BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 2 1943

A PROSPECT IN THERAPEUTICS*

BY

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One of the clear impressions left by my student days in hospital, at the beginning of the present century, is that of surprise and disappointment at the lack of conviction, and even of interest, which our teachers showed in medicinal treatment, in contrast to the care and enthusiasm which they devoted to diagnosis. I suppose that before I entered the wards I had assumed, with the world at large, that a correct diagnosis would regularly enable an appropriate and effective remedy to be applied. It was disappointing to discover that this was so rarely the result, and that treatment would so often be prescribed with no better hope than to make the patient easier by alleviation of his symptoms, leaving Nature to deal, if possible, with the cause of the trouble. It is clearer to me in retrospect than it was at the time that there were very few remedies then which were even expected to deal with the causes of disease; for knowledge of those causes was itself a new thing, though it was beginning to come in a flood from the fresh outburst of experimental medicine in the last quarter of the nineteenth century. I think, indeed, that the period which I thus recall probably represented about the low-water mark of interest and confidence in the remedial value of drugs. Some of the established remedies for symptoms were clearly invaluable for their own purposes—digitalis, for example, morphine, cocaine, the bromides, and the general anaesthetics. The same was true of the use of salicylates in acute rheumatism, though it was not clear whether this dealt with the symptoms only or with the cause of the disease; nor can it be so even now until the cause of acute rheumatism is established beyond doubt. There were, indeed, a few drugs even then in use of which we can say, in the light of present knowledge, that their remedial value was due to action on the infective causes of the diseases in which they were used—cinchona and quinine for malaria, ipecacuanha (though not yet emetine) for dysentery, mercury and the iodides for syphilis. But at that time the knowledge of the malaria parasites was almost a novelty, the dysenteries were imperfectly differentiated, and the discovery of the spirochaete of syphilis was still in the future. The use of these remedies, like that of so many others, was then still based on the tradition of past centuries.

Emergence of Remedies of New Types

Out of the experimental researches which were bringing to light the causes of disease, and thus weakening the credit of drugs which could do no more than modify or alleviate symptoms, there were emerging, on the other hand, remedies of new types—not drugs unnatural to the body, but natural principles which would directly repair its deficiencies, or would supplement and reinforce its protective responses to infections.

One brilliant example was already available of each of these two types of natural medication. These were the treatment of myxoedema and cretinism with thyroid gland substance, and the treatment of diphtheria with the specific antitoxin. Here, beyond doubt, were remedies dealing directly with causes; and it was very clear that the physician ordered these with a confidence in their efficacy that contrasted strongly with the vague hope of indirectly helping the curative action of Nature, on which so much of the older therapeutics seemed to depend. It was in this direction only, in the provision of such natural remedies for deficiencies and infections, that a prospect seemed then to be opening for a new therapeutics dealing with causes; and researches in a range of the sciences allied to medicine have indeed gone far beyond any prospect then visible, in providing the wealth of hormones, vitamins, and immunological remedies which are now available. These alone would have effected a therapeutic revolution; we have only to recall the treatment of diabetes without insulin, of pernicious anaemia without liver extract, of rickets without vitamin D, or, for those whose memories extend so far, of diphtheria without antitoxin, to recognize its magnitude. At the beginning of the century it might well have seemed, on the other hand, that the prospect of advance open to the older therapeutics, which used drugs and chemicals unnatural to the body, and to a pharmacology then concentrated on attempts to analyse the effects of these, would be limited to the discovery of new and perhaps better remedies for the alleviation of symptoms. This, indeed, has been achieved, and with an almost embarrassing profusion. But the prospect was not to be thus limited. Even before this century began, the idea of a new type of therapeutic enterprise, aiming by deliberate experiment at the discovery of new artificial remedies which would deal directly with the causes of infection, had already begun to germinate. The results of this undertaking, as we can see them to-day, figure at least as prominently as the discovery of the natural remedies in the tremendous change, since the beginning of the century, in the very objective as well as in the achievements of medicinal treatment. I invite you to consider with me, in brief review, the steps leading to the present position, and the prospect now visible of a more regular and ordered advance in this quest for new and specific remedies for infections, called "Chemotherapy" by Paul Ehrlich, the man of genius who first undertook it and led the way to its first achievements.

As long ago as 1891, just when antitoxins were being newly discovered, Ehrlich had already seen that some of the parasitic causes of infection then coming to knowledge in such an imposing sequence did not evoke any rapid or effective reaction of immunity. He himself was also then engaged in experiments on antitoxins and antigens, and for another decade he was to lead the world in researches which gave to these their practical applications; but he had seen that along those lines there was nothing in view for the treatment of malaria, for example. The use of quinine had provided one of the few historic examples of a remedy which was dealing with the parasitic cause of an infection long before this was known. But, as Ehrlich was apt to complain, although the parasites causing malaria were now well recognized, pharmacology could still offer only data about the action of quinine on frog's

* The Frederick Price Lecture delivered to the Royal College of Physicians, Edinburgh, on July 2, 1943.

muscle or on the mammalian nervous system, and nothing really relevant to its practical value in banishing the malaria parasites from the patient's blood. He embarked on a direct attempt to find a remedy for malarial infections better than quinine. He had been one of the first to use the aniline dyes in medical research, and had made extensive studies of the selective staining action of methylene blue. Since this dye stained the malarial parasites, and was not very poisonous for the higher animals, he was led to try it as a substitute for quinine. He obtained no more than a hint of success; methylene blue was certainly not better than quinine; but in that early observation was the germ of the method of research which he was later to call Chemotherapy. It is of interest, further, to note that when at length, many years after Ehrlich's death, the experimental use of malaria-like infections of birds led to the discovery of artificial remedies for human malaria itself, the structural analogy of methylene blue rather than that of quinine provided the point of departure for trials which led to success.

Dyes and Analogous Substances

When his studies on toxins and antitoxins were drawing to a close Ehrlich returned to this search for artificial remedies which would thus act directly on the causes of infection. The researches of Laveran and Mesnil had by this time brought to notice much more suitable conditions for such experimental work, in the use of trypanosomes which, unlike the parasites of human malaria, could be transmitted and retransmitted in indefinite series to small animals, such as rats and mice. Here were large actively motile protozoa, readily visible in a drop of blood under a moderate power of the microscope. It was easy to watch the effect on them of substances to be tested for curative action, to see whether the trypanosomes were stained by these if they were dyes, whether, in any case, their motility was abolished or impaired, and whether a treatment caused them to disappear from the peripheral blood of an infected animal. Early in these studies Ehrlich and the Japanese bacteriologist Shiga, who was then working with him, found a benzidine dye, which they called trypan red, that would

FIG. 1.—Trypan red.

stain the trypanosomes with which they were working, and would banish these from the blood of mice heavily infected with them and bring to an end an infection which, if untreated, was progressive and uniformly fatal. This, the first experimental success of the new method of approach to therapeutics, has a personal interest for me in that it was just coming to completion when I myself was privileged to enter Ehrlich's Institute in Frankfurt for a short period, in 1903. I do not think that anybody, except perhaps Ehrlich himself, foresaw at that time what the future might hold of development from this small beginning.

Trypan red had not itself a real success in practical therapeutics; neither in man nor in large domestic animals would it deal with natural trypanosome infections as it had dealt with the very acute artificial infections of mice and rats. It did provide, however, the starting-point for a series which reached the peak of its achievement, so far, in the production, many years after Ehrlich's death but under the inspiration of his former pupil Hermann Roehl, of the complex substance known first as "Bayer 205," and later as "germanin," "antrypol," and "suramin," the last being the unprotected name adopted for general use by the *British Pharmacopoeia*.

FIG. 2.—Bayer 205 (germanin, suramin, etc.).

This is a colourless substance, but in structure and substantive properties resembles the dyes, and is a remedy of real value. After trypan red, French workers had produced other dyes with remedial actions on infections with trypanosomes—trypan blue and afridol violet; and the latter, though not a practical success

in itself, contained already the central urea-linkage which characterized the structure of the later Bayer 205 (suramin).

The line of research which started with the benzidine dye, trypan red, had thus led to one clear practical success; but it had made a contribution of much wider importance to the future of this newer kind of therapeutics in the knowledge which it had afforded incidentally concerning the mode of action of some of these artificial remedies for infection. Ehrlich had, as was usual with him, picturesque conceptions of the mode of action of these curative substances. He thought of them as magic bullets, flying unerringly to the objective and avoiding anything else in their path. Their specific affinity must at least be strong, he maintained, for the infecting parasite, and weak or wanting for the cells of the infected subject—they must be maximally parasitotropic and minimally organotropic; and he attributed this selective affinity to the possession by the protoplasm of the parasite, and the absence from or paucity in the body cells, of "chemoreceptor" groups with which the curative substance would specifically combine. The action of an effective chemotherapeutic remedy, when thus fixed, he had thought of as a direct and rapid killing of the parasites, in a dose still harmless to the cells of the host. It has become, in fact, very doubtful whether the action of any successful chemotherapeutic agent conforms to this simple type. When Ehrlich and his co-workers watched the action, on trypanosomes swimming in diluted blood, of a dye which rapidly cured mice of an infection by the same species of trypanosomes, the effect observed was not of this directly lethal type. The organisms were seen to be stained by the dye, but their motility was unaffected, and the period of their survival under the artificial conditions was not perceptibly reduced. Yet when these stained and apparently still vigorous trypanosomes were injected into a normally susceptible mouse no infection followed. Ehrlich interpreted this observation as showing that the dye, while leaving the contractile activity of the trypanosomes unimpaired, had stopped their power of multiplication, so that when they were injected into the mouse they died without reproducing themselves, and no infection was established. At the time when it was first put forward this might well have been regarded as an explanation of the opportunist type, improvised to meet an observed anomaly. With trypanosomes it could not be put to the test of more critical experiment, since there is no method even yet available for inducing these parasites to multiply in artificial culture. Nevertheless subsequent evidence obtained with organisms of other types has abundantly confirmed this speculative idea. There can now be no doubt, I think, that a large proportion of chemotherapeutic cures are, in fact, produced in this way—that the infection is brought to an end by stopping the further multiplication of the parasites, rather than by killing them outright; and it will be convenient to mention at this point one of the observations in which this mode of action was directly demonstrated.

During the last war it was found by Dobell and myself that the visible action of emetine on the vitality of *Entamoeba histolytica*, during the limited period of its survival in a saline suspension after removal from the infected bowel, was remarkably weak. To immobilize and visibly to kill the amoebae under these conditions a concentration of emetine was required many times greater than any which the human body would tolerate. By contrast, a derivative of emetine—demethoxyemetine—which was much more toxic for the amoebae in such tests and much less toxic for man, was found by Dr. Carmichael Lowe to be devoid of curative action in cases of amoebic dysentery which yielded normally to emetine. It was clear that observation on surviving amoebae, made under such conditions, gave no measure at all of the therapeutic action of the substances tested. One of the possibilities which we considered was that the curative action of emetine might be due, not to such outright killing of the amoebae, but to suppression only of their growth and reproduction. It must be admitted that this had not seemed to us then to be very likely; but when Dobell and Laidlaw later devised a really good method for maintaining *Entamoeba histolytica* in artificial cultures it became possible for them to test a number of substances directly for their power to stop the multiplication of the amoebae. It was then revealed that emetine and its lower homologue, cephaeline, had a pre-eminent activity of

this kind, corresponding to their unique value, among alkaloids of that series, in curing amoebic dysentery; while, by contrast, the derivatives, like demethoxyemetine, which had appeared to have a more potent immediate action on the vitality of individual amoebae but had failed to cure the infection in man, failed altogether to stop the growth of cultures when applied in the high dilutions at which emetine was still effective.

Arsenical Compounds

Another important line of chemotherapeutic research had started in 1905 with the observation by Thomas and Breinl, in Liverpool, that an organic derivative of arsenic acid, which had been marketed under the trade name "atoxyl," had a significant curative action on trypanosome infections in small rodents. Ehrlich then discovered, with Berthelm, in 1907, the true structure of atoxyl, as *p*-aminophenyl-arsonic acid:

FIG. 3.

With Roehl he added, in 1909, the very important observation that atoxyl itself had little visible effect on trypanosomes outside the body, but that the arsenoxide produced from it by reduction, in which the pentavalent arsenic was reduced to the trivalent arsenious form, was rapidly lethal for them in high dilutions. Following these clues, and widening the biological scope of the quest to include pathogenic spirochaetes as well as trypanosomes, he ultimately arrived with his Japanese co-worker, Hata, at the earliest major triumph of what was then (1910) still the new research method of chemotherapy, in the discovery of salvarsan (arsphenamine), with its revolutionary effect on the treatment of syphilis and other spirochaetal infections.

My concern, however, is not with the now familiar record of this practical therapeutic achievement but rather with what could be learned from the actions of atoxyl, salvarsan, and related substances, such as the later tryparsamide, as to the nature of the chemotherapeutic process. Salvarsan, an arsenobenzene derivative, has its arsenic in the trivalent form, but in a stage of reduction beyond that of the arsenoxide. Ehrlich's co-workers tested its directly lethal action on spirochaetes, using the soluble derivative neosalvarsan, which can be applied in a solution of neutral reaction, and found that here again the motility and visible signs of vitality of the organisms were unimpaired, though again they failed to produce infection if they were injected into a susceptible animal after they had been so treated. We might suppose, as Ehrlich had suggested in the case of trypanosomes treated with dyes, that the action of salvarsan had been to deprive the spirochaetes of the power of reproducing themselves, without directly and immediately destroying all their vital properties.

We have seen, however, that reduction of the pentavalent arsenical group of atoxyl to the trivalent condition, producing the corresponding arsenoxide, made it immediately and intensely lethal for trypanosomes; and the same is true of the most important of these pentavalent arsenicals, the later-introduced tryparsamide. Now if the arseno-compound salvarsan is partially oxidized, so as to produce two molecules of the corresponding arsenoxide, the same appearance of an immediate

FIG. 4.

intense toxicity for trypanosomes or spirochaetes is observed. For salvarsan (arsphenamine), therefore, as for the pentavalent arsenicals, there was a likelihood that the curative action observed after injection into a man or an animal suffering from a trypanosomal or spirochaetal infection was due, not to the substance as injected, but to a much more active arsenoxide produced from it by a relatively slow process of partial oxidation or reduction, as the case might be. And this possibility was directly supported by experiments carried out by Voegtlin and his co-workers, demonstrating that the

relatively slow effects of either the arsenic acids or the arseno-derivatives, in causing the disappearance of the parasites from the blood, began after a definite interval, which was not present before the onset of the much more rapid action which the corresponding arsenoxides produced in much smaller doses. So that we have available two quite different explanations of the apparent lack of activity, outside the body, of arsenical derivatives which are so effective against the same parasites *in vivo*: on the one hand, the therapeutic process may, as in other cases, be due to the suppression of the reproduction of the parasites, rather than to the immediate destruction of all their vital activities; and, on the other hand, these arsenicals probably act after conversion, by contact with the body tissues, into the directly active form, by partial reduction or partial oxidation, as the case may be.

These considerations may naturally raise the question why, if the action is in any case due to the arsenoxides, these should not be directly administered, rather than the substances from which the body will produce them slowly and after an initial delay. Ehrlich, be it noted, had the arsenoxide corresponding to salvarsan in his hands before he had salvarsan itself, which he produced from it by reduction. Why did he introduce this additional step in production of the remedy, and impose on the body, apparently, the task of reversing it? The answer, I think, is to be found in the fact that the arsenoxide is not only much more potent in its action on the parasites but also, and to an equal extent, more poisonous for the tissues of the mammalian or other infected host. It can therefore only be given in a small dose at one injection; and, though the effect of this against the parasites is immediate and powerful, the concentration of the arsenoxide in the body fluids falls so rapidly from this low, tolerable concentration to a level below that at which it is effective, that the action is as evanescent as it is powerful. Even if only a few parasites have survived this brief action, these can rapidly renew their multiplication and re-establish the infection when the impediment has been removed. And there comes thus to our notice another factor in an effective chemotherapeutic action, which has not always, I suspect, received sufficient attention—namely, the need for a sufficiently prolonged and continuous action. This need is almost implied in the conception of the process as essentially an arrest of the multiplication of the parasites rather than an immediately lethal action on them. What is required is not the sudden attainment of a concentration sufficient to kill most of the parasites, at the risk of a concomitant injury to the host's tissues, but the long-continued maintenance of a much lower and safer concentration, just sufficient to suppress the propagation of the parasites, without harming the cells of the host.

It would seem reasonable, then, to attribute the special efficacy of salvarsan not to a special affinity for the parasites associated with the arsenobenzene structure but rather to the physical properties which enable it to form a depot in the tissues, or, perhaps, a film on the surface of the parasites, from which a steady low concentration of the much more toxic but directly active arsenoxide will be produced. Discussing this idea in a review, now some twenty years ago, I further raised the question whether the same result might be obtained by direct injection of the arsenoxide, if means could be devised for administering this by a slow, continuous infusion. Apparently this has now been achieved, and the hydrochloride of this precursor of salvarsan, which Ehrlich so long ago rejected as too immediately toxic, has, in fact, recently come into use in the U.S.A., and even more recently in this country, for intravenous administration by constant drip infusion, or by frequently repeated small injections, under the trade name "mapharsen," or "mapharside."

How the Action of Diamidines was Discovered

Hitherto the process of discovery of new therapeutic agents had been to a large extent empirical, involving the synthetic preparation and testing of a large variety of derivatives of some substance which had itself shown a promising degree of activity against some particular kind of infection. Such new starting-points have themselves, in notable instances, been reached with the help of theories which have had to be discarded. A striking example is provided by researches started

by Prof. v. Jancsó of Szeged, who set out to find substances which would deal with trypanosomes, not by killing them, or even affecting them directly, but by starving them out. So he treated the infected mice with decamethylene-diguanidine, which, under the name "synthalin," had been tried some years ago as a synthetic substitute for insulin. It had not been really successful for that purpose, but v. Jancsó found that it caused the trypanosomes to disappear rapidly from the blood of his mice, and naturally regarded this as confirming his conception of a chemotherapy based on depriving the trypanosomes of glucose by causing a disappearance of that substance from the

blood. Fortunately the late Prof. Warrington Yorke had the curiosity to test the matter for himself, and found that synthalin, applied even in extremely high dilutions, containing as little as 1 part in some 250 millions, would cause the death of trypanosomes in an artificial suspension in which there could be no question of its affecting the supply of glucose. And thus synthalin, the trypanocidal action of which had been discovered in a trial based on a completely erroneous hypothesis, became the starting-point of a new line of investigation, in which my former colleague Dr. Harold King and the late Prof. Warrington Yorke were led to the discovery of still more therapeutically potent and at the same time less toxic diamidines; and their discoveries led, in turn, to further developments in industrial laboratories which have given to therapeutics the substances called stilbamidine, which seems likely to establish itself as the most effective remedy for kala-azar yet produced, curing types of that infection in which antimonial remedies of all kinds had failed; pentamidine, which has proved to be effective in Babesia infections of cattle; and propamidine, which appears to be giving promising results even in the local treatment of certain bacterial infections of wounds. It is of interest to note that, as long ago as 1928, Staub and Küng had observed the powerful antiseptic action of synthalin, but regarded it as too toxic to have chemotherapeutic value.

Sulphanilamide and its Derivatives

The fact that v. Jancsó's conception, which led him to test the chemotherapeutic action of synthalin, had so little true relation to what he observed or to the discoveries which followed from the clue thus presented, should not prevent us from recognizing its essential novelty. He seems to have envisaged, for the first time, the possibility of a chemotherapy which eliminates the parasites, not by a direct action on them of any kind, but by depriving them of nutriment—not by a *Blitzkrieg*, or even by a war of attrition, but by a food-blockade. We shall see that this idea is not wholly unrelated to those which offer the most suggestive interpretation for one of the most recently discovered and probably the most widely recognized and celebrated of all chemotherapeutic actions—the action on bacterial infections of sulphanilamide and the growing family of its derivatives. The effect of introducing a sulphonamide ($-\text{SO}_2\text{NH}_2$) grouping into azo-dyes, in increasing the fastness of their staining properties for silk and wool, their so-called "substantive" properties, probably led to the first trial of such compounds for therapeutic effects on infections due to bacteria, on the lines of Ehrlich's conception that the remedy must be fixed by the parasite to exert its lethal, curative action. For many years there had been no clear and unqualified success in this field of antibacterial chemotherapy, and the view had even become prevalent that chemotherapy was unlikely to provide a successful remedy for diseases due to bacteria, and that we should have to depend on immunology to produce specific remedies for these, in contrast to the success of chemotherapy, and the failure of immunology, in finding remedies for infections due to protozoa and spirochaetes. Even in this period of long delay, however, there were more promising signs for those who could read them. Morgenroth's discovery of the cure of experimental pneumococcus infections in mice by optochin, an artificial homologue of quinine, had failed of practical application only because of an unforeseen toxic action of this alkaloid on a proportion of human patients.

Browning, during the last war, had discovered valuable anti-bacterial properties in acriflavine, an acridine dye which Ehrlich had earlier found to be effective in trypanosome infections of mice and had named trypaflavin in that connexion; but acriflavine and the simpler proflavine, while retaining their value as bactericidal agents for local applications, had not proved suitable for internal chemotherapy in the stricter sense. It appears, indeed, in retrospect, that the curative value of sulphanilamide itself might even have been discovered many years earlier, when Heidelberger and Jacobs, in New York, observed in 1919 that a sulphonamide derivative of the cinchona alkaloid cupreine had a potent antiseptic action on artificial cultures of certain bacteria. There was no practical development, however, of that observation; and the production of the red sulphonamide dye known as prontosil, a sulphonamide of the dye chrysoidine, by the German chemists Klarer and Mietsch in 1932, and Domagk's finding that this had a definite curative action on mice infected by an otherwise fatal injection of streptococci, was an essentially new discovery, and certainly one of the most important yet made in the short history of chemotherapy. Domagk published these observations in 1935, with the promising results of certain clinical trials of prontosil on cases of streptococcal infection in man. In the following year

Colebrook and Kenny, in London, completed an extended and carefully controlled clinical trial of its value, in a uniform series of cases; and the publication of their results first convinced the world that the discovery of prontosil and its action was an event of first-rate importance for practical therapeutics. Even while this trial was in progress, however, came evidence from Tréfouel, Nitti, and Bovet, in Paris, that sulphanilamide, a reduction cleavage-product of prontosil, was as effective against experimental infections as prontosil itself; and Colebrook and Kenny were thus able to confirm this finding also, and to extend it to the natural streptococcal infections of their series. And then it was found that prontosil undergoes this reduction cleavage in the body, giving rise there to sulphanilamide; it might be doubted, therefore, whether there was any ground for connecting the action of prontosil with its properties as a dye, or for regarding it as anything but a source of sulphanilamide.

When prontosil was tested on artificial cultures of streptococci, or of other organisms susceptible to its action *in vivo*, it had no effect upon them. Sulphanilamide, on the other hand, while it did not sterilize the culture in any reasonable concentration, stopped its growth in dilutions comparable to those which could be produced in the blood of the living patient. Here again, therefore, we have a case in which the substance originally administered was not chemotherapeutically active as such, and in which the direct agent, formed from it by action of the body tissues, produced its effect by stopping the growth of the infecting parasites and not by killing them directly—being in this instance "bacteriostatic" and not directly "disinfectant" in its action. There was not in this case, however, any clear advantage in administering the inactive precursor prontosil and allowing the directly active substance sulphanilamide to be formed from it as required; the cleavage proceeded so rapidly that the administration of the preformed sulphanilamide appeared to be practically equivalent.

From that point onwards the practical applications of sulphanilamide itself, and of the succession of more effective derivatives that have issued from the chemical laboratories of many countries, have been developed and extended with a rapidity new to experience. Week by week the medical press of the world has offered, and still offers, new evidence of the special value of one or another of these derivatives for the treatment of a particular type of infection; and the repute of their value has spread far beyond medical circles. There can be no doubt that their introduction has, in itself, transformed an important branch of curative medicine, or that the use of these substances by various methods, including that of

local application, has changed the aspect of many of the medical and surgical problems of war. The prospect, however, of an ordered development of this relatively new field must certainly depend on the extent to which a clearer conception can be attained of the nature of the chemotherapeutic process itself; and a new promise of such insight was offered by discoveries which were just becoming ripe for record, when the outbreak of war again brought an interruption of so many researches of the type requiring patient concentration and the long view.

New Links between Chemotherapy and Biochemistry

The conceptions which spring from these recent discoveries, in contrast to those which were earlier dominant, attempt to interpret the action of a drug on an infecting organism in terms of a cellular biochemistry which was not available to the earlier theorists. As long ago as 1923 Voegtlin and his co-workers, in considering the mode of action of the directly active arsenoxides produced in the body from arsphenamine and the other specifically curative arsenic derivatives, had pictured the arsenoxide radical as combining with the thiol radicals of glutathione in the protoplasm of the cells affected, and thereby interfering with their respiratory metabolism. In 1940 Fildes independently put forward a closely similar idea

FIG. 7.—Suggested mode of action of an arsenoxide (Voegtlin).

to explain the disinfectant action of mercury salts, suggesting that the mercuric ions combined with and inactivated the free thiol groups of bacterial cells. In both these investigations the suggestion was supported by evidence that reduced glutathione, or other substances offering free thiol radicals, injected into the animal or added to the culture medium, would neutralize the activities of arsenoxides and mercury salts respectively.

It will be noted that in both these cases the suggested action involved the fixation of the toxic agent to particular groups in the protoplasm of the affected cells; and to that extent the suggested thiol groups might be regarded as representing a type of Ehrlich's chemoreceptors in a chemically more definite form. Fildes, however, used his observation, and others which were being made in his laboratory, as the basis of a more general conception of the chemotherapeutic process, as essentially an interference with the action of some group or chemical factor required by the infecting organism for its normal life and growth. In such a generalized form this conception can be made applicable to the action of the sulphonamide derivatives on bacteria. There is no evidence, in this case, for any firm combination of the therapeutic agent with any group or constituent of the cells attacked. The chemoreceptor conception, in that original sense, is here not applicable. A sufficient concentration of the sulphonamide derivative must be maintained in the environment of the susceptible bacterial cells if their growth and multiplication are to be suppressed; and they begin to grow and multiply again as soon as the concentration falls below that minimum. We have then, apparently, not a combination but a labile antagonism to some substance or process needed for growth. Fildes referred to the substance, thus somehow put out of action, as an "essential metabolite," while others have spoken of a "growth factor" or pictured interference with an enzyme action. Further experiments gave a much greater precision to the idea. Stamp showed that a sterile extract of streptococci, added to the culture medium, would neutralize and nullify the bacteriostatic effect of sulphanilamide on the culture. Then Woods, in Fildes's laboratory, found that an extract of yeast would act similarly as an antagonist to the action of sulphanilamide; and, finally, he was able to identify the antagonistic constituent, to a high degree of probability, as *p*-aminobenzoic acid.

FIG. 8.

A casual inspection shows that the molecular configuration of *p*-aminobenzoic acid has a large measure of community with that of sulphanilamide. Woods accordingly suggests that the

mode of action of sulphanilamide is to preoccupy some cellular enzyme, the normal function of which involves an action upon *p*-aminobenzoic acid as one of the essential steps in the growth-metabolism of the bacteria.

The hypothesis is attractively suggestive, and there is additional evidence in its favour. Woods found a fairly constant proportion between the concentration of sulphanilamide in a medium and the concentration of *p*-aminobenzoic acid which must be added to neutralize its bacteriostatic effect. He found, further, that the concentration of *p*-aminobenzoic acid required to abolish the effects of different sulphonamide derivatives increased with the bacteriostatic activity: sulphapyridine, for example, required a greater concentration to neutralize its effect, corresponding to its greater activity, than did sulphanilamide itself. It must be admitted, however, that an inspection of their formulae does not yet afford any suggestion why sulphapyridine should be more effective than sulphanilamide in preoccupying an enzyme normally acting on *p*-aminobenzoic acid. Two other substances have already been detected in certain bacteria which similarly antagonize the action of sulphanilamide, and other attempts to find substances with bacteriostatic effects, attributable to similarities in molecular configuration with other known factors of bacterial growth, such as pantothenic acid, have already given results of promise. Whether these ingenious suggestions are confirmed by further knowledge or prove to be inadequate, I believe that they will have a permanent significance, as having brought the interpretation of a chemotherapeutic process for the first time into clear relation with a cellular metabolism which can be stated in terms of a known chemistry. I think that we are entitled to hope that chemotherapy may soon find that its means of discovery are no longer limited to the almost unselective trial of as many derivatives or analogues as possible of some substance which has shown an unexplained curative effect on a particular infection. A more fundamental knowledge of the conditions and factors required for the multiplication of the infecting organisms may give, at least, an invaluable direction and concentration of aim to the search for remedies. The bacteria, with the ready opportunities they afford for studying in artificial cultures the methods of supplying and interfering with their requirements for growth, or the factors promoting their pathogenicity, offer the most promising field as yet for thus exploiting the resources of biochemistry with a therapeutic aim. But it is tempting to speculate as to what the world might gain, for example, in the present emergency if means were available for growing the malaria parasites at the different stages of their complicated life cycles, under artificial conditions permitting factors requisite for their multiplication to be studied, as a guide to attempts to find other and better remedies than quinine—even better than mepacrine (atebrin), which has already come from one of the most brilliant and determined exploitations of the longer available methods.

Mycogenous Remedies for Infections

Meanwhile, we may note the creation and strengthening of another kind of link between biochemical studies and anti-bacterial therapeutics. Dubos, in New York, has trained a soil bacillus, by cultural restriction, to give a good yield of peculiar polypeptides having a high toxicity for certain pathogenic bacteria, one of them, gramicidin, being specific in its intense antiseptic action for the Gram-positive cocci. Much more brilliant, however, in its promise for the practical treatment of septicæmic conditions has been the recent progress in the purification and isolation of penicillin. After Fleming's chance observation of the production of this remarkable substance from a colony of *Penicillium notatum*, and his preliminary examination of its properties, and even of its therapeutic possibilities, in 1929, there was a long gap of years. Then, after the discovery of the action of sulphanilamide had shown the possibility of an antibacterial chemotherapy with agents of a low toxicity, the interest in penicillin was revived and rapidly extended by the work of Florey and his colleagues at Oxford. They showed how it could be extracted from a culture filtrate and purified so as to make it safe for intravenous administration; and they produced the first clinical evidence of its remarkable therapeutic possibilities. And now the chemists in Oxford and elsewhere are hot on the trail,

and manufacturing laboratories on both sides of the Atlantic are straining their resources in the attempt to make and to isolate penicillin, and to determine, perhaps, its constitution, or that of the penicillins if there should prove to be more than one active constituent. Then finally, perhaps, may come a synthesis of a penicillin, or of some simpler analogue with similar action. There seems to be a possibility, even a likelihood, that this remedy against infection from a new kind of source may approach, more nearly than any other yet available, to the ideal of toxicity for the infecting organism and complete absence of it for the patient. Here again, however, the effect on the organisms is merely to prevent their multiplication, and not to kill them outright. There can be little doubt that the first pages only are being turned, as yet, of a new and brilliant chapter in the specific treatment of infections.

Conclusion

If we are to form an idea of the prospect which I took as the subject of my lecture, I think that it is important to recognize how much of the change which I have been discussing has taken place in the latter part of the period covered by the survey—in the years, in fact, since the last war came to an end. This is not only true of the progress of chemotherapy, which I have considered in more detail, but equally so of exact knowledge of the natural remedies for deficiencies. At the end of the last war only one hormone, adrenaline, had yet been chemically identified, and no vitamins. It was still possible then to suggest of many of the hormones that their very existence was problematic, and that they, and the vitamins as well, might in any case be so labile and otherwise inaccessible that the methods of chemical isolation and structural study would never be applied to them. Yet before the present war broke out large and rapidly growing series of both hormones and vitamins had been isolated, identified, and made available for practical use by artificial synthesis; and a beginning had even been made of the discovery of simpler synthetic substitutes. In chemotherapy, though salvarsan had appeared before the last war began, there was still, when it ended, no clear prospect of further advance by routes which could be mapped on scientific principles. Wholesale and almost haphazard trial of derivatives or analogues of any substance which had shown a promise of activity seemed to be the only way open; the marvel was, and still is, that so much has been achieved by such methods.

I have tried to trace the progress since that time, from the picturesque but frankly empirical conceptions which Ehrlich had bequeathed, based apparently on his knowledge of selective staining with dyes and of the extreme specificity of immune reactions. From these ideas of magic bullets, bringing down only the parasites by virtue of a mysterious affinity for them alone, we passed to recognition of the effect as stopping their reproduction rather than slaying them outright, turning to their defeat the tide of battle with the host's natural resistance to infection. We came thus to the conception of the effect as usually a mild and persistent one, dependent for that persistence, in some cases, on affinity of the remedy for the host's tissues and not merely for the parasites. And, finally, we reached a conception, just emerging, of the nature of the effect on the reproduction of the parasites, stated in biochemical terms of an interference with known factors of their growth. Look back with me again to medicinal treatment at the opening of this century, and consider that the portentous change between then and now has been so largely an achievement of the years of uneasy armistice between two open wars, at a time when the full impact of research on practical medicine was only just beginning to be felt. Portentous, I said; but what does it portend? Does it not mean that, when we have won freedom from war and from fear of war, the prospect of further achievement in this field of experimental therapeutics, so newly opened to enterprise, is brilliant beyond anything that experience yet enables us to picture? What an opportunity, I am moved to exclaim, for those who still have the years before them in which to use it! I hope that it may fall to the lot of one of them, when the passage of years may justify another review, to tell again in one of these lectures how much of this present prospect has by then become a reality, and to survey yet further possibilities, beyond any that we can now imagine, which will then be coming into view.

CUTANEOUS AND CONJUNCTIVAL DIPHTHERIA

A SERIES OF CASES

BY

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Medical literature records that cutaneous diphtheria was not an uncommon condition in the nineteenth century, but nowadays it is so rarely recognized in this country that a series of cutaneous and conjunctival infections admitted to the Isolation Hospital, Southampton, seems worth recording. All the cases were admitted from a military hospital. Rolleston (1925), describing a large number of diphtheria admissions, found that approximately 1% showed cutaneous involvement, and Cameron and Muir (1942) reported a series of 66 cases occurring among troops in Palestine.

Details of Cases

During the eight months prior to April, 1943, there were admitted to the Isolation Hospital, Southampton, from a military hospital, 19 patients who had lesions of the skin or eyes from which *C. diphtheriae* had been isolated. Of these, 12 were infected with virulent and 4 with avirulent organisms; in the remaining 3 cases tests for virulence were not carried out.

The 12 infections with virulent organisms comprised: (i) 6 cases of cutaneous diphtheria; (ii) 2 cases of cutaneous and conjunctival diphtheria; (iii) 3 cases of conjunctival diphtheria with associated skin lesions from which *C. diphtheriae* was not isolated (one of these was also a faucial carrier); (iv) 1 case of cutaneous and faucial diphtheria. The avirulent infections were 3 cases of cutaneous infection and 1 of conjunctival infection. The 3 cases in which tests for virulence were not carried out were two of cutaneous and faucial infection, and one of conjunctival infection with an associated skin lesion from which diphtheria bacilli were not isolated. All 19 patients were males aged between 21 and 37 years.

Case 1.—Admitted to the military hospital for treatment of seborrhoea of the scalp and forehead and acute purulent conjunctivitis of four days' duration. An eye swab contained diphtheroid organisms. After two weeks the conjunctivitis became more purulent, and a few days later bilateral keratitis was present. The eye was again swabbed, and was found to be infected with *C. diphtheriae* (gravis type I). When admitted to the isolation hospital bilateral conjunctivitis and keratitis were present. There were a dense opacity of the right cornea and an ulcer of the left cornea with hypopyon. No membrane was seen. Treatment consisted of 20,000 units of diphtheria antitoxin i.m., and bland irrigations to the eyes. The inflammation settled down in two weeks, leaving corneae with a frosted-glass appearance. Two months later the vision of the right eye was 6/18 and the cornea was moderately scarred, especially in the lower segment. The pupil was active, but posterior synechiae were present. The left cornea was extensively scarred and staphylomatous in the lower quarter. Vision of left eye was C.F. at 6 in. only.

Case 2.—Admitted to military hospital with impetigo of face and ear. Skin swab reported positive five weeks after admission, but later reported avirulent.

Case 3.—Admitted to the military hospital with dermatitis affecting the "respirator" area. Two weeks later developed a sore throat, a swab from which proved to contain *C. diphtheriae*. A skin swab was also positive. On admission to the isolation hospital the dermatitis cleared up within a fortnight. 10,000 units of antitoxin were given, but later skin swabs showed the presence of diphtheroids only.

Case 4.—Admitted to the military hospital with purpura and pyoderma. A skin swab was reported positive for *C. diphtheriae* shortly before transfer to the isolation hospital, where on admission the patient was found to have an ulcer the size of a five-shilling piece on the outer aspect of the right thigh. The ulcer had a well-marked edge and a grey membranous slough covering its base. 10,000 units of antitoxin were injected, and sulphanilamide powder was applied after the ulcer had been soaked to remove slough. The ulcer healed in about two months.

Case 5.—Admitted to military hospital with impetigo of the face. Three weeks later a skin swab was found to contain virulent *C. diphtheriae* (gravis).

Case 6.—Admitted to military hospital with impetigo of the face and neck of four days' duration. Five days later he developed