century have been sketched. In 1900 the total membership of the Association was 1,925. In that year the weekly journal *Science*, owned and edited by Dr. J. McKeen Cattell, became the official publication of the Association. By 1908 the membership had become approximately 8,000, and it remained about the same until the close of the First World War, after which it increased rapidly for a decade. It exceeded 15,000 in 1928 and 18,000 in 1938. Now it exceeds 40,000.

There has been a geographical expansion of science, as well as a great increase in the number of men of science, in the United States. In 1916 the Pacific Division of the Association was organised by members of the A.A.A.S. who lived in the great and now populous States on the Pacific coast, about three thousand miles from the large cities in the East and two thousand miles from those in the Middle West, the regions in which meetings were generally held. In 1920 the South-western Division was similarly organised for the convenience of members who live in the south-western States. These Divisions of the Association are wholly autonomous in the election of their officers, the organisation of their annual meetings, and their relations with other scientific societies in their respective areas. The Pacific Division now has more than 4,600 A.A.A.S. members and the South-western Division nearly 800.

Perhaps the most effective way of describing the current policies and activities of the A.A.A.S. is to present a few statistics of the meeting that it held in Chicago during December 26–31, 1947. Although registration at the meetings of the Association has not been generally required, there were 4,940 registrants at the Chicago meeting, each of whom received a general programme. There were, in addition, several thousand persons for whom programmes were not available. As an illustration of the number of men of science at the meeting from a limited field, the official tabulated attendance at the annual "Biologists' Smoker" was 5,575—substantially greater than the total registration from all fields.

There were registrants at the Chicago meeting from every one of the forty-eight States, and also from Alaska and Hawaii. There were seventy-three registrants from Canada, twenty-eight from India and fourteen from China. There were 105 registrants from California, about 2,000 miles westward from Chicago, and 102 from Massachusetts, about 1,000 miles in the opposite direction.

A total of 2,019 papers were on the programme, of which 1,809 were presented orally, 46 consisted of demonstration and informal discussions, and 164 were read by title only. They were distributed among 340 sessions, the maximum number held simultaneously being 41.

It should not be assumed from the large number of men of science attending the "Biologists' Smoker" that the meeting was dominated by biologists or by scientific workers from any other particular field. Nor should it be assumed that the number of men of science was small from the fields in which there are large special scientific societies (the American Chemical Society, for example, or the American Medical Association), which hold their own large meetings quite independently of other scientific societies. At the Chicago meeting of the Association 4,288 registrants recorded their fields of specialization, in part, as follows: fields in which the special societies meet with the A.A.A.S.—zoology (822), biology (498), botany (903); fields in which the special societies hold independent meetings—chemistry (678), physics (296), medical sciences (354), agriculture (176), education (100).

As a consequence of the large attendance at the Association's meetings of specialists from every field of science, it is possible to organise and hold joint symposium programmes on subjects that are not limited by the usual boundaries of the various sciences.

An important feature of the Chicago meeting of the A.A.A.S. last December was the address of Dr. James B. Conant, retiring president of the Association and the president of Harvard University, entitled "The Role of Science in Our Unique Society". There were also ten scholarly addresses by retiring vice-presidents of the Association which in general were surveys or summaries of the fields of science of the Association's sections of which they were the respective chairmen.

In addition to these official programmes, there were seven sessions for special lectures or discussions of various aspects of the role of science in human affairs. Finally, there were fifty symposia on broad sectors of science of high current interest and importance.

As the present officers of the Association look back over the century of progress in organised American science made by their predecessors they have only feelings of gratitude and admiration for the high ideals which moved them and the success which crowned their efforts. As the present officers look with anxiety toward the future they can only say (to paraphrase the closing words of Milton's "Paradise Lost") that all the world is before them and Providence their guide. To paraphrase the opening invocation to "Paradise Lost", they can only say, to the Heavenly Muse, what in us is dark, illumine, what is low, raise and support, that we may rise to the height of the great opportunities before us and justify our confidence in the powers of science to improve the lot of man.

ANTIBIOTIC ACTIVITY OF GROWTH-FACTOR ANALOGUES*

THE most striking reactions associated with the growth of micro-organisms are synthetic ones, particularly those concerned with protein synthesis. The synthesis of each compound is a stepwise process, each step being catalysed by an enzyme; if the necessary enzyme is in some way inhibited or has been lost during evolution of the organism, then growth will be slowed down or stopped unless the product of the reaction in which that enzyme was involved is now provided in the external environment. When the synthesis of a compound essential for growth is impossible through natural loss of an enzyme system, then that compound becomes for the organism a 'growth-factor'. By this term it is not meant to imply that the compound in question is not essential for those organisms which do not require it to be present in the external environment; all growth-factors investigated have been shown to be synthesized by a wide variety of organisms which do

* Based on a discussion held at the Royal Society on June 17. There took part in the discussion, which was opened by Sir Paul Fildes, F.R.S., Dr. D. D. Woods, Dr. H. McIlwain, Dr. T. S. Work, Dr. H. N. Rydon and Dr. F. L. Rose; Dr. J. Walker, Dr. A. Albert and Prof. M. Stacey also contributed.

not require them pre-formed and, where these are known, to have the same functions as in the more exacting organisms. Thus it seems justifiable to consider all growth-factors as 'essential metabolites' and to regard the differences in requirements for them as differences in synthetic ability of the organisms investigated. On this basis, interference with the functioning or further utilization of the essential metabolite would produce the same metabolic lesion in those organisms which are able to synthesize it as already exists naturally in those which are not.

The concept of 'substrate competition' was already well established at the time that the mode of action of the sulphonamide drugs was demonstrated. For example, malonate was known to be sufficiently like succinate to be able to form a complex with succinic dehydrogenase, but to be unlike it enough not to go through the stages of dehydrogenation and dissociation. If enough malonate was present, most of the enzyme became involved in formation of the more stable complex; the enzyme was, in fact, inhibited by malonate which was competing with succinate for the attention of the enzyme. This inhibition could be reversed by increasing the concentration of succinate, renewed by adding more malonate, and so on. In just such a way the sulphonamides are held to compete with *p*-aminobenzoic acid, and to prevent its further utilization. Although a certain amount is known about the functions of *p*-aminobenzoic acid, little is known about the receptors with which the competition is concerned Possibly the sulphonamides form complexes with an enzyme or series of enzymes, analogous to the malonate–succinic dehydrogenase complex. Possibly they form complexes with a compound normally associated with *p*-aminobenzoic acid and thus interfere with its function; it has been suggested that reductone may be such a compound, and reductone-*p*-aminobenzoic acid and reductone-sulphonamide complexes have been demonstrated.

Whatever the exact point of attack of the sulphonamides, it was clear that they acted by competing with *p*-aminobenzoic acid, shown to be a growth-factor for a wide variety of organisms, and considered to be an essential metabolite at least for those which are sulphonamide-sensitive. Realization of this, together with the marked chemotherapeutic success of the sulphonamides, stimulated the development of the Fildes–Woods 'rational approach' to chemotherapy. Briefly, it was suggested that analogues of known bacterial growth-factors (or essential metabolites) might be found to bear the same relationship to the parent growth-factor as the sulphonamides bear to *p*-aminobenzoic acid and thus prevent their utilization (or synthesis). Such analogues should prevent or slow bacterial growth and might thus prove to be of use as chemotherapeutic agents.

Several hundred analogues of compounds known to be of importance to the cell have been synthesized and many of them possess the predicted bacteriostatic property *in vitro*. Of the active compounds many are inactive *in vivo*, and others are too toxic to the host to be considered as chemotherapeutic agents. Only certain analogues of pantothenic acid (for example, phenylpantothenone) retain their property of inhibiting growth of the micro-organism *in vivo* and at the same time fulfil the other requirements of a chemotherapeutic drug; had it not been for the development of other antimalarial drugs, phenylpantothenone might to-day be regarded as a practical achievement for the rational approach. Superficially, however, this approach has yielded nothing of chemotherapeutic value; but certain points must be considered before passing judgment. The first concerns the choice of factor. Such is the fundamental similarity of metabolic patterns in all cells that an agent which interferes with a given process in one cell is likely to interfere in a similar way with the metabolism of another cell, independent of species. The repetition of metabolic patterns is seen most clearly in the case of the B group of vitamins. Members of this group have an extremely wide distribution as essential metabolites, and analogues of them have been synthesized and tested in the greatest number. It is not difficult, therefore, to understand the failure of these analogues in chemotherapy, for they interfere with the same fundamental processes in both parasite and host; indeed, several analogues, such as those of aneurin, nicotinic acid and folic acid, have produced in mammals syndromes indistinguishable from those produced by simple dietary deficiencies of the factors concerned.

The relationship between an inhibitory compound and the growth-factor on which it is modelled tends rather to be taken for granted. An analysis of any existing relationship and of the metabolic effects produced by the antagonism is desirable not only for a proper understanding of the mode of action of the analogue (and thereby of the corresponding growth-factor) but also so that the development of more efficient inhibitors should proceed logically. Evidence for the existence of a relationship is of three main types. First, the actual existence of a mutual antagonism has to be demonstrated between growth-factor and analogue. This aspect has been very widely studied and has been the subject of several reviews. Second, even though such an antagonism is established, it should not be assumed that growth inhibition is solely due to it. Specificity of action should be examined in as wide a variety of organisms as possible, and reversal of the inhibition should be limited to the growth-factor in question and to any compounds which may replace it in the growth of organisms requiring it. Finally, the effects produced by the analogue should be just those which are seen as the result of a nutritional deficiency of the growth-factor.

It is desirable also that the exact point of attack of the inhibitor should be investigated. This presents a complex problem, for the various possible processes which can be interfered with are all closely interrelated, and it is not easy to separate primary and secondary effects. Of the three main points at which an analogue can cause a block, the first two, assimilation of the growth-factor and its elaboration into a functioning form, are particularly difficult to separate. No instance of an analogue preventing the assimilation of a growth-factor is known, though little work has been done on this aspect. Most work has been concentrated on the metabolism of the factor once it has entered the cell, and this includes studies on the further elaboration of the growth-factor molecule. As instances, the conversion of *p*-aminobenzoic acid to folic acid and its inhibition by sulphonamides, and the conversion of glutamic acid to glutamine and its inhibition by the sulphoxide of methionine might be cited as examples. In the second example it is also known that the methionine sulphoxide does not interfere with the assimilation of glutamic acid from the external environment but only with its conversion after entry into the cell. The third point at which

analogues may exert their effect is during the actual functioning of the growth-factor, that is, at the point of catalysis. Here again there is very little information available, although the hydrazide of glutamine has been shown to inhibit the breakdown of glutamine to glutamic acid and ammonia, and to be correlated with the inhibition of the growth of streptococci caused by the hydrazide.

To obtain by the rational approach analogues which possess the selective toxicity so necessary for successful chemotherapy, processes must be blocked which are quantitatively more important, or peculiar, to the parasite. It seems unlikely that the sulphonamides are the only analogues of an essential metabolite which fulfil this requirement; with increase in our knowledge of the metabolism of micro-organisms and of the parasite-host relationship, it is probable that more agents will become available. The sulphonamides owe their chemotherapeutic success to the inhibition of a reaction which seems to be absent from animals but essential to micro-organisms (and to members of a number of other species). In several bacteria the sole defect produced by the sulphonamides seems to be an inhibition of the incorporation of *p*-aminobenzoic acid into the folic acid molecule. Animals cannot carry out this synthesis and rely upon a supply of pre-formed folic acid being present in the diet; in other words, the biochemical lesion induced in sensitive organisms by the sulphonamides already exists in the host. Nevertheless, the host may be partially dependent on factors synthesized by its intestinal flora; the effect of sulphonamides on such syntheses may account in part for the toxicity sometimes seen in sulphonamide therapy. Moreover, it is known that much of the folic acid present in animal tissues and fluids is in the form of conjugates, a form not available to the parasite. The position, then, is that formation of folic acid by the host-dwelling parasite is blocked from below by the sulphonamides and from above by natural lack of the enzymes necessary for releasing folic acid from its conjugates; the host, on the other hand, relies on a supply of pre-formed folic acid and converts it largely to a form not available to the parasite.

A similar situation may explain the partial success of analogues of pantothenic acid, for this factor is known to exist in animals in the form of co-enzyme A, a more complex molecule. Although micro-organisms carry out a similar elaboration of pantothenic acid, they do not seem to be able—through inability to absorb it or through slight differences in structure—to make use of animal co-enzyme A to supply their own requirements.

The sulphonamides, and possibly also analogues of pantothenic acid, thus provide examples of compounds which interfere with reactions peculiar to the parasite and exploit qualitative differences in metabolism. Further inhibitors of this type, as already stated, will develop logically from the finding of further synthetic reactions of the parasite. A hopeful field, already opening out, is associated with peptide metabolism. Species specificity of structure is nowhere so marked as in the proteins, and it is in their synthesis that species-specific biochemical reactions are most likely to be found. For example, a certain amino-acid sequence in a simple peptide might be harmless or even favourable to one species, but toxic, through slight differences in sequence from normal peptides of that species, to another. It is interesting to note in this connexion that several of the naturally occurring antibiotics are peptides. One of them, lycomarasmin, is the tripeptide seryl-glycyl-aspartic acid. Its toxicity towards *Lactobacillus casei* and the tomato plant (it is the active agent of the tomato-wilt fungus *Fusarium lycopersici*) is annulled competitively by strepogenin, also a peptide and a growth-factor for a number of organisms. The observed biological relationship suggested a possible chemical relationship (using the rational approach 'in reverse'), and the synthesis of seryl-glycyl-glutamic acid yielded a compound with some strepogenin activity, both as growth-factor and as antagonist to lycomarasmin. On this and other grounds, it is not impossible that other natural antibiotics may turn out to be analogues of essential metabolites; there is already some evidence for this in the case of streptomycin.

For successful chemotherapy, though, it may not be necessary to restrict attention to reactions which are peculiar to the parasite. The temporary blocking of processes quantitatively more important to the parasite than to the host might prevent growth of the former for long enough to allow the natural defences of the host to overcome the invader without seriously damaging the host.

Another point to be considered is that, although the rational approach has yielded little in the way of chemotherapeutic agents, it has provided metabolic tools of the greatest value in investigating the actual function of the growth-factors. As they inhibit specifically the synthesis or utilization of the growth-factor, they provide a chance of tracing the primary effect of that factor. For example, γ-3,4-urylene-cyclohexylbutyric acid, an analogue of biotin, inhibits the growth of *Lactobacillus arabinosus*, and this inhibition is reversed competitively by biotin. The amount of biotin required to overcome the inhibition by a given concentration of the analogue is decreased ten-fold if oxaloacetic acid is present. This indicates that one function of biotin is in the synthesis of oxaloacetic acid.

Similarly, use of the sulphonamides has contributed greatly to knowledge of the function of *p*-aminobenzoic acid, which is involved in the synthesis of folic acid, purines, thymine, methionine, lysine, serine and possibly valine. Inhibition of the synthesis of folic acid by sulphonamides has been demonstrated in a direct way. Further use of the analogues in such researches may well reveal points suitable for chemotherapeutic interference.

When a suitable growth-factor or essential metabolite has been selected for modification, it has yet to be decided what changes are to be wrought in the original molecule to produce an efficient inhibitor. So far it has only been possible to predict in a very general way whether a given analogue is likely to prove inhibitory. Trial and error may be costly of time and fail completely to give an active compound, for it is not enough to go on simple pictorial analogy. It is not enough because an analogue may look like a metabolite and yet lack the chemical groupings necessary for combination with, for example, an enzyme; and because it leads to over-emphasis of the geometry and under-emphasis of the chemistry of the molecule. At the same time, pictorial analogy is too much, for it leads to possible overloading of the analogue with unnecessary groups which have nothing to do with the attachment of it or the metabolite to the enzyme. In order to model inhibitors more successfully on essential metabolites two things at least must be known: first, the structure of the metabolite to be imitated; and secondly,

the precise way in which it associates with the bacterial enzyme to which it is related as substrate, co-enzyme or product.

Many attempts have been made to explain the action of the antimalarial drugs on the basis of their being metabolite analogues, and there is much evidence, both chemical and biological, that quinine, 'Mepacrine' and 'Pamaquin' and possibly also the more recent anilinopyrimidines act as inhibitors of reactions involving riboflavin, at any rate in bacteria. As pointed out above, the fact that a substance chemically related to a growth-factor can compete with that growth-factor does not of necessity imply that an associated growth inhibition can be correlated with the metabolite analogy. Thus the final biological proof that these antimalarials are active solely because tinthey erfere with the action of flavin derivatives has yet to be provided. The newer and highly successful 'Paludrine' was developed by logical chemical steps from 'Mepacrine'; yet in no system has it been shown as a riboflavin competitor. There is no biological evidence for the mode of action of 'Paludrine', but it has been suggested that either by chelating a metal or by nature of the intrinsic size and shape of its cation (there is evidence that it exists as a pseudo-triazole involving a hydrogen-bond) it may possess a structure similar enough to that of the porphyrins to interfere with their metabolism. It is also possible that, as also in the case of the natural antibiotics, discovery of the mode of action of 'Paludrine' may disclose a new essential metabolite.

Had oxine (8-hydroxyquinoline) not already been well known as a chelating agent, the strong antibacterial activity of it and its derivatives might have been difficult to explain, particularly as the lethal action against Gram-positive organisms is far more powerful at low concentrations ($10^{-5}M$) than at higher ones ($3 \times 10^{-3}M$). The hypothesis advanced to explain their action against Gram-positive organisms, and backed by much experimental evidence, is this: at low concentrations the oxine chelates cobalt (cobalt is the only metal which will reverse the inhibition) and thus exposes the bacteria to the action of a second metal which catalyses the oxidation of a chemical group protected by the cobalt. Stronger solutions of oxine protect the organism by removing the injurious metal as well. The second metal has been identified as ferrous iron, and it seems that in the presence of lethal concentrations of oxine the organisms actually die of iron poisoning. Although oxine and its derivatives are not growth-factor analogues, their action is to prevent the normal functioning of essential ions, and a consideration of them is germane to any discussion on chemotherapy. Their mode of action, a disturbance of an existing balance with lethal results to the micro-organism, is worth considering in connexion with other antibacterial agents.

So the biochemist and the chemist, by producing changes in the structure of compounds already known to show a selective toxicity towards the parasite, may produce more and more efficient chemotherapeutic agents. But inevitably, as more is learned of the comparative biochemistry and physiology of micro-organism and potential host, and of those synthetic processes which are more peculiar to the micro-organism, the biologist will seek the closer co-operation of the chemist so that together they may plan, synthesize and test new chemotherapeutic agents.

PARTITION CHROMATOGRAPHY ON PAPER, ITS SCOPE AND APPLICATION

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WITHIN the last twenty years, chromatography has become increasingly important as an analytical tool. The purpose of this article is to review the uses of partition chromatography on paper, which, since its inception four years ago[1], has had a wide application and which promises to become as well established as the older forms of chromatography. It should be emphasized, however, that, as mixtures, especially from biological sources, may be very complex, preliminary separations[2–5] may often be necessary before paper chromatography can be used to the best possible advantage. For recent developments in all types of chromatography, the reader is referred to a number of articles indicated in footnotes 6–9.

Partition chromatography was first developed by Martin and Synge[10] at the laboratories of the Wool Industries Research Association. In this type of chromatogram, separations are achieved because of the differences in partition coefficients between aqueous and non-aqueous phases of the components of a mixture. Using silica gel as the supporting medium for the aqueous phase and eluting with certain water-saturated immiscible organic liquids, separation and estimation of a number of acetyl-amino-acids were effected. This provides a relatively simple and valuable method for the quantitative analysis of some half-a-dozen amino-acids in protein hydrolysates, after acetylation. With the free amino-acids themselves, it was found that owing to their strong adsorption by the silica gel, separations could not be achieved. However, cellulose, in the form of filter paper, was found to be suitable as the stationary support. In this method, a filter paper strip, carrying a mixture of amino-acids (a few micrograms of each) near its upper end, is hung from a trough containing water-saturated solvent (for example, *n*-butanol, phenol, *s*-collidine), the whole system being in an atmosphere saturated with respect to solvent and water vapours. The solvent syphons down the strip, and after a suitable time the amino-acids are revealed by drying the paper and spraying with a solution of ninhydrin and then gently heating. (A simplification of this method—'ascending' chromatography—has been recently reported[11].) It was found that the movement of amino-acids corresponded fairly closely with that calculated from their partition coefficients, thus demonstrating that the cellulose, as in the case of silica gel, acts as an inert support for the aqueous phase. This type of chromatogram is of value because of its simplicity and because many analyses may be carried out on one paper strip, using only very small amounts of material. An early application[12] was to show that *nor*-leucine was not present in spinal cord, as had been originally supposed.

No single solvent has been found which will separate all the common amino-acids on a paper strip; but a more complete separation can be obtained in the 'two-dimensional' technique in which the mixture applied near one corner of a filter paper sheet is chromatographed in one direction with one solvent and then in a direction at right angles with another