3 cm Tiefe ist die Dosis 3mal so groß als an der Oberfläche. Dieser überraschende Befund ist durch den Umstand bedingt, daß bei sehr hohen Spannungen die aus den bestrahlten Atomen ausgelösten Compton-Elektronen fast alle in Richtung der einfallenden Röntgenstrahlen emittiert werden. Von der Luft her fallen weniger Compton-Elektronen auf die Oberfläche, so daß hier die Dosis die geringste Erhöhung durch diesen Beitrag erfährt. Die Entfernung des Dosismaximums von der Oberfläche entspricht der durchschnittlichen Reichweite der Compton-Elektronen und vergrößert sich demgemäß mit wachsender Spannung.

Eine Tiefentherapie mit ultraharten Röntgenstrahlen ermöglicht es also ebenfalls, an den Herd eine Dosis zu bringen, die größer ist als an irgendeinem anderen Ort des durchstrahlten Körpers. Wegen des langsamen Abfalls der Dosis hinter dem Herd – bei einem Körper von 20 cm Dicke ist die Austrittsdosis noch die Hälfte des Höchstwertes – wird aber eine sehr hohe Volumendosis erreicht, was aus den früher erwähnten Gründen durchaus unerwünscht ist.

Bei Bestrahlung mit schnellen Elektronen werden die gesunden Körperpartien wegen der begrenzten Eindringungstiefe zweifellos mehr geschont. Als weitere Vorteile sind die viel größere Intensität zu nennen, die sehr kurze Behandlungszeiten ermöglicht, sowie die Vereinfachung des Strahlenschutzes. Man wird daher der weiteren technischen Entwicklung im Hinblick auf eine therapeutische Anwendung der schnellen Elektronenstrahlen mit gespannter Erwartung entgegensehen.

Summary

It is discussed which of the new rays of atomic physics (protons, neutrons, electrons) may be applicable for depth therapy. The range of protons is too short. Recent information from the U.S.A. shows that neutron therapy is not as successful as formerly supposed. Irradiation with fast electrons produced by the betatron may be superior to X-ray therapy. Some preliminary results of biological experiments with *Drosophila* eggs and ionization measurements are reported. The position of the maximum effect is some centimetres below the surface and not on it. Electrons have a definite range, so that the parts of the body beyond this range are not irradiated.

New Pathways of the Physiology of Infection and Immunity

By G. MANSFELD[1], Budapest

I. *The role of the nervous system in the genesis of infectious diseases*

H. BERGSON says that to understand you have to put yourself into the position of the one you try to understand. Trying to understand the genesis of an infectious disease, we do well to think ourselves into the position of the pathogenic germ that entered the organism and ask what we should do in its place. It is quite clear that the first and paramount task of such a primitive creature is to procure food. The way these protophytes get their food has been known since we learned to cultivate them. They split the ingredients of the media with their enzymes and feed upon these breakdown products. They have to procure their food in the same way in the living organism, with the difference that here they find not dead matter ready for them, as in the culture media, but living cells. Thus the first question arising is:—What happens to living cells when brought into contact with enzymes?

The literature is rather laconic on this problem, and I myself can say more about this only because, in my very young days, when the problem of life and death fascinated me, I wondered whether a well-defined difference between live and dead cells could be established. I performed an experiment that made a great impression on me and—maybe subconsciously—inspired me decades later to make a series of experiments on which I have been working now since 14 years, and the results of which I should like to report now for the first time.

If we prepare an emulsion of unicellular organisms, for instance bacteria (I used *Paratyphus* B), and subject them to the action of an enzyme that splits all their ingredients, proteins, fats, and carbohydrates—as does pancreatic juice—nothing happens at all, not even if we keep the mixture for hours in the thermostat. If, however, the cells have been heated previously to 60°C or treated with 1–2% carbolic acid, the same enzyme digests the cells in about half an hour and the opaque emulsion becomes transparent.

Analogously we have to suppose that the enzymes of bacteria can attack only cells which are, if not dead, at least damaged. The so-called pathogenic germs are ambitious and not content to live on predigested food like the saprophytes of the intestine; so they produce toxins to damage the cells of the organism. Once these toxins are neutralized, as seen in diphtheria, the germs become powerless and mostly leave the organism, looking for another, more suitable "climate".

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There are not many cases known in which detoxication of a toxin cures a disease, but the example of diphtheria is really exemplary, and I think that, when analysing the genesis of infections, we have to consider the problem of the toxins, that is their fate in the organism, more thoroughly than heretofore.

If we now examine the fate and the mode of action of these toxins not only from the standpoint of the bacteriologist, but also from that of the physiologist, then first of all we find that we have to do with substances of which infinitesimally small quantities have relatively very great effects, and that these effects are not produced immediately but after a certain latency. The significance of the fact that the great effect of small quantities and the latency are closely related to one another was bound to escape the attention of bacteriologists and also of physiologists until we found a well-defined crystalloid substance that exerts its effect after a period of latency in toxin-like small quantities. This substance is a hormone of the thyroid: *thyroxine*. It was first found by GUNNAR AHLGREN[1] that this hormone greatly increases oxidation in the cells, when administered in a concentration of 10^{-12}, that is one millionth of a microgram p. cc (i. e. thousand million molecules of thyroxine). After 2–3 years of incredibly hard work the knowledge of the specific qualities of thyroxine provided a deeper insight into the workshop of the organisms. Omitting details, this can be summarized as follows:—

First, it became clear that thyroxine, which was supposed to be a substance stimulating oxidation, does not act on combustions proceeding on the surface of the cells, but influences fermentative processes in the oxygen-poor interior of the cells, and thus only secondarily speeds up combustions[2].

Another fact revealed was that those exceedingly small quantities of thyroxine normally found to be active on the cells cannot exert their influence via the cell surface, where oxidations are in full vigour. That means that they cannot reach the cells from the blood but must get into the interior of the cells through a backdoor to become effective. This backdoor is the nerve fibre which connects every cell of the organism with the central nervous system[3].

That the axon conducting impulses can conduct chemical substances as well, was first shown for tetanus toxin by MEYER and RANSON[4], and has since then been demonstrated for several viruses also. Thus the supposition that thyroxine takes the same route to reach the interior of the cell did not seem impossible. The hypothesis was supported by an interesting finding of SCHITTENHELM and EISLER[5] according to which the iodine content of the brain of rabbits (especially that of the tuber cinereum) sharply increases after the intravenous injection of thyroxine. But another experiment shows even more clearly that thyroxine wanders along the nerves and reaches the cells in this way:—if we want to examine the distribution of thyroxine in the body and its mode of action, we take an organ (for instance a kidney) or fragments of an organ and determine its O_2-consumption. Repeating the procedure a certain time after the administration of thyroxine on the other kidney or on fresh fragments, we can determine the rate of increase of O_2-consumption. If we now compare the minimum time required for the appearance of the thyroxine effect in different organs, we see that this depends upon the distance that separates the particular organ from the central nervous system[1]. This finding excludes the possibility that thyroxine reaches the peripheral cells via the blood-stream, because its effect would then become apparent everywhere simultaneously, and can be explained only by assuming that thyroxine reaches the cells through the nerve fibres.

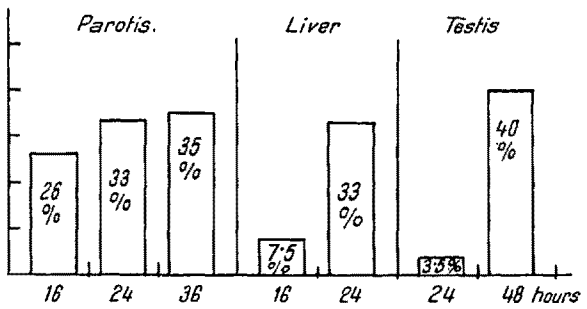

Fig. 1. – Increase of O_2-consumption in %.

It can be seen that while the thyroxine effect develops in the parotid after 16 hours, no effect is observed at that time in the liver. After 24 hours O_2-consumption of the liver is found to be increased, while the testicles, being further away from the brain, show an increase only 48 hours after the injection.

These experiments not only proved this peculiar wandering of thyroxine but also explained the long latent period of its action, a phenomenon similar to that called incubation in the case of infectious diseases. *This gave me the idea that the toxins of bacteria might have to travel the same way to be able to damage the cells effectively.*

We had first to test the supposition that toxins and viruses reach certain cells of organs by way of the nerves as thyroxine does, the different affinities of the nerve-cells to the different toxins and viruses thereby determining the localization of the disease. One fact which supports this possibility *a priori* is that infectious diseases are accompanied by fever which is known

[1] GUNNAR AHLGREN, Skand. Arch. Physiol. *47*, Suppl. 225 (1925).
[2] G. MANSFELD, Klin. Wschr. *14*, 884 (1935).
[3] G. MANSFELD and G. HORVÁTH, Pflügers Arch. *235*, 520 (1935).
[4] H. MEYER and FRED RANSOM, Arch. exp. Path. Pharm. *49*, 369 (1903).
[5] A. SCHITTENHELM and B. EISLER, Klin. Wschr. *6*, 1935 (1927).

[1] G. MANSFELD, Arch. exp. Path. Pharm. *193*, 241 (1939).

to be due to the toxins and viruses penetrating and stimulating certain cells of the brain. For the experimental tests of our working hypothesis, we chose the following method:— It is well known, that there are some drugs which can cure colds or other infectious diseases like polyarthritis, or may prevent them if given in proper time. These drugs are the salicylates or more generally the antipyretics, which act in the mid-brain on the heat-centres. It should be remembered that alcohol with its great affinity to nervous centres—and among these the heat-centres—is of great therapeutic value in case of some infections, puerperal fever for instance. Pharmacologists know that these drugs, if they have any bactericidal properties, display them only in concentrations that can never be attained inside the organism. But they are also acquainted with the type of drug-antagonism known as *displacement* (*Verdrängung*) that means that one drug expells another from the cell, or, when given in time, prevents the other one getting inside the cell. Thus it was no wonder that I first tried to prove my hypothesis this way.

At that time the latest news in medicine was the discovery of the action of sulfonamides (SA), and there arose a lively discussion whether these compounds are true chemotherapeutics, killing the bacteria themselves, or whether they cure in some other mysterious way called usually "increasing the resistance of the organism"? That the action of the SA is not directly bactericidal was clear to everyone versed in natural sciences, since the concentrations in which they have to be applied locally or *in vitro* to kill bacteria are about 8,000 times their curative concentration in the human organism. LEVADITI[1] was the first to suggest, that the action of the SA is directed *against the toxins* and not against the bacteria, and considering what was said above, we had to think *that the SA, being neurotropic substances themselves, enter the brain-cells and by blocking them exclude the toxins.* To begin with, I tested the correctness of my hypothesis in a model experiment, which proved decisive for the further work. If the SA can really block the brain-cells for other substances, then, administered in time and permanently, they should be able to prevent the effect of thyroxine, but should not influence the already developed thyroxine effect, as this takes place on the periphery after the thyroxine has arrived there along the nerves.

The following experiments confirmed this assumption in every point, but besides this they revealed another very interesting fact. It is known that different SA are not equally effective in different diseases, one being more effective against erysipelas, the other against pneumonia, the third against gonorrhœa. Examining the action of thyroxine on the liver, under the influence of different SA:— sulfapyridine (SPYR) proved to be ineffective, while paraaminobenzosulfamide (PBSA) completely inhibited the increase of O_2-consumption following thyroxine. I can only explain thie by assuming that while PBSA blocks the brain-cells through which thyroxine reaches the liver, SPYR leaves these cells unaffected, a fact that throws an interesting light on the problem of the specificity of these drugs.

Table I

O_2-consumption of liver-cells increased by thyroxine, in %

Without SA	Treated before thyroxine with		Treated 24 hours after thyroxine with PBSA
	SPYR	PBSA	
+ 30	+ 24	+ 7	+ 36
+ 39	+ 9	+ 12	+ 32
+ 24	+ 37	— 14	+ 34
+ 21	+ 13	— 19	Average
+ 42	+ 79	+ 9	+ 34%
+ 39	+ 33	+ 2	
+ 24	+ 24	Average	
+ 31	+ 40	— 3%	
+ 19	Average		
+ 14	+ 32%		
+ 35			
+ 26			
+ 27			
+ 23			
Average			
+ 27%			

Table I shows the effect of 1 mg thyroxine on O_2-consumption of liver-cells (rabbit) and the change of this effect caused by administering SA. The dose of SA was given first 4 hours *before* the administration of thyroxine and continued every 4 hours, day and night. Continuous administration is essential for obtaining positive results. This observation corresponds with clinical experience that only maintainance of a certain blood level secures satisfactory therapeutic results. The last column of the table shows that if we begin the administration of SA 24 hours *after* the injection of thyroxine, but otherwise just as in the other experiments, the action of thyroxine is not affected:—an unassailable proof that a simple destruction of the thyroxine effect is out of question.

Another interesting case of the specificity of SA was also found. As we saw, the thyroxine effect in the liver could only be prevented by the use of PBSA. SPYR, proved to be ineffective. It could be assumed that SPYR is altogether ineffective in preventing the development of the thyroxine effect. If, however, we repeat these experiments with the parotid and the testicles, we find the same SPYR that proved to be ineffective in the case of the liver, inhibiting completely any increase of O_2-consumption in the parotid and testicle cells—a very strong proof in favour of my theory that the specificity of the SA is due to a selective affinity of the different compounds towards different cell-groups of the brain, as is the case with bacterial toxins.

[1] C. LEVADITI and A VAISMAN, Presse médicale *45*, 1371 (1937).

Table II

Increase of O_2-consumption of liver, parotid, and testicle cells following administration of thyroxine after previous treatment with SPYR.

Liver		Parotis		Testis	
normal	SPYR	normal	SPYR	normal	SPYR
+ 30	+ 24	+ 105	+ 13	+ 13	+ 18
+ 39	+ 9	+ 34	— 9	+ 10	— 20
+ 24	+ 37	+ 28	+ 16	+ 18	+ 5
+ 21	+ 13	+ 73	+ 8	+ 9	— 22
+ 42	+ 79	+ 15	— 7	+ 12	— 19
+ 39	+ 33	+ 24	+ 28	+ 10	+ 17
+ 24	+ 24	+ 16	+ 6	+ 21	+ 6
+ 31	+ 40		— 4	+ 14	+ 8
+ 19		Average	— 5	+ 15	— 17
+ 35	Average	+ 42%			— 14
+ 26	+ 32%		Average	Average	— 15
+ 23			+ 4·4%	+ 13%	
+ 27					Average
					— 5%
Average					
+ 27%					

Similar experiments with antipyretics also gave very interesting results. 0·5 g of antipyrine, which inhibits or abolishes the rise of body temperature following puncture of the thermoregulatory centre of the brain (*Wärmestich*), proved to be completely ineffective on the liver effect of thyroxine. But the most interesting fact was that this effect could be prevented by microdoses of quinine—0·5 mg every four hours in rabbits—while doses of 0·10 g were ineffective, a fact which is the more interesting as it is universally known that only these microdoses have a preventive effect in the case of human influenza, for instance.

Table III

Increase of O_2-consumption of liver-cells following thyroxine administration, in %

Without previous treatment	Treated with		
	0·10 g quinine	0·0005g quinine	0·5 g antipyrine
+ 16	+ 62	+ 8	+ 33
+ 25	+ 36	+ 9	+ 123
+ 25	+ 28	+ 8	+ 35
+ 29	+ 32	+ 5	+ 29
+ 35		+ 9	
+ 66	Average	— 16	Average
	+ 39%		+ 55%
Average		Average	
+ 33%		+ 3%	

After these promising preliminaries we turned to the main-experiments to see whether we could inhibit or prevent the effect of *bacterial toxins* by SA. In these experiments filtrates of bouillon cultures were used which had been concentrated to $^1/_{10}$ of their original volume *in vacuo*. 2–3 cc of this preparation produced marked fever in rabbits. A combination containing 0·20 g of each of the three principal representatives of the SA:—PBSA, SPYR, and sulfathiazole, were administered to rabbits simultaneously with, or following an interval after the injection of toxins, and the effect on body temperature was observed.

Before I report on these experiments two problems have to be cleared up. First:—may not the SA paralyse the heat-centres directly and thus exhibit an antipyretic action like the antipyretics; secondly:—do the SA not bind the toxins chemically—that is *in vitro*—and thus inhibit their action without any blocking of the brain-cells.

The first problem was approached by investigating the effect of SA on fever caused by puncture of the thermoregulation centre. The following experiments show the results; it is obvious that the SA cannot influence the excitation of the heat-centre even in the massive doses mentioned (0·60 g).

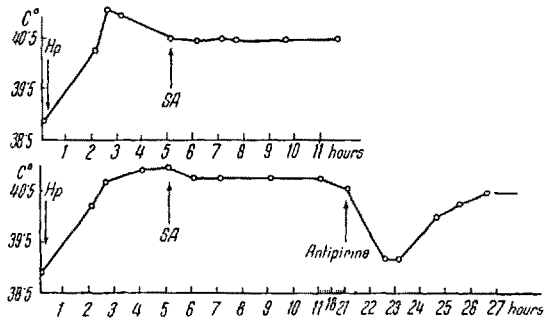

Fig. 2. – Puncture of the heat-puncture centre *Hp*.

Experiments aiming at detoxication of the toxins were also unsuccessful. In these we mixed the toxins with quantities of SA that we knew were too small to have any effect on the organism and kept the mixtures for 2–3 and sometimes up to 40 hours in the thermostat before injection. The results may be seen on the following charts.

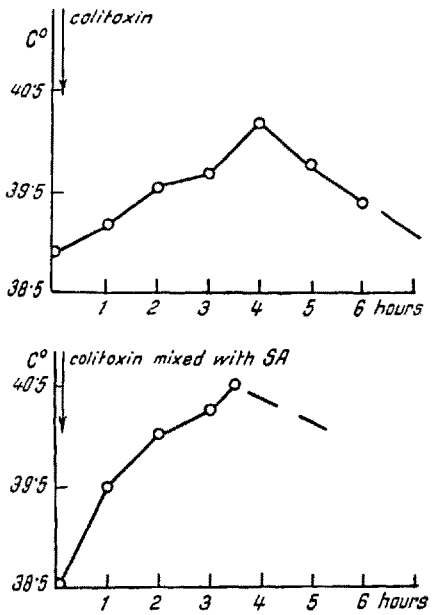

Fig. 3. – Effect of *in vitro* "detoxication" of colitoxin by SA. SA has no effect on pneumotoxin *in vitro*.

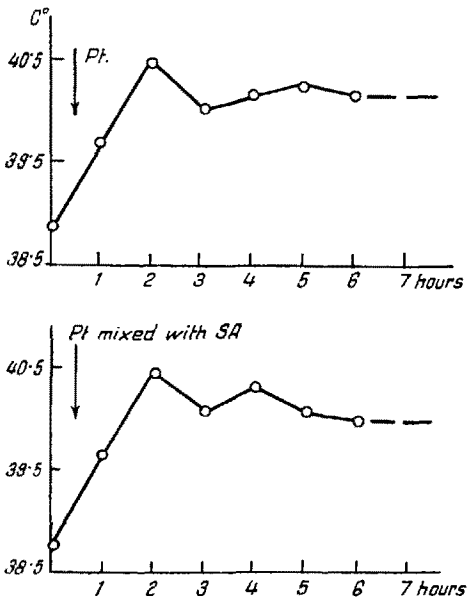

Fig. 4. Effect of *in vitro* "detoxication" of pneumococcus toxin (*Pt*) by SA.

The main-experiments were performed with 6 different toxins, obtained from coli, dysenteria, typhoid, streptococcus, staphylococcus, and pneumococcus strains. The SA proved to be effective against the colli streptococcus, and pneumococcus toxins, while they had no effect on staphylococcus, typhoid, and Shiga-Kruse toxins, a finding that corresponds with clinical

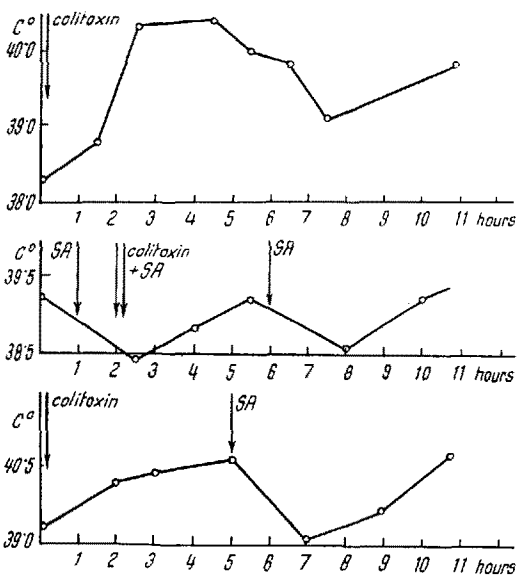

Fig. 5. Colitoxin

experience (Figs. 5–11). The SA were also totally ineffective in the case of tetanus and diphtheria toxins.

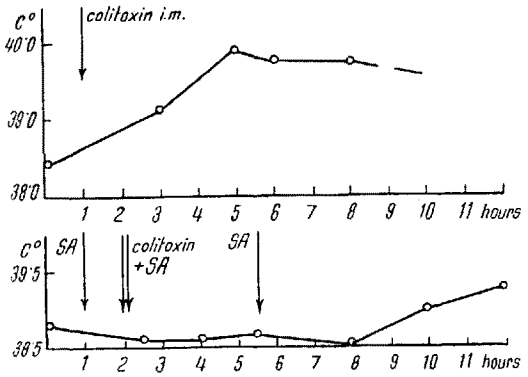

Fig. 6. Colitoxin

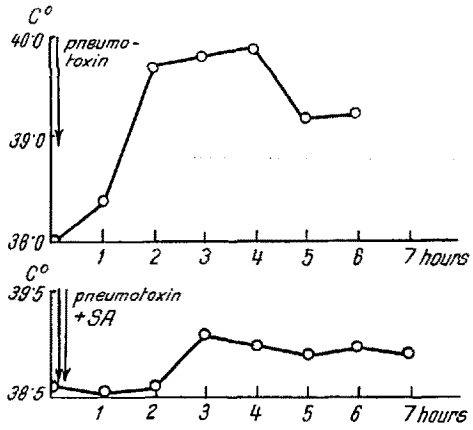

Fig. 7. Pneumococcustoxin

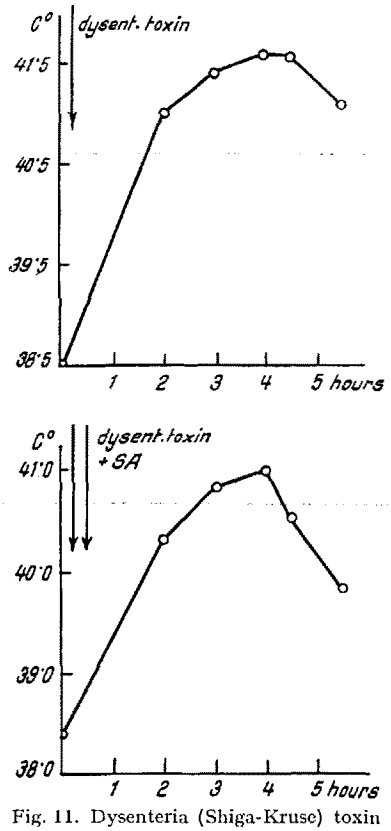

Fig. 11. Dysenteria (Shiga-Kruse) toxin

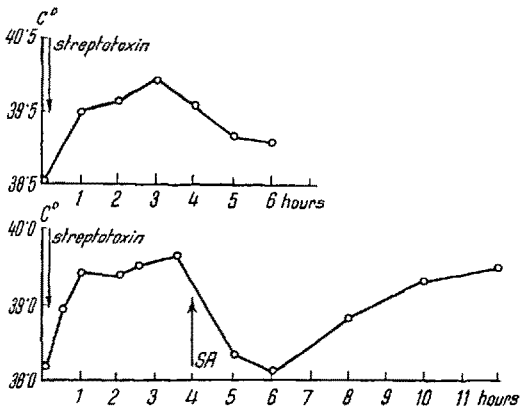

Fig. 8. Streptotoxin

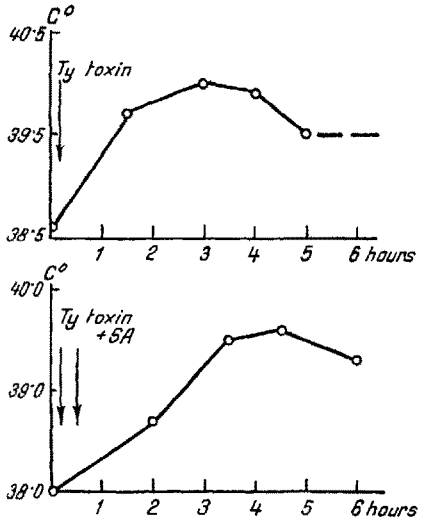

Fig. 9. Typhoid toxin

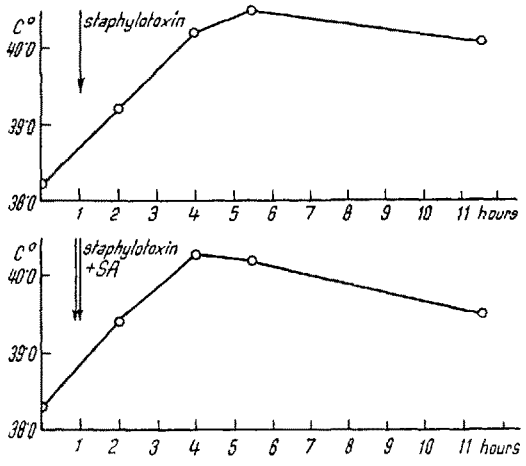

Fig. 10. Staphylotoxin

Our hypothesis that the antitoxic action of SA is located in the central nervous system and acts presumably by blocking the corresponding brain-cells for the toxins, was greatly strengthened by the following experiments:—

Minimal amounts (0·07 g for instance) of SA intracystically given inhibit completely the pyrogenic action of the colitoxin, while having no effect whatsoever on pyrexia due to puncture of the heat-centre (*Hp*).

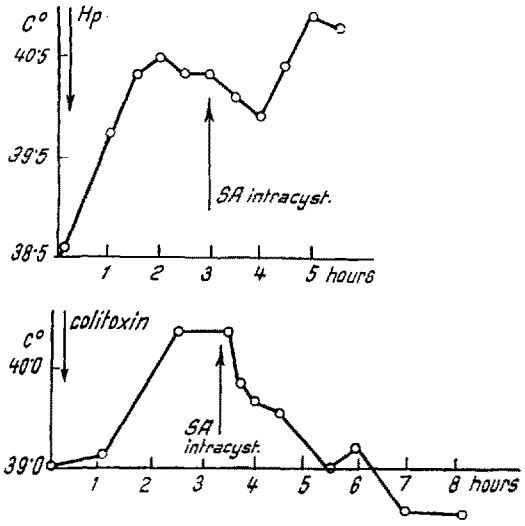

Fig. 12. SA intracysternally

II. *The difference between chemical and infectious inflammation*

We now turn to the question of whether a connection can be established between the genesis of an infection and the nervous system, with other words:—Can we find experimental proof of a decisive role of the nervous system in the genesis of infections, as expected by our hypothesis?

As the general reaction of the organism to pathogenic germs is inflammation, and necrosis is seen rather exceptionally, this question is closely connected with the problem of the role the nervous system—that is innervation—plays in inflammation. There is a discordance of views in this respect, yet careful analysis of the literature leads to interesting and important results.

The communication of the laryngologist SPIESS[1] of Frankfurt that inflammations heal quickly or do not develop at all if the sensorial nerves are severed attracted general attention. Thus the opinion became prevalent that of the four classical symptoms of inflammation *dolor* is the dominant one. This view was strengthened by a case of polyarthritis of GAISBÖCK[2], in which, following an intercurrent hemiplegia, the inflammation of the joints receded on the anæsthetic side. There was a somewhat similar case of EISELSBERG (personal communication) in which a transversal injury of the spinal cord, caused by a motor accident, was accompanied by an erysipelas, the margins of

[1] G. SPIESS, Münchner med. Wschr. *53*, 345 (1906).
[2] D. GAISBÖCK, Arch. klin. Wschr. *121*, 339 (1917).

which demarcated the border of the anæsthetic region clearer than any neurologist could have done.

These clinical observations were not in agreement with the findings of experimental medicine. BRUCE[1] investigated the problem experimentally by observing whether chemical substances that are known to cause inflammation, like mustard or croton oil, exert their action on tissues that have been anæsthetized by cutting the corresponding dorsal roots of the spinal cord, or which were completely disconnected from higher centres of the nervous system by transversal section of the spinal cord. The result of these investigations was that inflammation is not a reflex phenomenon and therefore in a very high grade independent of the central nervous system. This contradicted clinical experience and could be explained only by supposing that chemical inflammation and infectious inflammation are different processes.

But before I drew this conclusion, it seemed advisable to make sure whether inflammation can develop on denervated tissues. These investigations have been carried out by G. László in my institute on normal and denervated eyes of rabbits, with the result that chemical inflammation can be produced after extirpation of the ganglia Gasseri and even after degeneration of the sympathetic nerves.

This result seemed to me of great importance. The pathology of inflammation is well known, and we had no right to suppose that inflammation caused by dead matter differs from that caused by a living agent. The fact that, contrary to chemical inflammation, infectious inflammation does not develop if the nerves are severed, strongly supports our theory, according to which infectious viruses and toxins must wander along the nerves to the periphery to be able to attack the cells effectively. This conclusion was too far-reaching to be based solely on clinical observations, so that an experimental inquiry seemed necessary.

The most suitable object for this purpose seemed the small-pox vaccine, which is followed in rabbits unfailingly on the fifth day after vaccination by a characteristic pustule. We also know that rabbits once inoculated become immune against vaccine virus, and further inoculations remain ineffective. Our reasoning was, that if it is true that changes can be brought about by live germs only after they have reached the periphery through the nervous system, no pustule can develop on denervated skin. Our first experiments were performed on rabbits whose spinal cord had been sectioned in the cervical region. They were inoculated above and below this region, on their head and above their rump-bone.

The results were perfectly uniform and rather surprising. After the spinal cord has been severed at the level of the 7th cervical segment, we never saw a typical reaction, that is, a pustule in the lower region. There was sometimes a local reaction, mostly reddening of the skin, and in more severe cases necrosis of the superficial layers of the epidermis, but not once did we see pustules. As it might be conceivable that inoculation on the head might influence the result of the inoculation above the rump-bone, in some cases we inoculated only above the rump-bone, with the same result.

This method had its drawbacks. Section of the spinal cord is a rather severe operation, a great proportion of the animals die in post-operative shock, and their maximum term of life is 3–4 weeks; they have to be looked after constantly (e. g. catheterized), and the minor local reactions that were sometimes seen could be caused by the fact that the site of section, and even the origin of the nerves, is never quite the same in two animals. Therefore in the following my aim was to leave the central nervous system intact and denervate a part of the skin completely, dividing even the perivascular nervousc ommunications. We succeeded in doing so on the consideration that it takes months for a severed nerve to grow again into the damaged tissues, while the severed vessels do so in a considerably shorter time. We denervated a circular region of skin of 6–8 cm diameter over the rump-bone of a rabbit which was cut out in two sittings as shown in Fig. 13.

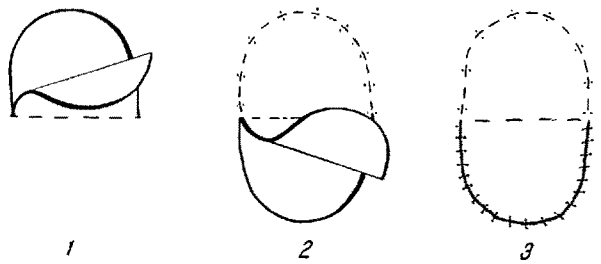

Fig. 13.

In the first operation the skin is cut in a semicircle and laid free with the subcutis down to the meridian (Fig. 13, No. 1), whereby blood-vessels and nerves coming from above the meridian are severed. This flap of skin is then laid back in its original position and sewn on as shown in the upper half of Fig. 13, No. 2. Then, after a pause of two weeks' time, the same operation is made on the lower half of the circle (Fig. 13, No. 2, lower half). Fig. 13, No. 3, shows the condition after the second operation.

The pause of two weeks is necessary, so that the blood supply of the upper semicircle can be reestablished from above, otherwise the cutting through the lower flap of skin would cause necrosis, as is the case if the whole circle is cut through at one time, freed and at once sewn on again.

Thus we were able to pursue our experiments on a very great number of rabbits, till the problem was wholly solved.

[1] N. BRUCE, Arch. exp. Path. Pharm. *63*, 633 (1910).

First we repeated the experiments described above with the same result, but in this series not even the slightest local reaction was ever seen. The crosses drawn with the lancet remained clearly discernible throughout.

These experiments proved beyond doubt that small-pox develops only on skin connected by nerves to the central nervous system.

This result seemed so important that we had to reexamine the reaction of perfectly denervated skin to chemical inflammatory agents. We applied strips of charta sinapisata and emplastrum cantharidatum on the border of denervated and normal skin to see whether there is any difference between the reaction of the two regions. As a result we saw flush, inflammatory œdema, and sometimes blisters develop in both regions. But we were not content with that either. EBBECKE[1] was the first to describe that if we inject histamine into the skin and then inject a negatively charged colloidal dye like congo-red or trypane-blue intravenously, the histaminized skin stores the dye and changes its colour. This phenomenon was confirmed by many authors, but while EBBECKE and HOFF[2] saw its cause in the increased permeability of the vascular walls, LESZCZYNSKI[3] and later JANCSÓ[4] recognized it as being essentially an action of histamine on the histiocytes, the importance of which in the defensive role of inflammation was stressed by JANCSÓ. Thus the question of whether the accumulation of dyes following histamine takes place also in denervated skin became of particular importance. Our experiments answered it in the affirmative, as denervated and normal skin accumulated congo-red identically following the administration of histamine.

Having proved that the denervated skin reacts normally regarding its readiness for inflammation, we felt we were entitled to draw from our vaccine experiments the somewhat bold conclusion that the central nervous system plays an essential part in the development of vaccine pustules.

I must mention here that the neurotropy of small-pox virus is long known, there are countries like Spain, where vaccination is performed not with vaccine won from the udder of a cow but from the brain of rabbits inoculated with this virus.

There are two possible explanations for the inactivity of small-pox vaccine on denervated skin:—(1) either the vaccine wanders through peripheral nerves centripetally into the brain and from there returns to the skin again by way of the nerves, or (2) it passes through the lymph and blood into the nervous system and from there it reaches the inoculated skin sites by way of the nerves. The second alternative seems the more probable, since CALMETTE and GUERIN have shown that if they inject vaccine virus intravenously and make a skin wound somewhere, the pustules occur on the wounded place.

To see which of the two possibilities is the right one, it must be tested whether an immunity occurs after inoculation on the denervated skin. For, if the inoculation is inactive after the denervation because the virus is not carried any further, then no immunity should occur. If, however, in spite of inactive inoculation, an immunity does occur, then it must be assumed that the virus has spread itself through the body, and the action has not appeared because, owing to lack of nerve-paths, it could not reach the periphery.

Our experiments led to the result that 10 days after the unsuccessful inoculation on the denervated skin the animals all became immune, since a second inoculation in any normal piece of skin was inactive.

Since the inactivity of the first inoculation was caused by the nerves being severed, the conclusion is justified that for the development of a pustule the vaccine virus must apparently penetrate the nervous system in order to damage the cells by way of the nerves. The fact that, after inactive inoculation, immunity can result, suggests that the nervous system plays a role in immunity, a point to which I shall return later.

First I wish to report some experiments which show a similar result to those just described. To see whether the prevention of the disease by denervation accompanied by immunity only occurred with vaccine virus, I wished to repeat the experiment with a second pathogenic germ and with a bacterium. To do so turned out to be more difficult than expected because neither dermatologists nor bacteriologists nor veterinarians know any bacteria that cause inflammation on the skin of rabbits. After a number of unsuccessful experiments I received from Prof. FENYVESSY a pneumococcus strain isolated from the pus of an abscess which caused an erysipelas-like inflammation on the skin of rabbits. Experiments with this strain led to the same result as those with vaccine virus:— on denervated skin, after its vascularization and therefore with its complete vitality has been restored, one could not provoke inflammation, but 8–10 days later the normal skin of the animal was immune as well.

III. *Experiments on the possible role of the nervous system in the genesis of immunity*

The marvellous discoveries of the facts of immunity are certainly one of the prides of medical research, and if we study the critical survey of the actual material

[1] U. EBBECKE, Klin. Wschr. *2*, 1341 (1923).
[2] T. HOFF, Z. klin. Med. *102*, 745 (1926).
[3] LESZCZYNSKI, IIIe Congrès de l'Union des dermatologistes slaves (Prague 1934). Quoted from: ST. L. KWIATKOWSKY, Arch. f. Dermat. u. Syphilis *171*, 444 (1935).
[4] N. JANCSÓ, Magyar Orvosi Archivum *42*, 367 (1941) and Orvosi Hetilap *85*, 386 (1941).

in a recently published work of R. DOERR[1], we can see how modern methods of research, such as electrophoresis, electron microscope, use of heavy nitrogen (N^{15}), etc., have advanced our knowledge of the mode of genesis and the nature of the immune body. But if we look at these fundamental researches from the point of view of the physiologist, we recognize that some important points need further investigation, for example whether the much-studied immune body of blood serum really increases our understanding of immunity—congenital or acquired—? It is more than questionable whether this immune globulin of the blood serum is in effect the carrier of what is often a life-long immunity. But the new results of research leave no doubt that the immune globulin arises not in the blood but in the cells of organs, and it is certainly unfortunate that immune biology has somewhat neglected the problem of immunity itself in comparison with the study of the serum immune body. This defect is grounded on technical reasons, but we must not fall into the same error as histology once did in believing that the most clearly visible and most easily stained parts of the cell were the most important for the life of the cell. It should never be forgotten, as DOERR[1] expressly notes, that we can only investigate the so-called *humoral* factor of the antibody that arises in the body, while the second factor, the "sessile" or "cell constant" antibody is hardly considered owing to the technical difficulties, and we content ourselves with the assumption that "no important differences between the two forms need be assumed" (DOERR, l. c. p. 15).

Even if this is true, we must still raise the question, whether this sessile antibody is not the actual carrier of immunity. In regard to our experiments already described on the role of the nervous system in the genesis of infectious diseases, its role in the genesis of immunity should also be tested. It seems not improbable, and in any case worth testing, that the sessile antibodies are formed in the brain-cells under the influence of toxins and viruses, thus preventing a further, or rather a later, entrance of more of the poisonous substance, and that this blocking of the brain-cells would produce the immunity. That the central nervous system had something to do with immunity or, to be more exact, with the formation of antibodies, has been suggested by many authors before myself, to mention only BESREDKA[2], REITLER[3], BELÁK[4], SPERANSKY[5], and SCHLAUK[6], and I would not pass over the fact that the hypothesis described above is very similar to the so-called MAGRASSI[1] phenomenon, which led to DOERR's[2] concept of *Schienenimmunität* (rail-immunity). According to this the mild or mitigated virus of herpes, which causes no encephalitis, hinders the encephalitogenic virus in reaching the brain when applied previously to the skin or cornea, because the mild virus wandering along the nerve robs this nerve, the "rail", of its ability to conduct the virus.

But we had still to test whether the antibody circulating in the blood and body fluids plays in fact the determining role usually ascribed to it in the genesis of immunity.

I approached this problem by the following experiments. Vaccinating a rabbit (A) in the usual way against small-pox, we waited till the reaction receded and the animal became immune, which normally takes 10 days. After waiting another day or two to be sure that there was no more virus present in the immune animal, it was brought into parabiosis with another animal (B) of the same litter *with communicating peritoneal cavities and the omenta cut and sewn to one another*. After 3–4 days both animals were inoculated with the result that no reaction was found—naturally—on the already immunized animal (A), but the animal B did not show the slightest trace of immunity, though living in joint circulation with an immune animal for days.

To see whether the parabiosis had really ensured the exchange of blood between the two animals, we first made the parabiosis in two pairs of animals and then inoculated the one animal A with vaccine virus and three days later separated the two animals again. After 8–10 more days, the second animal (B) was inoculated but without result, a sign that the virus with which the animal A had been inoculated had gone over with the blood to animal B and had immunized it. Thus we are justified in drawing the conclusion, from the first series of experiments carried out on three pairs of animals, that the small-pox immunity does not pass humorally to a second animal.

This experimental result has, however, a precursor. GRASSET described in 1902 (Gaz. méd. de Paris) that one of the Siamese twins whose circulation communicated suffered from severe tuberculosis while the other remained perfectly healthy. GRASSET concludes from this:—"...le grand appareil de défense n'est pas l'appareil circulatoire, mais le système nerveux", thus stating 46 years ago that the nervous system plays a part in immunity.

GRASSET had of course no right to draw this conclusion, for his clinical observations did not give the slightest grounds for believing that immunity had anything to do with the nervous system.

[1] R. DOERR, *Die Immunitätsforschung*, Vol. 1, Antikörper. Teil 1 (Springer, Wien, 1947).

[2] A. BESREDKA, Handb. Technik u. Methodik d. Immunitätsforschung, Suppl. 1 (1911).

[3] R. REITLER, Wiener klin. Wschr. *37*, 267 (1924).

[4] A. BELÁK und L. CSERESZNYÉS, Z. exp. Med. *52*, 572 (1926).

[5] A. D. SPERANSKY, *Eine Grundlage für die Theorie der Medizin*, Allg.-path. Schriftenreihe, H. *2*, 18–37 (1941); Kongreßblatt f. d. ges. innere Med. *112*, 196 (1942).

[6] A. SCHLAUK, Deutsche med. Wschr. *64*, 1407 (1938).

[1] F. MAGRASSI, Boll. Acad. med., Roma *62*, 53 (1936).

[2] R. DOERR, Klin. Wschr. *15*, 1062 (1936).

Having regard to our experiments, which showed that the nervous system was important for the occurence of infection, we are now more justified in supposing that immunity operates in the nervous system. But we content ourselves with drawing from our parabiosis experiments the negative conclusion that acquired small-pox immunity is not transferable humorally and we have tried in another way to prove the significance of the nervous system for immunity:—

Our first reported experiments showed that a displacement of thyroxine, and also of the toxins, from the brain-cells is possible through those chemical substances which cure the corresponding diseases, namely the SA the neurotropy of which was first emphasized by MARX. If the nervous system should be the site of immunity, one might imagine that *in the brain-cells of immune animals sessile antibodies make the entrance or effectiveness of toxins and viruses impossible.*

Two facts support this possibility. We know that many infectious diseases, such as the well-known children's diseases, and also spotted fever, yellow fever, etc., leave a life-long immunity. Immunology supposes that in these cases the "immune bodies", which can no longer be traced in the blood, are "anchored" to the cells of different organs and are liberated in case of emergency. But immunologists forget that the cells of the organism perish continually and are replaced by new ones, so that in later years there is not even a trace of the cells to which in childhood the immune bodies might have become attached. The physiologist on the other hand knows that there is one organ, the cells of which are unable to regenerate, and this is the brain. Its cells escort us from the cradle to the grave. It is very interesting that an old master of immunology once likened immunity to memory, little knowing how near he came perhaps to the essence of this phenomenon.

The second fact which suggested the role of the nervous system in immunity was the effectiveness of PASTEUR'S protective inoculation against rabies, even when the virus is killed in the brain, as we now know.

It therefore had to be tested whether animals injected with brain substance were protected against the same disease instigator. For these experiments we used rabbits, and as infectious material always the same strain of bovine tuberculosis bacillus. The results of our first series of experiments were astonishing:— we injected 10 rabbits intravenously with 1·5 mg each of our very virulent Tb bacillus, and we killed them after 2 months. The lungs showed very severe tuberculosis. We then made a 50% emulsion of the brain, which was removed sterilely, in a sterile solution of sodium chloride. The emulsion, like all other brain substances which we used, was tested by experiments on guinea-pigs and found to be free of Tb bacilli. Then 14 rabbits were given *a single dose only* of 2 cc of brain emulsion subcutaneously, and 3 weeks later they were each given 1·5 mg Tb bacilli intravenously of the same strain with which the brain donor was injected. Out of 14 animals, 12 died within 3–10 days without demonstrable changes in the organs.

Control animals, which had not previously been treated, remained alive and only died of tuberculosis after some months. This seemed to us important, for it suggested that the brain of the infected animal contained something which reacted against the Tb bacillus.

In order to test whether it was not a coincidence, I repeated the experiment with coli bacilli in the following way:—3 rabbits were given an intravenous injection of living coli bacilli. The thickness of the emulsion corresponded to that used for vaccinations. The animals reacted with high fever (up to 42°C) but showed no other symptoms of disease. After 14 days, they were killed and the brain prepared, with the addition of 0·3% tricresol, as a 33% emulsion and injected subcutaneously into 3 rabbits in a single dose. After 3 weeks the animals were given an intravenous injection of the same coli culture as the brain donor. The result was that, out of 3 animals, one died 2 hours after the injection, the second one 24 hours after, and the third survived. Here also there seemed to be some substance in the brain of the infected animals which made the receiver animals over-sensitive to the coli bacilli used. That the brain substance itself does not cause over-sensitization we shall see later.

The fact that the brain substance of tuberculous animals apparently contains an antigen or antibody that reacts with the bacilli in the organism of the animal prompted us to repeat our Tb experiment in such a way that we injected the brain emulsion every second day for 3 weeks to desensitize the animals. The brain was from rabbits infected intravenously with Tb bacilli 2 months before their death, and all without exception showing very severe changes of the lungs. The 33% brain emulsion with 0·3% tricresol added was shown by tests on guinea-pigs to be free from Tb bacilli. Our brain emulsions were sufficient for 10 animals which each received 2 cc per dose, that is to say within 3 weeks each animal had a total amount of 20 cc with about 6 g of wet brain substance. One animal died during the injections. At the end of the pre-treatment, the 9 previously treated and 12 control animals (of which one died) were infected intravenously with 1·5 mg of Tb bacilli each. All 20 animals were killed after 2 months, at a time when experience shows that the disease is fully developed. When evaluating the results of these experiments, it should be remembered that the infection was carried out with very massive doses (suspension of 1·5 mg Tb bacilli intravenously), producing without exception most severe alterations in the lungs, which seem to be strewn with tubercles. The severity of the process has been marked

with 3–5 crosses according to number and size of the tubercles and the degree of proliferation, while disseminated tubercles have been marked with two, and single tubercles with one cross (see Table IV).

Table IV

Untreated rabbits	Rabbits treated previously with brain emulsion of tuberculous rabbits
1. ++++	1. +
2. ++++	2. ø
3. +++	3. ++
4. +++	4. +
5. ++++	5. +
6. ++++	6. ++
7. +++++	7. +++
8. +++++	8. ++++ +
9. +++++	9. +
10. ++++	
11. ++++	

The difference is very marked and suggests that the brain of infected animals when administered over a longer period can produce a protective action in the majority of cases.

In view of the fact that this experimental series was too small to permit a final conclusion, I had the intention of making a further series of experiments with 50 rabbits. But for this an equal number of brain donors and control animals would be necessary, making 150 animals in all. In order to save animals within 2–3 months' time, I used the brain of severely tuberculotic beasts from the slaughter-house, the brains of one buffalo and one ox being prepared as an emulsion in the laboratory of the National Serum Institute in Budapest, in the same way as brains of sheep used for lyssa inoculation. This brain substance which was found in tests on guinea-pigs to be sterile, was now injected subcutaneously every second day for 3 weeks into 45 rabbits, while 5 rabbits were given one single dose of brain emulsion subcutaneously.

I shall first report on these last 5 animals, which only received a single dose of brain emulsion. Three weeks after this pre-treatment, they received 1·5 mg intravenously of Tb bacilli of the bovine strain which we usually used. In contrast to the experiments described previously, in which the brain donor and receiver were infected with the same bacillus culture, all 5 animals remained alive. Repetition of the experiment on 5 guinea-pigs gave the same result.

This experiment suggests that even amongst the bovine strains different types of Tb bacillus exist, and that the over-sensitization achieved with the brain substance in the previous experiments seems to be type-specific. No less interesting was the fate of the 45 previously treated rabbits which had received brain emulsion from the slaughter-house beasts over a period of 3 weeks.

After the pre-treatment, these animals, together with an equal number of control animals, were injected intravenously with 1·5 mg of our Tb bacilli. *The brain substance of the slaughter-house beasts had not produced a protective action in one single case.* Previously treated and control animals all showed equally the most severe alterations of the lungs.

It seems, therefore, that a protective action is only to be expected from such brain substance as is also able to produce over-sensitization with a single dose, and that both characteristics are apparently type-specific.

These experiments show also that the brain substance *per se* has no protective action, but only when it is gained from animals previously treated with disease instigators, and only, as we saw, with those with which the brain receiver was infected.

The discovery that the brain of infected animals contains type-specific substances must in any case be recognized as a step forwards. Admittedly it has still to be proved whether only the brain or also other organs contain such substances. If it should be found that this is a characteristic of the brain alone, it would be a strong support for our supposition that the central nervous system plays a central part in both the genesis of infectious diseases and in the process of acquired immunity.

Whether our experiments point in the right direction for a generally applicable organ therapy for infectious diseases, such that the Pasteur lyssa inoculation would be only a special case of a general law, cannot as yet be said. In any case, we see a new path opening for the testing of so-called sessile immune bodies, which have as yet been too much ignored by research on immunity. There seems to be no reason why bactericidal toxins or viruses taken up by the brain-cells should not transform certain protein substances, as has been shown in the case of the globulins of blood serum. From the total picture of our experimental results, it seems probable that the brain has a greater importance than hitherto suspected in the process of infection and of immunity, and that closer research on the sessile antibodies, especially of this organ, might lead to long-desired completion of the very successful serum research, and thus to the solution of many puzzling problems in our knowledge of immunity.

Zusammenfassung

Es wird zunächst die Arbeitshypothese geprüft, ob nicht die Entstehung und Lokalisation infektiöser Krankheiten erst dadurch entsteht, daß Bakterientoxine und Viren ähnlich dem Thyroxin von bestimmten Hirnzellen aufgenommen werden (Fieber!) und durch periphere Nerven in die Organzellen gelangen, um dort durch ihre Schädigung die Lebensbedingungen für die Krankheitserreger zu schaffen. Es konnte Folgendes gezeigt werden:

1. Sowohl die Thyroxinwirkung als auch jene der Bakterientoxine werden durch zentrale Wirkung der

Sulfonamide (SA) vernichtet. Für die zentrale Wirkung spricht, daß sehr geringe Mengen intrazystal gegeben volle Wirkung entfalten. Entsprechend den therapeutischen Erfahrungen waren die SA nur gegen die Toxine von Koli, Pneumokokken und Streptokokken wirksam und versagten vollkommen gegenüber Staphylococcus-, Typhus- und Dysenterietoxinen.

2. Während an vollkommen entnervten Hautpartien – wie bekannt – chemische Entzündung zustandekommt, konnte sowohl durch Pockenvakzine als auch durch einen hautentzündungserregenden Pneumokokkenstamm keine Entzündung erzielt werden, was dafür sprach, daß nicht die Entzündung selbst, sondern die Infektion durch die Entnervung verhindert wird.

3. Die weitere Tatsache, daß sich trotz Ausbleibens der Infektion Immunität entwickelte, führte zu der Möglichkeit, daß der eigentliche Sitz der Immunität jene Hirnzellen sind, von welchen das Toxin aufgenommen wurde und daß die in diesen entstandenen sessilen Antikörper das spätere Eindringen, also das Wirksamwerden des Toxins verhindern.

4. Durch Parabioseversuche an Kaninchen, welche zeigten, daß bei gemeinsamem Blutkreislauf an zwei Geschwistern die Impfung des einen Tiers auch im zweiten Immunität hervorruft, wurde diese Annahme gestützt. Wird aber ein schon immunisiertes Tier mit einem zweiten zusammengenäht, so erlangt letzteres trotz gemeinsamen Kreislaufes keine Immunität.

5. Zur Prüfung, ob in den Hirnzellen «sessile Antikörper» vorhanden sind, wurde Hirnsubstanz von tuberkulösen Kaninchen an normale Tiere verimpft. Dabei zeigte sich:

a) *Einmalige* Injektion der (sterilen) Hirnsubstanz macht die Tiere spezifisch gegenüber jenem Tbc-Stamm mit welchem das Spendertier infiziertwurde, überempfindlich. Dasselbe konnte mit dem Hirn koliinfizierter Kaninchen erzielt werden.

b) Nach *wiederholter* Impfung mit Hirnsubstanz (3 Wochen lang jeden 2. Tag) tuberkulöser Kaninchen zeigten dagegen unsere Versuchstiere entschiedene Resistenz gegen eine nachfolgende Infektion, aber nur in dem Fall, wenn die Infektion mit dem gleichen Tbc-Stamm erfolgte, der auch für die Infizierung der Hirnspender verwendet wurde. Es scheint demnach nicht ausgeschlossen, daß die PASTEURsche Lyssaimpfung nur einen speziellen Fall einer allgemeinen Gesetzmäßigkeit darstellt.

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

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Die Dehydrierung primärer und sekundärer Alkohole mit N-Chlorsuccinimid

Kürzlich berichteten HEBBELYNCK und MARTIN an dieser Stelle[1] über die Chlorierung von Toluol und der Xylole mit N-Chlorsuccinimid. Diese Autoren beobachteten ferner die dehydrierende Wirkung dieser Verbindung auf Benzylalkohol und Benzhydrol, wobei sie in guter Ausbeute Benzaldehyd bzw. Benzophenon erhielten. Da in unserem Laboratorium seit einiger Zeit die Verwendung von N-Chlorsuccinimid zur präparativen Herstellung von Aldehyden und Ketonen studiert wird, sehen wir uns durch die erwähnte Mitteilung veranlaßt, jetzt schon über unsere bisherigen Untersuchungen zu berichten.

Zwecks Auffindung neuer Methoden zur Dehydrierung primärer und sekundärer Alkohole haben wir eine Reihe von N-halogenierten Säureamiden auf ihre Eignung als Dehydrierungsmittel untersucht. Wir fanden, daß N-Bromsuccinimid in Tetrachlorkohlenstofflösung Benzylalkohol in guter Ausbeute zu Benzaldehyd dehydriert, besonders in Gegenwart von Pyridin, welches zur Bindung des entstehenden Bromwasserstoffs dient. Da die bekannte bromierende Wirkung des N-Bromsuccinimids auf ungesättigte Verbindungen für unsere Zwecke störend in Erscheinung trat, untersuchten wir das N-Chlorsuccinimid, welches nach ZIEGLER[1] keine analoge Chlorierung zur Folge hat.

Die oxydierende Wirkung von N-Chlorsuccinimid wurde schon vor langer Zeit von SELIWANOW[2] beschrieben. Dieser Autor stellte fest, daß primäre und sekundäre Alkohole, nicht aber die tertiären, von diesem Reagens angegriffen werden und schlug dieses unterschiedliche Verhalten zur Erkennung der tertiären Natur von Alkoholen vor, ohne aber irgendwelche Einzelheiten bekanntzugeben. Daß auch andere N-halogenierte Säureamide auf Alkohole dehydrierend wirken können, geht aus einer Beobachtung von CHATTAWAY und ORTON[3], welche unter den Produkten der Einwirkung von N-Bromacylaniliden auf Äthanol Acetaldehyd nachwiesen, hervor. Ferner nahm TSCHERNIAK[4] auf Grund seiner Versuche an, daß die oxydierende Wirkung der N-Chlor-Säureimide, wie N-Chlorsuccinimid und N-Chlorphthalimid, nicht auf die vorübergehende Bildung von unterchloriger Säure zurückzuführen sei, wie es SELIWANOW annahm, sondern daß diese Verbindungen Oxydationsmittel für sich seien.

Wir sind damit beschäftigt, die dehydrierende Wirkung von N-Chlorsuccinimid auf primäre und sekundäre

[1] M. F. HEBBELYNCK und R. H. MARTIN, Exper. *5*, 69 (1949).

[1] K. ZIEGLER, A. SPÄTH, E. SCHAAF, W. SCHUMANN und E. WINKELMANN, Annalen *551*, 80 (1942).

[2] TH. SELIWANOW, Ber. Dtsch. chem. Ges. *25*, 3617 (1892).

[3] F. D. CHATTAWAY und K. J. P. ORTON, Ber. Dtsch. chem. Ges. *32*, 3573 (1899).

[4] J. TSCHERNIAK, Ber. Dtsch. chem. Ges. *34*, 4209 (1901).