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Partial cell functions

Nobel Lecture, December 11, 1908

The history of the knowledge of the phenomena of life and of the organized world can be divided into two main periods. For a long time anatomy, and particularly the anatomy of the human body, was the α and ω of scientific knowledge. Further progress only became possible with the discovery of the microscope. A long time had yet to pass until through Schwann the cell was established as the final biological unit. It would mean bringing coals to Newcastle were I to describe here the immeasurable progress which biology in all its branches owes to the introduction of this concept of the cell. For this concept is the axis around which the whole of the modern science of life revolves.

It is, I think, a generally acknowledged and undisputed fact that everything which happens in the body, assimilation, disassimilation, must ultimately be attributed to the cell alone; and furthermore, that the cells of different organs are differentiated from each other in a specific way and only perform their different functions by means of this differentiation.

The results produced here are mainly based on histological examinations of dead and living tissues, with, of course, most valuable contributions from the neighbouring sciences - physiology, toxicology, and particularly comparative anatomy and biology. Yet I am inclined to think that the limit of what the microscope could and has done for us is now approaching and that for a further penetration into the important, all-governing *problem of cell life* even the most highly refined optical aids will be of no use to us. Now, at this moment, the time has come to penetrate into the most *subtle chemism* of cell life and to break down the concept of the cell *as a unit* into that of a *great number* of individual specific *partial functions.* But since what happens in the cell is *chiefly* of a *chemical* nature and since the configuration of chemical structures lies beyond the limits of the eye's perception we shall have to find other methods of investigation for this. This approach is not only of great importance for a *real* understanding of the life processes, but also the basis for a truly rational use of medicinal substances.

The *first* advance in this complicated field came about, as so frequently happens, in a roundabout way. After Behring's renowned discovery of the

antitoxins I had set myself the task of penetrating further into the mysterious nature of this process, and after long labours I have succeeded in finding the key to it.

As you know, the function of stimulating the formation of antibodies belongs to one *particular* group of poisonous substances only, to the so-called *toxins.* These are metabolic products of animal and plant cells: diphtherial toxin, tetanus toxin, the phytotoxin of jequirity, ricin, snake venom, e tutti quanti. None of these substances can be made to crystallize, and they obviously belong to the class of protein substances. The toxin is generally characterized by two properties: (1) by its poisonousness; and (2) by its ability to stimulate the production of the specific antitoxin in the animal body.

My quantitative investigations of this process have shown that the toxins, especially the solutions of diphtherial toxin, will - either spontaneously if left standing for some time, or through the action of thermal influences or certain chemicals (iodine) - change in such a way that they are more or less deprived of their toxicity but retain their ability to produce antibodies. Furthermore, it has become obvious that the products of this transformation which I call *toxoids,* and which my honoured friend Professor Arrhenius has also encountered in his numerous experiments, have still retained the ability to neutralize the antitoxin in a specific way. Indeed, in favourable cases I and others have succeeded in proving that the transformation of toxin into toxoid can be a perfectly quantitative one, so that a given toxic solution will combine with exactly the same amount of antitoxin before and after the transformation into toxoid.

These facts permit only one explanation, namely that there must be two differently functioning groups present in the toxin. One of these, which has been preserved also in the "*toxoid*" and must therefore be considered the more stable one, must be allowed the ability on the one hand to stimulate the formation of antibodies in the animal body by immunization, and on the other to neutralize antibodies in the test tube and in vivo. The relations between toxin and its antitoxin are *strictly specific* - tetanus antitoxin neutralizes exclusively tetanus toxin, diphtheria serum only diphtherial toxin, snake serum only snake venom, to mention just a few examples out of hundreds. For this reason it must be assumed that the antipodes enter into a *chemical* bond which, in view of the *strict specificity* is most easily explained by the existence of two groups of distinctive configuration - of groups which according to the comparison made by Emil Fischer fit each other "like lock and key". Considering the stability of the bond on the one hand and the fact on the other that neu-

tralization occurs even in very great dilutions and without the help of chemical agents, it must be assumed that this process is to be attributed to a close chemical relationship and probably represents an analogue to actual chemical syntheses.

More recent investigations have in fact shown that it is possible by chemical actions to break up the product of the union, the neutral combination toxin-antitoxin, into its original components. For instance Morgenroth in particular has proved in the case of a number of toxins - I shall just mention snake venom and diphtherial toxin - that under the action of hydrochloric acid the compound can be separated again into its original constituents, in the same way that in pure chemistry stable compounds, such as the glycosides, can through the action of acids be broken down into their two components: sugar and the basal aromatic complex. These investigations have shown that the *stable* group of the toxin molecule, which I call *haptophore,* can exercise great chemical activity of a specific kind, and thus the obvious assumption was that it must be precisely this group which causes the adhesion of the toxin to the cell. When we see how some bacterial poisons produce disturbances only after weeks of incubation and then damage the heart or kidney or nerves, when we see how animals suffering from tetanus present contractions and spasms for months, we are forced to the direct conclusion that all these phenomena can only be caused by the *adhesion of the toxic substance to quite definite cell complexes.*

I therefore assumed that the tetanus toxin for instance must unite with certain chemical groupings in the protoplasm of cells, particularly the motor ganglion cells, and that this chemical union represents the prerequisite and cause of the disease. I have therefore simply called such cell groupings "*poison receptors*" or just "*receptors*". Wassermann has been able to prove my view correct in every detail in his noted experiments in which he was the first to produce evidence that normal brain is able to render innocuous given quantities of tetanus toxin which are introduced. Many objections have been made to these experiments, but none have proved valid, and I believe that I may now pronounce it as a definite fact that certain specific groupings must in fact exist in the cells which fix the poison. That these, the cell's *receptors, which produce the fixation,* react to the *haptophore* part of the toxin can be deduced from the immunizations through toxoids, where the haptophore group is the only one which has been preserved. But since this haptophore grouping of the toxin must have a highly complex and peculiar stereochemical structure, and since it reacts *simultaneously* and *in the same sense* to the cell receptors

and the antitoxin, it must be concluded from this that the group in the protoplasm, the *cell receptor,* must be identical with the "*antitoxin*" which is contained in solution in the serum of immunized animals, for a *really well-made key* will not open different locks at the same time. As the cell receptor is obviously pre-formed, and the artificially-produced antitoxin only the consequence, i.e. secondary, one can hardly fail to assume that the antitoxin is *nothing else* but *discharged* components of the cell, namely receptors discharged in excess. The explanation of this fact was a very obvious one. One only needs to assume that the various specific cell receptors which take up the snake venom, the diphtherial toxin, the tetanus toxin, the botulin poison, etc. are not *properly speaking* designed for the purpose of serving as *toxic receptors* for substances with which the animal under the normal conditions of its life might perhaps never come into contact, but that they exist, in actual fact, in order to combine chemically with *normal* products of metabolism, i.e. to *assimilate* them. As these receptors, which may be regarded as *lateral chains* ("Seitenketten") of the protoplasm, capable of assimilation, become occupied by the toxin, the relevant normal function of this group is eliminated. Now *that* element comes in which was to be expected from the *fundamental law* of tissue defect and its compensation, discovered by Karl Weigert - the deficiency is not merely *exactly compensated,* but *made up to excess*, i.e. there is hyperregeneration. Finally, if the injections are increased and repeated, so many such groupings are formed in the body of the cells that they inhibit as it were the normal functions and the cell gets rid of the disturbing excess by discharging them into the blood.

The colossal difference between the amount of poison injected and the antitoxin produced is probably the most characteristic feature of this process and this is best illuminated by Knorr's statement that *one* part of toxin produces an amount of antitoxin capable of neutralizing *millions of times* the amount of the poison which started the process.

There are however many minds which consider the process a much more simple one. Straub is of the opinion that it is on the whole analogous to simpler processes of vital detoxification, e.g. to the forming of a sulphuric acid ester from injected phenol, and that these processes only differ in that the phenol-sulphuric acid remains stable in the organism, whilst the toxin-antitoxin complex in the organism is not held but is partially destroyed. But only one component, the injected toxin, is said to perish, while the *other,* the product of the reaction of the organism - as something which has developed in the body and thus is not foreign to it - escapes elimination and remains

preserved in the blood and body fluids. By systematic repetition of the poisoning it would then be possible to accumulate protective power in the blood, so that when it is introduced into other organisms it can also protect these from toxic diseases and would thus be acting as a curative serum.

So far Straub. Faced with such a simple explanation it can only be surprising that this problem has occupied the great army of researchers studying immunity for so many years. But in fact the author has *completely* missed the *vital clue,* namely that according to his theory a certain amount of toxin would produce only exactly the *equivalent* amount of antitoxin! In actual fact this is fortunately not the case in immunization. On the contrary, it has been proved much more conclusively - and I refer to my statement about Knorr above - that one part of poison can produce so much antibody that a millionfold multiple of the equivalent is achieved. This should prove Straub's view untenable.

It is much more important that from the evidence of this hyperregeneration the *pre-formation and the chemical individuality of the toxin receptors concerned is proved.* That which can be constantly formed anew in the cell and mixed with the blood like a secretion *must* have a chemical "*individuality*", and with realization of *this* the *first* step had been taken which led to the differentiation of the concept of the cell into that of *a great number of separate, individual functions.* I had assumed right from the beginning that the toxin represents *nothing more* than a nutritive substance capable of assimilation, to which in addition - by some sort of accident - is attached a *lateral grouping,* usually of an *unstable* nature, which causes the toxic action as such.

This view, which I have held from the beginning, has subsequently very quickly found confirmation many times over. It has in fact been possible to prove the complete independence of the haptophore and toxophore groups, as substances were discovered which had the ability to produce antibodies, and therefore were antigens, without at the same time having a toxic effect. Perhaps I may remind you in the first place of the precipitins, which were first discovered by Kraus, Tschistowitsch and Bordet. Through the important discovery that even the genuine protein substances of animal and plant organism are able, irrespective of whether they have a toxic effect or not, to produce antibodies with a specific chemical reaction, an antigenic nature could be proved also of actual nutritive substances, just as could previously be expected after my observations. But even among the poisons produced by nature some have been found which will readily demonstrate the independence of the haptophore and the toxophore apparatus. These are the cytotoxins

which are normally found in the blood serum of higher animals or can be produced arbitrarily through immunization with any type of cell. They differ from all other poisons known to us in their extraordinary specificity, in their monotropic action, which so far distinguishes only these poisons which are fabricated in the living animal body. Because of the complexity of their constitution a differentiation between the haptophoric and the toxophoric principle is palpably obvious, so that here the distributive component, the amboceptor, is given the function of concentrating the actual active substances on the affected substratum, through the increase in avidity which follows localization. The fact that the animal cells are antigens, although they have no toxic action, proves simultaneously, not only the possibility of immunization with protein substances in solution, but also the sole responsibility on the part of the haptophore group for the formation of antibodies.

It is precisely this discovery and analysis of the specific relations between haptophore antibody groups and receptors which has become of the highest theoretical and practical importance for the more recent serum diagnosis. I mention only the determining of the agglutination titre which has found its most important use in Widal's typhoid reaction; the differentiation of proteins established by Wassermann and Uhlenhuth which is so important for forensic blood tests; the measuring of the opsonic index inaugurated by Wright, not to mention the manifold uses which have been found for the process of complement fixation - the scientific foundation of which likewise rests on the principle of the adhesion of the antibody to the haptophore group.

I will not go into this any further now and will only draw "*the*" conclusion from it that there are a series of nutritive substances, probably mostly of protein nature, which find *specific* receptors in the cells and that it is thus possible, *through immunization,* to lure into the blood these structures in great abundance and in the form of typical varieties - as represented by the agglutinins, the precipitins, the amboceptors, the opsonins on the one hand, and the antitoxins and antiferments on the other. They can then be accumulated there to such an extent that a thorough study of these substances, which within the cell-formation is quite impossible, can now actually be undertaken. How far the analysis of such processes can be taken is shown by the study of the type of link between toxin and antitoxin and the discovery of the very complicated action of the amboceptors.

Of course this does not solve the secret of life itself, which may be compared with the complicated organism of a mechanical work of art, but nevertheless the possibility of taking out *individual* wheels and studying them exactly sig-

nifies an advance compared with the old method of *breaking into pieces* the whole work and then trying to deduce something from the mixture of broken pieces.

I describe all the receptors which are able to and designed to assimilate nutritive substances as "*nutriceptors*" and would regard these nutriceptors as the source of the *antibodies* which are theoretically and practically so important, and which I have enumerated above. Obviously anyone adhering to the pluralistic point of view - and considering the complicated system of the organism, the almost illimitable variety and specificity of cell functions, this seems to me absolutely inescapable - must assume that there exists a whole *range* of nutriceptors of different types. From the point of view of immunization these can be differentiated into three types:

(1) Those which do *not* enter the blood in the form of antibodies. It may be assumed that this will probably be the case with those nutriceptors which serve the *very simplest* functions, for instance the assimilation of simple fat substances or of types of sugar.

(2) Those which enter the blood in the form of the antibodies mentioned and characterized above, and the development of which corresponds to a *hyperregeneration.*

(3) The third form presents a contrast to this in so far as it is not a case of new formations, but of a *decrease* in receptors. Experimental proof of this occurrence has however so far been only very rare. The only known instance is probably the evidence produced by H. Kossel that after prolonged immunization of rabbits with the haemotoxic eel serum the blood corpuscles as such did finally become insensitive to this agent, as though they had lost the specific receptors.

Now I, in company with my colleagues, Dr. Röhl and Miss Gulbransen, have succeeded in penetrating further into the nature of the artificial loss of receptors and in illuminating the whole mechanism. Our work will shortly be published in a more extensive form; here I would like to emphasize that the experiments were done on trypanosomes. Franke had at one time infected a monkey at my Institute with a certain species of trypanosome, then brought about its cure through chemotherapeutic agents, and then again, in order to test the immunity of the animal, reinfected it with the original strain. But contrary to expectation it turned out that the monkey was not immune, but that it sickened again after a very prolonged period of incubation. If mice were treated with blood coming from the infected animal, i.e. containing trypanosomes, they fell ill and died. But if the trypanosomes were first re-

moved from the blood of the monkey it became apparent that the serum thus produced was capable of killing off the *original parasites.* This revealed that a variety of the parasites had developed in the monkey which in contrast to the original strain was no longer affected by the serum - a *serum-resistant strain.* Similar observations were at the same time recorded by Kleine and lately also by Mesnil.

Now if experimental animals which have been infected with a certain species of trypanosome are treated not with a full sterilizing dose of a suitable substance (arsanil, arsacetin, arsenophenylglycin), but with a somewhat smaller one, trypanosomes disappear from the blood for a greater or lesser period of time. The formation of antibodies has occurred in this case too, as can be easily proved. The few parasites which have escaped death now remain in the organs for a greater or lesser period of time, gradually adapt themselves to the anti-substances in the serum, and then, as soon as this has happened, return to the blood where they increase rapidly and lead to the death of the animal. If the trypanosomes obtained by this method are transferred to one group of mice which have been previously infected with the original strain, have been cured through the administration of suitable doses and have thus become carriers of the specific antibodies, and to a second group of normal mice, one becomes convinced that the parasites grow equally quickly in both groups. The parasites of the recidive strain have therefore undergone a biological change in that they have become *serum-resistant.** The change which has thus been produced in the parasites is not a superficial one, but may *be reproduced unchanged for many months* by passage through normal animals. The recidive strain retains unchanged its property of being *resistant* to the antibodies produced by the *original strain* and can thus be identified with absolute *certainty.*

It was now our concern to obtain an insight into the nature of this process. The explanation for this which we have found after many and varied experiments is the following : the original strain contains an abundance of a certain uniform type of nutriceptor which we shall call group "A". When the parasites are killed and dissolved within the organism of the mice the "A" grouping acts as an antigen and now produces an antibody which originates by virtue

* It is, by the way, possible to get exactly the same strain in another, much simpler way which consists of infecting the mice with the original strain, fully curing them on the second day with a full dose, and then reinfecting them 2-3 days later with the same strain. After a greater or lesser period of time parasites will appear in the blood which fully correspond to those of the recidive strain.

of its relationship to group "A". If living parasites are now brought into contact with this antibody, either in the test tube or in vivo, it will be adhered to by the trypanosomes. The effect on the parasites in this way is that they undergo in vivo the biological change which leads to the development of the recidive strain. This change occurs in that in the new strain the original "A" grouping disappears and a new grouping, which we shall call "B", appears instead. That there is a new grouping in the recidive strain can be shown as follows : if two mice are infected with the recidive strain - carrier of the "B" grouping - and then completely healed; if one mouse is then infected with the original strain, and the other with the recidive strain itself, the reinoculation with the original strain - carrier of "A" grouping - proceeds smoothly, while reinfection with the recidive strain fails at first. This shows that the original strain and the recidive strain are not identical, or must possess *two differently functioning groups. We therefore have a typical case of immunization producing loss of receptors while developing a completely new type of receptor.*

Whether one calls this change a mutation or a variation is really of little significance; the main thing is that it can be produced intentionally and artificially and that it is hereditary. But in view of the great interest which this particular problem has for biology and the theory of evolution, we have tried to get a fuller understanding of the process.

First of all it was necessary to determine how the trypanosome-antibodies influence the parasites. In accordance with the assumption common in immunology it might be accepted, that these antibodies produce direct toxic actions, i.e. contain toxophoric or trypanolytic groups, and that therefore the adhesion as such would necessarily produce damage to or death of the cell. But my colleagues and I have become convinced that this is not the case. In contrast to the usual species of trypanosomes, which contain only one uniform grouping "A", "B", or "C", etc. and which may therefore be called "*unios*", other types present themselves, which have two groups in the protoplasm at the same time, e.g. "A" and "B", and may therefore be called "*binios*". If one such binio "A" - "B" is acted upon by the isolated antibody "A" or "B", this does not cause the slightest damage to growth. This arises only if the parasite is occupied by both anti-substances at the same time. It follows from this that the presence of antibodies does not have a direct toxic effect on the trypanosomes, and it seems to follow from this triple experiment that the antibody only has an effect in so far as it prevents the intake of nutritive substances through occupation of the group concerned. If in the binio "A" - "B" the grouping "A" is obstructed by the antibody, the parasite can continue

to vegetate through its grouping "B". This also proves that the groupings "A" and "B" must be chiefly regarded as nutriceptors.

If the amount of antibody is very large the parasite can no longer feed itself at all and dies. It is easiest to convince oneself of this by mixing parasites with varying amounts of antiserum in the test tube. With the high concentrations which stop the intake of food altogether the death of the parasites follows, while with *weaker* concentrations which permit a *vita minima* in which mutation is possible a recidive strain develops. This mutation must therefore be entirely due to *starvation of the protoplasm,* under the influence of which new *potential* structures of the trypanosome develop. Antibodies like those which we have just been considering, and which have a purely *anti-nutritive* action, I call "*atrepsirls*" and I believe that these *probably play an extraordinarily important role* not only for bacteria, but in *biology* in general.

Most of my colleagues in this field will probably find it easy to accept the idea that there are certain chemical groupings in the cell for the reception of the various nutritive substances, once their existence has been definitely proved by the presence of the antibodies. But what is much more difficult is the question whether for the reception of other, less complicated substances too there are analogous functional groups. For the simplest further function of the cell, the *absorption of oxygen,* the problem is in my opinion already solved. We know that in the haemoglobin molecule it is exclusively the organically associated *iron residue* which provides the loose link between oxygen on *the one hand* and carbon dioxide and hydrocyanic acid on *the other.* It will therefore be necessary to assume certain groupings in the protoplasm of the red blood corpuscles, which have a maximal relationship to iron, and form a *complex* compound with it which has the characteristic functional properties. The protoplasm of the red blood corpuscles would thus be characterized by the abundant presence of "*ferroceptors*" which complemented with iron would lead to the finished haemoglobin molecule. In a similar way it will also be necessary to assume that the blue respiratory pigment of crayfish contains "*cuproceptors*", and others probably "*manganoceptors*". Also, the localization of *iodine* in certain glandular systems, particularly the thyroid gland, and the evidence that iodine is arranged in certain aromatic lateral chains will have to be interpreted in this way.

Much more difficult, however, is the question whether such preformed chemoreceptors may also be assumed to exist in the cell in the case of the great number of actual *medicaments.* This question takes us into the important field of the relation between *constitution* and *action,* which represents the basis

for a rational development of therapy. Only when we really know the points where the parasites attack, only when we have established what I call the *therapeutic biology of the parasites,* will it be possible to combat the infective agents successfully.

I have therefore carried out these studies of mine on the detection of definite chemoreceptors, first on monocellular living beings - protists - because the *conditions* there are much more favourable to a clear understanding than those in the infinitely complicated machinery of the higher organisms. I therefore asked myself the question: do the *trypanosomes* possess in their protoplasm definite *groupings* which govern the captivation of definite chemical substances ?

If a certain substance is able to kill trypanosomes or other parasites in the test tube or in the animal body, this can *only* happen because an accumulation of it takes place in these parasites, but the *process* itself is not explained by the establishment of these *bare facts.* There are *very many* explanations for this and only when it is possible to prove that we have here a *function* which is open to *specific* changes and variations, will we have proof of a *preformed* formation.

Unfortunately it appears that the way in which it was so easy to produce proof of preformation for the *nutriceptors,* namely by the *transfer* of the cast-off receptors into the blood, does not apply for the chemoreceptors, as they are much more simply constructed and remain attached to the cell - that is, they *are not rejected.*

Here it was only possible to see clearly in a *roundabout way,* which took us via the *drug-resistant* strains of the trypanosomes. Together with my faithful colleagues Franke, Browning, and Röhl, I have shown that it is possible to obtain by a systematic treatment trypanosome strains which are resistant to the three substances which so far are known to be inimical to trypanosomes: compounds of the arsenic series, fuchsine, and the acid azo-dye from the benzopurpurine series, trypan red. These resistant strains have the following characteristics :

(1) Stability of the acquired property. This is so great that for instance our arsenic strain, after it has passed in 2½ years about *380 times through mice,* is now even today *equally resistant to* drugs as the original strain.

(2) A principal characteristic of drug resistance is its *strict specificity* which is distinctive in that it relates not to one specific compound but to the *whole chemical grouping* to which this specific compound belongs. The strain resistant to *fuchsine,* for instance, is not only resistant to this, but also to a whole series of related triphenylmethane dyes, e.g. *malachite green, ethyl green, hexaethyl*

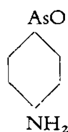
violet. On the other hand it has remained sensitive to both the other types, that is, to trypan red and an arsenical substance. A corresponding specificity is shown by the strain resistant to trypan red and also that resistant to arsenicals. That there are in fact three different functions here is furthermore apparent from the fact that by successive treatment of *one* and the same strain of trypanosomes with the three above-named substances it is possible to obtain a triple-resistant strain, i.e. a strain which is resistant to representatives of all three classes. Such a strain, assuming *maximal* stability, is extremely valuable for the discovery of new types of trypanocidal agents. If, for instance, some new substance is obtained which as such is capable of destroying the normal trypanosomes, it is only necessary to let this substance act upon the triple-resistant strain to find out whether it is a new type of remedial substance or not. If not, the triple-resistant parasites will *not disappear* with this treatment, but continue to flourish; but if they *do disappear* then the substance under test does not correspond to any of the three types of remedial substance mentioned, and a *representative of a new class of remedial substance* is being dealt with. The triple-resistant strain is therefore so to speak the cribrum therapeuticurn, the *therapeutic sieve*, with the aid of which it is possible to recognize what is *homologous,* and separate what is *different.*

A further important question was then to determine in what way this *specific* drug resistance comes about. Here it was the atoxyl strain which I used for the experiments. To get an exact picture it seemed necessary to investigate the behaviour of the arsenic resistant parasites in the test tube, removed from all the disturbances and complications of the organism. In this a great difficulty soon arose, as the remedial substance used most often in therapy, atoxyl (*p*-aminophenyl arsenic acid) does not have the slightest lethal effect on trypanosomes in *the test tube;* even solutions of a higher percentage were not sufficient for this. This phenomenon was all the more striking since according to Koch's investigations the parasites could be made to vanish within the human body in a few hours after injections of 0.5 grams of atoxyl; a lethal effect had therefore been achieved with a concentration of 1:120,000.

This was a process which more recently has been named "indirect effect". It was not difficult for me to find the reason for this phenomenon as I had in previous years made a thorough study of the reducing power of the body. We know that arsenic acid in the body is reduced to arsenious acid; we also know that cacodylic acid is reduced to that *foul-smelling* cacodyl; it was therefore obvious to think of reduction first of all. In atoxyl, *p*-aminophenyl arsenic acid, the arsenic residue is *penta*valent, while in the two products of

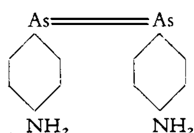
the reduction of it the arsenic residue only has a trivalent action - as in arsenious acid. We thus obtained two different products :

(1) monomolecular *p*-aminophenyl arsenic oxide



and

(2) arising from the reduction of the latter, the yellow diaminoarsenobenzene



In contrast to atoxyl, these substances proved to be highly trypanocidal both in the test tube and in the animal body. Even solutions of 1:1,000,000 of the arsenic oxide compound destroyed the trypanosomes within one hour. The closely related *p*-hydroxyphenyl arsenic oxide has an even stronger effect: 1:10,000,000.

Through this it was proved that the pentavalent arsenic residue releases no trypanocidal function whatsoever, but that this function is exclusively connected with the trivalent unsaturated state.

More than 60 years ago Bunsen, with prophetic clarity of perception, pointed out that cacodyl, the product of reduction, is so poisonous in comparison with the almost non-poisonous cacodylic acid, and deduced from this the chemical character of the binding of the cacodyl. It also tallies extraordinarily well with this that *unsaturated* carbon monoxide, for instance, and a number of other unsaturated compounds are so much more toxic than the corresponding saturated radicals. We shall therefore have to assume that the *arsenoceptor* of the cells is only able to take up the arsenic residue which is unsaturated and therefore eager to adhere.

With the aid of such reduced compounds it was now quite easy to examine the atoxyl strain in the test tube. It became apparent that suitable concentrations of the chemicals would still destroy it, i.e. that this was not a case of

loss of *receptors,* as we had proved with regard to the recidive strain. But a comparison of the *lethal* dose with *that* necessary to destroy the *normal* strain showed that the resistant strain required a much higher *concentration,* and that an amount which would destroy the *normal* strain at once did not, even after one hour, show the slightest effect on the *viability* of the resistant parasites.

These test-tube investigations seemed to indicate that the arsenoceptor had been preserved in the atoxyl-resistant trypanosome strain, but that its avidity had decreased, which could be seen from the fact that only through the use of much stronger solutions could the *toxic concentration* necessary for *lethal effects* be achieved; the normal arsenoceptor of the original strain will attract the same amount to itself from weaker solutions because of its initially higher avidity.

We have now been able to prove biologically quite clearly that the *arsenoceptor* does in fact represent a certain *function,* the avidity of which can be *systematically* and *successively* decreased through *immunization.* So far we have been able to reach *three* different stages of relationship. Stage I was achieved by subjecting the parasites systematically to the treatment of *p*-aminophenyl arsenic acid and its acetyl product. We continued the treatment *ad maximum* for years, until there was no further increase. The resistant strain thus obtained was *at the same time* also resistant to a whole series of other arsenic compounds, from among which I would particularly like to mention the p-oxide compound, the urea compound, the benzylidene compound, a number of acid derivatives, etc.

As there is the possibility - and in animal experiments this happens very frequently - that arsenic-resistant strains develop during therapeutic processes in animal and in man and these do of course completely prevent a successful continuation of therapy, it was now necessary to find substances which were still able to attack the resistant strain and combine with its receptors. After a long search we found altogether three compounds, the most important of which is arsenophenylglycine. With the help of this compound it was possible to bring even the *arsenic strain* I characterized above to a cure, which can only be thus explained that the substance seizes the avidity stump of the arsenoceptor like a *pair of pincers.* With this anchorage, however, the possibility opens to obtain a still higher resistance to arsenic. We did in fact succeed in this, though not without considerable trouble, and derived from the arsenic strain I at a higher level, *arsenic strain II*, which was completely resistant to *arsenophenylglycine.*

Now Plimmer has recently discovered a preparation, tartar emetic, which

in high dilutions also destroys trypanosomes. Tartar emetic is the salt of an antimony compound which is closely related chemically to arsenic. When we thereupon tested tartar emetic on the arsenic strain II we found that the latter was destroyed by the *tartar emetic.* Furthermore we succeeded in going a stage further by treating the arsenic strain II with *arsenious acid,* so that there now developed the third strain, *arsenic strain III,* which had now also become resistant to tartar emetic. I would like to emphasize particularly that this arsenic strain III, which was bred only under the *influence* of *arsenious acid,* was resistant to *tartar emetic,* but not to *arsenious acid.* This result can only be explained by the assumption that it is arsenious acid which, of *all* conceivable arsenic compounds, has the *maximal* relationship to the arsenoceptor, and that it will probably require the greatest effort or even be entirely impossible to produce a strain - and this would be *arsenic strain IV* - which would be resistant to arsenious acid as well.

To support my view that under the influence and attack of selected compounds there is a successive avidity restriction of the same receptor, I could produce many additional interesting facts, as for instance the phenomenon that the trypanosome can of course also be made resistant directly, with a more strongly effective reagent, i.e. arsenophenylglycine. A strain produced in this way proved as expected resistant also to the class of less avid substances, that is, atoxyl, acetyl arsenilate, etc. A panresistant strain would thus be obtained if one were to start producing resistance with right away the most highly effective agents - and these are tartar emetic and arsenious acid. According to our researches, however, it seems unfortunately impossible to produce resistance directly with these substances; it is only possible to do this in the roundabout way via the previous treatment of strains with phenyl arsenic acid derivatives.

The restriction of avidity is of course a chemical process which obviously allows the interpretation that in the vicinity of the arsenic grouping concerned, *other groups develop* or *disappear* which reduce the capacity to react. Perhaps I may give a chemical example. Benzyl cyanide reacts to nitrosodimethylaniline. But in order that the reaction may take place, heat and a stronger condensation agent, the free alkali, are necessary. If on the other hand a nitro-group is introduced into the benzene nucleus, the reacting power of the methylen group is heightened tremendously: the two substances, nitro-benzyl cyanide and nitrosodimethylaniline, react even in the cold. The introduction of the nitro-group has therefore had an accelerating influence on the reaction. If the nitro-compound is reduced to *p*-aminobenzyl cyanide it is less capable

of reaction than the original material; the amino-group has therefore had a diminishing influence on the reaction, while the acetyl product of the amino-compound reacts more or less like the original material.

We can see from this simple example that three different groupings, attached to the benzene nucleus in the paraposition, will either have no influence whatsoever on the methylene group, or *strengthen* it or *weaken* it. The *weakening would in our case correspond to the restriction of avidity.*

In my opinion the protoplasm can therefore be divided into a large number of individual functions which are interspersed among the *nutriceptors* in the form of different *chemoceptors.* But in my opinion these two main groups must be closely interconnected. This becomes apparent from the following consideration:

Trypanosomes of different origin, bred in different laboratories, usually show a different behaviour right from the beginning towards a certain curative substance. For instance, the trypanosome strain Mal de Caderas which I tried first had no resistance to trypan red, and I was therefore able to get a cure with this substance. This is still possible even today. Jakimov has had similarly good cures in Russia, while Uhlenhuth could not observe any influence on his strains. These are therefore *innate* differences; but that these are not *wholly fortuitous* is obvious from the fact that even *today,* after it has passed through normal mice for *many years,* my strain shows the same *curability* through trypan red as before. In contrast to this my Nagana strain could not be cured by trypan red and is still the same today. But when we made this strain into a *recidive strain* then it became apparent that within *14 days* this property which had been continued and maintained for years had altered. This proves that the *chemoceptors* are connected with the constitution of the *protoplasm* and undergo *alterations* if we alter the constitution of the protoplasm by mutation.

The reverse, i.e. whether a change of the cell substance, and particularly its nutriceptors, can be achieved by influencing the chemoceptors, has on the other hand not yet been definitely established. Browning had indeed observed and reported that through the serum reaction the fuchsine and atoxyl strains differ from each other and from the original strain. But more detailed investigation has shown further that these were not specific changes, in connection with *fuchsine* or *arsenic,* but changes corresponding to the recidive mutation described above; changes which are due to the fact that during the treatment the mice have frequently undergone recidivations which then led to the development of recidive strains.

I have thus come to the end. I am conscious of the fact that there are gaps in the work I have presented. But how could this be otherwise with a subject a truly exhaustive study of which would require the recapitulation of long and wearisome labours? But I did want to show you that we are getting to grips with the problem of obtaining an insight into the nature of action of therapeutic substances, the conception of which must consist in the recognition de sedibus et causis pharmacorum. I also hope that if these aspects are followed up systematically, it will be easier than heretofore to develop a rational drug synthesis, and I may mention that in this respect arsenophenylglycine has so far proved an entirely ideal remedy in animal experiments.

For with the help of this substance it is really possible in every animal species and with every kind of trypanosome infection to achieve a *complete cure* with *one* injection, a result which corresponds to what I call *therapia sterilisans magna.*