

## CONCLUDING COMMENTS

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In this symposium sixteen review lectures were given by the leading experts in their respective fields, all of the highest quality and presenting a vast mass of experimental material. In these short concluding remarks which I have been asked to make, I shall not attempt to summarize the papers—this would seem not only an unsatisfactory, but also a wholly unnecessary task—but I shall try to bring out the most important general trends of the research as they delineated themselves from the reports, and to evaluate them from the point of view of what we may expect from them so far as the development of new drugs is concerned. This approach will probably be criticized by some as too aimbound and utilitarian, but would seem to me legitimate in a symposium dedicated to pharmaceutical chemistry; I am, of course, well aware that there exist regions of research bordering on this field which are of the greatest importance for its development and well worthwhile exploring, though they may not lead directly to the discovery of new drugs.

Drugs act on cellular metabolic processes in animals and bacteria, and it was appropriate that the first two lectures of the symposium, by Professor Bücher and Dr Ing, were devoted to the general problems of the pattern and coordination of metabolic reactions and of the drug-receptor concept.

While great progress has been made in the elucidation of the biosynthetic pathways of most of the major metabolites, our knowledge of the relative rates of the different metabolic reactions in the cell and the mechanism of their regulation is still very limited indeed.

Metabolic schemes give us only a qualitative picture of the pathways of biosynthesis, but tell us nothing, or very little, about the quantitative aspects, on which, after all, the metabolic pattern of a specific tissue depends. The pathways of biosynthesis are more-or-less the same in all tissues, yet the relative rates of the metabolic reactions vary widely from tissue to tissue.

The situation is further complicated by the fact that in many instances enzymic activities are compartmentalized inside the cell, and interactions between metabolic reactions which can theoretically occur and in fact, have been shown to occur *in vitro*, may not at all occur *in vivo* because of physical separation of the enzyme system concerned by internal permeability barriers. Furthermore, enzyme concentrations do not always remain constant in the cell, but may be increased by substrates, for instance by induction, or may be diminished.

For these and other reasons any metabolic scheme, however complex and intricate, is still extremely primitive in relation to reality, and can at

best only give us an indication of some of the possible interactions between different metabolic reactions, but the chances also exist that it may give us a very distorted picture of the events which actually occur in the cell.

In the present state of knowledge it would, therefore, seem quite impossible to predict the effects on the metabolic pattern of a tissue, and even less the physiological and pharmacological consequences, of interfering with a specific enzyme reaction, except in some very simple cases. It is thus clear that at present we are still far from the state where we shall be able, on the basis of metabolic schemes, to design drugs endowed with specific pharmacological properties. This does not mean that we may never reach this stage, and it is obviously of the greatest importance to extend our knowledge of the reactions of intermediate metabolism, particularly from the quantitative aspect. In recent years, some progress has been made towards some aspects of the biochemical mode of action of a few regulating substances, such as adrenalin, thyroxin, and the oestrogens. The biochemical effects of these substances are concerned mainly with reactions of oxido-reduction and carbohydrate metabolism, and Professor Bücher, who, with his co-workers, has made valuable contributions to the field, has ably reviewed the present state of knowledge. It must be realized, however, that despite the biochemical progress made, for the reasons outlined above, we are still a long way from understanding the physiological and pharmacological effects of these substances on a biochemical basis.

Dr Ing, discussing the drug-receptor problem, reviewed in a thoughtful presentation some of the experimental and conceptual difficulties encountered in attempts to explain certain aspects of drug actions, such as excitation, inhibition and dose-response, as the result of the interaction of the drug with a specific receptor in the cell. The main inherent weakness of all general theories designed to establish a quantitative basis for the drug-receptor concept is the fact that the concentration of the supposed reactant in the cells cannot be measured directly, but must be deduced by the very indirect method of quantitative measurements of the pharmacological response. This is unsatisfactory in view of the complexity of the conditions in the cell and the interactions of the different metabolic reactions, as outlined above. From the fact that the experimental quantitative pharmacological findings agree with a theory it does not necessarily follow that this theory is correct; in systems of a complexity such as characterize a living cell many theoretical possibilities can be visualized which will fit the experimental findings. In view of the interwoven pattern of the enzymic reactions in the cell it is more than likely that the pharmacological effect of a drug, except in a few very special cases, is not only the result of its interaction with one single cell constituent, but is caused by triggering off a whole chain of reactions all coupled with each other by common reactants.

At present, there does not seem to exist a substitute to the old established way of rational empiricism for the development of new drugs, *i.e.* to look for substances, natural or synthetic, with pharmacological activity and to try to synthesize analogues and establish in each series empirically rules between chemical structure and biological activity.

A class of natural compounds, which has revealed itself as a rich source of new biologically active compounds, is that of the peptides, both of higher

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and low molecular weight, and it justly occupied a central position in the symposium.

The field of polypeptides which only a short time ago were considered substances with structures of such complexity as to be practically inaccessible to experimental approach, has seen an astonishing development in the last two decades. This has been a consequence of the possibility of establishing the complete amino-acid sequence even of very complex polypeptides by new and sensitive analytical techniques, such as chromatography and electrophoresis in its various forms, counter-current distribution, and labelling techniques for end-group determinations. A recent interesting addition to the chromatographic techniques which has shown itself particularly useful for the separation of polypeptides is the method of molecular filtration on gels, developed by Porath and his colleagues at Uppsala. Dr Porath described the theoretical basis of this method and its application to the separation of the nonapeptide hormones, vasopressin and oxytocin, and to the purification of the much more complex thyrotropic hormone, which possesses a molecular weight of about 30,000.

The peptide field has received a big stimulus from the intensive research on antibiotics which has brought to light many novel types of polypeptides exhibiting antibacterial properties. In fact, the first polypeptide of which the complete structure was established, was the cyclic antibacterial decapeptide gramicidin S. Though of limited practical importance in clinical medicine, the polypeptide antibiotics have played a most important historical rôle in the development of the techniques for the determination of the complete sequence of amino-acids in complex polypeptides and proteins. Furthermore, the study of their structure has brought to light many new amino-acids as their constituents. Dr Sheehan, in a report of his own work in this field on the structure of the new polypeptide antibiotic telomycin, has demonstrated the presence in this molecule of the new amino-acids 3-hydroxyproline and erythro- $\beta$ -hydroxyvaline. He also referred briefly to his previous work on the antibiotic etamycin which was shown to contain the new amino-acids L- $\alpha$ -phenylsarcosine and L- $\beta$ -N-dimethylleucine. Professor Prelog gave a review of the work of his group on iron-containing polypeptides and polypeptide-like substances from various fungal sources. This widely distributed interesting class of compounds, termed siderochromes, consists of polypeptides with antibacterial activity, termed sideromycins, and of growth-promoting polypeptides, the sideramines. Elucidation of the structure of some members of the second group, the ferrioxamines B, D, G, and E, showed that they were all differently N-substituted trihydroxamates, with 1-amino-5-hydroxylaminopentane as the characteristic structural unit, which has not been encountered before in natural products.

One member of the ferromycin group was shown to yield a ferrioxamine on hydrolysis, showing a close structural relationship. The structure of the ferrioxamines was confirmed by synthesis.

A very interesting group of cyclic polypeptides with very powerful toxic actions are the *Amanita phalloides* poisons which have been studied extensively by Professor Wieland and his group. Professor Wieland gave an account of the structure of the two major groups of these poisons, the phalloidins and amanitins, both of which are bicyclic heptapeptides of unusual steric

configuration containing some uncommon hydroxyamino-acids. From the results of the synthetic work in this series it would seem that not only the presence of the intact ring system is essential for the biological activity, but also the steric configuration of the ring system as it occurs in the natural isomer. Professor Wieland's studies represent a new and important contribution towards the understanding of structure-activity relations in biologically active peptides.

The penicillins may be considered to belong to the group of low molecular peptides. Though of relatively simple structure, they have resisted for many years the concerted efforts of large groups of chemists of achieving their synthesis in reasonable yields.

It was easy enough to synthesize the penicilloic acids, but in all attempts with the then known ring-closing agents to bring about ring closure of the  $\beta$ -lactam ring the penicilloic acids behaved as acylated amino-acids would be expected to behave, *i.e.* they gave the corresponding azlactones with the simultaneous opening of the thiazolidine ring. It was clear at the time that the chemist who would develop a novel type of ring-closing agent bringing about the  $\beta$ -lactam ring formation in the penicilloic acids under conditions where the penicillin molecule could survive, *i.e.* in neutral aqueous solution, would at the same time make a notable contribution to synthetic peptide chemistry in general. This turned out to be the case. The introduction by Sheehan of the substituted carbodi-imides as condensing agents not only made possible the synthesis of the penicillin molecule, and of the nucleus of the penicillin molecule, 6-amino penicillanic acid, (from which many new penicillins can be derived by acylation of the amino group and which is now readily accessible by fermentation and enzymatic hydrolysis of benzylpenicillin), but represented the discovery of a new powerful tool for the synthesis of much more complex polypeptides which could not be achieved by other means; the carbodi-imide condensation process has become one of the basic and most widely used methods in the field of peptide synthesis.

Two very interesting communications dealt with the synthesis of polypeptides with hormonal activity. Spectacular progress has been made in this field in recent years, and it is astonishing to think that the synthesis of the octapeptides pituitary hormones vasopressin and oxytocin, which formed the subject of du Vigneaud's Nobel lecture in 1956, is now considered a routine procedure for which several methods are available.

Drs Hoffman and Schwyzer reported on their work of the synthesis of adrenocorticotrophic hormones (ACTH) and melanocyte-stimulating hormones (MSH).

ACTH is a polypeptide of 39 amino-acids, the complete sequence of which has been established, and MSH is one part of this sequence, consisting of the first 13 amino-acids. The synthesis of the latter was reported by both speakers, as well as the synthesis of a polypeptide containing 10 further amino-acids of the ACTH amino-acid chain, *i.e.* a total of 23, and displaying the full biological activity of ACTH. Professor Schwyzer also gave a review of synthesis of the tissue hormones bradykinine (a nonapeptide), angiotensin I and II (deca and octapeptides) and a large series of analogues of these hormones, accomplished in his own, as well as in other, laboratories.

These syntheses have been achieved by suitable combination of classical

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methods of amino group protection, classical condensation methods and the application of the new carbodi-imide method.

The synthesis of polypeptide hormones has made accessible synthetic substances with the same or very similar pharmacological activities as those of the natural products, though in the case of the higher polypeptides the cost of the synthetic product may not yet be able to compete with the natural; future improvements of synthetic techniques may, however, lead to a considerable reduction in cost. The many possibilities of variations and permutations of the single amino-acid constituents or groups of amino-acids in the peptide chain raise the hope that substances with new pharmacological properties, possibly antagonistic to the natural hormones, or, at least, with improved pharmacological properties, may result from the intensive synthetic work which at present is carried out in many laboratories; however, up to the present time, the synthetic peptides have displayed only quantitative differences in respect of the natural ones. In any case, there is no doubt that the field of peptide synthesis is in a very active phase of development, and hope is justified that we may witness some very interesting, and perhaps even spectacular, results, particularly with regard to the synthesis of peptides of higher molecular weight and, perhaps, of some enzymes.

The work on polypeptide synthesis is of a very time-consuming nature and requires a considerable organizational effort. For this reason it is particularly well suited for the industrial research laboratories from which already many important contributions have come in this field, as they have come in the past in other fields of similar complexity, for example, the steroids and antibiotics, which require resources of man-power and an organization not normally available in academic laboratories.

Since the beginning of pharmaceutical chemistry, the alkaloids have been a rich source of new drugs, which does not yet seem to have been exhausted, though it may perhaps be too optimistic to hope for the repetition of a discovery with such spectacular consequences as was that of reserpine. For this reason, structural studies on new alkaloids continue to be of great interest for pharmaceutical chemistry.

Two lectures by Professors Janot and Battersby dealt with the chemical structure of alkaloids. Professor Janot gave a comprehensive review of the extensive structural and synthetic work of his group on Apocynaceae steroid alkaloids and indole alkaloids, which has resulted in the elucidation of a large number of members of this group. He demonstrated impressively the enormous saving of time and material which can be achieved in the determination of alkaloid structures by judicious application, in conjunction with the classical methods of degradation, of the modern physical methods, such as infra-red spectroscopy, nuclear magnetic resonance, and mass spectroscopy.

It would seem that for the elucidation of structures of natural products the rôle of the organic chemist is becoming increasingly less important, and that, ultimately, organic structural work of the classical degradation type may be substituted almost completely by machines. In the future, the chemist interested in natural products will concentrate his main effort not, as in the past, on degradation (except, of course, in the particular case of degradation of substances labelled in specific positions for the elucidation of biosynthetic

pathways—often a great help for the understanding of the structures of natural products) but on the synthesis of the complex structures shown to exist in nature, and their analogues. There is plenty of scope for development in this area and for display of skill and ingenuity. Progress will be conditioned by the discovery of new methods, particularly of the kind capable of specifically directing reactions towards obtaining one of the several theoretically possible stereoisomers, and introducing groups in specific positions in complex polycyclic systems; in these respects the introduction of biochemical techniques, such as the use of micro-organisms and isolated specific enzymes, has proved of great value.

Professor Battersby reported on his work on the structure of alkaloids from *Strychnos toxifera* and *Pleiocarpa* which have revealed many interesting novel structural features, among them the existence of novel polycyclic ring systems.

Studies of the relations between structure and pharmacological activity have been one of the classical methods of pharmaceutical chemistry towards the development of new drugs, which still gives valuable results. We heard four examples of this approach in the lectures of Professors Pratesi, Shemyakin, Musajo and Jucker. Professor Pratesi, in a study of structure-activity relations in the catecholamine series, showed that the laevorotatory forms of adrenaline, noradrenaline and isopropyl-noradrenaline were sterically related to D-(—)-mandelic acid, and, furthermore, was able to demonstrate that the basic function was of primary importance in determining the type and intensity of biological activity ( $\alpha$  or  $\beta$ ) of compounds of this group, whereas the catechol function served the more general purpose of anchoring the catechol amines to the receptors. Professor Shemyakin gave a report on structure-activity relations in the field of antibiotics, based mainly on the large volume of synthetic work carried out in his laboratory in the chloramphenicol, cycloserine, sarcomycin and depsipeptide groups of antibiotics. With regard to the latter, he affirmed that synthetic studies of his group did not support some of the structural formulae proposed by other authors, for instance those of the enniatines and the valinomycins.

It was of great interest to obtain a first-hand comprehensive account from as well informed and competent a source as Professor Shemyakin, of the state in the Soviet Union of this kind of work which, of course, has also been the subject of intensive study in many laboratories elsewhere.

An interesting class of natural substances which have found applications in therapy are the furocumarins. Some of these, especially psoralene derivatives such as xanthotoxin, bergaptene, angelicine and others, have photodynamic activity producing erythema in skin after exposure to the light and have been used extensively in the treatment of the skin disease vitiligo which is characterized by the appearance of white patches devoid of the normal skin pigment. Professor Musajo, who has dedicated many years to the study of this class of compounds, gave a report of his work. He and his group have synthesized a large number of furocumarins which have enabled them to establish structure-activity relationship in this group. Studying the biochemical mechanism of the photodynamic action, Professor Musajo and his group discovered interactions between furocumarins and flavin mononucleotides, leading to the formation of photolytic products of FMN such

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as lumiflavin, lumichrome, and others, as yet unidentified, which contain bound cumarine moieties from which on acid hydrolysis FMN can be regenerated. A number of the oxidation products of the furocumarines, which are formed in this reaction, have been isolated and identified. Professor Musajo postulates that the photodynamic effect on the furocumarines may be related to their interaction with the flavin nucleotides, known to be involved as coenzymes in many important oxidative enzymic reactions.

Among the synthetic drugs hydrazine derivatives have occupied a prominent position since the discovery of the antipyretic properties of the pyrazolone derivatives at the end of last century, and continue to do so. Among the well-established hydrazine derivatives which have found wide clinical applications are the potent antitubercular agent isoniazide and the antirheumatic phenylbutazone.

Hydrazines owe their importance in pharmaceutical chemistry to their property to undergo readily cyclization to a variety of heterocyclic systems, with 4, 5, and 6-membered rings endowed with interesting pharmacological properties, thus making available a very large number of differently substituted heterocyclic derivatives; but straight hydrazines and hydrazones were found to possess important therapeutic properties.

Dr Jucker gave a general review of this field and described in particular the work of his own group on the development of new hydrazides azetinediones, amino-pyrazoles, pyrazolones, pyrazolidone-diones, pyridozones and phtalazones, which were shown to be endowed with a wide range of pharmacological activities, including diuretic, anti-inflammatory, serotonin inhibiting, amino-oxidase inhibiting, analgesic, spasmolytic, anti-histaminic and tranquillizing properties. It is evident that this field lends itself particularly well to structure-activity studies.

Two fields of chemotherapy in which a particularly large effort is made in numerous laboratories all over the world were reviewed in Symposium lectures, that of cancer chemotherapy by Professor Bergel, and that of psychopharmacotherapy, by Professor Bovet.

Professor Bergel gave a broad and competent survey of the different lines of cancer chemotherapy which are at present actively explored and include the use of alkylating agents of various types, hormones, enzyme inhibitors, anti-metabolites interfering with various metabolic pathways such as nucleotide and nucleic acid synthesis, destruction of essential metabolites and cytostatic antibiotics. As is generally realized, despite the intensity of the research effort and the great ingenuity which has gone into the synthesis of some of these substances the success hitherto obtained gave results of only limited extent; at best, it has been possible to prolong the life of the patient for a few years, except in the special case of tumours of the prostate where the administration of stilboestrol has occasionally kept patients alive for longer periods. This may in itself, of course, be considered a major achievement and the forerunner of better products to come. However, despite the considerable progress which has been made in recent years towards the understanding of the causes of cancer, it must be admitted that our knowledge is still very rudimentary and, in the reviewer's opinion, may in many ways be compared to the state of knowledge of infectious diseases before Pasteur's discovery of micro-organisms as the causative agents, or

that of the structure of the atom before the advent of Madame Curie, Rutherford, Einstein and Niels Bohr. Much more will have to be learned about the fundamental biology of cell organization, cell growth and cell differentiation before real progress can be made in the cancer field, and it would seem most important to encourage this kind of biological research even if it has no direct bearing on the cancer problem. Biological research has been much neglected at the expense of the chemical and biochemical approach, but the time may not yet be mature for this kind of approach, and our understanding of the cancer problem may be promoted to a much greater extent by pertinent fundamental biological studies than by a vast programme of organized chemical research.

Professor Bovet surveyed from a pharmacologist's point of view the field of psychotropic drugs which has undergone an almost explosive expansion since the discovery of the "tranquillizing" actions of reserpine and some of the antihistamines.

A whole range of synthetic drugs with stimulating or depressing action on mental activity is now available, and some of these have been used with success for the clinical treatment of some psychopathological conditions. In animal behaviour tests some substances have even been shown capable of improving memory and the capacity of learning, and Professor Bovet gave an interesting example from the work of his own group on the "cretinizing" effect of the antithyroid substance methylthiouracil and the memory improving action of thyroxin.

Many of the psychotropic drugs belong to the group of autonomic stimulants or blocking agents; for instance, some of the most effective energizing agents are catecholamines or monoaminoxidase inhibitors, some tranquillizing agents are histamine, catechol or acetylcholine antagonists. Others are related to the big groups of hypnotics and convulsants. However, Professor Bovet pointed out that the relations between chemical structure, pharmacological action and psychotropic effect of these drugs were complex and in many cases still obscure; because of this no rigid classification of the psychotropic drugs according to their pharmacological and biochemical action was as yet possible.

In several cases, documented by Professor Bovet with examples of work of his own group, the analysis of the action of psychotropic drugs by different techniques has revealed a multiplicity of effects. For this reason Professor Bovet advocates the use of animal performance tests with simultaneous registration of different behaviour parameters. During the past few years, in fact, many new and ingenious techniques for the study and the automatic recording of animal behaviour have become available. However, even with the most elaborate animal behaviour tests the difficulties of translating animal behaviour experiments to man remain great; for example, mental fatigue in man cannot be adequately represented by physical fatigue in animals, and the same applies to intelligence, memory and emotional behaviour. Therefore, progress in the field of psychotropic drugs will depend on the close collaboration of the psychiatrist, the experimental psychologist, the pharmacologist, the organic chemist and the biochemist. The biochemical approach, *i.e.* a better understanding of the fundamental biochemical processes in brain and nervous tissue in normal



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and pathological conditions, as well as the qualitative and quantitative studies of the hormonal pattern in psychological stress and mental diseases promises to be of great value in providing new leads to the development of new types of psychotropic drugs which, at present, despite the vast number of products on the market, are limited to only a few groups of compounds.

While in some limited areas of psychopharmacotherapy considerable, and even impressive, success has been achieved without doubt, yet the field is still in its infancy. Although it is certain that with the application of the new techniques many valuable discoveries will come to light from further research in this interesting new border between several disciplines of science, it would seem wise not to pin one's expectations too high for the immediate and, even the more distant, future. We are still very far from the stage when we shall be able to improve human intelligence or influence human character and more likely than not—and perhaps this is just as well—we shall never reach it.

According to the introductory remarks of Professor Pratesi the scientific programme of the Symposium was designed to give a cross-section through some of the most topical trends of modern pharmaceutical research, with special emphasis on the polypeptide hormones, chemotherapeutic agents and substances acting on the nervous system.

There can be no doubt that the aim of the Symposium has been amply achieved, and I think that I speak on behalf of all present, if I express to Professor Pratesi, Professor Soldi and their colleagues our sincere gratitude for their great organizational effort which has made the Symposium an unqualified success from the scientific as well as the social point of view.