate of breathing. At the same time buzzing in the ears (tinnitus), which has previously sometimes been taken as an indication that an effective salicylate concentration has been reached, slowing of the pulse rate, loss of appetite, deafness and drowsiness began to appear. These latter symptoms and signs taken together were so typical that they may be characterised as the "special salicylate syndrome". They can justly be regarded as the incipient symptoms of salicylate poisoning, but although these less desirable effects of salicylate therapy may be observed to some slight extent even when the plasma salicylate concentration is around the usually safe figure of 35-40 mg. %, at this concentration they were more mildly troublesome than serious. At this stage in the treatment the E.S.R. as may be seen from Table I had not begun to decrease, and was in some cases even showing an appreciable increase.

Table I.

The Erythrocyte Sedimentation Rate and Salicylate Administration.

Case	Initial Value (day 1) mm/hr.	Value during administration (day 3-5) mm/hr.	Value on last day of dosage (day 9-19) mm/hr.	Final Value (day 16-35) mm/hr.
1	125	117	83	48
2	118	104	76	35
3	107	121	-	40
4	111	119	9	10
5	105	100	50	15
6	81	117	85	66
7	60	108	70	-

Cases 3 and 6 were given sodium bicarbonate and sodium salicylate, the others received sodium salicylate only.

In the second stage of the treatment there was a marked divergence of condition among the patients. In the first group of four, who maintained a salicylate concentration of 30-38 mg. %, the symptoms of the salicylate syndrome abated, while the E.S.R. began to fall fairly slowly and steadily. But in the second group of three patients, whose plasma salicylate concentrations rose to 65-72 mg. % the symptoms of the syndrome were considerably

intensified and some mental confusion developed, so that by the tenth day salicylate had to be discontinued for a few days until treatment could safely be resumed.

When treatment was completed, the E.S.R. had fallen considerably with accompanying signs of marked clinical improvement, and the final values measured one to three weeks after withdrawal of the drug, when the condition of the patients remained much improved, showed some further fall towards more normal values (10-20 mm./hr).

Absorption, excretion and degradation of sodium salicylate.

The absorption of salicylate throughout the period of treatment was found to be remarkably high. The total salicylate excreted in the urine and faeces of the four patients who received sodium salicylate alone was estimated colorimetrically and the completeness of absorption from the gastro-intestinal tract was assessed by comparing the total oral dose with the amount of salicylate recovered from the faeces. Total excretion was taken as the sum of the salicylate present in the urine and in the faeces, since it has been shown (13) that the loss in sweat, the other possible method of excretion, is negligible. It is evident from the figures given in Table II that only a small fraction of the total dose is excreted in the faeces. Since incubation experiments involving addition of known quantities of salicylate to faeces indicated that there was but slight possibility that the drug might be destroyed

Table II.

Urinary and Faecal Salicylate in Relation to dose.

	Total dose	Total urinary salicylate		Total faecal salicylate	
Case	g.	g.	% of dose	g.	% of dose
1	60	12.2	20.3	1.2	2.0
4	135	41.5	30.7	2.0	1.5
5	147	35.9	24.4	2.0	1.3
7	135	34.5	25.5	1.2	0.9

in the bowel, it follows that the total salicylate excreted into the urine and faeces totals only 22-32 % of the total dose. Thus even after prolonged oral administration practically all administered salicylate is absorbed, and of the total absorbed 68-78 % is degraded in the body. This confirms the short term studies of Hanzlik[13] and indicates further that rate of absorption and excretion do not change appreciably with prolonged dosage. It also seems possible that the variations in plasma concentration resulting from the same dosage of sodium salicylate must be explained not by varying rate of absorption, but by varying rate of degradation.

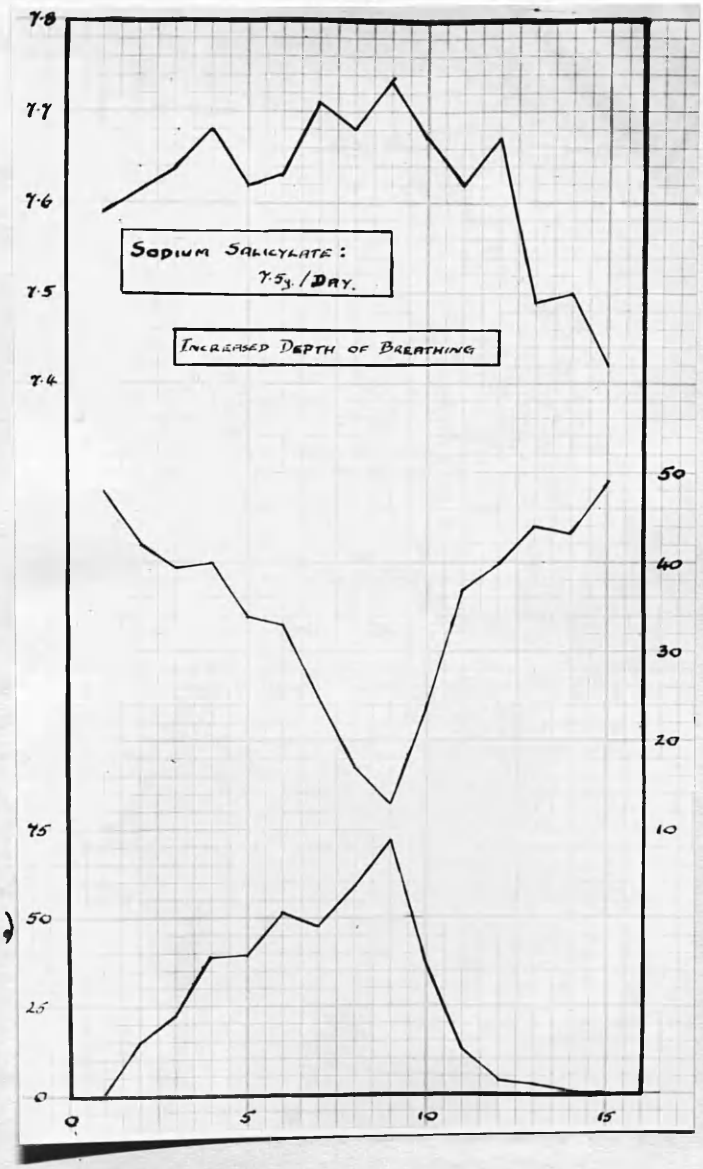
Acid-base changes in plasma. The plasma pH primarily depends on the H_2CO_3 : $NaHCO_3$ ratio in the blood. Accordingly, to increase the overall accuracy of the examination of acid-base changes in the blood, both a direct plasma pH measurement and a measure of the plasma CO_2 content were made in the four patients receiving sodium salicylate only, while in the patients receiving both sodium salicylate

and sodium bicarbonate the plasma pH determination only was made. On starting salicylate treatment the plasma pH rose from high initial values of 7.50 to 7.62 to peak values of 7.58 to 7.75 within the first ten days. Thereafter the pH value fell slowly, settling at a value somewhat lower than the initial value some days after salicylate dosage had ceased. The indications are therefore that an alkalaemia is a natural tendency in rheumatic fever and is intensified by sodium salicylate treatment.

Individual variations in plasma CO_2 content were greater, but the general trend was clear. The initial values of 48-55 vols. of CO_2 from 100 ml. of plasma fell to 23-39 vols. per 100 ml. during the first week, but decreased farther only if the higher range of plasma salicylate concentration (68-75 mg. %) was reached. The intensity of the salicylate syndrome was inversely proportional to the plasma CO_2 content. When salicylate treatment was terminated the plasma CO_2 content of all four cases rose again within a few days to 49-56 vols. per 100 ml. It is thus indicated that one effect of salicylate treatment is to lower the plasma CO_2 content.

Consideration of these results is most interesting. A rise in plasma pH accompanied by a fall in plasma CO_2 content shows that CO_2 is removed from the plasma by the lungs much more rapidly than sodium is excreted by the kidneys, so that the H_2CO_3:$NaHCO_3$ ratio is diminished and plasma pH rises. The outward manifestation of this

Fig.1.



The relationship between salicylate concentration, plasma carbon dioxide content, and plasma pH in a case of rheumatic fever treated with sodium salicylate.

respiratory alkalosis is the deep breathing observed in all patients. The relationship is conveniently expressed in Fig. I.

Plasma Volume. From unusually high initial values of 3.3 to 5 L. the plasma volumes of the four patients who avoided excessively high plasma salicylate concentrations rose sharply to 4.4 to 5.5 L., then fell gradually till a few days after salicylate treatment had ceased a final value considerably lower than the initial value was reached. A similar, though less marked, change was noted by York and Fischer in 1947[14]. In the three patients in whom the salicylate syndrome developed, initial values were markedly high, so that a gradual fall in plasma volume occurred throughout the treatment. It would seem, therefore, that a high plasma volume is another natural feature of rheumatic fever which is, where possible, first intensified, then removed on treatment with sodium salicylate. In attempting to explain these changes in plasma volume the protein and mineral content of plasma must be considered, and these will now be discussed in turn.

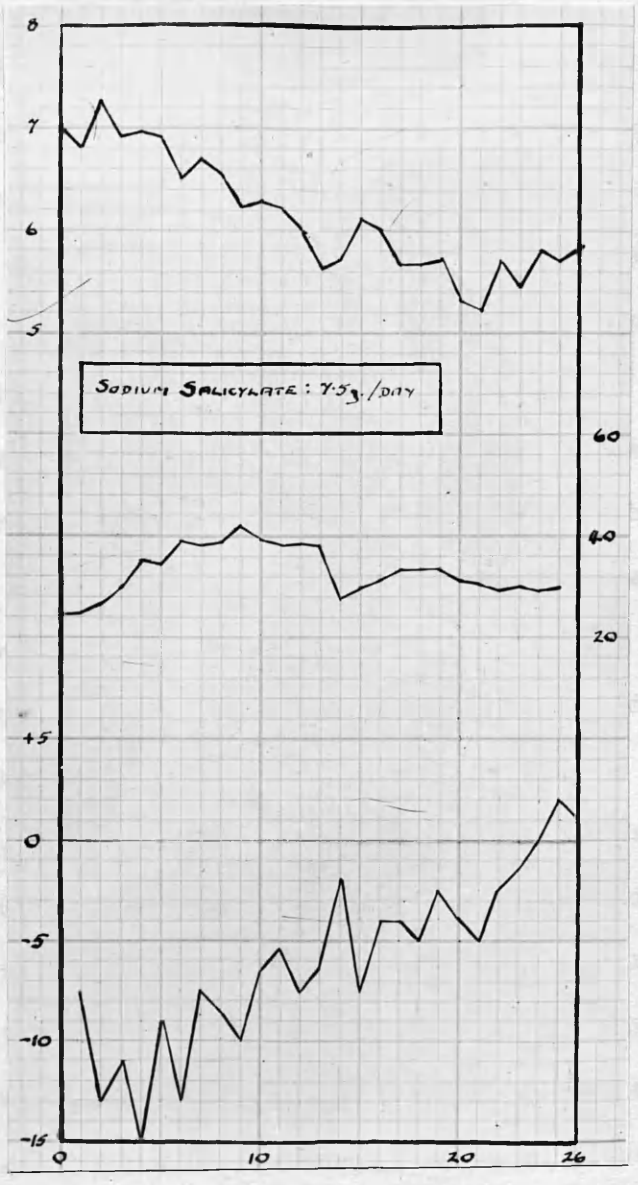
Plasma protein and nitrogen balance. After the first few days of salicylate therapy, the plasma protein concentration likewise fell fairly steadily, the fall continuing until seven to nine days after the completion of salicylate dosage. Moreover, it will be appreciated from the data given in Table III that the loss of protein was relatively much greater than the change in plasma volume, indicating that there was a true fall in total plasma protein and not merely a dilution of the initial quantity present.

Table III.

Case		Immediately before salicylate adminstration	During salicylate administration			After salicylate adminstration.		
			4-5th day	9-12th day	16-20th day	7-9th day	14th day	20th day
1	plasma-volume (litres)	4.0	3.2	3.7	-	3.2	3.4	3.1
	plasma-protein(g/100ml)	6.83	7.46	6.78	-	6.38	7.09	6.61
2	plasma-volume (litres)	4.0	3.6	3.2	-	3.4	-	3.0
	plasma-protein (g/100ml)	7.06	7.18	6.43	-	5.60	-	6.90
3	plasma-volume (litres)	5.0	4.8	-	-	4.5	3.4	2.8
	plasma-protein(g/100ml)	7.45	7.50	-	-	5.70	7.10	7.10
4	plasma-volume (litres)	4.2	5.3	4.0	4.4	3.3	-	-
	plasma-protein(g/100ml)	6.78	6.81	5.89	5.57	5.92	-	-
5	plasma-volume (litres)	3.6	4.1	5.5	4.1	3.6	2.8	-
	plasma-protein(g/100ml)	7.26	7.48	7.00	6.27	6.00	8.10	-
6	plasma-volume (litres)	3.3	4.4	4.3	3.8	3.1	-	-
	plasma-protein(g/100ml)	7.90	6.80	6.60	7.04	7.00	-	-
7	plasma-volume (litres)	4.8	4.1	5.4	4.2	4.1	-	-
	plasma-protein(g/100ml)	8.20	7.94	7.66	6.53	6.81	-	-
Aver-age	plasma-volume (litres)	4.1	4.2	4.3	4.1	3.6	-	-
	plasma-protein(g/100ml)	7.37	7.31	6.72	6.35	6.20	-	-

Cases 1, 2 and 3 suffered severely from the toxic effects of salicylate adminstration so that dosage had to be discontinued.

Fig 11



The results of the plasma protein, plasma non-protein nitrogen and nitrogen "balance" determinations in a case of rheumatic fever treated with sodium salicylate.

Thus the total plasma protein, calculated from the observed concentration and the plasma volume, fell from an initial average of 304g. to an average of 222g. 7-9 days after treatment had begun, an average reduction of about 82g. protein. A consideration of the plasma non-protein nitrogen and nitrogen "balance" figures develops the picture of protein breakdown still farther. During the period of effective salicylate therapy nitrogen output was consistently greater than nitrogen intake, while the fall in non-protein nitrogen on withdrawal of salicylate, indicating cessation of protein breakdown, was most marked. The progressive breakdown of plasma protein and high nitrogen excretion is clearly demonstrated in the typical graphs shown in Fig II. The postulate of Madden and Whipple [15] that an equilibrium between circulating and cellular protein will exist even while total body nitrogen is being reduced, is considerably strengthened by these observations.

Total body water. As may be seen from Table IV a fall in body weight does occur during salicylate therapy. The progressive fall in body weight was associated with a steady rise in urine volume, so that there is little doubt that a diminution of total body water occurs. The marked increase in urine output and decrease in body weight immediately after cessation of therapy is a characteristic feature of patients under treatment with sodium salicylate alone, and constitutes the final removal of excess water from the body.

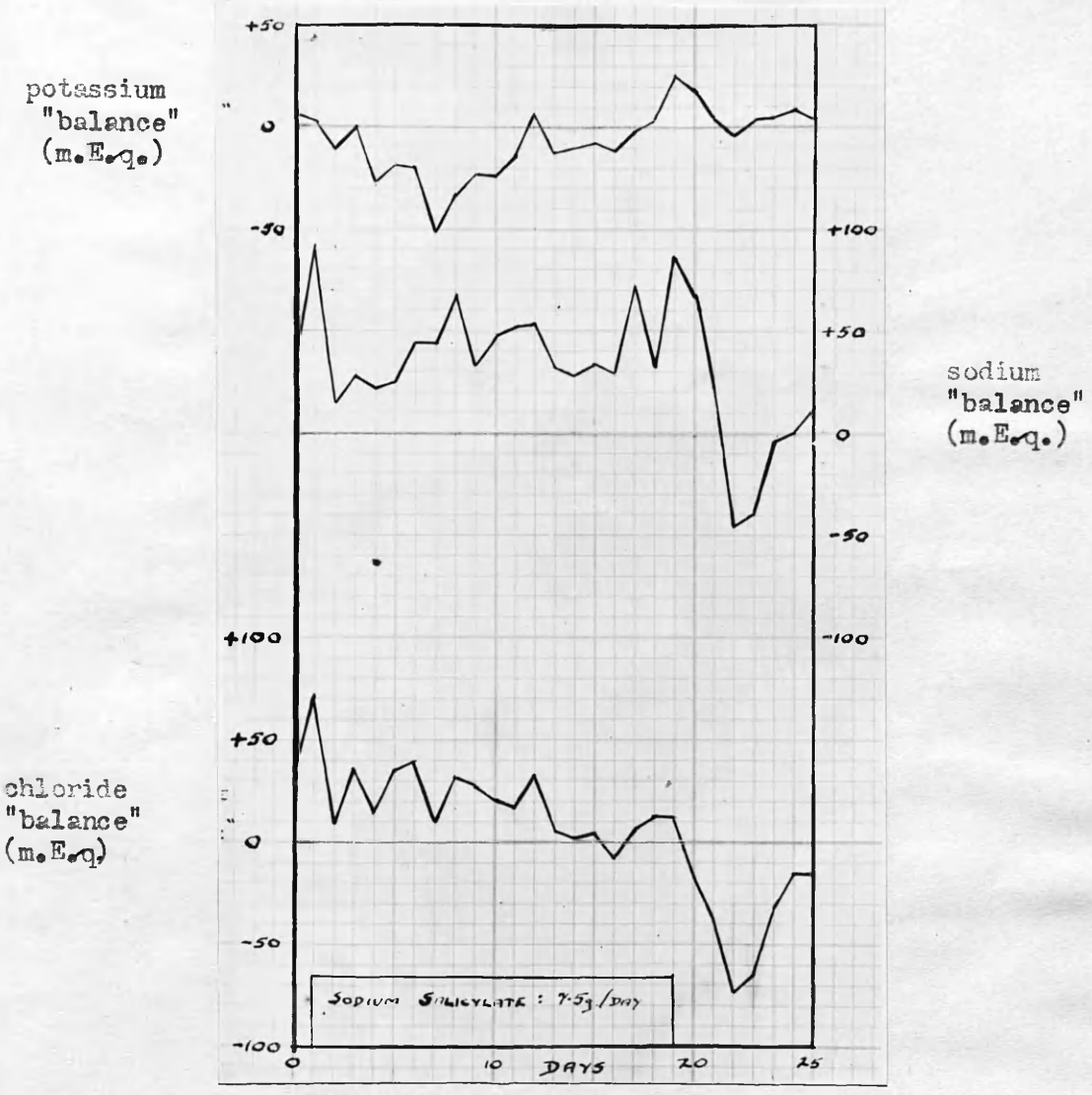
Table IV.

Body Weight and Salicylate Therapy.

Case	Weight before salicylate administration (Kg.)	Weight on last day of salicylate administration (Kg.)	Weight 7-14 days after cessation of therapy (Kg.)	Total reduction in weight. (Kg.)
2	73.4	-	68.4	5.0
4	56.2	67.8	52.0	4.2
5	71.0	67.8	67.0	4.0
7	55.4	53.2	53.0	2.4

Extracellular water. The variations in the sodium and chloride "balances" and thus in extracellular water, of a typical patient receiving sodium salicylate only are represented in Fig. III. It will be seen that urinary sodium and chloride was considerably in excess of the intake of sodium and chloride only during the period of high urine output immediately following the cessation of salicylate therapy. During the period of salicylate administration, on the other hand, the intake of sodium and chloride was constantly greater than the urinary output. The indications are therefore of a probable build up of the volume of extracellular fluid during the course of treatment, followed on cessation of salicylate administration by a sudden and drastic return to a more normal volume by excretion of the excess extracellular fluid.

Fig 111



The difference between dietary intake and urinary out put of potassium, sodium and chloride in a case of rheumatic fever treated with sodium salicylate.

Intracellular water. The variation in the potassium intake less potassium output figures, and thus of intracellular water, of the same patient is also represented in Fig. III. The results indicate that after an initial period of 10-20 days during which potassium output is greater than intake, a normal balance is restored. It is probable, therefore, that in the case of intracellular water there is an initial drop in volume during the period of salicylate administration, and thereafter little change.

It will be seen from the foregoing data that the remission of rheumatic fever under treatment by sodium salicylate forms a well ordered pattern.

Within five days of the beginning of treatment at an effective plasma concentration, fever, tachycardia, joint pain and swelling are completely relieved. Meanwhile, the "Special salicylate syndrome" may make some appearance with slight outward signs of increased depth of breathing, slowing of pulse rate, reddening and increased heat loss at the skin surface, nausea, vomiting, ringing in the ears, deafness and drowsiness. Where the plasma salicylate concentration remains within the safe therapeutic range of 30-40 mg. % these signs either remain slight or tend to subside and disappear, but if the plasma salicylate concentration continues to rise until it reaches 60-70 mg. % the syndrome becomes well defined, and salicylate poisoning may be severe enough to necessitate temporary cessation of therapy. Thus although the relief of symptoms of rheumatic fever

may be more speedy at this higher salicylate concentration it is certain that the 30-40 mg. % concentration is much more commendable even if slightly slower in action.

The study of the acid-base changes in plasma show that not only the relief of symptoms, but also the depth of breathing is directly proportional to the plasma salicylate concentration. This had previously been noted clinically, but these results show that the slight alkalaemia always present in rheumatic fever patients is increased in direct proportion to the plasma salicylate concentration. Since deep breathing causes excessive loss of carbon dioxide, and compensation by renal excretion of sodium is not complete, the ratio of H_2CO_3 : Na HCO_3 increases and plasma pH rises. Thus what had been an interesting clinical observation becomes an important pointer as to the mode of action of salicylate in rheumatic fever. It is therefore possible that in this respect the plasma salicylate concentration of 30-40 mg. % is important in causing a degree of alkalosis great enough to speed the remission of the rheumatic process, but not enough to stimulate dangerous side reactions.

Considered together with the study of acid-base changes, the protein and water balance studies show that the principal pharmacological actions of the drug are the stimulation of protein breakdown and the aggravation of respiratory alkalosis. The increased excretion of nitrogenous product in the urine induced almost from the first day of salicylate therapy affects first the intracellular water, as shown by the excessive excretion of potassium, then the extracellular water as shown by the high sodium and parallel chloride excretion.

It is probable therefore that the initial protein breakdown occurs within the cell, with subsequent shift of fluid partly as a result of the initial actionon intracellular protein , and partly as a result of the appearance of similar protein breakdown affecting the extracellular protein. Finally, when the salicylate therapy is stopped, the excess fluid is removed in the course of a few days during which the urinary output is much greater than fluid intake.

Strong evidence in favour of the use of the erythrocyte sedimentation rate as an index of the cure of rheumatic fever is given by the fact that although in the initial stages of removal of intracellular water the inflammation in the joints is temporarily relieved and the patient is subjectively much encouraged, it is only when the excess sodium and chloride in the extracellular fluid are being excreted that the E.S.R. begins to fall. Although the salicylate concentration is maintained at the 30-40 mg. % value throughout the course of the observations, it is only when a more fundamental change occurs that the E.S.R. indicates that the disease process is regressing.

The final values for the erythrocyte sedimentation rate offer fairly conclusive evidence that the effect of sodium salicylate on the E.S.R. in vivo is not ascribable solely to direct action. If this were so, the E.S.R. might reasonably be expected to rise again as the plasma salicylate concentration falls,but these determinations show that this rise does not occur, so that the sedimentation rate may indeed be taken as a measure of the clinical condition of a

patient suffering from rheumatic fever. Lichty and Hooker [16] had observed some rise in the E.S.R. on withdrawal of sodium salicylate, but it is probable that the fall was ascribable to too early withdrawal of sodium salicylate and resulting return of symptoms of rheumatic fever rather than to the ending of direct action by salicylate in the blood.

Thus the action of sodium salicylate in rheumatic fever may be a two stage process. Firstly, increased protein katabolism and the associated shift of potassium lead to the transfer of water from the affected swollen joints to the extracellular fluid. In the second stage the excess water and retained sodium is excreted, and so extracellular and intracellular fluid balance restored to normal.

During the decrease in volume of intracellular fluid, however, there is a rise in plasma pH and a fall in plasma CO_2 content, indicating a respiratory alkalosis. It is as yet uncertain whether the protein breakdown or the respiratory alkalosis is the primary action, but it has been suggested[17] that the action of salicylate in cats is to stimulate the vagal nerve endings thus indicating a respiratory alkalosis as the primary effect. It is, however, probable that further experiments will be necessary before the initial action can be definitely determined.

These data together indicate that the cure of an attack of rheumatic fever by sodium salicylate and the self cure by spontaneous remission may follow an identical route. It is naturally difficult to

obtain full data concerning a natural remission, but it is noticeable that alkalaemia is already present in patients suffering from rheumatic fever, and plasma volume is already high. On treatment with sodium salicylate, the plasma pH and plasma volume do not at once begin to return to normal. On the contrary, the aberration is first intensified, then when the process is complete, normal conditions prevail. Sodium salicylate would therefore seem to act in rheumatic fever by speeding up and intensifying the natural course of the disease.

Thus sodium salicylate has the advantage that at the recommended therapeutic plasma concentration of 30-40 mg. % in the great majority of cases natural remission is accelerated, and the initial transfer of intracellular water to the extracellular fluid, then the loss of this excess total body water in increased urine output, is accomplished without the development of dangerous side effects. Protein breakdown is accelerated at a rate commensurate with the rate of removal of the breakdown products. There is further the valuable feature that the antipyretic action of salicylate in rheumatic fever is so characteristic as to have been suggested on several occasions as a specific test for the disease.

Variation in plasma concentration in patients resulting from the same dosage of sodium salicylate is however, great enough to limit somewhat the use of the drug in treatment of rheumatic fever. While

the symptoms of the "special salicylate syndrome" give sufficient warning of any unduly high plasma concentration, t o obviate the possibility of fatalities due to salicylate administration, these symptoms themselves can cause the patient considerable discomfort, and may necessitate at least temporary withdrawal of the drug before remission of the disease is complete. When frequent plasma or urinary salicylate concentration determinations are carried out, the probability of avoiding the development of mildly toxic symptoms is greater, but unless these analyses are made at least daily it is very doubtful if the effective 30-40 mg.% plasma concentration, as opposed to the more troublesome 60-70 mg.% plasma concentration, could be ensured in every case. Only by frequent variations of the dosage in accordance with the determinations made can complete control of the plasma salicylate concentration be effected. Thus although sodium salicylate is therapeutically active, it is not an ideal drug, so that an alternative medicinal would undoubtedly be most welcome.

Experimental.

Plasma and urinary salicylate. The method of estimation of Reid[7] was followed. A specimen of plasma or urine (0.5ml.) was added to pH2 buffer (14.5ml.) and extracted with amyl alcohol (30ml.) The amyl alcohol phase (20ml.) was extracted with pH 8.6 buffer (30 ml.), the layers again separated by use of the centrifuge, and the amount of salicylate in the aqueous layer (20ml.) determined colorimetrically by the reaction of Jorissen as described by Sherman[18].

Erythrocyte Sedimentation Rate. The degree of settling of the erythrocytes of whole blood in a Westergren tube[19] was measured after one and two hours.

Plasma pH. A Cambridge potentiometer with calomel and glass electrodes was used for all pH determinations. Blood samples were collected and kept under paraffin to prevent loss of CO_2.

Plasma CO_2 content. The total CO_2 available in the blood was measured manometrically by the method of Van Slyke and Neill[20]. A specimen of whole blood (1 ml.) was acidified with lactic acid, and the pressure of CO_2 and O_2 released measured at constant volume. The CO_2 was then absorbed by sodium hydroxide, and the pressure again measured. The difference gave a satisfactory measure of the CO_2 content of the blood under investigation.

Total plasma volume. The dye dilution method of Gregersen[12] was used to estimate the total plasma volume. An aqueous solution of the dye T 1824 (15 mg. in 1.5 ml.) was injected intravenously, and four or five blood samples taken at 15 min. intervals. The concentration of dye in each specimen was estimated with a Beckman spectrophotometer, standards being provided from a blood sample taken immediately before injection of the dye, with known amounts of dye added.

Urinary nitrogen. The estimation was made by the method of Kjeldahl[21]. The specimen was digested with concentrated sulphuric acid then rendered alkaline with sodium hydroxide solution, the ammonia boiled off, re-absorbed in sodium hydroxide solution and the excess sodium hydroxide titrated with hydrochloric acid, using bromo-phenol blue as indicator.

Plasma protein and non-protein nitrogen. A specimen of plasma was treated with trichloroacetic acid as described by Greenwald[22,23] in order to precipitate out the protein. The nitrogen present in the filtrate and precipitate was then separately determined by the same method employed in urinary nitrogen determinations.

Urinary Sodium. Rather surprisingly, a most satisfactory method of estimation of sodium was developed from the modification of the titrimetric determination of sodium in a pyroantimonate precipitate described by Balint[24]. An outline of the procedure used is as follows: A specimen of urine (1 ml.) was ashed, the residue dissolved in water, and an aqueous solution of potassium pyroantimonate (5 ml. of 2%) added. On introduction of ethyl alcohol (1.5 ml. of 95%) a precipitate settled out, was washed several times with ethyl alcohol (5 ml. of 30%) and finally dissolved in hydrochloric acid (5 ml. of 5 N). Potassium iodide solution was added, and the excess iodine titrated with sodium thiosulphate solution in presence of starch.

The chief disadvantage of the method lies in the reagent itself. Roscoe and Schorlemmer [25] have shown that there is considerable

doubt as to the degree of separation of pyro-and met-antimonates so far achieved, so that the "purity" of the reagent is rather arbitrary. However, Kramer and Gittlemann [26] have suggested that if the potassium pyroantimonate forms a perfectly clear aqueous solution(2%) and is not precipitated out by the addition of ethyl alcohol (one fifth the volume of 95%) a fairly good result will be obtained. It has now been found that the pH of the solution is also important. Potassium hydroxide solution should be added when necessary to adjust the pH of the solution to 9.00. The most practicable check on the purity of the reagent is, however, given by an initial series of determinations with each batch of reagent prepared. Nine tubes containing an aqueous sodium chloride solution of known strength (1 ml. of approximately 145 m. E/L.) are treated with the reagent (5 ml. to each) followed by varying quantities of ethyl alcohol (0.5 ml. to 2.5 ml. of 95%). If the % sodium determined is graphed against the volume of alcohol added, and a reasonably low gradient is found where the curve crosses the 100% line, the reagent may be accepted for use. After further tests with different concentrations of sodium verifying the quantity of alcohol required, this particular volume is used in every estimation made with the batch of pyroantimonate reagent in question.

It is most surprising, therefore, that so arbitrary a method should prove so satisfactory in practise. In the course of the estimations carried out with adherence to the above principles and

attention to detail, the accuracy of the method was within 0.5%. This was a higher degree of accuracy than was consistently maintained in the gravimetric determination of sodium as sodium zinc uranyl acetate by Butler and Tuthill's modification [27] of the method of Barber and Kolthoff [28], or as sodium magnesium uranyl acetate by the method of Caley[29] and had the advantage of being considerably quicker and more convenient when a large number of determinations were required.

Urinary potassium. The standard gravimetric method of estimation of potassium in urine recommended by Peters and Van Slyke[30] relies on unpublished work by Mackay and Butler and involves deproteinising urine (120ml.), digesting the filtrate in nitric acid, precipitating calcium, magnesium, sulphate and phosphate with ammonia, barium chloride and ammonium carbonate, evaporating the filtrate, redissolving, and precipitating the potassium with chloroplatinic acid. It was found that the method as described was not entirely satisfactory in removing final traces of barium, calcium and iron, so that standard test solutions, prepared by adding known quantities of potassium chloride to urine samples, tended to yield high results. Accordingly, the following modification was devised : The urine specimen (10 ml.) was digested with concentrated hydrochloric acid (30 ml.), evaporated to dryness, and the residue dissolved in water (30ml.). Aqueous barium chloride solution (4 ml. of 10 %) was added, and the mixture heated almost to boiling and treated with concentrated ammonia (4 ml.)

and aqueous ammonium carbonate (4 ml. of 20%). The filtrate was evaporated to dryness, then heated in a muffle furnace to 600°C. The residue was redissolved in water and concentrated ammonia (0.5 ml.), aqueous ammonium carbonate solution (0.5 ml. of 20%) and saturated aqueous ammonium oxalate solution (0.5 ml.) added. On evaporating the filtrate and again heating to 600°C. in the furnace, a white residue suitable for potassium precipitation as potassium chloroplatinate was obtained. Aqueous chloroplatinic acid solution (3 ml. containing 10% platinum) was added, and the mixture carefully evaporated to dryness on the water bath. The residue was then well washed with ethyl alcohol (95 %) dried carefully at 100°, and weighed as K_2PtCl_6. Over an unusually wide range of urinary potassium concentrations, from 15 to 90 m.E./L, the error in the method as shown by addition standards and duplicates was not greater than 1 %.

Urinary chloride. Since it is, in general, necessary to digest biological fluids to remove interfering protein and ensure maximum homogeneity of the specimen, it will be apparent that some form of Volhard's method [31] is most likely to be advantageous in the estimation of the chloride ion concentration of such media. In the first stage of this method the anion is removed from solution as insoluble silver chloride, so that the drastic digestion following has no harmful effect on the accuracy of the method. Furthermore, the titration is carried out in acid solution so that the need for careful neutralisation is avoided. It was found that a modification of the procedure of Wilson and Ball [32] may be applied equally readily to urine, plasma and whole blood. In its original form the

method involved the digestion for 30 mins. on the steam bath of a specimen of whole blood (1 ml.) with concentrated nitric acid (3 ml.) in presence of aqueous silver nitrate solution (1 ml. of 0.15 N) cooling to room temperature, adding aqueous ferric alum solution (6ml. of 5 %) and titrating the excess silver nitrate with ammonium thiocyanate($\frac{N}{50}$).

The method actually employed was as follows: A specimen of whole blood, plasma or urine (1 ml.) was digested 2 hrs. on the steam bath with aqueous silver nitrate solution (2 ml. of 0.1N) and concentrated nitric acid (5 ml.), the mixture cooled in an ice bath, aqueous ferric alum solution (5 ml. of 5 %) added, and the excess silver nitrate titrated with ammonium thiocyanate ($\frac{N}{20}$). In this way a complete initial precipitation of chloride ion was more readily assured, a wider range of chloride ion concentration was covered, and salicylate interference was removed. This cooling of the solution in an ice bath immediately before titration was found to be not a refinement of dubious value, but a necessity if a consistently sharp end-point was sought.

The same method is applicable to estimation of chloride in faeces, when necessary. The stool is first stirred to a homogenous suspension in water, the volume made up to 1 L. and an aliquot (25 ml.) digested with nitric acid (50 ml.) and aqueous silver nitrate solution (2 ml. of 0.1 N).

Sodium 2:6 - Dihydroxybenzoate.

It has been shown that sodium salicylate, while remarkably specific in its action in the treatment of rheumatic fever, and while having the great advantage of acting by increasing the rate of natural remission of the disease rather than by introducing a further series of disease relief factors, is not altogether suitable for continued general use. The difficulty of achieving accurate control of the plasma salicylate concentration, and the development of symptoms of salicylate poisoning, make it difficult to commend the drug except when careful clinical and laboratory assessment is possible. However, the data accumulated on the pharmacological changes produced in the course of salicylate therapy, and the clinical observations made throughout the treatment, were such as to indicate both the desirability and the practicability of introducing some new drug which might have the same action as salicylate without inducing the troublesome signs of salicylate poisoning. Either a lowering of the effective therapeutic concentration or a raising of the concentration at which toxic symptoms appeared would achieve this aim.

Stockman in 1920[33] had noted the almost complete inactivity of *m* and *p*-hydroxy benzoic acids in the treatment of rheumatic fever, in direct contrast to the specific action of sodium salicylate. It was probable, therefore, that the activity of salicylate was at least to some extent dependent on the *o*-positioning of the hydroxyl

group relative to the carboxyl. A drug having the same mode of action as salicylate, but allowing a greater safety margin in its use, might thus be expected to have the salicylate nucleus with some substituent or substituents in the benzene nucleus to produce the desired slight modification in action.

In 1942 Kapp and Coburn [34] verified the suspected occurrence of gentisic acid (2:5-dihydroxybenzoic acid) in the urine of patients under treatment with sodium salicylate. The amount present in the urine was only 4-8% of the salicylate dose, but there remained the possibility that the first stage in the action of sodium salicylate was its conversion to gentisic acid, in which case initial treatment with sodium gentisate might induce therapeutic activity in much smaller doses. A quantity of gentisic acid was therefore prepared by the oxidation of salicylic acid with potassium persulphate [35] and initial clinical trials instituted, but results were not encouraging. It was quite clear that with an oral dosage comparable to that of sodium salicylate (10-12g. per day) sodium gentisate was much less effective in reducing the symptoms of rheumatic fever. Recent work [36, 37] has confirmed this finding, as there seems to be no doubt that while sodium gentisate is less toxic than sodium salicylate - indeed the LD_{50} for rats (the dose causing death in 50% of the tested animals) is 3.1g/K for sodium gentisate, but 0.88g/K for sodium salicylate [38] - it is not an effective anti-rheumatic agent. It is possible that both the high toleration and the low activity of sodium gentisate are

explained by its higher solubility, but Meade and Smith [39] have shown that a plasma concentration of 25 mg. % is not unusual. In marked contrast to observations on the excretion of sodium salicylate however, Mainardi and Semenza[40] found that 60-80 % sodium gentisate was excreted unchanged within 6 hrs. of oral administration.

p-amino salicylic acid was shown by Lehman[41] in 1946 to have a high tuberculostatic activity. Since then many analogues have been tested, but to date there is remarkable unanimity of opinion regarding the maximum effectiveness of the primary compound[e.g.42,43,44], and it was therefore thought that a preliminary trial of anti-rheumatic activity of this compound would give a fair indication of whether the amino substituent in the salicylic acid nucleus was likely to be of value in enhancing the action of sodium salicylate. A dose of 10-15 g. per day of sodium p-amino-salicylate did not, however, produce any marked remission of symptoms in patients suffering from rheumatic fever so that this compound too was discarded.

It seemed therefore that some attention might be paid to the physio-chemical properties of salicylic acid itself. Most striking is the greatly enhanced ionisation constant of salicylic acid relative to benzoic acid and to m- and p- hydroxybenzoic acids. Consideration of the ionisation constants of benzoic acid, the hydroxybenzoic acids, and the dihydroxybenzoic acids as given in Table V shows that in 2:6-dihydroxybenzoic acid the same greatly enhanced ionisation constant over the other dihydroxybenzoic acids has been noted. Thus in the

hydroxy and dihydroxy benzoic acids in which there is no substitution o to the carboxyl group, the ionisation constants are of the same order of magnitude as that of benzoic acid. Indeed, substitution of a hydroxyl group in the 4-position actually decreases the degree of ionisation somewhat. Again, in the acids in which there is a hydroxyl group in the o position to the carboxyl group, the ionisation constants are of the same magnitude - about 20 times greater than those of the first group, - but that of 2:4- dihydroxybenzoic acid is lower than that of salicylic acid. With 2:6-dihydroxybenzoic acid, however, the ionisation constant is almost 50 times that of salicylic acid and 800 times that of benzoic acid.

Table V.

Ionisation constants of Benzoic acid and of Hydroxybenzoic acids[45]

Acid	Ionisation Constant (K_c)
Benzoic acid	0.006
o-Hydroxybenzoic acid (salicylic acid)	0.102
m-Hydroxybenzoic acid	0.008
p-Hydroxybenzoic acid	0.003
2:3-dihydroxybenzoic acid	0.114
2:4-dihydroxybenzoic acid (β-resorcylic acid)	0.052
2:5-dihydroxybenzoic acid (gentisic acid)	0.108
2:6-dihydroxybenzoic acid (γ-resorcylic acid)	5.0
3:4-dihydroxybenzoic acid (protocatechinic acid)	0.003
3:5-dihydroxybenzoic acid (α-resorcylic acid)	0.003

The greatly enhanced acidity of the salicylic acid derivatives over the other hydroxy benzoic acids was attributed by

Baker[45] to hydrogen bond formation between the o-hydroxyl groups and the oxygen atoms of the carboxylic group. In this way the anion is stabilised, and the return of the proton hindered, so that the degree of ionisation is increased. In 2:6-dihydroxybenzoic acid there is even greater opportunity for chelation and the strong formal ressemblance of the ionised carboxyl group to the nitro group prompted Baker to suggest from analogy with previous work on 2-nitroresorcinol[46], that the carboxyl group might even chelate to both hydroxyl groups simultaneously. Whether this is actually the case or whether the increased chelation of the compound is due to the increased probability that any given molecule at any given time will contain a single chelate link is not yet determined, in spite of the accumulation of much indirect physical evidence[e.g. 47-50], but as a working hypothesis, the concept of chelation is most useful in the hydroxy carboxylic acid field as in so many others.

With 2:6-dihydroxybenzoic acid thus in an analogous position with regard to salicylic acid as salicylic acid with regard to benzoic acid, it was thought that a clinical trial of its effect on treatment of patients suffering from rheumatic fever would be of interest. A quantity was therefore prepared by the method of Limaye and Kelkar[51]: Resorcinol was condensed with acetoacetic ester in presence of ortho-phosphoric acid to give 4-methyl-7-hydroxycoumarin which was acetylated and subjected to rearrangement by treatment with anhydrous aluminium chloride. The 4-methyl-7-hydroxy-8-acetylcoumarin obtained

by fractional crystallisation of the product was treated with sodium acetate and acetic anhydride, when 2-methyl-3-acetyl-5-hydroxychromone was formed, which was hydrolysed by N sodium hydroxide solution to give 2:6-dihydroxybenzoic acid. The yield obtained was as claimed by Limaye.

It is interesting to note in this connection that although there is no record of any previous account of a trial of 2:6-dihydroxybenzoic acid as a therapeutic agent, an investigation by Clewer, Green and Tutin[52] of the tubers of the Indian lily Gloriosa superba had revealed that the principal alkali-soluble constituent was 2-hydroxy-6-methoxybenzoic acid, and not, as had been previously thought[53], salicylic acid. The dried, powdered tuber of the lily was a traditional Indian treatment for the pain of childbirth, and was in fact used in most treatment in which a general analgesic action was desired.

Meanwhile, a second great step had been taken in the progress of rheumatic fever therapy. Just as the discovery of the specific and characteristic action of sodium salicylate in rheumatic conditions by Buss[4] and MacLagan[5] had marked the first great development since the introduction of willow and birch bark extracts, so the discovery of the high degree of activity of cortisone by Hench and his associates[54,55] revealed entirely new possibilities of methods of action of anti-rheumatic drugs and of common modes of action by what appeared at first sight largely divergent types of drugs.

Even in these initial papers, however, there were indicatiions that although cortisone was a most active and useful drug it would not be a completely satisfactory therapeutic agent. Thus although in patients suffering from rheumatic conditions treatment with 100 mg. per day of 17-hydroxy-11-dehydrocorticosterone (compound E, or cortisone) relieved symptoms in 80-90 % of the cases, and occasionally even promoted prolonged remission of the disease, so that symptoms did not return immediately on cessation of treatment, disturbing side effects were observed. The effects which were recorded included rounding of the facial contours, hirsutism, acne, formation of a pad of fat across the upper spine, excretion of glucose in the urine and diminished ability to metabolise glucose, sodium retention leading to swelling of the tissues by excess water, alkalaemia, increased blood pressure, and personality change, and were sufficiently characteristic of the symptoms of Cushing's disease[56] - a disease of the pituitary gland - to be designated a mild Cushing's syndrome. The effects are usually mild, and not always all present in any one patient, but they do represent the introduction of a syndrome not necessarily directly connected with the relief of the disease, and therefore a complication not strictly desirable in a therapeutic agent. The biological changes observed in the course of treatment with cortisone and A.C.T.H. may be summarised as an initial retention and later liberation of sodium chloride and body water, reduction of plasma potassium, and urinary output of nitrogenous material greater than nitrogen intake.

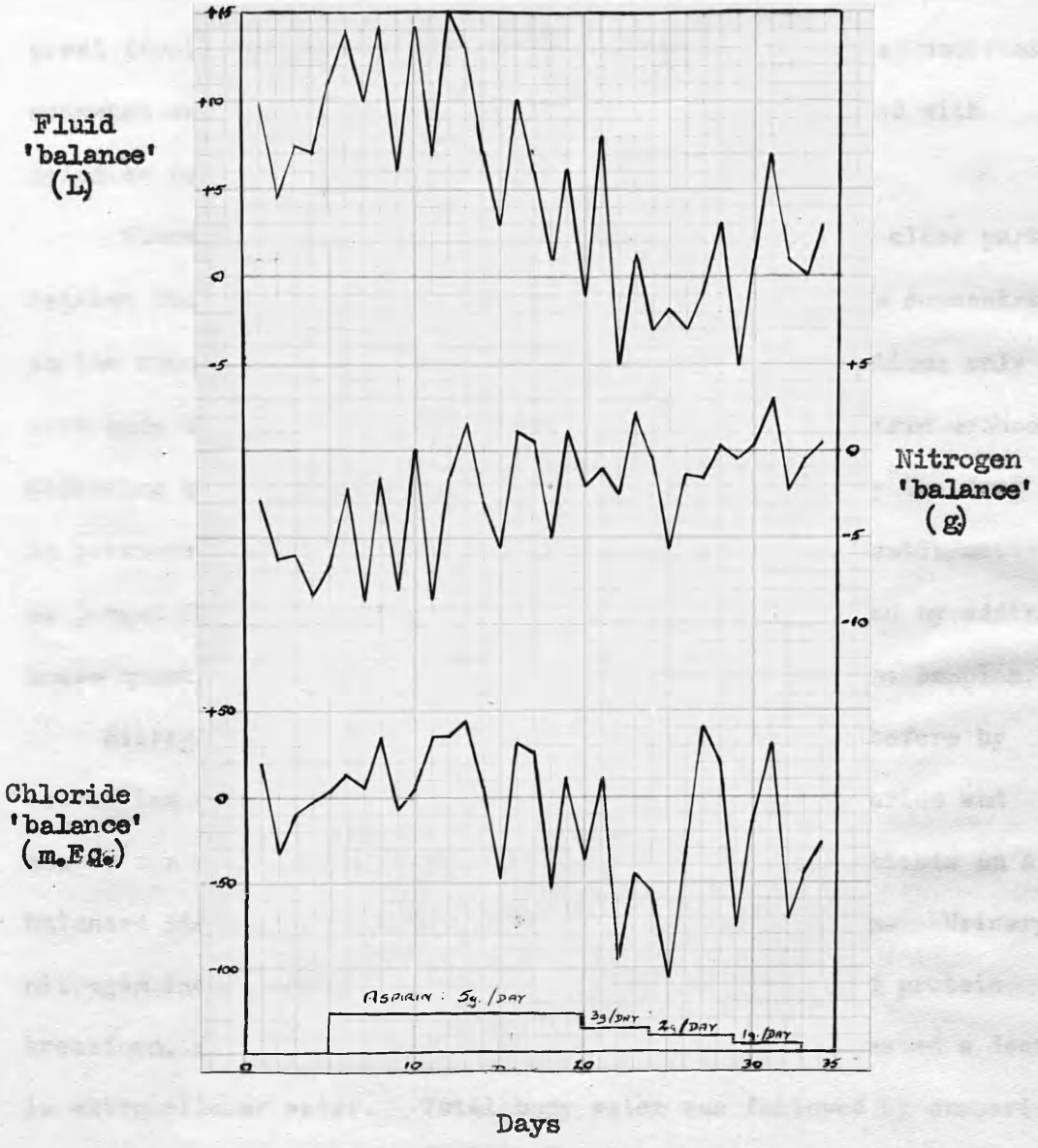
on shifts
This latter series of observations/ in body water and effect on nitrogen balance induced by cortisone therapy bore a sufficiently close resemblance to the observations already made on the biological results of salicylate therapy to render it most natural that close watch should be maintained for any further signs of analogy between cortisone and salicylate administration. In the course of this series of observations of patients under treatment with aspirin, it was found that the rounding of facial contours, acne and depression of mental state constituting the outward symptoms of a mild Cushing's syndrome were visible in one patient. Determination of ability to metabolise glucose and haemoglobin count confirmed that a mild Cushing's syndrome had developed. Fig. IV emphasises the normality of the other biological results obtained. The initial retention, followed by later liberation, of chloride and body water and the high urinary nitrogen values are clearly shown just as already described (Ch.I) for salicylate therapy, and as found by Hench in cortisone therapy.

This observation, published by Cochran, Watson and Reid (Brit. Med.J., 1950, 2 , 1411) was most important in providing further evidence in support of a common mode of action by cortisone, A.C.T.H. and salicylate. It appears that to some extent at least the method of action, or the body defence mechanism induced to act, is identical for the cortisone group of drugs and the salicylate group. The mildness of the Cushing's syndrome, and the activity of the "special salicylate syndrome" which will frequently be present simultaneously, make it not unnatural that the former should not previously have been

noted, but when attention was drawn, the evidence was there for correlation. The observation has since been confirmed in several private communications.

There is no doubt at all from the work of Hench that in cortisone a therapeutic agent for the treatment of rheumatic conditions active in much lower concentrations than salicylate has been discovered. It is probable, however, that the difference between effective therapeutic plasma concentrations and concentration resulting in the appearance of mild toxic symptoms is not appreciably greater than for salicylate itself. But the greatest disadvantage to the use and development of cortisone therapy is the scarcity and cost of the drug. Cortisone was originally obtained by the extraction of cattle or hog adrenals, but, quite apart from the low yield and the laboriousness of the process, it is very doubtful if supplies from this source could ever be adequate. Rheumatic fever and allied disorders are too widespread and frequent in occurrence for such an essentially limited source of medicament to be at all satisfactory. Naturally a great deal of work is being carried out regarding its possible complete synthesis from readily obtainable materials, but to date the main source remains its partial synthesis from other naturally occurring steroids, and the cost remains high. The introduction of a drug with something in common with both salicylate and cortisone, but with the disadvantages of neither, would therefore seem most desirable. It is possible that the action of both is similar, and it might be reasonable to expect that a drug possessing

Fig. IV



The fluid, chloride and nitrogen 'balances' in a case of rheumatic fever treated with acetyl salicylic acid and exhibiting the symptoms of a mild Cushing's syndrome.

the therapeutic activity, but not causing the toxic symptoms of either, might exist.

Preliminary trials of sodium 2:6-dihydroxybenzoate had shown great promise, so that a more detailed investigation was undertaken, somewhat analogous to the investigation clearly completed with relation to sodium salicylate.

Since the work with sodium salicylate had shown a close parallel between the concentration of the drug in plasma, and the concentration in the urine, for greater convenience urinary determinations only were made of sodium 2:6-dihydroxybenzoate. A colorimetric method depending on measurement of the blue colour developed by the drug in presence of aqueous ferric chloride was found quite satisfactory, as judged from the values obtained for standards prepared by adding known quantities of sodium 2:6-dihydroxybenzoate to urine samples.

Nitrogen and chloride "balance" was determined as before by estimation of the nitrogen and chloride content of the urine and comparison with the known intake maintained with the patients on a balanced diet throughout the course of the investigation. Urinary nitrogen excretion greater than dietary intake indicated protein breakdown, similarly a negative chloride "balance" suggested a decrease in extracellular water. Total body water was followed by comparison of urine volume excreted and of total body weight with the fluid intake. A decrease in weight coupled with urinary excretion greater than fluid intake indicated a drop in total body water.

Since the drug was being used for the first time, some investigation was first made of its effects on healthy adults. A dose of 200 mg. sodium 2:6-dihydroxybenzoate was given orally to each of eight healthy adult males having the same balanced diet and fluid intake, and the urine collected for 24 hours. There were no visible objective or subjective reactions to this dose. The excretion in the urine is shown in Table VI. Considerable quantities appeared in the urine in the first two hours after the dose, and the average peak excretion was reached within the second two hour period. Thereafter the rate of excretion steadily fell until at 24 hours only small quantities were present. The average amount excreted in 24 hours was about 67 % of the dose given. The indications are therefore that sodium 2:6-dihydroxybenzoate is rapidly absorbed, and that the peak blood levels are probably reached within two to four hours of oral administration.

A single dose of 200 mg. did not therefore appear to have any harmful effects. Since the drug was not previously known, and the most effective dosage indeterminate, the amount administered orally daily varied considerably more than in the investigation with sodium salicylate. Thus six patients received amounts varying from 1.8g. in three days to 10g. in ten days, an average of 0.8g. per day. This was not a considerable variation, so that the six patients could be treated as a group receiving a dosage of sodium 2:6-dihydroxybenzoate equal to about one tenth the usual therapeutic dose of sodium salicylate.

Table VI.

Urinary excretion of sodium 2:6-dihydroxybenzoate by healthy males.

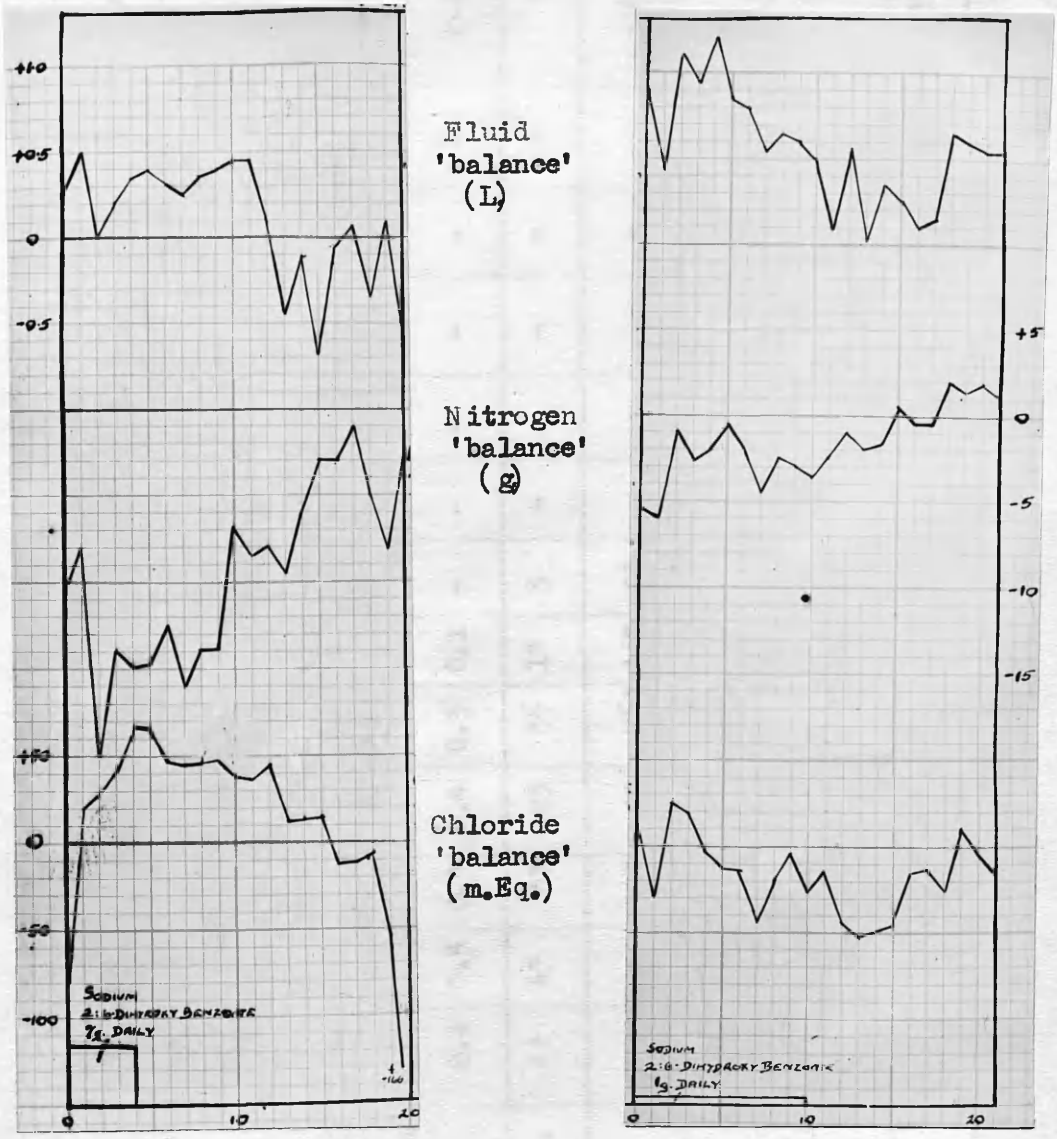
Case	Urinary excretion of 2:6-dihydroxybenzoate (mg.) at intervals after 200 mg. by mouth.					Total 24 hrs.
	0-2 hrs.	2-4 hrs.	4-6 hrs.	6-13 hrs.	13-24 hrs.	
1	19	30	27	45	14	135
2	23	46	36	41	13	159
3	33	60	43	36	18	190
4	13	18	24	34	0	89
5	22	41	37	54	8	162
6	23	33	31	-	-	-
7	18	34	27	10	10	99
8	20	19	24	32	10	105
Range	13-33	18-60	24-43	10-54	0-18	89-190
Average	21	35	31	36	10	134

Table VII.

Effect of sodium salicylate and sodium 2:6-dihydroxybenzoate on the E.S.R.

Case	Sodium salicylate E.S.R.(mm./hr.) group.			Sodium 2:6-dihydroxybenzoate (E.S.R. mm./hr) group.		
	Immediately before salicylate	Peak Value during first week	Value after 24-31 days	Immediatlely before 2:6-dihydroxy-benzoate	Peak value during first week	Value after 25-32 days.
1	125	117	51	111	117	54
2	118	104	35	91	125	58
3	111	119	8	92	92	54
4	107	121	40	90	110	54
5	105	100	46	85	91	-
6	81	117	66	74	75	34
7	60	108	71	71	106	11
Average	101	112	45	88	102	47

Fig. V



Days

Days

The fluid, nitrogen and chloride 'balances' in a case of rheumatic fever treated with sodium 2: 6-dihyroxybenzoate.

Table VIII.

Urinary excretion of sodium 2:6-dihydroxybenzoate in Rheumatic Fever.

Case	Day		1	2	3	4	5	6	7	8	9	10	11	12	Total	% dose excreted
1	dose (g.)		0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.2	-	10	
	urinary excretion	mg./100ml.	46	52	73	81	87	60	64	52	46	55	7	0		63
		mg./24 hrs.	466	702	588	580	538	625	675	726	588	713	103	0	6304	
2	dose (g.)		0.4	0.5	0.5	0.4	0.3	0.1	-	-	-	-	-	-	2.2	
	urinary excretion	mg./100ml.	42	47	41	25	36	18	3	-	-	-	-	-		73
		mg./24hrs.	193	338	375	256	263	148	23	-	-	-	-	-	1596	
3	dose (g.)		1.0	0.8	0.8	0.8	0.6	0.6	0.1	-	-	-	-	-	4.7	
	urinary excretion	mg./100ml.	66	76	71	68	68	67	50	19	9	-	-	-		72
		mg./24hrs.	732	167	426	537	559	207	600	137	74	-	-	-	3439	

For comparison purposes the seventh patient was given a dose of 7g. per day, an amount about equal to the usual dose of sodium salicylate. The daily dose in each case was divided into five equal portions and given at four hourly intervals, starting at 8 a.m.

E.S.R. and clinical condition: The most striking feature after administration of sodium 2:6-dihydroxybenzoate was the promptness of relief of symptoms. Within a few hours three of the patients were able to move acutely affected joints which had been practically immobile. In the other four patients the same stage was reached in two to three days. The changes in the E.S.R. are shown on Table VIII with the corresponding figures for a group of patients treated with 7.5 - 10g. sodium salicylate given alongside for comparison purposes. It will be seen that the drugs affect the E.S.R. to about the same extent and at about the same rate regardless of the much lower dosage of sodium 2:6-dihydroxybenzoate. In some patients however, symptoms of the "special salicylate syndrome" made an appearance, and disappeared only when administration of the drug was stopped. In two patients a fairly well characterised Cushing's syndrome developed, and was confirmed by verification of the low ability of the patient to metabolise glucose.

Urinary excretion of sodium 2:6-dihydroxybenzoate: The amounts of drug excreted in the urine was estimated daily for three of the seven patients, and the results summarised in Table VIII. Rather surprisingly, the amount excreted (63-73% of the dose given) was within the limits excreted by healthy individuals.

Nitrogen "balance": The results for a typical patient in the group receiving the smaller dose, and for the patient receiving the larger dose are shown in Fig. V. The findings were the same in all cases, differing only in degree. During the period of drug administration a negative nitrogen balance, indicating protein breakdown, was induced. On cessation of therapy a normal balance was again established.

Total body water: The results are also summarised in Fig V. Initial retention of water during treatment with the drug is followed by a diuresis on stopping drug administration.

Extracellular water: As explained previously, the chloride balance usually parallels the sodium figure, and therefore gives a measure of the changes in extracellular water. As shown in Fig V the extracellular water parallels the general fluid balance.

It is possible to see from these data that the same pattern of clinical and biochemical changes develops under therapy with sodium 2:6-dihydroxybenzoate as under sodium salicylate and cortisone. The characteristic "special salicylate syndrome" made its appearance in several patients, while the appearance in two of symptoms of the almost equally specific Cushing's syndrome was noted with great interest. At the same time the nitrogen and chloride balance and fluid shift figures are in very close agreement with the observations already made on patients under treatment with sodium salicylate and with the observations of Hench on treatment with cortisone.

It seems probable, therefore, that sodium 2:6-dihydroxybenzoate is

considerably more active than sodium salicylate, and acts in a similar manner. Similar results with one tenth the dose is a fairly reliable criterion of greater activity. Unfortunately, however, the increased activity is not solely with reference to the effect in reducing the symptoms of rheumatic fever. The toxic features of the action of sodium salicylate are also reproduced in patients when the dosage of sodium 2:6-dihydroxybenzoate is one tenth the usual salicylate dosage. Just as Cushing's syndrome appears in patients under treatment with 10g. sodium salicylate per day, and 100 mg. cortisone per day, so a 1 g. per day dose of sodium 2:6-dihydroxybenzoate is sufficient to induce the less desirable side effects. The effective therapeutic concentration of sodium 2:6-dihydroxybenzoate does not therefore, seem to be sufficiently different from the concentration producing toxic symptoms to permit it to be regarded as an ideal therapeutic agent.

The increased interest in sodium 2:6-dihydroxybenzoate and the increased demand for samples, did however, lead to some considerable research into possible methods of preparation. The method of preparation by Limaye and Kelkar[51] depending on the Nidhone process[57] already outlined was effective but laborious. The necessity for fractional crystallisation of the rearrangement products of 4-methyl-7-acetoxycoumarin in presence of anhydrous aluminium chloride in order to separate the 4-methyl-7-hydroxy-8-acetylcoumarin from the 4-methyl-7-hydroxy-6-acetylcoumarin also formed reduced the quantity of product ultimately obtained and greatly increased the time required.

An average of 4-6 recrystallisations from dilute alcohol was necessary to obtain a product melting 167-8°. The modification of Russell and Frye[58] for the preparation of 2:6-dihydroxyacetophenone was not found to show any appreciable improvement. The use of sulphuric acid in place of phosphoric acid for the initial condensation of resorcinol with acetoacetic ester required great care to avoid high colour and charring, so that both the quantity and quality of the 7-acetoxy-4-methyl coumarin obtained were lower than that of the product prepared according to the method of Limaye. Constant stirring during the addition of either acid to the resorcinol, acetoacetic mixture, and for 2-3 hours thereafter was, however, advantageous. The best overall yield of 2:6-dihydroxybenzoic acid from resorcinol by the method of Limaye was only 7 %.

Of the other methods known, direct carboxylation by the Kolbe[59] process was attractive. 2:6-dihydroxybenzoic acid was first prepared by this method in low yield by Senhofer and Brunner[60] by heating resorcinol with a concentrated solution of ammonium carbonate at 120-130°. A mixture of 2:4-dihydroxybenzoic acid, 2:6-dihydroxybenzoic acid and resorcinol 2:4-dicarboxylic acid was obtained, the 2:6-dihydroxybenzoic acid being present only in small quantity. Brunner[61] later obtained a 40% yield of almost pure 2:6-dihydroxybenzoic acid by heating resorcinol with sodium bicarbonate in glycerol at 135° with a stream of carbon dioxide passing through the mixture. Mauthner[62] failed to confirm this result, the product in his case consisting

of 2:4-dihydroxybenzoic acid. Clibbens and Nierenstein[63] showed that 2:4-dihydroxybenzeic acid could be prepared in 60% yield by the treatment of resorcinol in aqueous sodium or potassium bicarbonate in a stream of carbon dioxide.

Experiment confirmed the difficulty of duplicating the results claimed by Brunner[61]. In several experiments after 6 hrs. heating at 135° an approximately 30% yield of predominantly 2:4-dihydroxbenzoic acid was always obtained. The amount of 2:6-dihydroxybenzoic acid separated by fractional crystallisation from warm water, in which it is very soluble, was never greater than 1 %. On increasing the reaction time with continued passage of carbon dioxide first to 12 hrs., then to 24 hrs., the yield of purified 2:6-dihydroxybenzoic acid obtained did not exceed 2 %. Replacement of sodium bicarbonate by potassium bicarbonate and of glycerine by water likewise did not appear to affect the course of the reaction. With ammonium carbonate in place of sodium carbonate the yield of both isomers was slightly lowered.

It appeared, therefore, that the earlier investigators were justified in discarding this attractive direct method of preparation, and it was accordingly abandoned. Even had the yield of mixed 2:4-dihydroxybenzoic acid and 2:6-dihydroxybenzoic acid been considerably increased it is doubtful if the constant fractional crystallisation required and constant danger of decarboxylation due to over heating would justify continued adherence to the method.

The method of Mauthner[64, 62], though at first sight rather

unwieldy in the early stages, was soon developed into a practical and convenient method of laboratory preparation of 2:6-dihydroxybenzoic acid. The synthesis is based on the observation of de Bruyn[65] on the reaction of m-dinitrobenzene and potassium cyanide in alcoholic solution. De Bruyn showed that on the addition of a cold concentrated aqueous potassium cyanide solution to a solution of m-dinitrobenzene in methyl alcohol or in ethyl alcohol, a nitrile group was introduced to the 2-position and one of the nitro groups replaced by a methoxy or ethoxy group respectively. On refluxing the product with a solution of potassium hydroxide in the appropriate alcohol yields of 2:6-dimethoxy benzonitrile, 2:6-diethoxybenzonitrile or 2-methoxy-6-ethoxybenzonitrile were obtained. Mauthner showed that 2:6-dimethoxybenzonitrile so obtained could be hydrolysed by aqueous potassium hydroxide solution and demethylated by anhydrous aluminium chloride in benzene to give 2:6- dihydroxybenzoic acid. Russell and Tebbens[66] have modified somewhat the method of preparation of 2:6-dimethoxybenzonitrile but the yield was not greatly increased compared with that claimed by Mauthner.

The overall yield of 2:6-dihydroxybenzoic acid from m-dinitrobenzene by the method outlined above was around 6% but this was soon improved to 12-15% The improvement in the first three stages was largely confined to technique and will be dealt with later. In connection with the demethylation of the 2:6-dimethoxybenzoic acid, however, there seemed considerable probability of an improvement in the method.

The method as described by Mauthner gives a 40% yield of rather impure 2:6-dihydroxybenzoic acid, the impurity of which seemed to be enhanced rather than otherwise by his technique of steam distilling off the benzene solvent. In dealing with 2:6-dihydroxybenzoic acid, in which there is noticeable decarboxylation if the solid or its solution remain over 80° for any length of time, it seemed most desirable to keep the reaction temperature down as much as possible. Accordingly, a method relying on filtration and separation of the benzene phase rather than on steam distillation was soon developed and the yield from the demethylation increased to 85-95% of 2:6-dihydroxybenzoic acid m.p. 158-160° from 2:6-dimethoxybenzoic acid. A pure specimen of the acid could be obtained when necessary by recrystallisation from water or benzene, but it was found that sample specimens of acid recovered from sodium 2:6-dihydroxybenzoate made from acid of the above standard of purity had a melting point of 164-165°, so that most material was not further purified before conversion into salt.

However, a considerably wider investigation into possible alternative methods of demethylation yielded some interesting results. Treatment with anhydrous aluminium chloride in boiling chloro-benzene and in nitrobenzene in place of benzene yielded only resorcinol. The demethylation was just as successful as with benzene as a solvent, but the higher temperature resulted in prompt decarboxylation of the

2:6-dihydroxybenzoic acid formed. Treatment with anhydrous aluminium chloride in chlorobenzene and in nitrobenzene at 80° with stirring produced quantities of 2:6-dihydroxybenzoic acid comparable to those obtained in boiling benzene solution. The demethylation was also successful with anhydrous aluminium bromide in place of anhydrous aluminium chloride, but the yield of the same order of magnitude, so that a change to the reagent was not justified.

Some investigation into the possible use of bound hydrogen halide acids as demethylating agents was also carried out. Although it is possible that the use of such compounds, suggested by Prey[67] as a means of achieving high temperature reaction with hydrogen halides without the need of pressure tubes, might not seem particularly apposite to the problem of demethylation of 2:6-dimethoxybenzoic acid, it was thought that it might be possible by lowering the reaction temperature, to initiate a smooth, steady, convenient reaction. Thus pyridine hydrochloride, used by Prey as a convenient reagent between its m.p./144° and its b.p. without decomposition at 218°, was heated with 2:6-dimethoxybenzoic acid at 200° and at 150°. Resorcinol only was obtained in each case. With quinoline hydriodide in boiling quinoline (b.p. 240°) as used by Baddar[68], and with quinoline hydriodide just above its melting point (135°), similar results were obtained. Warming on the steam bath with moist pyridine, suggested by Cahn[69] as a possible means of demethylation under mild conditions,

yielded only unchanged starting material.

Finally, the low temperature demethylation method of Freudenberg and his associates[70] was investigated with respect to 2:6-dimethoxybenzoic acid. A solution of the acid in liquid ammonia on treatment with metallic potassium yielded material soluble in sodium bicarbonate which proved on crystallisation from benzene to be a mixture of unchanged 2:6-dimethoxybenzoic acid together with some 2-hydroxy-6-methoxybenzoic acid. An increase in reaction time from 3 to 6 and finally to 12 hrs. did not increase the proportion of 2-hydroxy-6-methoxybenzoic acid above 25 % On treating this acid itself with potassium in liquid ammonia, the starting material was recovered unchanged. On treating it with anhydrous aluminium chloride in benzene solution, 2:6-dihydroxybenzoic acid was obtained.

It seemed, therefore, that the improved method of demethylation in benzene on treatment with anhydrous aluminium chloride was by far the most satisfactory, and it was therefore adhered to for the demethylation of such other 2:6-dimethoxybenzoic acid as was prepared.

Since the sodium salt rather than the free acid was administered to patients, sodium 2:6-dihydroxybenzoate was always prepared by addition of the free acid to N-sodium bicarbonate solution at 60-70°.

The ethyl and n-propyl esters of 2:6-dihydroxybenzoic acid and the ethyl, n-propyl, n-amyl, and n-hexyl esters of 2:6-dimethoxybenzoic acid were also prepared with a view to examination in order to find a drug of slower and more gradual action than sodium

2:6-dihydroxybenzoate itself (Watson, J.C.S., 1952, 2940).

It has since been shown[71] that direct carboxylation of resorcinol by treatment with an aqueous solution of potassium bicarbonate under carbon dioxide at 27 atmospheres yields a mixture of equal parts of 2:4- and 2:6-dihydroxybenzoic acids which may be separated by ion exchange chromatography.

Experimental.

Urinary 2:6-dihydroxybenzoate. The concentration of sodium 2:6-dihydroxybenzoate in the urine was measured by a simple colorimetric method. Urine (1 ml.) was diluted to 25 ml. with a pH 2 buffer and saturated aqueous ferric chloride solution (0.1 ml.) added. A blue colour developed, the intensity of which was promptly measured on a colorimeter. Comparison with standards made by the addition of known quantities of sodium 2:6-dihydroxybenzoate to the urine indicated the amount of drug present. The blue colour is not stable, and soon turns mauve or brown, but satisfactory results were obtained when the colorimeter reading was taken immediately after the iron solution is added.

E.S.R. The sedimentation rate of erythrocytes in whole blood was measured as before[19].

Urinary nitrogen. The concentration of chloride in the urine was determined as already described[21].

Sodium 2:5-dihydroxybenzoate. Gentisic acid was prepared by the method of Mauthner[35]. Salicylic acid was oxidised in alkaline solution by potassium persulphate in presence of ferrous sulphate, when on extraction of the acidified, filtered reaction mixture with ether, the gentisic acid was obtained. On recrystallisation of the product from water, a yield of 38% of acid m.p. 197-199° was obtained (cf. 196°) [35]. The sodium salt was prepared in the same way as that of 2:6-dihydroxybenzoic acid.

Sodium 2:6-dihydroxybenzoate.

(a) Synthesis from resorcinol through 2:6-dihydroxyacetophenone: The modified method found most satisfactory was as follows:-

(I) 4-methyl-7-hydroxycoumarin: Resorcinol (22g.) was added to ortho-phosphoric acid (d. 1.75, 75 g.) in a round bottomed flask fitted with mechanical stirrer, glycerol seal and calcium chloride drying tube, and the mixture cooled in an ice bath. Acetoacetic ester (52 g.) was added over 1 hr. with constant stirring, and stirring continued for a further 3 hrs. The mixture was left overnight then poured into water (500ml.) stirred for 15 mins., and the precipitate collected and again stirred with water (500ml.) for 15 mins. The material thus obtained was refluxed with 80% ethyl alcohol and activated charcoal and recrystallised as colourless needles m.p. 186-7° (cf. 185-6°)[51]. Weights and melting points of yields obtained from resorcinol (22g. 0.2 g.m.) as starting material are given in Table IX.

Table IX.

4-methyl-7-hydroxycoumarin from resorcinol (22 g., 0.2 g.m.)

Condensing agent.	Without mechanical stirring.	With mechanical stirring.
ortho-phospheric acid.	(a) 25.3g. m.p. 185-6° (b) 24.5g. m.p. 184-6°	(a) 28.7g. m.p. 186-7° (b) 28.4g. m.p. 186-7°
sulphuric acid.	(a) 24.1g. m.p. 183-6° (b) 24.8g. m.p. 184-6°	(a) 27.6g. m.p. 185-7° (b) 26.9g. m.p. 186-7°

It was found that the quantity of phosphoric acid recommended by Limaye[51] was rather meagre, but stirring improved both the quantity and quality of the yield, even after the quantity of phosphoric acid had been increased. With sulphuric acid as condensing agent, as recommended by Russell[58], great care was necessary to avoid the development of high colour in the product.

II. 4-methyl-7-acetoxycoumarin: The acetyl derivative was prepared by refluxing 4-methyl-7-hydroxycoumarin (25 g.) and fused sodium acetate (25 g.) with acetic anhydride (50 g.) for 2 hrs. The reaction mixture was cooled, poured into water (500 ml.), stirred for 15 mins., and the precipitate collected and again stirred with water (500 ml.) for 15 mins. The 4-methyl-7-acetoxycoumarin thus obtained was recrystallised from 90% ethyl alcohol and obtained as colourless needles, m.p. 150-151° (cf. 151°)[51]. The yields obtained varied from 25.9 g. to 27.1 g. It was felt that recrystallisation

was advisable to ensure a uniform purity of starting material for the next stage.

III. 4-methyl-7-hydroxy-8-acetylcoumarin: 4-methyl-7-acetoxycoumarin (20 g.) was well mixed with anhydrous aluminium chloride (45 g.) in a 1 L. round bottomed flask. The temperature was raised to 160° over 1 hr. and maintained at that temperature for a further hour. The reaction mass was then cooled, broken up somewhat and water at 0° (250 ml.) added over 30 mins. followed by hydrochloric acid (250 ml. of 2 N). The mixture was then heated on the steam bath for 30 mins. with mechanical stirring. The product obtained was filtered, washed with water and recrystallised from 95 % ethyl alcohol, when greenish yellow crystals melting 154-158° were obtained. Four further recrystallisations from the same solvent yielded pale green crystals m.p. 166-7° (cf. 167-8°)[61]. The yields obtained from 7-acetoxy-4-methylcoumarin (20 g.) by this method and by the procedures of Limaye[51] and Russell[58] are indicated in Table X.

The method of extraction of the product in small quantities of 10% sodium carbonate solution was not found to serve any useful purpose. Neither quantity nor quality of yield seemed to be affected. The method of Russell was not found to give a greatly improved product. The melting point of the initial mixture obtained from the reaction was somewhat higher, but the final yield of pure 7-hydroxy-8-acetyl-4-methylcoumarin was not considerably enhanced.

Table X.

4-methyl-7-hydroxy-8 acetylcoumarin from 4-methyl-7-acetoxycoumarin (20g.)

Method	Melting pt. after one re-crystallisation	Melting pt. after five re-crystallisations	Weight after five re-crystallisations
as given by Limaye.	(a) 149 - 154° (b) 150 - 154° (c) 150 - 155°	165 - 166° 166 - 167° 164 - 166°	3.1 g. 4.0 g. 3.4 g.
as given by Russell.	(a) 154 - 158° (b) 154 - 159° (c) 153 - 158°	165 - 166° 165 - 166° 166 - 167°	4.3 g. 4.5 g. 4.6 g.
as given above .	(a) 154 - 158° (b) 153 - 158° (c) 154 - 159°	166 - 167° 165 - 166° 166 - 167°	4.6 g. 4.8 g. 4.5 g.

IV. 2:6-dihydroxyacetophenone: 7-hydroxy-8-acetyl-4-methylcoumarin (9.0g.) was placed in a 250 ml. round bottomed three necked flask, fitted with mechanical stirrer with mercury seal, dropping funnel, reflux condenser, gas inlet and gas outlet with mercury tap. Water (25 ml.) was added, and nitrogen passed through the apparatus for 15 mins. then sodium hydroxide solution (37.5 ml. of 20%) added, and the reaction mixture heated for 6 hrs. on the steam bath, with constant mechanical stirring. At the end of this time the mixture was cooled, acidified with 4 N hydrochloric acid and the precipitated reddish brown mass, collected, washed with water, and recrystallised. from water when a yield of 5.3 g. pale yellow thick needles m.p. 155-156° was obtained.

If a nitrogen atmosphere was not maintained, the purity of the yield deteriorated somewhat, though the quality was little affected (5.2 g., m.p. 153-5°). Increasing the length of time the mixture was heated on the steam bath to 12 hrs. also resulted in no significant improvement in yield (5.4 g., m.p. 154-6°) (cf. m.p. 157-8°)[58].

V. 2-methyl-3-acetyl-5-hydroxychromone: 2:6-dihydroxyacetophenone (5g.) was heated with fused sodium acetate (5 g.) and acetic anhydride (10 g.) at 160° for 5 hrs. The mixture was then cooled and poured into water and the brown powder so obtained dissolved in ether (200 ml.) This ether extract was washed twice with N sodium hydroxide solution (40 ml.) and twice with water (40 ml.) On acidification of the combined aqueous extract with 4 N hydrochloric acid, a pale brown powder was precipitated, which yielded 4.8 g. colourless crystals m.p. 122° on recrystallisation from 20% acetic acid (cf. m.p. 122°)[51].

VI. 2:6-dihydroxybenzoic acid: 2-methyl-3-acetyl-5-hydroxychromone (5 g.) was warmed on the steam bath for 2 hrs. with N caustic soda (50 ml.). The mixture was then cooled in an ice bath and acidified with cooled 4 N hydrochloric acid. The powder precipitated out was redissolved in sodium carbonate solution (35 ml. of 10%) and reprecipitated with 4 N hydrochloric acid. The pink product so obtained was recrystallised from water to give 3 g. colourless crystals m.p. 165-6° (cf. 166-7°)[51].

2 methyl-3-acetyl-5-acetoxychromone: 2 methyl-3-acetyl-5-hydroxychromone (2 g.) was heated with fused sodium acetate (2 g.) and acetic anhydride (15 g.) at 150° for 4 hrs. The mixture was then

cooled, and the powder so obtained dissolved in ether (100 ml.). This ether extract was washed twice with N sodium hydroxide (20 ml.) and twice with water (20 ml.), then dried over anhydrous sodium sulphate, and the ether evaporated off. On recrystallisation of the residue from water, 0.9 g. colourless crystals m.p. 108-9° were obtained (cf. m.p. 109°)(51).

The same product was obtained directly from 2:6-dihydroxyacetophenone (2 g.) by heating at 160° for 6 hrs. with fused sodium acetate (2 g.) and acetic anhydride (20 g.) On working up the reaction mixture in the same way, 0.7 g. 2-methyl-3-acetyl-5-acetoxychromone m.p. 108-9° was obtained. Acidification of the alkaline washings with 4 N hydrochloric acid, and recrystallisation of the precipitate from 20% acetic acid, yielded 0.1 g. 2-methyl-3-acetyl-5-hydroxychromone m.p. 121-2°.

(b) Synthesis from resorcinol by direct carboxylation: Dry resorcinol (22 g.) was placed in a 500 ml. round bottomed flask fitted with gas inlet to bottom of flask, reflux condenser and gas outlet with mercury trap. Sodium bicarbonate (44 g.) and glycerol (44 g.) were added, and the mixture heated to 135° for 6 hrs. with constant passage of carbon dioxide. The mixture was then cooled and water (150 ml.) added, and the whole well stirred, filtered and extracted twice with ether (300 ml.) The aqueous solution was then acidified with 4 N hydrochloric acid, and extracted four times with ether (500 ml.) The combined ethereal solution was dried over

anhydrous sodium sulphate, the ether removed, and 13.5 g. material melting 146-194° obtained. This material was recrystallised from water (50 ml.). The less soluble fraction after three further recrystallisations from water, weighed 9.8 g. and proved to be 2:4-dihydroxybenzoic acid. From the aqueous solution after saturation with sodium chloride and extraction with ether a residue was obtained from which after further fractional crystallisation from water 0.3 g. 2:6-dihydroxybenzoic acid m.p. 163-5° was finally separated. A similar technique was employed in the other experiments on direct carboxylation, the results of which are summarised on Table XI.

The statistical significance of these results is doubtful, but the yield seemed consistently too low, and too laboriously gained, to justify the accumulation of further data on the direct carboxylation of resorcinol at atmospheric pressure.

(c) Synthesis from m-dinitrobenzene through 2:6-dimethoxybenzonitrile: The method finally adopted was as follows:

I. 2-nitro-6-methoxy benzonitrile: m-dinitrobenzene (500 g.) was dissolved in methyl alcohol (7.5 L.) in a 10 gallon earthenware crock placed in a sink supplied with hot and cold water to serve as thermotank. The temperature was raised to 40° and a solution of potassium cyanide (230 g.) in water (400 ml.) added over a period of 15 mins., with constant mechanical stirring throughout the addition. The mixture was maintained at 40° for a further 2 hrs., vigorous stirring being continued during this period. The dark purple mixture

Table XI.

Dihydroxybenzoic acids by direct carbonation of resorcinol (22g.,0.2g.m.)

Starting material	Experimental conditions.	Initial bicarbonate soluble product.		2:4-dihydroxy benzoic acid (isolated) g.	2:6- dihydroxy benzoic acid (isolated) g.
		weight g.	m.p. °C.		
resorcinol (22 g.) sodium bicarbonate (44 g.)	6 hrs. at 135°	(a) 14.4 (b) 13.5 (c) 15.3	141-198 146-194 142-195	9.2 9.8 9.5	- 0.3 0.7
glycerol (44 g.)	12 hrs. at 135°	15.6	140-189	9.0	0.9
	24 hrs. at 135°	15.2	143-197	9.1	1.7
resorcinol (22 g.) potassium bicarbonate (44 g.) glycerol (44 g.)	6 hrs. at 135°	14.9	148-199	10.3	-
resorcinol (22 g.) sodium bicarbonate (44 g.) water (44 g.)	6 hrs. at 100°	15.1	144-196	10.1	0.4
resorcinol (22 g.) ammonium carbonate (44 g.) water (44 g.)	6 hrs. at 100°	12.8	149-191	9.7	0.2

was then allowed to stand at room temperature for 3 days, at the end of which period the black precipitate was filtered off under reduced pressure, and water (60 L.) added to the filtrate, and the mixture stirred for 1 hr., then allowed to stand overnight, when a further quantity of dark brown precipitate was obtained. The combined precipitates were dried as much as possible on the Buchner funnel for 3 days, then exhaustively extracted with chloroform in a Soxhlet apparatus. The combined chloroform extracts were then concentrated to 500 ml. by distillation and 60-80° petroleum ether (1 L.) added to precipitate out the crude 2-nitro-6-methoxybenzanitrile. This red powder was filtered and dried on the Buchner funnel. Yields obtained from m-dinitrobenzene were 154 g., 163 g., 172 g., 168 g., representing an average of 34 %. This material was not further purified before proceeding to the next stage.

(2) 2:6-dimethoxybenzonitrile : 2-nitro-6-methoxybenzonitrile (8 g.) was refluxed for 2 hrs. with a solution of potassium hydroxide (64 g.) in methyl alcohol (1.5 L.). The solution was then concentrated by distillation to a volume of 320 ml. and poured into water (3 L.). The brown solid so obtained was washed with cold water, dried and refluxed with chloroform (240 ml.) and activated charcoal. 60-80° petroleum ether (400 ml.) was added to the filtrate and the light brown needles precipitated, filtered, and dried, m.p. 116-7°. (cf. 116-7°).[66] Yields obtained were 56 g., 54 g., 57 g., 56., 58 g., 59 g., representing an average of 64 % calculated from 2-nitro-6-methoxybenzonitrile as starting material, or 19.1 % calculated from m-dinitrobenzene.

III. 2:6-dimethoxybenzoic acid: 2:6-dimethoxybenzonitrile (8 g.) was mixed with a solution of potassium hydroxide (200 g.) and water (400 ml.) in a Pyrex flask and the mixture boiled on a sand bath under a reflux condenser for 48 hrs. The solution was then cooled, and the potassium salt of 2:6-dimethoxybenzoic acid collected on a sintered glass filter. It was converted to the acid by dissolving in the minimum quantity of water (approx. 15 ml.) and acidifying the solution with concentrated hydrochloric acid. It was then filtered, washed with water, dried, and purified by extraction with benzene in a Soxhlet apparatus. Yields ranging from 6.0 to 6.8 g. acid m.p. 186-7° (cf. 186-7°)[64] were obtained, representing an average yield of 76 % from 2:6-dimethoxybenzonitrile or 14.9 % from m-dinitrobenzene.

IV. 2:6-dihydroxybenzoic acid: 2:6-dimethoxybenzoic acid (6 g.) was dissolved in dry benzene (360 ml.), powdered anhydrous aluminium chloride (22.4 g.) added, and the mixture heated on the water bath for 2 hrs. under reflux with constant stirring. The solution was then cooled, poured on to powdered ice (150 g.), filtered, and the benzene layer separated off. The aqueous layer was then acidified with concentrated hydrochloric acid (approx. 15 ml.) and the crystals filtered off and washed with benzene (10-20 ml.). Yields of from 4.3 to 4.8 g. acid m.p. 159-161° were obtained, representing an average yield of 90 % of 2:6-dihydroxybenzoic acid from 2:6-dimethoxybenzoic acid or 13.1 % from m-dinitrobenzene.

V. Sodium 2:6-dihydroxybenzoate: 2:6-dihydroxybenzoic acid (10g.) was dissolved in N sodium bicarbonate solution (30 ml.) by heating on a water bath at 70-80° with stirring. Solid sodium bicarbonate (2.5 g.) was then added, the solution filtered hot and cooled to 0°. The sodium salt was filtered and recrystallised from 0.5 N sodium bicarbonate solution (10-15 ml.) again heating only to 70-80° and cooling to 0°.

Experiments on demethylation of 2:6-dimethoxybenzoic acid.

(a) 2:6-dimethoxybenzoic acid (2 g., 0.11 g.m.) was dissolved in chlorobenzene (20 ml. dried over magnesium sulphate, b.p. 131-2°) and powdered anhydrous aluminium chloride (7.35 g., 0.055 g.m.) added. The mixture was refluxed with mechanical stirring for 1 hr., cooled, poured on to powdered ice (50 g.), filtered, and the chlorobenzene removed by steam distillation. The residual aqueous layer was acidified with concentrated hydrochloric acid (approx. 5 ml.) and extracted twice with ether (200 ml.) The combined ether extract was washed twice with N sodium bicarbonate solution (10 ml.) then twice with N sodium hydroxide solution (10 ml.) On acidification and recrystallisation of the products obtained, the bicarbonate extract yielded 2:6-dihydroxybenzoic acid (0.1 g.) and the sodium hydroxide extract yielded resorcinol (0.8 g.)

On repeating the experiment using the same quantities and conditions but continuing the refluxing for 2 hrs., resorcinol (1.2 g.) only was obtained.

On repeating the experiments with the same quantities, but stirring for 2 hrs. at 80° instead of refluxing, 2:6-dihydroxybenzoic acid (0.9 g.) and resorcinol (0.2 g.) were obtained.

(b) When the above experiment was repeated with nitrobenzene (20 ml.) in place of chlorobenzene, after 1 hr. reflux resorcinol(1.0 g.) only was recovered.

On repeating the experiment with the same quantities but stirring for 1 hr. at 80° instead of refluxing, 2:6-dihydroxybenzoic acid (0.8 g.) and resorcinol (0.1 g.) were obtained.

(c) 2:6-dimethoxybenzoic acid (2g., 0.011 g.m.) was dissolved in benzene (100 ml.) and anhydrous aluminium bromide (14.7 g., 0.055 g.m.) added. The mixture was refluxed for 2 hrs. with constant stirring, then cooled and poured on to powdered ice (50 g.), filtered, and the benzene separated off. The aqueous layer was acidified with concentrated hydrochloric acid (approximately 5 ml.) and the precipitate filtered off and washed with a little benzene, when 2:6-dihydroxybenzoic acid (1.1 g.) m.p. 157-160° was obtained.

(d) 2:6-dimethoxybenzoic acid (2 g., 0.011 g.m.) and pyridine hydrochloride (3.85 g., 0.033 g.m.) were heated for 6 hrs. at 200° in a round bottomed flask fitted with reflux condenser and calcium chloride drying tube. At the end of this time, the mixture was cooled and dissolved in ether (200 ml.). The ethereal solution was washed twice with 2 N hydrochloric acid (20 ml.) and twice with water (20 ml.). On evaporation of the dried ether, resorcinol (0.8 g.) was obtained.

On repeating the experiment with the same quantities and with the same conditions except that the temperature was maintained at 150° instead of 200°, 0.6 g. resorcinol was obtained.

(e) 2:6-dimethoxybenzoic acid (2 g., 0.011 g.m.) was dissolved in quinoline (10 ml., b.p. 237-238°) and quinoline hydriodide (8.5 g., 0.033 g.m.) added. The mixture was refluxed for 6 hrs.under anhydrous conditions, then cooled and dissolved in ether (200 ml.). The ethereal solution was washed twice with 2 N hydrochloric acid (20 ml.) and twice with water (20 ml.). On evaporation of the dried ether, resorcinol (0.9 g.) was obtained.

On repeating the experiment omitting the quinoline, and maintaining the mixture at 150°, resorcinol (0.6 g.) only was eventually recovered.

(f) 2:6-dimethoxybenzoic acid (2 g., 0.011 g.m.) was dissolved in piperidine (10 ml.) in a round bottomed flask fitted with mechanical stirrer and reflux condenser, water (0.5 ml.) added and the solution warmed on the water bath with constant stirring for 30 mins. At the end of this time, the whole was dissolved in ether (200 ml.), washed twice with 2 N hydrochloric acid (20 ml.) and twice with water (20 ml.). On evaporation of the dried ether, only unchanged 2:6-dimethoxybenzoic acid was again recovered.

On repeating the experiment but extending the time of heating to 6 hrs. only unchanged 2:6-dimethoxybenzoic acid was again recovered.

When the experiment was repeated using pyridine (10 ml.) in place of piperidine, unchanged starting material only was recovered.

(g) 2:6-dimethoxybenzoic acid (3 g.) was dissolved in liquid ammonia (150-200 ml.) in a 500 ml. round bottomed flask, fitted with inlet from an inverted ammonia cylinder, stirrer with paraffin seal, and reflux condenser with mercury trap at top. Potassium (3 g.) was added and the ammonia volume maintained during 3 hrs. stirring at room temperature. At the end of this time the ammonia cylinder was replaced by a nitrogen cylinder, and the ammonia evaporated off under a stream of nitrogen. Ether(50 ml.) was then added, and the potassium decomposed by careful addition of water (20 ml.). The ether layer was separated off, diluted up to 200 ml., and washed twice with water (50 ml.) From the mixture obtained on evaporating down from the ether, crystallisation from water finally isolated unchanged 2:6-dimethoxybenzoic acid (1.0 g.) and 2-hydroxy-6-methoxybenzoic acid (0.2 g.) m.p. 134°(cf. 135°)[52].

On repeating the experiment, but extending the reaction time first to 6 hrs. then to 12 hrs., the yield of 2-hydroxy-6-methoxybenzoic acid rose to 0.3 g. and 0.4 g. respectively.

The experiment was then repeated using the pure 2-hydroxy-6-methoxy benzoic acid (0.5 g.) as starting material, but only the unchanged acid was recovered after 12 hrs.

2-hydroxy-6-methoxybenzoic acid (0.5 g.) was dissolved in benzene (30 ml.) and powdered anhydrous aluminium chloride (1.72 g.) added, and the mixture heated on the steam bath for 2 hrs. under reflux

conditions with constant mechanical stirring. The solution was then cooled, poured on to powdered ice (12 g.), filtered, and the benzene layer separated off. The aqueous layer on acidification with concentrated hydrochloric acid yielded 2:6-dihydroxybenzoic acid (0.28 g.) m.p. 158-160°.

Esterification of 2:6-dihydroxybenzoic acid.

Methyl 2:6-dihydroxybenzoate:

(a) 2:6-dihydroxybenzoic acid (5 g.) was dissolved in methyl alcohol (20 ml. dried over sodium), concentrated sulphuric acid (0.2 ml.) added and the solution refluxed for 8 hrs. on the steam bath. The excess alcohol was then distilled off, water (50 ml.) added and the aqueous solution extracted, twice with ether (100 ml.). The combined ethereal solution was washed twice with N sodium bicarbonate solution (20 ml.) and twice with water (20 ml.), dried over anhydrous sodium sulphate and the ether evaporated off. The residue on recrystallisation from 80% methyl alcohol yielded methyl 2:6-dihydroxybenzoate (2.1g.) m.p. 67-68° (cf. 67-68°)[62].

(b) 2:6-dihydroxybenzoic acid (5 g.) was dissolved in methyl alcohol (100 ml. dried over sodium), the solution saturated with dry hydrogen chloride and refluxed 6 hrs. on the steam bath. This process of saturating with hydrogen chloride and refluxing for 6 hrs. was repeated twice more. The alcohol was then distilled off, water (50 ml.) added and the mixture extracted twice with ether (100 ml.). The combined ethereal solution was washed twice with N sodium bicarbonate

solution (20 ml.) and twice with water (20 ml.), dried over anhydrous sodium sulphate, and the ether evaporated off. The residue on recrystallisation from 80% methyl alcohol yielded methyl 2:6-dihydroxybenzoate (3.1 g.).

Ethyl 2:6-dihydroxybenzoate: On dissolving 2:6-dihydroxybenzoic acid (5 g.) in ethyl alcohol (20 ml.) and treating as in process (a) above, ethyl 2:6-dihydroxybenzoate (1.5 g.) was obtained.

When 2:6-dihydroxybenzoic acid (5 g.) in ethyl alcohol (100 ml.) was treated as in process (b) above, ethyl 2:6-dihydroxybenzoate (2.4 g.) was obtained.

The melting points and analyses of ethyl 2:6-dihydroxybenzoate and of the other esters of 2:6-dihydroxybenzoic aand 2:6-dimethoxybenzoic acids of which no record was to be found in the literature, are given in Table XII..

n- propyl 2:6-dihydroxybenzoate. On dissolving 2:6-dihydroxybenzoic acid (5 g.) in n-propyl alcohol (20 ml.) and treating as in process (a) n- propyl 2:6-dihydroxybenzoate (0.6 g.) was obtained.

When 2:6-dihydroxybenzoic acid (5 g.) in n-propyl alcohol (100 ml.) was treated as in process (b) n-propyl 2:6-dihydroxybenzoate (1.8g.) was obtained.

Esterification of 2:6-dimethoxybenzoic acid.

The method was identical with that already described for 2:6-dihydroxybenzoic acid (a). Yields from 2:6-dimethoxybenzoic acid varied from 3.7 g. for the methyl ester to 1.6 g. for the n-hexyl ester.

80 % methyl alcohol was used as solvent for recrystallisation in every case.

Table XII.

Esters of 2:6-dihydroxybenzoic acid and of 2:6-dimethoxybenzoic acid.

Ester	m.p. °C	Found C,%	Found H,%	Formula	Required C,%	Required H,%
ethyl 2:6-dihydroxybenzoate	50-51	59.2	5.5	$C_9H_{10}O_4$	59.3	5.5
n-propyl 2:6-dihydroxybenzoate	38-39	61.0	6.5	$C_{10}H_{12}O_4$	61.2	6.2
ethyl 2: -dimethoxybenzoate	71-72	62.8	6.7	$C_{11}H_{14}O_4$	62.8	6.7
n-propyl 2:6-dimethoxybenzoate	37-38	64.4	7.1	$C_{12}H_{16}O_4$	64.2	7.2
n-amyl 2:6-dimethoxybenzoate	47-48	66.4	7.8	$C_{14}H_{20}O_4$	66.6	8.0
n-hexyl 2:6-dimethoxybenzoate	48-49	67.4	8.2	$C_{15}H_{22}O_4$	67.6	8.3

3-Alkyl-2:6-dihydroxybenzoic acids.

It has been shown that sodium 2:6-dihydroxybenzoate , while considerably more active than sodium salicylate in that it produces comparable results with one tenth the dose, also produces comparable side effects at this dosage. In patients receiving a dose equal to one tenth the usual sodium salicylate dose, or ten times the customary cortisone dose, the "special salicylate syndrome" which is the frequent concommittant to salicylate therapy, and the Cushing's syndrome which has frequently been observed in patients under treatment with cortisone and A.C.T.H. have both been observed. It seems probable therefore, that like salicylate and also cortisone itself, sodium 2:6-dihydroxybenzoate must be regarded not as the ultimate therapeutic agent in a particular field, but as a practical interim drug whose pharmacological and biological properties may be correlated with its chemical properties to give some indication of the characteristics required in an improved substitute. A drug with the therapeutic activity of sodium 2:6-dihydroxybenzoate but which did not induce toxic symptoms except in a dose perhaps four or five times the normal therapeutic dose, would be satisfactory. One which could combine the slow rate of absorption and steady action of cortisone with a more permanent effect on the patient in order to avoid the frequent relapses on cessation of therapy, would represent a great advance.

It is probable that with oral administration of sodium 2:6-dihydroxybenzoate rate of absorption is rather too great. Clinical observations made on patients undergoing treatment with sodium 2:6-dihydroxybenzoate indicated that a four hourly dosage was barely sufficient, as even within this relatively short interval there appeared on occasion to be immediate initial remission followed by partial return of some of the more obtrusive symptoms of rheumatic fever, such as joint pain and general stiffness. Dosage more frequently than four hourly would obviously be most inconvenient so that a slower acting drug would be preferred.

It was decided, therefore to attempt to assess the therapeutic value in the treatment of rheumatic fever of a number of 2:6-dihydroxybenzoic acid homologues, with the chief features sought being an activity equivalent to, or greater than, that of sodium 2:6-dihydroxybenzoate but less obtrusive side effects and at the same time a lower rate of absorption. Such a compound if found in this series, would constitute a replacement for sodium salicylate and a substitute for cortisone at once commendable for general use and relatively readily available in virtually any quantity required. Attention turned in the first instance to 2:6-dihydroxy-3-alkylbenzoic acids, of which only slight mention of the 2:6-dihydroxy-3-ethylbenzoic acid[72] has as yet appeared in the literature. 2:6-dimethoxy-3 methylbenzoic acid[73,74] has however, been prepared by a route based on

the synthesis of Limaye for 2:6-dihydroxybenzoic acid, using 3-ethylresorcinol as starting material, and methyl 2:6-dihydroxy-3-methylbenzoate has been prepared by a Gattermann reaction on methyl 2:6-dihydroxybenzoate[75].

It seemed probable, however, that a more direct route for the preparation of these acids than the syntheses of either Limaye[51] or Mauthner[62,64] might be profitable, in order to reduce to a minimum the quantity of 4-alkylresorcinol or 2:4-dinitro-alkylbenzene required as starting material, and attention was therefore given to the possible introduction of 3-alkyl-2:6-dimethoxyphenyllithium or 3-alkyl-2:6-dihydroxyphenyllithium as intermediaries.

The value of organolithium compounds in organic chemistry lies in their relatively high stability as compared with the corresponding organosodium and organopotassium compounds. As might be expected from the position of lithium in theperiodic table, the chemical behaviour of organolithium compounds is intermediate between that of the other organoalkali-metal compounds and the organomagnesium derivatives comprising Grignard reagents. Thus although the sensitivity of these organolithium compounds to oxygen and to water remains high, so that reactions must be carried out in an inert atmosphere, in an atmosphere of dry nitrogen a considerable range of reactions may be effected.

Organolithium compounds may be prepared directly by the action of metallic lithium on an alkyl or aryl halide in a variety of solvents including diethyl ether, benzene and the various petroleum ether fractions, the preparation being represented by the equation:

$$RX + 2\,Li \rightarrow R\,Li + Li\,X.$$

In practice however, the number of reagents made directly by the above method is relatively small with the vast bulk of the published work in this field depending initially on the preparation of n-butyl or phenyllithium, and to a lesser extent methyl, ethyl and n-propyllithium, all of which may readily be prepared in a high yield. These organolithium compounds are then reacted with a suitable starting material to yield the organolithium derivative actually required, either by hydrogen-metal interconversion or metalation as may be represented:

$$R\,Li + R'H \rightarrow R'Li + RH.$$

or by halogen-metal interconversion which may be represented:

$$R\,Li + R'X \rightarrow R'Li + RX.$$

These hydrogen-metal and halogen-metal interconversions greatly increase the range and usefulness of organolithium compounds, since not only are most organolithium compounds more conveniently prepared in this way, but many which might not be formed at all by direct action of lithium with an initial hydrocarbon or halide, may be formed in this way by hydrogen-metal or halogen-metal/conversion with one of the more readily prepared organolithium compounds. Thus o-hydroxyphenyllithium cannot be obtained by direct reaction of o-bromophenol with metallic lithium, but under suitable conditions o-bromophenol reacts with n-butyllithium to give a high yield of the lithium salt of o-hydroxyphenyllithium[76].

Where hydrogen-metal interconversion occurs, in general lithium will attach itself to the more nucleophilic radical. Thus 9-fluorenyllithium is formed in ether almost quantitatively from fluorene and ethyllithium[77]. Where halogen-metal interconversion occurs, again equilibrium is reached with the lithium attached to the more electro-negative group. Thus α-napthyllithium is formed in 95% yield from α-bromophthalene and n- propyllithium[78]. If the two radicals R and R' are of approximately equal electronegativity, however, the yield of R'Li is of the order of 50%. This is clearly demonstrated by examination of the mixture obtained on treating equimolecular quantities of phenyllithium with p-iodotoluene or of p-tolylithium with iodobenzene when approximately equal quantities of the four substances phenyllithium, p-iodotoluene, p-tolylithium and iodobenzene are obtained in either case[79].

The required organolithium compound having been prepared by one of the reactions outlined above, addition of a further reagent may give a product difficult to obtain by any other route. Thus direct carboxylation either by passing a stream of carbon dioxide gas through a solution of the organolithium compound or by pouring the solution gradually on to solid carbon dioxide, readily converts the organolithium compound to an organic acid. Addition of the organolithium compounds to unsaturated molecules takes place readily in the majority of cases and reaction is usually complete. An alternative route of reaction of the organolithium compound involves

coupling with a further compound, such as an organic halide, which may be simply represented:

$$RLi + R'X \rightarrow RR' + LiX.$$

The products obtained on treating an organolithium compound with an alkyl halide frequently indicate that the reaction process has not been simple, but that hydrogen-metal interconversion, halogen-metal interconversion and addition may proceed simultaneously. Thus the reaction of o- chloroanisole and phenyllithium yields o-methoxyphenyllithium, chlorobenzene and 2-methoxy-diphenyl[80], indicating that halogen-metal interconversion and addition have both occurred. On the other hand, the reaction of p-bromoanisole and phenyllithium yields p-methoxyphenyllithium, chlorobenzene, 2-methoxy-4-bromophenyllithium and benzene, indicating that halogen-metal interconversion and addition have occurred. In general, however, due care in determination of the most suitable solvent and halide will ensure that the required product is obtained in good yield and with minimum contamination from alternative reaction processes of the reactants. What is an undesirable side reaction in one synthesis, becomes the required reaction route in another, but by careful manipulation of conditions it is frequently possible to ensure control of the end product.

The reaction of phenyllithium and m-dimethoxybenzene had already been studied by Wittig and Pockels[81] and a yield of pure 2:6-dimethoxybenzoic acid uncontaminated by 2:4-dimethoxybenzoic acid obtained on carbonation, indicating that only 2:6-dimethoxyphenyllithium

and no 2:4-dimethoxyphenyllithium was formed. A similar observation with regard to the reaction of n-butyllithium and m-dimethoxybenzene was made by Gilman[82] who also obtained only 2:6-dimethoxybenzoic acid on carbonation, but in higher yield than was claimed by Wittig (55% in place of 14%). On treating resorcinol with n-butyllithium, however, he obtained a mixture of 2:6-dihydroxybenzoic acid and 2:4-dihydroxybenzoic acid on carbonation indicating that 2:6-dihydroxyphenyllithium and 2:4-dihydroxyphenyllithium both occurred as intermediaries. The total yield remained high, but the mixed nature of the product rendered the reaction less valuable. The increase in yield as compared with that obtained by Wittig, Gilman ascribed to carbonation by spraying the aryl lithium solution on to solid carbon dioxide, rather than by passing dry carbon dioxide gas through the solution. It seemed more probable therefore, that a yield of pure 2:6-dimethoxy-3-alkylbenzoic acids would be afforded by the treatment of 2:4-dimethoxyalkylbenzenes with n-butyllithium and carbonation of the product, rather than that a yield of pure 2:6-dihydroxy-3-alkylbenzoic acids would be obtained by similar treatment of 4-alkyl resorcinols. In the latter method the saving of the additional stages of methylation and demethylation would be outweighed by the necessity for fractional crystallisation to separate the 2:6-dihydroxy-3-alkylbenzoic acids from the 2:4-dihydroxy-5-alkylbenzoic acids likely to be formed simultaneously.

A supply of 4-methyl, 4-ethyl, 4-n-propyl, 4-n-butyl, 4-n-amyl and 4-n-hexyl resorcinols was therefore prepared and methylated to give the corresponding 2:4-dimethoxyalkylbenzenes to be used as starting materials.

4-methylresorcinol was prepared by reduction of 2:4-dihydroxybenzaldehyde, which was itself prepared by two modifications of the method of Gattermann[83]. In the first of these[84] dry hydrogen cyanide was absorbed into an ethereal solution of resorcinol, the solution then saturated with dry hydrogen chloride, and when the reaction was complete, the precipitated aldimine hydrochloride decomposed with boiling water. It was found that great care had to be taken to avoid too rapid inflow of hydrogen chloride, as there was always a strong tendency for formation of a bright red compound, probably as a result of condensation of 2:4-dihydroxybenzaldehyde with unreacted resorcinol. In the second method, based on an analogous preparation of Radha and Shah[75] dry hydrogen chloride was passed slowly through a constantly stirred suspension of zinc cyanide in a solution of resorcinol in ether, and when the reaction was complete, the product again decomposed with warm water. The second method had the advantage of not requiring the use of free hydrogen cyanide gas, but the yields obtained were somewhat lower. A slightly modified form of this method, giving yields after one recrystallisation from hot water approximately equal in weight and melting point to those obtained by the first method, was however, developed and adopted as

the most convenient method of preparation of 2:4-dihydroxybenzaldehyde. The reduction of the 2:4-dihydroxybenzaldehyde to 4-methylresorcinol was carried out by a modification of the Clemmensen method[85,86]. The red oil obtained was twice distilled under reduced pressure, then crystallised four times from 60-80° petroleum ether, when whitish needles of constant melting point were obtained. This methyl resorcinol was methylated by dimethylsulphate in caustic soda solution in the usual manner, to give 2:4-dimethoxytoluene as a pale yellow oil.

2:4-dihydroxyacetophenone was prepared from resorcinol by condensation with glacial acetic acid in presence of anhydrous zinc chloride[87] and reduced to 2:4-dihydroxyethylbenzene by the method of Clemmensen[85]. In the preparation of 2:4-dimethoxybenzene from 2:4-dihydroxyethylbenzene the method of Skraup and Bohm[88] did not prove satisfactory, but the methods of Twiss[89] and of Mauthner[90] both gave high yields. It was found that distillation of 2:4-dihydroxyacetophenone under reduced pressure had to be carried out with care, since any undue passage of air through the molten material was likely to induce irreversible polymerisation.

4-n-Propionyl, 4-n-butyryl, 4-n-amylyl and 4-n-hexylyl resorcinols were prepared in good yield by condensation of resorcinol with the appropriate fatty acid in presence of anhydrous zinc chloride, and reduced to 4-n-propyl, 4-n-butyl, 4-n-amyl, and 4-n-hexyl resorcinol respectively by a modification of the Clemmensen method[91].

The method of Twiss[89], as used for the methylation of 4-methylresorcinol, proved satisfactory for methylation to the corresponding 2:4-dimethoxyalkylbenzenes, whose boiling points agreed to a large extent with those already found in the literature. Dimethylsulphate with potassium carbonate in acetone as a medium for methylation[92] was also employed with satisfactory results when it was felt necessary to prepare a methylated product by a slightly different route in order to obtain corroborative evidence of its purity.

Meanwhile an investigation was made into the conditions of reaction most likely to be favourable for the purpose of preparing 2:6-dimethoxy-3-alkylbenzoic acids by carbonation of the corresponding 2:6-dimethoxy-3-alkylphenyllithium compounds. The preparation of 2:6-dimethoxybenzoic acid by carbonation of 2:6-dimethoxyphenyllithium was selected as a suitable reaction for the purpose of the investigation chiefly on the grounds of the ease of access of the starting material, and on the assumption that the optimum conditions for the reaction with this compound would not differ greatly in the case of the 2:6-dimethoxy-3-alkylbenzoic acids, or at least would differ only quantitatively rather than qualitatively, so that in the essential features of choice of solvent and of initial organolithium compound, the conditions would remain identical. By comparison of the work of Gilman and Wittig, a set of conditions which might be expected to induce a high yield from both n-butyllithium and phenyllithium was drawn up. Lithium metal was reacted with a slight excess of n-butyl

chloride or bromobenzene in diethyl ether as solvent, and refluxing of the solution continued for three hours, after the completion of addition of the halide. m-dimethoxybenzene was then added to the cooled solution of 2:6-dimethoxyphenyllithium, and the solution stirred for one hour, then refluxed for four hours. The solution was then cooled, dry carbon dioxide passed through for one hour, then the whole poured slowly on to solid carbon dioxide. The amount of m-dimethoxybenzene added represented a slight excess assuming a 75% yield of n-butylchloride, but it was found in practice that a slight excess of any of the reactants did not significantly affect the quality of the product

A comparison was also made of the results obtained by using 60-80° petroleum ether in place of diethyl ether as solvent while maintaining the other conditions of reaction constant. Tucker and Whalley[93], investigating the preparation of 9-fluorenecarboxylic acid by carbonation of 9-fluorenyllithium, had found that little or no yield of 9-fluorenyllithium was obtained on treating fluorene with methyllithium in diethyl ether , or with n-butyllithium in sulphur-free benzene. The low yield of 9-fluorenecarboxylic acid in the latter case was accompanied by low yields of butane gas , indicating that little n-butyllithium had been formed, and therefore that benzene was not a suitable solvent for the preparation of n-butyllithium. The preparation of n-butyllithium by reaction of n-butyl chloride and lithium in 67-69° petroleum ether was however, entirely successful and treatment of fluorene with the product gave satisfactory yields

of 9-fluorenyllithium and hence, on carbonation, of 9-fluorenyl - carboxylic acid. It was therefore felt probable that the use of petroleum ether as solvent for the reaction of n-butyl chloride and lithium, and for the reaction of the resulting n-butyllithium with m-dimethoxybenzene, might afford good results, so that petroleum ether was included in the investigation as an alternative solvent. It was confirmed that commercial 60-80° petroleum ether purified by extraction with concentrated sulphuric acid and with a potassium permanganate solution in 10% sulphuric acid, and dried over calcium hydride, boiled almost entirely within the range of 65-69°, so that this fraction only was in fact used in all experiments in order to increase the uniformity of the conditions.

In all initial experiments, the lithium metal used was prepared by forcing scraped lithium through a sodium press, so that it was obtained as fine wire[94] or by hammering pieces of the metal kept moist with toluene into thin sheets, and cutting the sheets into thin strips[95]. The purpose of both these procedures was to obtain as large a surface of fresh, newly exposed lithium metal as possible, to ensure maximum reactivity, but it was found that the yields of 2:6-dimethoxybenzoic acid obtained from m-dimethoxybenzene and n-butyllithium formed by the reaction of n-butyl chloride and lithium prepared by either of these methods tended to vary considerably. Since it was obviously important in assessing the relative values of the methods tried to have initially minimum variance from experiment to experiment, these methods of preparing the lithium were discarded.

The use of lithium sand, or lithium powder, or atomised lithium as it has variously been called, as described by Woodward[96] was then introduced and consistent results obtained. In this method the scraped lithium metal is melted in purified, dry liquid paraffin and stirred strongly in a flask fitted with a nickel Herschberg stirrer[97], the entire apparatus being swept by a current of dry nitrogen throughout. On cooling and washing with diethyl ether or 65-69° petroleum ether as required, fine lustrous reactive particles of lithium are obtained. The method was initially introduced as a means of preparing lithium reactive enough to enable formation of organolithium compounds from halides such as t-butyl chloride in which steric hindrance might render reaction difficult, but the results afforded were such as to make it apparent that the greater consistency of reaction resulting from its use would make it a profitable method for general application.

The course of reaction of n-butylchloride and lithium was followed by measuring the butyllithium formed by the method of Gilman and Haubein[98]. The method is similar in principle to that already outlinedby Ziegler and his associates[99], both depending essentially on an initial titration to estimate the total alkali present, followed by a second titration after treatment with a reagent to remove all organolithium compound present. In Gilman's method an aliquot is removed, hydrolysed, and titrated with standard hydrochloric acid, thus determining the total alkali

present. The difference of the two titres thus gives an accurate measure of the amount of alkyllithium present. An investigation of the products formed on treating n-butyllithium with benzyl chloride indicates that the following equations may represent the reactions which occur:

$$\underline{n}\text{-}C_4H_9Li + C_6H_5.\ CH_2Cl \rightarrow C_6H_5.\ CH_2.\ \underline{n}\ C_4H_9 + LiCl$$

$$\underline{n}\text{-}C_4H_9Li + C_6H_5.\ CH_2Cl \rightarrow C_6H_5.\ CH_2\ Li + \underline{n}\text{-}C_4H_9\ Cl$$

$$\underline{n}\text{-}C_4H_9Li + \underline{n}\text{-}C_4H_9Cl \rightarrow \underline{n}\text{-}C_4H_9.\underline{n}\ C_4H_9 + LiCl$$

$$C_6H_5.\ CH_2Li + C_6H_5.CH_2Cl \rightarrow C_6H_5CH_2.\ CH_2C_6H_5 + LiCl$$

It will be seen that in this case halogen-metal interconversion and addition reactions are occurring simultaneously, the reaction being complete within one minute. The method is not, however, applicable to aryllithium compounds, so that assessment of the phenyllithium formed was less accurate, being merely a measure of the total alkali present in the hydrolysed solution.

The reaction used by Ziegler[99] in removing the organolithium compound formed to permit a titre of the non-organolithium alkali present is also of interest. In this method the second aliquot is treated with excess n-butyl bromide and dibenzyl mercury before hydrolysis. The reactions occurring may be represented by the following equations:

$$(C_6H_5CH_2)_2Hg + 2\ \underline{n}\text{-}C_4H_9\ Li \rightarrow 2\ C_6H_5.\ CH_2\ Li + (\underline{n}\text{-}C_4H_9)_2\ Hg$$

$$C_6H_5.\ CH_2Li + \underline{n}\text{-}C_4H_9Br \rightarrow C_6H_5.\ CH_2.\underline{n}\text{-}C_4H_9\ LiBr$$

$$(\underline{n}\text{-}C_4H_9)_2\ Hg + 2\ \underline{n}\text{-}C_4H_9\ Br \rightarrow 2\ \underline{n}\text{-}C_4H_9.\ \underline{n}\text{-}C_4H_9 + HgBr_2$$

It will be seen that in this case metal - metal interconversion, halogen-metal interconversion, and addition reactions occur. The method is, however, also applicable to alkyl organolithium compounds only, and is rather less convenient than the procedure of Gilman, so that the latter method was used throughout in all estimations of n-butyllithium formed.

The results obtained left no doubt that the reaction of n-butyl chloride with lithium powder in 65-69° petroleum ether as solvent to give n-butyllithium, addition of m-dimethoxybenzene to form 2:6-dimethoxyphenyllithium, and carbonation by passage of carbon dioxide then by pouring the mixture on to solid carbon dioxide was the most satisfactory method of preparation of 2:6-dimethoxybenzoic acid. With diethyl ether as solvent the yield of 2:6-dimethoxybenzoic acid obtained was much less steady (41-50% overall or 66-79% on unrecovered 2:4-dimethoxybenzene), and the titration figures indicate a higher proportion of non-organolithium alkali present, possibly at least partly due to lithium ethoxide formed by cleavage of the diethyl ether. With bromobenzene in place of n-butyl chloride as reactant halide, the yields both of phenyllithium and of 2:6-dimethoxybenzoic acid were rather low in diethyl ether as solvent. Thus even ignoring the part of the titre taken by non-organolithium alkali, the amount of phenyllithium formed was only 34-43% of the initial bromobenzene, while the 2:6-dimethoxybenzoic acid was formed in 21-33% yield overall, or 62-69% calculated on the unrecovered m-dimethoxybenzene.

In 65-69% petroleum ether, bromobenzene yielded only 2-8% 2:6-dimethoxybenzoic acid or 20-50% calculated on unrecovered m-dimethoxybenzene, largely due to the very slight degree of formation of phenyllithium (7-20%). With n-butyl chloride in 65-69° petroleum ether, however, yields of 62-65% 2:6-dimethoxybenzoic acid (75-77% calculated on reacted m-dimethoxybenzene) were consistently attained. It was therefore decided to attempt the preparation of 2:6-dimethoxy-3-alkylbenzoic acid in the first instance under these conditions.

When the method was applied to 2:4-dimethoxyalkylbenzenes, however, somewhat lower yields were obtained in most cases. 2:4-dimethoxyethylbenzene afforded approximately the same yields as m-dimethoxybenzene with formation of 55-65% 2:6-dimethoxy-3-ethylbenzoic acid, overall, or 80-85 % calculated on unrecovered 2:4-dimethoxyethylbenzene, but the yields obtained fell progressively as the length of the alkyl side chain increased, until from 2:4-dimethoxy-n-hexylbenzene only 17-20% 2:6-dimethoxy-3-n-hexylbenzoic acid, or 35-39% calculated on unrecovered 2:4-dimethoxy-n-hexylbenzene, was obtained. A regretable accompaniement to this fall in yield was the rather lower recoveries of unreacted 2:4-dimethoxy-alkylbenzenes achieved in most cases. The yield obtained from 2:4-dimethoxymethylbenzene (2:4-dimethoxytoluene) was also rather surprisingly significantly lower than that from either m-dimethoxybenzene or 2:4-dimethoxyethylbenzene. Thus

2:4-dimethoxytoluene afforded yields of 20-26% overall or 33-37% calculated on the unreacted 2:4-dimethoxytoluene. The failure to recover a higher proportion of unreacted material may have been accounted for to some extent by the crudeness of the acid products initially obtained on evaporation of their ethereal solutions. The crude acids from 2:4-dimethoxytoluene, 2:4-dimethoxy-n-propylbenzene, 2:4-dimethoxy-n-butylbenzene, 2:4-dimethoxy-n-amylbenzene and 2:4-dimethoxy-n-hexylbenzene were all obtained in the first instance as thick red oils which solidified over a period varying from several days to several weeks, and were then pressed on porous plate and dried in vacuo. Sharp melting products were then obtained by recrystallisation from a mixture of benzene and 65-69° petroleum ether, but the material loss before the red oil was purified to colourless crystals was not inconsiderable. With each reaction of 2:4-dimethoxyalkylbenzene with n-butyllithium, a parallel reaction with m-dimethoxybenzene was carried through in order to check the continued consistency and purity of the reagents used, but fairly constant yields of 2:6-dimethoxybenzoic acid were obtained throughout.

In the demethylation of 2:6-dimethoxy-3-alkylbenzoic acids to 2:6-dihydroxy-3-alkylbenzoic acids, in benzene solution in presence of powdered anhydrous aluminium chloride under the same conditions, as those found satisfactory for the demethylation of 2:6-dimethoxybenzoic acid to 2:6-dihydroxybenzoic acid, similar

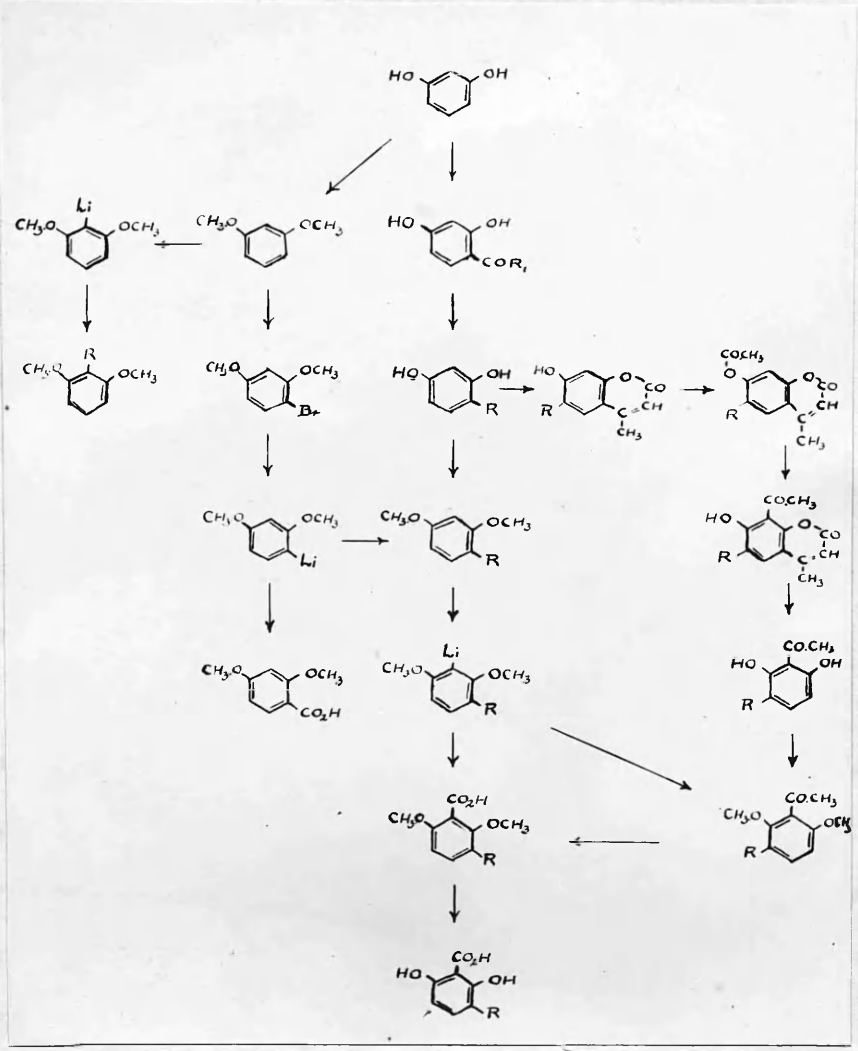
findings prevailed. Thus with 2:6-dimethoxy-3-ethylbenzoic acid a 91% yield of fairly sharp melting acid, melting without further purification only a few degrees lower than the melting point given in the literature[72] was readily obtained, but from 2:6-dimethoxy-3-n-hexylbenzoic acid even on increasing the reaction time, a yield of only 15% 2:6-dihydroxy-3-n-hexylbenzoic acid was obtained. It is probable that in this case steric hindrance constitutes the chief difficulty. Thus Sprenger and Rouff[73] in attempting to methylate the analogous 2:4-dihydroxy-3-ethylbenzoic acid, found that even with a large excess of dimethylsulphate in alkaline solution and prolonged boiling, only 2-hydroxy-4-methoxy-3-ethylbenzoic acid and no 2:4-dimethoxy-3-ethylbenzoic acid could be obtained.

It is probable, however, that in spite of these difficulties this method still constitutes a suitable and practicable route for the laboratory preparation of these 2:6-dihydroxy-3-alkylbenzoic acids. Thus the yield previously claimed of 2:6-dimethoxy-3-methylbenzoic acid from resorcinol[73] is around 3-5% and this figure has not in practice been increased beyond 10%, but 2:6-dimethoxy-3-methylbenzoic acid was prepared from resorcinol by the above method in shorter time and in fewer stages in 25-30% yield.

The orientation of the acids obtained was confirmed by the synthesis by a more unequivocal route of the methyl and ethyl homologues as outlined in Fig. VI. It will be appreciated that in a preparation by the route of Limaye[51] if a 4-methyl-6-alkyl-7-acetoxycoumarin is formed rather than 4-methyl-7-acetoxycoumarin

as from resorcinol, there is a greatly increased probability of rearrangement solely to the 8-position, and therefore only 2:6-dihydroxy-3-alkylacetophenone can result. 2:6-dihydroxy-3-methylacetophenone and 2:6-dihydroxy-3-ethylacetophenone were therefore prepared by the method of Limaye[51] from 4-methyl and 4-ethyl resorcinol respectively. The alkyl resorcinol in each case was condensed with acetoacetic ester in presence of phosphoric acid, the coumarin obtained acetylated, subjected to rearrangement in presence of anhydrous aluminium chloride and the resulting 4-methyl-6-alkyl-7-hydroxy-8-acetylcoumarin hydrolysed to give the corresponding acetophenone. This was methylated, then oxidised by sodium hypo-chlorite solution by a procedure resulting in a 75% yield of 2:6-dimethoxy-3-methylbenzoic acid from 2:6-dimethoxy-3-methylacetophenone, and a 70% yield of 2:6-dimethoxy-3-ethylbenzoic acid from 2:6-dimethoxy-3-ethylacetophenone, which represented an appreciable improvement on the method of Sprenger and Ruoff[73] who obtained a 50% yield of 2:6-dimethoxy-3-methylbenzoic acid from 2:6-dimethoxy-3-methylacetophenone. Most of the intermediate products obtained in the course of this confirmatory synthesis had already been characterised[72,73,74,100] and for these similar melting points were found to these already given in the literature. The preparation of 2:6-dihydroxy-3-n-hexylacetophenone by the method of Limaye[51] was however, unsuccessful. By varying the quantities of reagents and increasing the time of reaction

Fig. VI



Preparation of 2:6-dihydroxy-3-alkylbenzoic acids.

7-hydroxy-4-methyl-6-n-hexylcoumarin was obtained in a yield showing some improvement over that claimed for 7-hydroxy-4:6-dimethylcoumarin[74]. 7-acetoxy-4-methyl-6-n-hexylcoumarin was then obtained by acetylation without difficulty, but no pure crystalline material could be separated from the viscous mass obtained when the acetyl derivative was rearranged in presence of anhydrous aluminium chloride. Nor could any pure sharp melting product be obtained from the product of alkaline hydrolysis of this crude rearranged material. The explanation of this tardiness of rearrangement probably again lies in steric considerations, but the confirmation of the formulae of the two lower members of the series may probably be regarded as sufficient evidence to establish the configuration of the whole series.

Some further confirmation of the orientation of the organolithium compound was given by the preparation of 2:6-dimethoxy-3-methylacetophenone[73], and 2:6-dimethoxy-3-ethylacetophene from the corresponding organolithium compounds. 2:6-dimethoxy-3-methylphenyllithium and 2:6-dimethoxy-3-ethylphenyllithium were formed under largely the same conditions as in the previous experiments by reaction of n-butyl chloride and lithium powder to give n-butyllithium in diethyl ether solution. The 2:6-dimethoxy-3-alkyllithium compound having been obtained as before, it was treated with excess acetyl chloride instead of being subjected to direct carbonation by carbon dioxide, when after long refluxing the acetyl

derivatives were formed. These were identical with the product obtained on methylation of the 2:6-dihydroxy-3-alkylacetophenones prepared by the route of Limaye[51], and on oxidation with sodium hypochlorite solution also yielded the corresponding 2:6-dihydroxy-3-alkylbenzoic acids. The position of metalation in these 2:4-dimethoxyalkylbenzenes is thus further confirmed. An interesting feature of these experiments, and of similar initial experiments using m-dimethoxybenzene as starting material, was that although the reaction of the organolithium compound with acetyl chloride took place slowly in diethyl ether, as compared with the rate of indirect carbonation, it occurred not all in 65-69° petroleum ether. In repeated experiments using n-butyllithium and m-dimethoxybenzene in 65-69° petroleum ether and reacting with acetyl chloride no 2:6-dimethoxyacetophenone was obtained. In diethyl ether however, the products were obtained in fairly pure state in 60-65% yield after twenty four hours refluxing.

The same position obtained with regard to the reaction of 2:6-dimethoxyphenyllithium with methyl iodide and ethyl iodide. 2:6-dimethoxyphenyllithium prepared by the reaction of n-butyllithium from n-butyl chloride and lithium powder with m-dimethoxybenzene in 65-69° petroleum ether did not appear to react at all with methyl iodide or ethyl iodide, even on twenty four hours refluxing and twenty one days at room temperature to give 2:6-dimethoxytoluene or 2:6-dimethoxyethyl benzene. The starting material was recovered

unchanged in each case. In diethyl ether, however, under the same conditions, the yields of 2:6-dimethoxytoluene and 2:6-dimethoxyethylbenzene were 45-55% of pure material agreeing in melting point with the published figures[101,102]. Oxidation of the 2:6-dimethoxytoluene by aqueous potassium permanganate yielded 2:6-dimethoxybenzoic acid.

The reaction was not attempted with any of the 2:4-dimethoxy-3-alkylbenzenes prepared since it was not felt that any further confirmation of molecular structure would be obtained thereby, but it is thought that the method will provide a ready means of preparation of 2-alkylresorcinols and 2:6-dimethoxyalkylbenzenes generally, since these are not usually available except by rather indirect routes such as the Limaye method as applied by Adams and his associates[103]. The high price of lithium at the moment may limit its usefulness in large scale work, but for laboratory scale preparation it is suggested that the ease of formation of 2:6-dimethoxyphenyllithium in the pure state uncontaminated by isomers is a most useful feature. The fact that the organolithium compound so formed will react with alkyl halide, acyl halide or carbon dioxide makes the reaction of wide application. The metalation of methylated resorcinols in the 2-postion to the exclusion of the 4-position, has been confirmed, and the utility of the observation is stressed.

With a view to considering possible alternate routes for

preparation of 2:4-dimethoxyalkylbenzenes, the reaction of 2:4-dimethoxyphenyllithium with alkyl iodides was briefly studied. Wittig[81] had shown that 4-bromoresorcinol dimethyl ether (2:4-dimethoxybromobenzene) on treatment with phenyllithium, and carbonation of the resulting 2:4-dimethoxyphenyllithium, yielded pure 2:4-dimethoxybenzoic acid in 4% yield. He had, however, found also that the purity of the 2:4-dimethoxybromobenzene used was important, since a mixture of 2:4-dimethoxybromobenzene and 4:6-dibromoresorcinol dimethyl ether not only lowered the quantity of the ultimate yield of 2:4-dimethoxybenzoic acid, but also served to slow the reaction rate very considerably. The most certain method of obtaining pure 2:4-dimethoxybromobenzene is by the direct bromination of gentisic acid in acetic acid as solvent, decarboxylation of the resulting 5-bromo-2:4-dihydroxybenzoic acid and methylation of the product[104]. A similarly indirect method of obtaining 4-iodoresorcinol dimethyl ether (2:4-dimethoxy-iodobenzene) which might be used as an alternative reagent, depends on direct nitration of resorcinol with a nitric acid - sulphuric acid mixture, to give 4-nitro resorcinol, which is purified, methylated and the nitro-group reduced and diazotised, when treatment with potassium iodide yields 2:4-dimethoxyiodobenzene[105]. However, direct bromination of resorcinol dimethyl ether with N-bromosuccinimide had been found by Ziegler and his associates[106] to give a

satisfactory yield of 2:4-dimethoxybromobenzene and this finding was confirmed. This use of N-bromosuccinimide has since been found applicable for monobromination of a wide range of compounds[107] and usually provides a more complete reaction than N-bromoacetamide.[108] The reagent itself was readily prepared by direct bromination of an alkaline solution of N-bromosuccinimide in 70-75% yield. On bromination of m-dimethoxybenzene yields of 35-43% of a yellow oil boiling over a few degrees range are obtained, which on further purification yields 31-40% of pure 2:4-dimethoxybromobenzene.

Since it was found that the yield of 2:4-dimethoxybenzoic acid obtained on carbonation of the product from the reaction of 2:4-dimethoxybromobenzene in diethyl ether with n-butyllithium was higher (62%) than that obtained by carbonation of the product obtained by treating 2:4-dimethoxybromobenzene in diethyl ether with phenyllithium (34%), the former conditions were adopted for the attempted preparation of 2:4-dimethoxyalkylbenzenes. With methyl iodide, boiling in ether for twenty four hours, followed by fourteen days at room temperature, resulted in a 30% yield of 2:4-dimethoxytoluene. With addition of ethyl iodide under the same conditions, a 44% yield of 2:4-dimethoxyethylbenzene was obtained.

While it is not suggested that these yields are sufficiently high to warrant general preparation of 2:4-dimethoxyalkylbenzenes by this method, it is possible that the optimum conditions have not

yet been determined. It is also possible that the method may lend itself to the preparation of some of the 2:4-dimethoxyalkylbenzenes not so readily available by condensation of a fatty acid with resorcinol in presence of anhydrous zinc chloride. Furthermore, the rather unsatisfactory preparation of 2:4-dimethoxytoluene to which allusion has already been made, may well be displaced by this more ready and convenient method which largely obviates the danger of condensation and high colour development inherent in any method of nuclear methylation dependent on the Gattermann condensation and reduction of the aldehyde obtained.

It is hoped that physiological and clinical trials of some of the acids and other compounds prepared may reveal further signs of the properties sought in a safe, reliable therapeutic agent for rheumatic fever. Alternatively, it may be that the new data acquired in the course of trial will be sufficient to indicate some other feature necessary in such a drug. In either case, whether the signs are of direct improvement in the means of therapy available or of indirect indication of the characteristics which will be required in an improved drug, it is probable that some new indication of the mode of treatment of rheumatic fever will be provided.

Experimental.

2:4-dimethoxytoluene through 2:4-dihydroxybenzaldehyde.

I. 2:4-dihydroxybenzaldehyde: (a) [84] Resorcinol (50 g.) was dissolved in diethyl ether (150 ml.) in a 500 ml. round bottomed flask, which was then cooled to 5°, and a slow stream of hydrogen cyanide bubbled through. The hydrogen cyanide was generated by reaction of sulphuric acid (400 ml. of 35%) on potassium ferrocyanide (200 g.), and was dried by passage through calcium chloride tubes maintained at 35-40°. When the increase in weight of flask and contents showed a 50% excess of hydrogen cyanide (an increase of approximately 18.5 g.) the passage of hydrogen cyanide was stopped, and a slow stream of dry hydrogen chloride substituted, the solution temperature being maintained at 5°. Slow passage of hydrogen chloride was continued for 2 hrs., by which time the solution was saturated, then the reaction mixture left overnight and decomposed with boiling water (200 ml.) and the mixture filtered hot. On cooling, crystals of 2:4-dihydroxybenzaldehyde m.p. 131-4° were obtained in 40-51 % yield calculated on the initial weight of resorcinol (cf. m.p. 135-6°)[116].

(b) [75] Resorcinol (15 g., 0.136 g.m.) was dissolved in diethyl ether (100 ml.) in a 500 ml. round bottomed flask, and zinc cyanide (33.1 g., 0.272 g.m.) added. Dry hydrogen chloride was then passed through the cooled solution for 6 hrs., with frequent

shaking. At the end of this period the ether was decanted off, water (200 ml.) added, and the mixture heated on the steam bath for 2 hrs., then filtered hot, when crystals of 2:4-dihydroxybenzaldehyde m.p. 129-133° were obtained in 31-42 % yield.

(c) Resorcinol (25 g. 0.227 g.m.) was dissolved in diethyl ether (100 ml.) in a 500 ml. round bottomed flask, and zinc cyanide (53.4g., 0.440 g.m.) added. Dry hydrogen chloride was passed slowly through the solution maintained at 5° for 6 hrs. with frequent shaking. Boiling water (200 ml.) was then added, the mixture filtered hot, and the residue again taken up with boiling water (100 ml.) and again filtered hot. The combined yield of 2:4-dihydroxybenzaldehyde from the two aqueous extracts was 38-47% of crystals m.p. 130-133°.

A pure specimen of 2:4-dihydroxybenzaldehyde was obtained by refluxing the above material three times with water, with activated charcoal added, as pale cream needles m.p. 134-5°. A specimen of the diacetate prepared by heating 2:4-dihydroxybenzaldehyde (1 g.) with sodium acetate (1 g.) in acetic anhydride (5 ml.) had m.p. 68-9° (cf. 69°)(115).

A comparison of the yields obtained by the various procedures is given in Table XIII.

Table XIII.

2:4-Dihydroxybenzaldehyde from resorcinol.

Method	m.p. of product as obtained from hot water	Weight of 2:4-dihydroxy benzaldehyde	% yield calculated on initial weight of resorcinol.
as given by Johnson and Lane (method a)	(a) 131-134° (b) 130-134° (c) 131-133°	25.4g. 27.6g. 32.1g.	40.5 44.1 51.2
as given by Radha and Shah (method b)	(a) 130-133° (b) 129-133° (c) 130-133°	5.3g. 4.7g., 6.3g.	35.3 31.4 42.0
as given above (method c)	(a) 130-133° (b) 131-134° (c) 130-134°	9.5g. 12.0g. 11.3g.	38.0 47.1 45.2

II. 2:4-dihydroxytoluene: 2:4-dihydroxybenzaldehyde (6g.) dissolved in ethyl alcohol (35 ml.) was added gradually over 1 hr. to a mixture of zinc amalgam prepared from zinc turnings (60 g.) by treatment with mercuric chloride (6 g.) and hydrochloric acid (120 ml. of 10%) heated on a steam bath under reflux conditions. Ethyl alcohol (25 ml.) and concentrated hydrochloric acid (25 ml.) were then added, and heating continued for a further 2 hrs.. After cooling, the reaction mixture was extracted with diethyl ether, and from the ethereal extract dried over anhydrous sodium sulphate 2:4-dihydroxytoluene obtained as a red oil which

was twice redistilled under reduced pressure (b.p. 120-125°/2.5 mm. in the first distillation, then 123-125° at 2.5 mm. in the second distillation). The pale yellow oil thus obtained soon solidified and was poured on to porous plate and recrystallised from 60-80° petroleum ether to give pale yellow crystals (2.5 g., 46-55%) m.p. 103-7°. Three more recrystallisations from 60-80° petroleum ether did not alter this melting point. The melting point given in the literature[84] is 103-4°.

III. 2:4-dimethoxytoluene: 2:4-dihydroxytoluene (12.4g., 0.1 g.m.) was dissolved in sodium hydroxide solution (100 ml. of 20%) and dimethylsulphate (40 ml., 53g., 0.42 g.m.) added over 1 hr. with constant stirring. The stirring was continued for a further hour, then the mixture refluxed for 2 hrs., cooled, and extracted with diethyl ether. The 2:4-dimethoxytoluene was obtained from this ether extract after two distillations as a pale yellow oil (10.7 g., 70%) b.p. 127° at 30 mm. (cf. 211°/760)[109].

The overall yield of pure 2:4-dimethoxytoluene from resorcinol was thus 12-15%.

2:4-dimethoxyethylbenzene through 2:4-dihydroxyacetophenone.

I. 2:4-dihydroxyacetophenone[87]: Resorcinol (110 g., 1 g.m.) was added to a well stirred solution of anhydrous zinc chloride (165 g., 1.2 g.m.) in glacial acetic acid (165 g., 2.7 g.m.) at 140° and the solution maintained just at boiling point (about 160°) for 1 hr. On diluting with hydrochloric acid (500 ml. of 50%)

and cooling to 5°, a precipitate of dark red, crude 2:4-dihydroxyacetophenone was obtained. On purifying by distilling twice under reduced pressure, a pale tan powder (90-95 g., 59-63 %) m.p. 143-144° was obtained in most experiments.(cf. m.p. 142-144°).[87] In two distillations in which "bumping" prolonged the period of heating in presence of air, almost all the material polymerised to a thick, non-volatile mass.

II. 2:4-dihydroxyethylbenzene: 2:4-dihydroxyacetophenone (50 g.) was heated with amalgamated zinc (200 g.) and hydrochloric acid (600 ml. of 33%) until the reaction set in strongly. Thereafter heating was applied to maintain the mixture just at boiling point for 3 hrs. The mixture was then saturated with sodium chloride and extracted with diethyl ether, from which extract a pale yellow powder yielding on recrystallisation from chloroform colourless prisms (37.8 - 41 g., 85-89 %) m.p. 95-99° was obtained.(cf. 98-99°)[110].

III.2:4-dimethoxyethylbenzene: (a)[88] 4-ethylresorcinol (6 g., 0.045g.m.) was dissolved in sodium hydroxide solution (17 ml. of 10%) and treated over 1 hr. with dimethyl sulphate (20 g., 0.16 g.m.). The stirring was continued for a further hour, then the mixture refluxed for 2 hrs., when a diethyl ether extract yielded only 0.5 g. (7%) pure 2:4-dimethoxyethylbenzene.

(b)[cf.89] 4-ethylresorcinol (13.8 g., 0.1 g.m.) was dissolved in sodium hydroxide solution (100 ml. of 20%) and dimethyl sulphate

(53 g., 0.429 g.m.) added over 1 hr. with constant mechanical stirring. The stirring was continued for 1 hr. more, then the mixture refluxed for 2 hrs., cooled and extracted with diethyl ether. From the diethyl ether extract dried over anhydrous sodium sulphate 2:4-dimethoxyethylbenzene was obtained after two redistillations under reduced pressure in 61-65% yield (10.1 - 10.8 g.) b.p. 110-111°/11mm.(88).

(c) (cf. 90) 4-ethylresorcinol (13.8 g., 0.1 g.m.) was dissolved in sodium hydroxide solution (95 ml. of 16%) and dimethyl sulphate (22.7 g., 0.18 g.m.) added over 30 mins. with stirring and cooling so that the temperature remained between 30 and 35°. Then further dimethyl sulphate (22.7 g., 0.18 g.m.) was added over 20 mins. with stirring as before, and the temperature maintained between 40 and 45°. The mixture was then refluxed for 20 hrs., cooled, and extracted with ether when 2:4-dimethoxyethylbenzene (10.5 - 11.0 g., 65%) b.p. 110-111°/11 mm. was obtained.

The overall yield of 2:4-dimethoxyethyl benzene from resorcinol was thus 31-37 % excepting, of course, when methylation was carried out by method (a)(88) which was not repeated.

2:4-dimethoxy-n-propyl, n-butyl, n-amyl and n-hexylbenzenes through the corresponding 4-acylresorcinols:

I. 4-acylresorcinol: Resorcinol (55 g., 0.5 g.m.) was added over 30 mins. to a solution of anhydrous zinc chloride (72.5 g., 0.53 g.m.) in the appropriate fatty acid (1.5 g.) at 125-135° with constant mechanical stirring throughout the addition. The temperature was

maintained, and stirring continued, for a further 2 hrs., when the mixture was cooled, diluted with water (1 L.), stirred, and filtered. The residue was washed with diethyl ether and the filtrate extracted with the diethyl ether, from which combined ethereal solution the appropriate acyl resorcinol was obtained, and purified by distillation under reduced pressure.

II. 4-alkylresorcinol: The 4-acylresorcinol (0.25 g.m.) was added to amalgamated zinc (130 g.) in hydrochloric acid (390 ml.). The mixture was refluxed and stirred for 12 hrs., then cooled and extracted with diethyl ether, the ethereal solution well washed with water and dried over anhydrous sodium sulphate, when the 4-alkylresorcinol was obtained and purified by distillation under reduced pressure.

III. 2:4-dimethoxyalkylbenzene; (a)(cf. 89) The 4-alkylresorcinol (0.5 g.m.) was dissolved in sodium hydroxide solution(500 ml. of 20%) and dimethylsulphate (265 g., 2.1 g.m.) added over 30 mins. with constant mechanical stirring throughout the addition, the stirring continued for a further 30 mins., then the mixture refluxed for 2 hrs., cooled and extracted with diethyl ether. From the ether extract the 2:4-dimethoxyalkylbenzene was obtained as a pale yellow oil, and purified by redistillation.

(b)(cf. 92) The 4-alkylresorcinol (5 g.) was dissolved in dry acetone (200 ml.) and anhydrous potassium carbonate (10 g.) and dimethyl sulphate (10 g.) added. The mixture was refluxed for 8 hrs., then

the acetone distilled off, water (200 ml.) and diethyl ether (200 ml.) added, and the mixture well stirred and filtered. From the ethereal solution after washing with sodium hydroxide solution (10%) and water, pure 2:4-dimethoxyalkylbenzene was obtained.

In Table XIV are indicated the approximate yields obtained by the above methods, the products in each case being purified by at least one vacuum distillation to the degree of purity indicated by the melting point or boiling point given. The second method of methylation was used only as a means of verifying the purity of the final products but yields of around 80% were consistently attained.

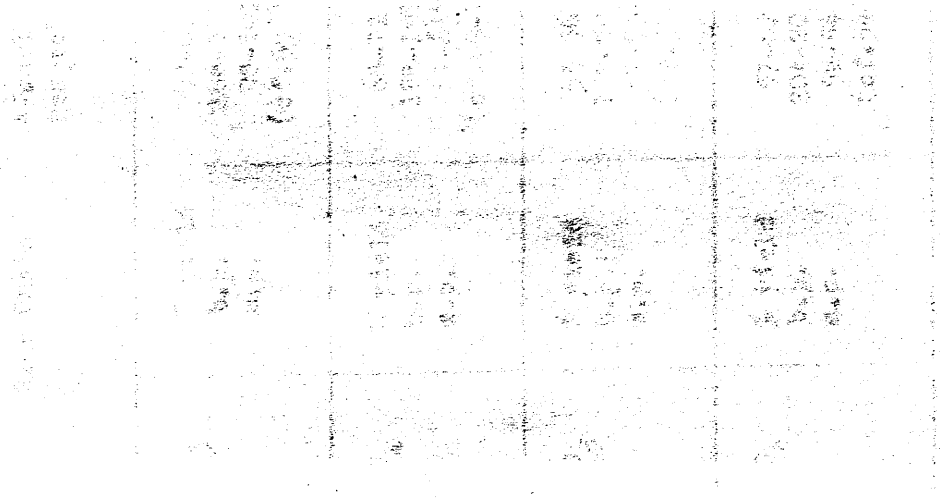


Table XIV.

2:4-Dimethoxyalkylbenzenes from resorcinol.

Side Chain		4-acyl resorcinol	4-alkyl resorcinol	2:4-dimethoxy alkyl	% overall yield.
C_3	% yield b.p. m.p.	58-62 186-188°/14mm 98-101° (cf.95°)(111)	58-65 170-172°/14mm. 79-81° (cf.82-83°)(110)	62-71 127-128°/18mm. (cf. 250°/710mm)(112)	21-29
C_4	% yield b.p. m.p.	68-71 188-192°/10mm 61-63° (cf.69-70°)(84)	73-80 186-188°/15mm. 48-50° (cf.47-48°)(91)	65-69 134-135°/11mm. (cf.264°/710mm)(112)	32-39
C_5	% yield b.p. m.p.	53-56 198-201°/10mm 51-53° (cf.58-60°)(91)	76-80 178°/10mm 68-70° (cf.71-73°)(91)	70-72 142-144°/8mm.	28-32
C_6	% yield b.p. m.p.	57-62 204-206°/10mm. 52-54° (cf.54-56°)(91)	78-81 180°/8mm. 65-66° (cf.67-69°)(91)	70-76 158-160°/9mm. (cf.164°/12mm)(89)	31-38

2:6-dimethoxybenzoic acid through 2:6-dimethoxyphenyllithium.

The conditions applied throughout the initial investigation, apart from some early rather unsatisfactory experiments using pressed lithium wire or hammer lithium sheet were as follows:

(a) Using 60-80° petroleum ether as solvent and n-butyl chloride as reactant halide:

I. Lithium powder:[96] Liquid paraffin (B.P.) was purified by shaking three times with approximately one quarter of its volume of concentrated sulphuric acid, then by shaking three times with one quarter its volume of 0.5 N sodium bicarbonate, and three times with an equal volume of water. It was then dried, first over quicklime at 200°, then over metallic sodium at 200° with mechanical stirring in the latter case. Finally, it was distilled from sodium under reduced pressure, and the fraction, boiling at 190-200° at 2 mm. collected.

60-80° petroleum ether was purified by shaking three times with approximately one fifth of its volume of concentrated sulphuric acid, and three times with one fifth of its volume of potassium permanganate solution (5% in 10% sulphuric acid) and three times with an equal volume of water. It was dried first over quicklime, then by refluxing over sodium metal. Finally, it was distilled from sodium and the major fraction boiling at 65-69° collected.

The purified paraffin (150 ml.) was introduced into a dry 1 L round bottomed, three necked flask fitted with dropping funnel, reflux condenser, Herschberg nickel wire stirrer with mercury seal, and gas inlet and outlet with mercury trap. Nitrogen, with oxygen removed by bubbling through fresh alkaline pyrogallol and dried by bubbling through concentrated sulphuric acid and passage over potassium hydroxide pellets and phosphorous pentoxide, was passed through the apparatus for 15 mins. to displace all air and water vapour from the system, then lithium metal (3g.,0.43 g.m.), freshly scraped under paraffin, introduced and the temperature of the mixture raised to 250° with a slow stream of nitrogen maintained. The stirrer was then started and the lithium quickly whipped into very fine shining particles. To prevent any possible recoagulation of the particles, stirring was continued while the mixture was allowed to cool to room temperature. Purified dry 65-69° petroleum ether (about 150 ml.) was then introduced through the dropping funnel and the mixture well stirred. The paraffin-petroleum ether mixture was then drawn off under slight negative pressure through a tube with a glass wool plug which prevented the withdrawal of any of the lithium powder. The flask was washed out twice more with petroleum ether (150 ml.) by which time the paraffin was almost completely removed, and the lithium particles were left in a finely divided state covered by petroleum ether (50 ml.)

II. n-butyllithium: A solution of n-butyl chloride (20.4 g., 0.22 g.m.) in 65-69° petroleum ether (150 ml.) was then slowly added with mechanical stirring, until the reaction began, and the rate of addition adjusted to maintain a gentle refluxing of the solvent. Slow passage of dry nitrogen was continued, throughout the experiment. When the last of the reagent was added (about 30 mins.) heating was applied, and the solution refluxed for a further 3 hrs.

At the end of this period petroleum ether was added until the mixture reached a 250 ml. mark on the side of the flask, the whole well stirred, then allowed to settle. An aliquot (5 ml.) of the solution was taken and hydrolysed in distilled water (10 ml.). It was then titrated with 0.1 N hydrochloric acid, using phenol phthalein as indicator, thus determining the total alkali. A further aliquot (5 ml.) was withdrawn and added to a solution of benzyl chloride (1 ml.) in dry ether (10 ml.), the mixture allowed to stand for 1 minute, then hydrolysed and titrated as before, thus determining the non-organolithium alkali present.[98] The quantity of n-butyllithium formed was thus estimated.

III. 2:6-dimethoxyphenyllithium: Mechanical stirring was restarted and m-dimethoxybenzene (22.5 g., 0.165 g.m.) in petroleum ether (50 ml.) added to the cold mixture over 30 mins., stirring continued for a further 30 mins., then the heating reapplied and refluxing continued for a further 4 hrs., after which the solution was again allowed to cool down.

IV. 2:6-dimethoxybenzoic acid: A slow stream of carbon dioxide, dried by passage over anhydrous calcium chloride, was then passed through the solution for 1 hr., after which the mixture was slowly poured on to solid carbon dioxide (approximately 1.5 K.), the flask washed out with a further solution of petroleum ether (250 ml.), and the washings added to the solid carbon dioxide mixture. The whole was then left for 12 hrs., by which time the solid carbon dioxide had gone, and hydrolysed by the addition of water (500 ml.) with stirring. The mixture was then filtered and separated, when unreacted m-dimethoxybenzene was recovered from the petroleum ether phase, and 2:6-dimethoxybenzoic acid obtained by ether extraction of the aqueous layer acidified with hydrochloric acid.

(b) Using 65-69° petroleum ether as solvent and bromobenzene as reactant halide:

The conditions applied were exactly as described above, but for the use of bromobenzene (35 g., 0.22 g.m.) in place of n-butyl chloride. Since the titration method did not apply to aryl lithium compounds, only the titration of total alkali was possible.

(c) Using diethyl ether as solvent and n-butyl chloride as reactant halide:

The diethyl ether was purified by shaking three times with one fifth its volume of ferrous sulphate solution (5% in 1% sulphuric acid), then three times with an equal volume of water, and

Table XV.

2:6-dimethoxybenzoic acid from m-dimethoxybenzene (22.5 g., 0.165 g.m.)

Reagents		2:6-dimethoxy-benzoic acid		m-dimethoxy benzene		N/10 hydrochloric titre (ml.)			% n-butyl lithium formed.
		g.	%	g.	%	total alkali	non organo-lithium alkali	n-butyl lithium	
n-butyl chloride	diethyl ether	13	44	10.1	74	34.1	2.3	31.8	74
		12.1	41	10.5	66	31.0	4.6	26.4	61
		14.8	50	7.6	75	34.7	1.1	33.6	78
	65-69° petroleum ether	18.3	62	3.9	75	31.7	1.5	30.2	70
		19.1	64	3.7	77	33.0	0.9	32.1	75
		18.5	63	3.9	75	31.9	0.5	31.4	73
		18.4	62	4.0	76	32.2	1.7	30.5	71
		19.2	65	3.6	77	34.8	2.4	32.4	75
		18.8	63	3.8	76	31.1	0.6	30.5	71
bromo-benzene	diethyl ether	7.5	33	13.4	62	18.8			44
		6.2	21	15.7	69	15.5	-	-	36
		6.9	23	14.8	68	15.3			36
	65-69° petroleum ether	1.1	4	20.3	38	6.4			14
		0.5	2	20.6	20	3.1	-	-	7
		2.3	8	19.1	51	8.9			21

Preparation of 2:6-dimethoxybenzoic acid through 2: -dimethoxyphenyllithium.

drying over caustic soda flakes and sodium metal. Finally it was distilled from metallic sodium and collected at 34°C.

Diethyl ether purified in this way was used in place of petroleum ether, but otherwise conditions were identical with those already described.

(d) Using diethyl ether as solvent and bromobenzene as reactant halide:

The same conditions were again maintained, but purified diethyl ether was used in place of petroleum ether, and bromobenzene (35 g., 0.22 g.m.) in place of n-butyl chloride.

The results of this series of experiments are shown in Table XV.

2:6-dihydroxy-3-alkylbenzoic acids through 2:6-dimethoxy-3-alkyl - phenyllithium compounds.

I. 2:6-dimethoxy-3-alkylbenzoic acids: n-butyllithium from lithium powder (3 g., 0.43 g.m.) and n-butyl chloride (20.4 g.,0.22 g.m.) was reacted with the appropriate 2:4-dimethoxyalkylbenzene (0.165 g.m.) in 65-69° petroleum ether as solvent, and the product subjected to direct carbonation,when the acid was formed. The conditions were as described previously (section a). The yields obtained are shown in Table XVI.

II. 2:6-dihyroxy-3-alkylbenzoic acid: The 2:6-dimethoxy-3-alkyl - benzoic acid (0.033 g.m.) was dissolved in dry benzene (360 ml.) powdered anhydrous aluminium chloride (22.4 g.) added and the mixture on the steam bath/heated under reflux with constant mechanical

Table XVI.

2:6-dimethoxy-3-alkylbenzoic acids from 2:4-dimethoxyalkylbenzenes.

Starting material.	2:6-dimethoxy-3-alkylbenzoic acid.			2:4-dimethoxy-alkylbenzene recovered.
	weight g.	overall yield %	yield calculated on reacted 2:4-dimethoxy-alkylbenzene %	g.
2:4-dimethoxy-toluene. (16.5g.,0.11 g.m.)	5.2 4.1 5.4	25 20 26	35 33 37	5.0 6.5 5.3
2:4-dimethoxy ethylbenzene (27.1g.,0.165 g.m.)	18.7 22.1 19.8	55 65 57	85 82 80	9.5 5.7 8.2
2:4-dimethoxy n-propylbenzene (19.5g.,0.11 g.m.)	6.2 5.6 5.8	26 23 24	40 42 43	7.1 8.9 8.8
2:4-dimethoxy n-butylbenzene (21.5g.,0.11 g.m.)	6.7 6.4 5.5	26 22 21	54 48 38	11.2 10.6 8.9
2:4-dimethoxy n-amylbenzene (22.6g.,0.11 g.m.)	5.3 5.8 5.1	19 21 19	35 37 35	10.5 9.9 11.1
2:4-dimethoxy n-hexylbenzene (24.0g.,0.11 g.m.)	5.2 4.9 5.7	18 17 19	37 35 39	9.8 10.0 9.5

Preparation of 2:6-dimethoxy-3-alkylbenzoic acids through 2:6-dimethoxy-3-alkylphenyllithium compounds.

Table XVII.

2:6-dihydroxy-3-alkylbenzoic acids from 2:6-dimethoxy-3-alkylbenzoic acids.

Starting material	2:6-dihydroxy-3-alkylbenzoic acid.	
	Weight g.	Overall yield. %.
2:6-dimethoxy -3-methylbenzoic acid	4.6	83
2:6-dimethoxy -3-ethylbenzoic acid	5.5	91
2:6-dimethoxy -3-n-propylbenzoic acid	3.0	46
2:6-dimethoxy -3-n-butylbenzoic acid	1.8	26
2:6-dimethoxy -3-n-amylbenzoic acid	2.1	28
2:6-dimethoxy -3-n-hexylbenzoic acid	1.2	15.

Demethylation of 2:6-dimethoxy-3-alkylbenzoic acids.

Table XVIII.

2:6-dimethoxy-3-alkylbenzoic acids and 2:6-dihydroxy-3-alkylbenzoic acids.

Alkylbenzoic acid	Melting point	Found			Required	
		C,%	H,%		C,%	H,%
2:6-dimethoxy-3-methyl	115-6° (cf.117-8°)(73)	60.9	5.9	$C_{10}H_{12}O_4$	61.1	6.1
2:6-dimethoxy-3-ethyl	95-6°	63.2	6.5	$C_{11}H_{14}O_4$	62.9	6.7
2:6-dimethoxy-3-n propyl	73-4°	64.1	7.1	$C_{12}H_{16}O_4$	64.3	7.1
2:6-dimethoxy-3-n butyl	83-4°	65.0	7.3	$C_{13}H_{18}O_4$	65.3	7.5
2:6-dimethoxy-3-n amyl	65-6°	66.3	7.8	$C_{14}H_{20}O_4$	66.6	7.9
2:6-dimethoxy-3-n hexyl	45-6°	67.7	8.3	$C_{15}H_{22}O_4$	67.7	8.3
2:6-dihydroxy-3-methyl	163-5°	56.7	4.7	$C_8H_8O_4$	57.1	4.8
2:6-dihydroxy-3-ethyl	148-9° (cf.150°)(72)	59.2	5.5	$C_9H_{10}O_4$	59.3	5.5
2:6-dihydroxy-3-n propyl	128-130°	61.0	6.0	$C_{10}H_{12}O_4$	61.2	6.1
2:6-dihydroxy-3-n butyl	117-8°	62.5	6.4	$C_{11}H_{14}O_4$	62.8	6.7
2:6-dihydroxy-3-n amyl	106-8°	64.7	7.3	$C_{12}H_{16}O_4$	64.3	7.1
2:6-dihydroxy-3-n hexyl	114-5°			$C_{13}H_{18}O_4$	65.5	7.5

All acids listed recrystallised either from 60-80° petroleum ether or from benzene-petroleum ether mixture.

stirring. The mixture was then cooled, poured onto powdered ice (150 g.) well stirred, filtered and the benzene separated off. On acidification with concentrated hydrochloric acid (approximately 15 ml.) a precipitate of the 2:6-dihydroxy-3-alkylbenzoic acid was obtained, and purified by recrystallisation from petroleum ether. The yields obtained are shown in Table XVII.

2:6-dimethoxy-3-methylbenzoic acid through 2:6-dimethoxy-3-methyl - acetophenone.

(a) Synthesis through coumarin intermediates:

I. 4:6-dimethyl-7-hydroxycoumarin: Resorcinol (11 g., 0.1 g.m.) and acetoacetic ester (14.3 g., 0.11 g.m.) were mixed in a 250 ml. round bottomed flask immersed in an ice bath and treated with phosphoric acid(d. 1.75, 45 ml.) with mechanical stirring, the stirring continued for 2 hrs., then the mixture allowed to stand overnight at room temperature. It was then poured into water (250 ml.) and the precipitate recrystallised from dilute methyl alcohol to give 4:6-dimethyl-7-hydroxycoumarin (13.8 g., 79%) m.p. 253 - 255° (cf. 254-5°)(74).

II. 4:6-dimethyl-7-acetoxycoumarin: 4:6-dimethyl-7-hydroxycoumarin (10g.) and fused sodium acetate (10 g.) were refluxed for 3 hrs. with acetic anhydride (25 g.), and mixture poured into water (250 ml.),

the precipitate well washed with sodium hydroxide solution(10%) then with water. The 4:6-dimethyl-7-acetoxycoumarin obtained was recrystallised from dilute methyl alcohol to give colourless needles (10.9 g., 87%) m.p. 158-9° (cf. 159°)[74].

III. 4:6-dimethyl-7-hydroxy-8-acetylcoumarin: An intimate mixture of 4:6-dimethyl-7-acetoxycoumarin (10 g.) and powdered anhydrous aluminium chloride (25 g.) was heated to 160° over 1 hr., then maintained at that temperature for a further hour. The reaction mass was then cooled in an ice bath, and cold 2N hydrochloric acid (250 ml.) slowly added. The precipitate was filtered, dissolved in sodium carbonate solution (10 ml. of 10%) and reprecipitated with hydrochloric acid. The 4,6-dimethyl-7-hydroxy-8-acetylcoumarin so obtained was recrystallised from dilute methyl alcohol as pale yellow crystals (6.4 g. 64%) m.p. 213-4° Found : C,67.1%; H,5.4 %. $C_{13}H_{12}O_4$ requires C,67.2%: H,5.2% .

IV. 2:6-dihydroxy-3-methylacetophenone: 4,6-dimethyl-7-hydroxy-8-acetylcoumarin (5 g.) was dissolved in sodium hydroxide solution (50 ml. of 20%) and the solution warmed on the steam bath for 3 hrs., a slow stream of nitrogen being maintained. It was then cooled and acidified with hydrochloric acid and the precipitate of 2:6-dihydroxy-3-methylacetophenone recrystallised from water as orange plates (2.9 g., 81%) m.p. 137-8° (cf. 138°)[74].

V. 2:6-dimethoxy-3-methylacetophenone: 2:6-dihydroxy-3-methyl - acetophenone (2.5 g., 0.015 g.m.) was dissolved in sodium hydroxide solution (15 ml. of 20%) and dimethyl sulphate (8g.) added over 15 mins. The mixture was then stirred for a further hour, then heated on the steam bath for 2 hrs. On extraction with ether pale yellow crystals of 2:6-dimethoxy-3-methylacetophenone (1.1g., 38%) m.p. 22-23°, b.p. 120°/5 mm. were obtained (cf. m.p. 20-22°)[73]. On acidification of the alkaline solution, 2-hydroxy-6-methoxy-3-methylacetophenone (0.5 g.) was obtained as pale yellow crystals m.p. 30-32° b.p. 110-111°/2 mm. Found: C,66.6%; H, 6.8%. $C_{10}H_{12}O_3$ required C, 66.7%, H.6.7%.

VI. 2:6-dimethoxy-3-methylbenzoic acid: A solution of sodium hydroxide (5.5 ml. of 40%) was added to powdered ice (20 g.) and chlorine prepared by the action of concentrated hydrochloric acid on potassium permanganate passed through until the solution was neutral to litmus. Further sodium hydroxide solution (5 ml. of 20%) was then added. The temperature was raised to 85° and 2:6-dimethoxy-3-methylacetophenone (1.5 g.) added over 15 mins., with constant mechanical stirring. The temperature was maintained at 85° and the stirring continued for a further 2 hrs., then the solution cooled and sodium bisulphite (2 g.) added to destroy unchanged sodium hypochlorite. When the excess hypochlorite was removed, the solution was acidified with concentrated hydrochloric acid and the precipitated acid recrystallised from benzene to give colourless crystals (1.05 g., 75%) m.p. 115-6° identical with those obtained by carbonation of 2:6-dimethoxy-3-methylphenyllithium. (cf. m.p. 117-8°)[73].

(b) Synthesis through 2:6-dimethoxy-3-methylphenyllithium.

2:6-dimethoxy-3-methylacetophenone: 2:6-dimethoxy-3-methylphenyllithium was formed as before in diethyl ether by reaction of n-butyllithium from lithium powder (1 g., 0.144 g.m.) and n-butyl chloride (6.8 g., 0.073 g.m.) with 2:4-dimethoxy toluene (8.4 g., 0.055 g.m.) and acetyl chloride (17.4 g., 0.22 g.m.) added to the cold solution. The solution was then refluxed with constant stirring for 24 hours then allowed to stand at room temperature for 21 days, when pale yellow crystals (6.9 g., 65 %) identical to those obtained by methylation of 2:6-dihydroxy-3-methylacetophenone, were obtained on hydrolysis.

When the same experiment was repeated in 65-69° petroleum ether only unchanged 2:4-dimethoxytoluene was obtained.

The 2:6-dimethoxy-3-methylacetophenone was oxidised as before with sodium hypochlorite to give 2:6-dimethoxy-3-methylbenzoic acid.

2:6-dimethoxy-3-ethylbenzoic acid through 2:6-dimethoxy-3-ethyl-acetophenone.

(a) Synthesis through coumarin intermediates.

The procedure was identical with that already described for the methyl isomer.

I. 4-methyl-6-ethyl-7-hydroxycoumarin: On recrystallisation from dilute ethyl alcohol, a yield of 74% colourless crystals, m.p. 212-3° was obtained (cf. m.p. 213°)(100).

II. 4-methyl-6-ethyl-7-acetoxycoumarin: Acetylation yielded 83% colourless crystals m.p. 142° (cf. 144°)[100].

III. 4-methyl-6-ethyl-7-hydroxy-8-acetylcoumarin: On recrystallisation from dilute ethyl alcohol 68% pale yellow crystals m.p. 138-9° were obtained (cf. m.p. 139°)[100].

IV. 2:6-dihydroxy-3-ethylacetophenone: A 73% yield of pale yellow needles m.p. 132-4° was obtained on recrystallisation from water (cf. m.p. 135°)[100].

V. 2:6-dimethoxy-3-ethylacetophenone: Methylation yielded 35% pale yellow oil b.p. 130-131°/5 mm. Found C, 68.6 %; H, 7.4%. $C_{12}H_{16}O_3$ required C, 69.0; H, 7.7%.

VI. 2:6-dimethoxy-3-ethylbenzoic acid: Oxidation yielded 69% acid m.p. 95-6° on recrystallisation from 60-80° petroleum ether, the melting point of which was not changed by mixing with the product obtained by direct carbonation of 2:6-dimethoxy-3-ethylphenyllithium.

(b) Synthesis through 2:6-dimethoxy-3-ethylphenyllithium.

The same conditions applied as in the preparation of the methyl isomer. When 2:4-dimethoxyethylbenzene (9.1 g., 0.055 g.m.) was reacted with n-butyllithium followed by acetyl chloride in 65-9° petroleum ether, starting material was recovered unchanged, but in diethyl ether a yield of 2:6-dimethoxy-3-ethylacetophenone (6.9 g., 60%) identical with that obtained by methylation of 2:6-dihydroxy-3-ethyl acetophenone was afforded.

2:6-dimethoxy-3-n-hexylbenzoic acid through 2:6-dimethoxy-3-n-hexyl - acetophenone.

The procedure was identical with that already described for the methyl isomer.

I. 4-methyl-6-n-hexyl-7-hydroxycoumarin: A yield of colourless crystals (84%) m.p. 165-166° was obtained on recrystallisation from dilute ethyl alcohol.

II. 4-methyl-6-n-hexyl-7-acetoxycoumarin: A yield of colourless needles (85%) m.p. 110-111° was obtained on recrystallisation from dilute ethyl alcohol. Found, C,71·7%; H,6·8 %. $C_{18}H_{22}O_4$ requires C, 71.5%; H, 7.2%.

III. 4-methyl-6-n-hexyl-7-hydroxy-8-acetylcoumarin: No crystalline material was isolated from the mixture.

IV. 2:6-dihydroxy-3-n-hexylacetophenone: An attempt to hydrolyse some of the crude rearrangement product did not yield any pure substance.

2:6-dimethoxyacetophenone through 2:6-dimethoxyphenyllithium.

The conditions were the same as those already described for the preparation of 2:6-dimethoxy-3-methylacetophenone through 2:6-dimethoxy-3-methylphenyllithium. m-dimethoxybenzene (22.5 g., 0.165 g.m.) was reacted in diethyl ether with the n-butyllithium from lithium powder, (3 g., 0.43 g.m.) and n-butyl chloride (20.4 g., 0.22 g.m.) and acetyl chloride (52 g., 0.66 g.m.) added to the cold solution. The mixture was refluxed for 24 hrs., with

constant mechanical stirring, then allowed to stand at room temperature for 21 days, when colourless crystals of 2:6-dimethoxyacetophenone (19.5 g., 60%) m.p. 72-73° were obtained on recrystallisation from 60-80° petroleum ether (cf. m.p. 68-9°)(113).

When the same experiment was repeated using 65-68° petroleum ether in place of diethyl ether as solvent, only unchanged m-dimethoxybenzene was recovered.

2:6-dimethoxytoluene through 2:6-dimethoxyphenyllithium.

2:6-dimethoxyphenyllithium was formed as before in 65-69° petroleum ether by reaction of n-butyllithium from lithium powder (3 g., 0.43 g.m.) and n-butyl chloride (20.4 g., 0.22 g.m.) with m-dimethoxybenzene (22.5 g., 0.165 g.m.), and methyl iodide (93.5 g., 0.66 g.m.) added to the cold solution. After 1 hr. at room temperature with mechanical stirring, heating was applied, and the solution refluxed for 24 hrs., then left at room temperature for 21 days. Water (250 ml.) was then added, the mixture stirred for 15 mins., then filtered. Only unreacted m-dimethoxybenzene was isolated.

When the same experiment was repeated in pure dry diethyl ether in place of petroleum ether as solvent, a yield of pure 2:6-dimethoxytoluene (10.5 g., 45 %) m.p. 37-38° was obtained (cf. 39°)(101).

2:6-dimethoxyethylbenzene through 2:6-dimethoxyphenyllithium.

When the above experiments were repeated using ethyl iodide (103 g., 0.66 g.m.) in place of methyl iodide, a yield of 2:6-dimethoxyethylbenzene (13.6.g., 55%) m.p. 58-59° (cf. m.p. 60°)[102] was obtained in diethyl ether solution, but in 65-69° petroleum ether solution again only unreacted m-dimethoxybenzene was recovered.

Oxidation of 2:6-dimethoxytoluene to 2:6-dimethoxybenzoic acid.

2:6-dimethoxytoluene (5 g.) in water (100 ml.) at 80° was treated with potassium permanganate (10.4 g.) over 1 hr. with constant mechanical stirring. After standing overnight, the solution was washed with water, when on acidification of the filtrate with concentrated hydrochloric acid, a precipitate was obtained, which on recrystallisation from benzene yielded 2:6-dimethoxybenzoic acid (4.3 g., 71%) m.p. 186-7°.

2:4-dimethoxytoluene through 2:4-dimethoxyphenyllithium.

I. 2:4-dimethoxybromobenzene: Succinimide was prepared as white crystals m.p. 123-5° by thermal condensation of ammonium succinate formed by the addition of ammonia (28%) to succinic acid[114].

Succinimide prepared as above (80g.) was dissolved in sodium hydroxide solution (200 ml. of 30%) and powdered ice (150 g.) added. Bromine (42.5 ml.) was then added quickly to the well stirred solution, and stirring continued for 5 mins. The N-bromosuccinimide formed was then filtered, well washed with ice

cold water, and dried at 40°, when 105 g. material sufficiently pure for use as a reagent was obtained.

m-dimethoxybenzene (15 g.) was dissolved in carbon tetrachloride (10 ml.) and bromosuccinimide (8 g.) added. The mixture was refluxed for 6 hrs., on the steam bath, then fractionally distilled under reduced pressure to give 2:4-dimethoxybromobenzene, which was redistilled several times to yield a pale yellow oil b.p. 156-158°/30 mm. (cf. 152°/25 mm.)[81].

The quantity of carbon tetrachloride used as solvent was varied and the yield increased somewhat as shown in Table IXX.

Table IXX.

2:4-dimethoxybromobenzene from m-dimethoxybenzene (cf. 107).

Carbon tetrachloride	2:4-dimethoxy bromobenzene			Recovered m-dimethoxy benzene
	Weight g.	Overall yield %	Yield calculated on unrecovered m dimethoxy benzene. %	g.
10	2.5	10	31	10.0
	2.3	10	31	10.3
	2.9	12	30	9.8
50	5.2	21	44	7.6
	8.1	34	48	4.4
	6.3	26	60	8.5
100	6.5	27	50	6.7
	4.8	20	38	7.1
	4.9	20	35	6.2

(b) 2:4-dimethoxytoluene: 2:4-dimethoxybromobenzene (23.4 g., 0.11 g.m.) was added to a solution of n-butyllithium formed from lithium powder (2 g., 0.29 g.m.) and n-butylchloride (13.6 g., 0.15 g.m.) in diethyl ether, and the solution refluxed for 6 hrs., with constant mechanical stirring, and the passage of a slow stream of dry nitrogen. The mixture was then cooled, methyl iodide (62.4 g., 0.66 g.m.) added, and the whole stirred for 1 hr., then refluxed for 24 hrs., and allowed to stand at room temperature for 21 days. At the end of this period the mixture was hydrolysed and 2:4-dimethoxytoluene (5.1 g., 30%) b.p. 127°/30 mm. obtained (cf. 211°/760 mm.)[109].

2:4-dimethoxyethylbenzene from 2:4-dimethoxyphenyllithium.

The conditions were exactly as described above for the preparation of 2:4-dimethoxytoluene, but ethyl iodide (69 g., 0.44 g.m.) was added in place of methyl ioidide. A yield of 2:4-dimethoxyethylbenzene (9.7 g., 44 %) b.p. 110°/11 mm. was obtained (cf. 113°/13 mm.)[88].

2:4-dimethoxybenzoic acid through 2:4-dimethoxyphenyllithium.

2:4-dimethoxyphenyllithium was formed in diethyl ether solution as already described. It was then subjected to direct carbonation by passage of carbon dioxide and pouring on to solid carbon dioxide, when 2:4-dimethoxybenzoic acid (12.9 g., 62%) m.p. 108-9° was obtained on hydrolysis (cf. 109°)[81].

When the experiment was repeated using phenyllithium from lithium (2 g., 0.29 g.m.) and bromobenzene (23.6 g., 0.15 g.m.) in place of n-butyllithium, 2:4-dimethoxybenzoic acid (6.9g., 34%) was obtained.

Conclusion.

It was suggested in the introduction that the chemist engaged in the field of chemotherapy must not be too highly specialised since he may be called upon not only to isolate the active principle of naturally occurring products or to synthesis new products, but also to develop methods of estimating the new drugs in biological fluids, to investigate the metabolism of the drugs in the body, and to determine their physical properties. This also applies equally to other scientists engaged in the work. For each there is a specialised section, but the bulk of the work constitutes a common pool to which all may contribute and from which all may draw. In this way it is possible to have a greater total knowledge applied to any one problem, so that the problem is approached from several points of view, and thus the possibility of its solution is increased.

In the initial investigation into the mode of action of sodium salicylate in rheumatic fever it was confirmed that remission of the disease was determined by the plasma concentration of the drug. This concentration was, however, much less closely related to the dosage, so that there was considerable variation in response to the same dose. It was also found that the secondary symptoms produced by salicylate therapy were, taken together, sufficiently characteristic to be designated the "special salicylate syndrome" and this syndrome was found to develop in patients with a plasma salicylate concentration

not sufficiently far removed from the effective therapeutic plasma concentration to ensure that an effective dosage for one patient would not constitute a mildly toxic dose for another. The development of a mild but unmistakable Cushing's syndrome in a patient receiving sodium salicylate treatment was observed with interest, since these symptoms have frequently been noted in patients during administration of cortisone and A.C.T.H. The biochemical changes following salicylate treatment of acute rheumatism are, in fact, similar in several respects to those resulting from cortisone administration.

Salicylate was quite effective in inducing remission of rheumatic fever, but was not entirely satisfactory as a drug because of the frequency of occurrence of distressing side effects. It was felt therefore, that some other compound chemically similar to salicylate might be found with a greater margin of safety between the therapeutic and toxic doses. The most prominent chemical feature of the salicylate molecule, the chelation between the hydroxyl and carboxyl groups and the resulting increase in acid strength as compared with the therapeutically inactive m- and p- hydroxybenzoic acids, is further enhanced in 2:6-dihydroxybenzoic acid. It was therefore felt that a trial of the sodium salt of this acid as an anti-rheumatic drug might be of interest. A survey was made of the various methods of preparation of 2:6-dihydroxybenzoic acid published in the literature, and a practical laboratory method developed.

Although the symptoms of rheumatic fever were relieved by approximately one tenth of the dose of sodium salicylate, symptoms of overdosage including the development of a mild Cushing's syndrome were also observed, so that it seemed apparent that sodium 2:6-dihydroxybenzoate possessed at least to some extent a common mode of action with both sodium salicylate and cortisone. Thus although the trial was not successful in introducing a safer anti-rheumatic drug, it indicated that further research into o-hydroxy substituted benzoic acids might be profitable. A further disadvantage of sodium 2:6-dihydroxybenzoate was its rapidity of excretion in the urine after oral or parenteral administration. In 2:6-dihydroxy-3-alkylbenzoic acids the degree of chelation might be anticipated to be of the same order as in 2:6-dihydroxybenzoic acid, and a longer period of action might be expected.

A series of 2:6-dihydroxy-3-alkylbenzoic acids was therefore prepared by direct carbonation of the corresponding organolithium compounds and some investigation of the optimum conditions for the reaction carried out. The configuration of the acids was verified by synthesis of two members of the series by a more unequivocal route, through the 4-methyl-6-alkyl-7-hydroxy-^{8}-acetylcoumarin, and the common identity of the two final products established. Further applications of the organolithium compounds in formation of 2:6-dimethoxy acyl and alkyl benzenes were investigated and the practicability of use of 2:6-dimethoxyphenyllithium as an intermediary established. The

formation of 2:4-dimethoxy acyl and alkyl benzenes through reaction of an appropriate reagent with 2:4-dimethoxyphenyllithium obtained from 2:4-dimethoxybromobenzene was also investigated, and the formation of 2:4-dimethoxybenzoic acid by direct carbonation of 2:4-dimethoxyphenyllithium verified. Clinical and pharmacological investigation of this series of acids is now to be carried out.

Bibliography

(1) Cullen, 'Synopsis Nosolgiae Methodicae', Edinburgh, 1769; quoted by Thomson,'Life of William Cullen', Blackwood, Edinburgh, 1859.

(2) Piria, Compt. rend., 1838, 6, 620.

(3) Kolbe, J. pr. Chem., 1874, 10, 89.

(4) Buss, Arch. klin, Med., 1875, 15, 457.

(5) MacLagan, Brit. Med. J., 1876, 627.

(6) Coburn, Bull. Johns Hopkins Hosp., 1943, 73, 435.

(7) Reid, Quart. J. Med., 1948, 17, 139.

(8) Bendien, Neuburg and Snapper, Biochem. Z., 1932, 247, 306.

(9) Homb urger, Amer. J. Med. Sci., 1945, 210, 168.

(10) Smull, Wegria,and Leland, J. Amer. Med. Assoc., 1944, 125, 1173.

(11) Smith, Gleason, Stoll and Orgorzalek, J. Pharmacol., 1946, 87, 237.

(12) Gregersen and Stewart, Amer. J. Physiol., 1939, 125, 142.

(13) Hanzlik, Scott and Thobrun, J. Pharmacol., 1917, 9, 247.

(14) York and Fischer, New England J. med., 1947, 237, 477.

(15) Madden and Whipple, Physiol. Reviews, 1940, 20, 194.

(16) Lichty and Hooker, Proc. Soc. Exp. Biol, N.Y., 1941, 48, 69.

(17) Graham and Parker, Quart. J. Med., 1948, 17, 153.

(18) Sherman and Gross, J. Ind. Eng. Chem., 1911, 3, 492.

(19) Westergren, Acta Med. Scand., 1921, 54, 247.

(20) Van Slyke and Neill, J. Biol. Chem., 1924, 61, 523.

(21) Kjeldahl, Z. anal. Chem., 1883, 22, 366.

(22) Greenwald, J. Biol. Chem., 1915, 21, 61.

(23) Greenwald, J. Biol. Chem., 1918, 34, 97.

(24) Balint, Biochem. Z., 1924, 150, 425.

(25) Roscoe and Schorlemmer, 'A Treatise on Chemistry', MacMillan, London, 1923.

(26) Kramer and Gittleman, J. Biol. Chem., 1924, 62, 353.

(27) Butler and Tuthill, J. Biol. Chem., 1931, 93, 171.

(28) Barber and Kolthoff, J. Amer. Chem. Soc., 1928, 50, 1625.

(29) Caley, J. Amer. Chem. Soc., 1932, 54, 432.

(30) Peters and Van Slyke,'Quantitative Clinical Chemistry',Balliere, Tindall and Cox, London, 1932.

(31) Volhard, Z. anal. Chem., 1878, 17, 482.

(32) Wilson and Ball, J. Biol. Chem., 1928, 79, 221.

(33) Stockman,'Rheumatism and Arthritis', Green, Edinburgh, 1920.

(34) Kapp and Coburn, J. Biol. Chem., 1942, 145, 549.

(35) Mauthner, J. pr. Chem., 1940, 156, 154.

(36) Rosenberg, Krevsky and Kagan, Ann. Intern. Med., 1952, 36, 1513.

(37) Capretti and Arduini, Giorn. clin. med., 1950, 31, 677, quoted in Chem. Abs., 1950, 44, 10933.

(38) Ginoulhiac, Semenza and Mainardi, Boll. Soc. ital. Biol. sperim., 1950, 26, 583.

(39) Meade and Smith, Lancet, 1951, 260, 236.

(40) Mainardia and Semenza, Boll. Soc. ital. Biol. sperim., 1951, 27, 352.

(41) Lehman, Lancet, 1946, 1, 15.

(42) Doub, Schaefer, Bambas and Walker, J. Amer. Chem. Soc., 1951, 73, 903.

(43) Bersch and Dopp, Arch. Pharm., 1951, 284, 30.

(44) Drain, Goodacre and Seymour. J. Pharm. Pharmacol., 1949, 1, 784.

(45) Baker, Nature, 1936, 137, 236.

(46) Baker, J.C.S., 1934, 1684.

(47) Martin, Trans. Faraday Soc., 1937, 33, 191.

(48) Coggeshall, J. Amer. Chem. Soc., 1947, 69, 1620.

(49) Flett, J.C.S., 1948, 1441.

(50) Flett, J.C.S., 1951, 962.

(51) Limaye and Kelkar, J. Indian Chem. Soc., 1935, 12, 788.

(52) Clewer, Green and Tutin, J.C.S., 1915, 835.

(53) Warden, Indian Med. Gaz., 1880, 15, 253; 1881, 16, 138, quoted in (52)

(54) Hench, Kendall, Slocumb and Polley, Proc. Mayo Clin., 1949, 24, 181.

(55) Hench, Kendall, Slocumb and Polley, Arch. intern. Med., 1950, 85, 545.

(56) Cushing, Bull. Johns Hopkins Hosp., 1932, 50, 137.

(57) Limaye, Ber., 1934, 67, 12.

(58) Russell, Frye and Mauldin, J. Amer. Chem. Soc., 1940, 62, 1441.

(59) Kolbe, Annalen, 1860, 113, 125.

(60) Senhofer and Brunner, Sitzungsber. Akad. Wiss. Wien, 1879, 80, 504.

(61) Brunner, Annalen, 1907, 351, 313.

(62) Mauthner, J. pr. Chem., 1930, 124, 319.

(63) Clibbens and Nierenstein, J.C.S., 1915, 1491.

(64) Mauthner, J. pr. Chem., 1929, 121, 259.

(65) de Bruyn, Rec. Trav. chim., 1883, 2, 205.

(66) Russell and Tebbens, Org. Synth., 22, 35.

(67) Prey, Ber., 1941, 74, 1219.

(68) Badder, Nature, 1944, 153, 377.

(69) Cahn, J.C.S., 1931, 630.

(70) Freudenberg, Lautsch and Piazolo, Ber., 1941, 74, 1879.

(71) Hale, Hawdon, Jones and Packham, J.C.S. 1952, 3503.

(72) Fujikawa, Nakajima and Fujii, J. Pharm. Soc. Japan, 1950, 70, 22.

(73) Sprenger and Ruoff, J. Org. Chem., 1946, 11, 189.

(74) Yanagita, Ber., 1938, 71, 2269.

(75) Radha and Shah, J. Indian Chem. Soc., 1942, 19, 393.

(76) Gilman and Arntzen, J. Amer. Chem. Soc., 1947, 69, 1537.

(77) Ziegler and Jakob, Annalen, 1934, 511, 45.

(78) Gilman and Moore, J. Amer. Chem. Soc., 1940, 62, 1843.

(79) Gilman and Jones, J. Amer. Chem. Soc., 1941, 63, 1441.

(80) Wittig and Fuhrmann, Ber., 1940, 73, 1197.

(81) Wittig and Pockels, Ber., 1939, 72, 89.

(82) Gilman, Willis, Cook, Webb and Meals, J. Amer. Chem. Soc., 1940, 62, 667.

(83) Gattermann, Annalen, 1907, 357, 313.

(84) Johnson and Lane, J. Amer. Chem. Soc., 1921, 43, 348.

(85) Clemmensen, Ber., 1914, 47, 51.

(86) Robinson and Shah, J.C.S. 1934, 1491.

(87) Cooper, Org. Synth., 21, 103.

(88) Skraup and Bohm, Ber., 1926, 59, 1007.

(89) Twiss, J. Amer. Chem. Soc., 1926, 48, 2206.

(90) Mauthner, Org. Synth., 6, 96.

(91) Dohme, Cox and Miller, J. Amer. Chem. Soc., 1926, 48, 1688.

(92) Dean and Robertson, J.C.S., 1953, 1241.

(93) Tucker and Whalley, J.C.S., 1949, 50.

(94) Fieser and Herschberg, J. Amer. Chem. Soc., 1937, 59, 394.

(95) Gilman, Langham and Moore, J. Amer. Chem. Soc., 1940, 62, 2327.

(96) Bartlett, Swain and Woodward, J. Amer. Chem. Soc., 1941, 63,3229.

(97) Herschberg, Ind. Eng. Chem. Anal., 1936, 8, 313.

(98) Gilman and Haubein,J. Amer. Chem. Soc., 1944, 66,1515.

(99) Ziegler, Crossmann, Kleiner and Schafer, Annalen., 1929, 473, 1.

(100) Desai and Ekhlas, Proc. Indian Acad. Sci., 1938, 8, 194.

(101) Sibata, J. Pharm. Soc. Japan, 1939, 59, 323.

(102) Limaye and Ghate, Rasayanam, 1936,1, 39; quoted in Chem. Abs., 1937, 31, 2182.

(103) Adams, Cain and Baker, J. Amer. Chem. Soc., 1940, 62, 2201.

(104) Sandin and McKee, Org. Synth., 17, 23.

(105) Simada and Hata, Sci. Papers Inst. Phys. Chem. Res., Tokyo, 1939, 35, 365; quoted in Chem. Abs., 1939, 33, 4594.

(106) Ziegler, Spath, Schaaf, Schumann and Winkelmann, Annalen, 1942, 551, 90.

(107) Buu-Hoi, Annalen,, 1944, 556, 1.

(108) Steinkopf and Otto, Annalen., 1921, 424, 61.

(109) Gattermann, Annalen., 1907, 357, 372.

(110) Johnson and Hodge, J. Amer. Chem. Soc., 1913, 35, 1019.

(111) Goldsweig and Kaiser, J. pr. Chem., 1891, 43, 86.

(112) Irani, Phalnikar and Nargund, J. Univ. Bombay, 1948, 16, 41; quoted in Chem. Abs., 1949, 43, 1033.

(113) Sugasawa, J.C.S., 1934, 1483.

(114) Clarke and Behr, Org. Synth., 16, 75.

(115) Karrer, Helv. Chim. Acta, 1919, 2, 89.