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ON POISONS AND DISEASE AND SOME EXPERIMENTS WITH THE TOXIN OF THE BACILLUS TETANI.[1]

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II

EXPERIMENTS WITH THE TOXIN OF THE BACILLUS TETANI[2]

I MAY now be permitted to give a brief account of some experiments with the causative principles of the disease known as tetanus or lockjaw, a truly frightful disease of the central nervous system terminating in exhausting and fatal convulsions. It is fortunately one of the rarer diseases of man, but in time of war and on certain occasions, such as Fourth of July celebrations, it occurs more frequently. It has long afflicted the race, and historians of medicine find it described in the Hippocratic writings and other early sources of medical knowledge. Of all our domestic animals, the horse is more subject to it than we, and the loss of these animals from tetanus, more especially in tropical countries, is considerable.

Until 1884 the cause and true nature of the disease remained unknown. In that year two Italian investigators demonstrated the transmissibility of the disease to animals by injecting them with a little purulent material from a small lesion of an individual with a fatal attack of tetanus. In the years 1884–1890, bacteriologists definitely established the infective nature of the disease by isolating the causative organism in pure culture. One ordinarily speaks of this organism as the bacillus tetani, but it is known to the specialist as clostridium tetani, one of the family of

[1] Address of the president of the American Association for the Advancement of Science, Boston, December 27, 1933.

[2] This part of the address was not presented in its entirety.

the bacillaceae of the general class of plant organisms known as Schizomycetes.

My associates, Dr. B. Hampil, of the Department of Bacteriology of the School of Hygiene and Public Health of the Johns Hopkins University, Professors F. C. Lee and W. M. Firor, of the surgical department of the Johns Hopkins Medical School, Mr. E. A. Evans, Jr., and I have been occupied for more than a year with experiments relating to certain aspects of this disease.

As there is only time for a very concise presentation, I shall summarize the main characteristics of the disease and the results of our own experiments and those of others in the following pages.

(1) That the disease may appear, the bacillus tetani or its spores must be introduced beneath the skin. Here the bacillus readily multiplies, if it meets with the proper conditions for its growth, such as association with relatively harmless pus-forming organisms as is generally the case, and a diminished tension of oxygen, or a necrosis of cells when spores only are introduced. The bacilli are not disposed throughout the body but continue to multiply for a time at the site of inoculation. Only very rarely and under special circumstances are they or their spores found elsewhere in the body.

(2) During the time of bacillary growth in the infected area a highly poisonous substance called tetanus toxin appears. This exceedingly potent, water-soluble substance is now known to be the true cause of the disease. A few bacteriologists believe that the bacillus does not itself produce a toxin, but rather a relatively harmless pro-toxin, which is transformed into the deadly toxin in culture media or at the site of infection in the presence of albumose-like substances. Nothing whatever is known of the chemical nature of the toxin. It is being constantly removed from its place of origin by lymphatic vessels and by the blood capillaries and is then distributed throughout the body by the arterial current. A boy with "lockjaw" is as truly poisoned as if he had been bitten by a rattlesnake.

(3) It is thought that this toxin of entirely unknown character finally reaches certain cells of the spinal cord and brain and by an action on them induces generalized convulsions of the most violent nature. The disease is now generally defined as clearly and solely one of the central nervous system. Certain of its symptoms, however, as the so-called local tetanus and the long-continued rigid states of certain muscles, lasting for weeks or months, that are occasionally observed in man, can not be brought into line with this concise definition of the disease. I can not enter here into a more detailed and accurate description of the course and extent of the alterations that finally affect the nervous and muscular systems.

(4) Our toxin, which is known only by its action in the animal organism, and this is true also of many other principles of its class, is of an exceedingly great potency, excelled only by the botulinus toxin found in meats and canned vegetables infected with the botulinus bacillus (Botulus—a sausage). It has been found that crude precipitates of the tetanus toxin will kill a mouse weighing 15 grams in the minute quantity of 0.00000005 gram. I think it more than likely that such precipitates contain only about 10 per cent., or even less, of the poison. Assuming the sensitivity of man to be equal to that of the monkey, which again is about equal to that of the highly sensitive horse, I may hazard the calculation that it would require only 0.01 mgm of the really pure poison to kill a man weighing 150 pounds, on the supposition that my calculations have any factual basis.

(5) During the entire time the toxin is being absorbed from its place of production, and often for some days following the cessation of its production, or after the excision of the infected organ as in animal experiments, there is observed a so-called period of incubation, that is to say, a time during which there are no observable signs of illness. This time of incubation varies in human beings and in such very susceptible animals as the horse from a few to many days. When this period of incubation in human beings is not more than five or six days, the mortality is nearly 90 per cent.; when it is longer, say 10 days or many more, the mortality is still about 50 per cent. The period of incubation in the very susceptible animals is considerably reduced when larger doses of the toxin are injected into them or when, in the natural disease, a very large amount of the poison is produced in the original lesion. It is never in any case, except after the intravenous injection of immense doses, reduced to less than three to four days. In the relatively insusceptible rabbit, in which the period is about 18 hours, it can be shortened by a few hours only with enormously large doses of the toxin. French observers have found that the subcutaneous injection of thirty thousand minimal lethal doses shortened the period of incubation by four hours, while the enormous dose of ninety thousand fatal doses shortened it by two hours only. Long periods of incubation are not confined to the infectious diseases. Pharmacological literature contains many examples of this phenomenon after the injection of poisons of known chemical nature, and in the forage diseases reported from South Africa we find examples of "periods of incubation lasting as long as eighty to ninety days in horses, after the last occasion on which the poisonous plant was eaten."

(6) By what mechanism is the toxin transported to the reacting cells of the spinal cord and brain? Is it taken up by them from the blood stream, as is the

case with alcohol, numerous alkaloids and many other substances? One of the most revolutionary theories ever proposed in medicine asserts that the toxin can not be taken up from the blood stream by the reactive cells of the central nervous system, but can be absorbed by them only when (1) it is transported to them in the nerve fibrils or axons of the motor nerves, or (2) when it is transported to them in the endo- and peri-neural lymphatic vessels of these nerves. According to these theories, then, the motor nerves of the vertebrates have the hitherto unsuspected ability to transport a soluble substance from the periphery into the spinal cord. Some of the latest converts to the theory believe that the cerebrospinal fluid also receives its share of the toxin. The experiments that have been made during the past thirty years or more in support of this nerve transport theory have been so well designed and so skilfully performed by men of the highest standing, and have appeared to be so valid and irrefutable, that this unusual and exceptional mode of transport of a soluble substance has been universally accepted as an entirely satisfactory explanation of the facts of the case. Speransky and his coworkers, Ponomarew and others, have recently performed a great number of ingenious experiments with the toxin and have interpreted their findings in a manner that encouraged them to announce a third variant of the neural transport theory. They appear to be firmly convinced that the toxin and other substances that may be absorbed by the terminals of a mixed nerve, such as the sciatic, are conveyed to the cerebrospinal fluid and the substance of the cord in the tissue spaces of the nerve. I can not pause here to give an analysis of their experiments. We should have to take into consideration known facts in respect to the pressure of the cerebrospinal fluid which in the lumbar theca of a man in the recumbent position varies from 80 to 120 cm, in terms of a Locke's solution, and then inquire whether an individual with the natural disease, due to a strictly localized infection, say of the foot, would ever develop such an enormous increase of the normal tissue pressure of his foot (2 to 6 cm) as would be necessary to propel the toxin from the foot to the subarachnoid spaces of the cord—even if there were a direct and open pathway or channel to the cord. But we are not dealing in normal tissue spaces with uninterrupted continuous channels. This matter will be considered more at length in a later section of this address. We have now three variants of the neural transport theory of tetanus toxin, and it is hardly likely that the ingenuity of investigators will enable them to invent a fourth. During the world war the attention of bacteriologists, pathologists and surgeons was again directed to various aspects of tetanus. Much of the earlier experimental work on which the neural transport theory is based was repeated, with the result that some scientists now accepted carriage of the toxin both by way of axis cylinders and neural lymphatics, while others were led to believe that the latter mode of transport suffices and is indeed the only one that can be accepted. In recent years a Japanese investigator has also published a paper in favor of this mode of transfer of the toxin to the spinal cord.

(7) It has just been stated that the theory of transport of toxin to the central nervous system by motor nerves has three variants. Let us consider first the variant that assumes that the axis cylinder is the pathway. It is evident that the toxin can not travel this pathway by diffusion, as this process is so very slow that it can not be made to explain the latent period or its variations in time. The proponents of the theory therefore assume that the toxin is propelled in the axis cylinder by a "protoplasmic streaming." As this process is also assumed to be one of slow character, it appears to account very nicely for the varying periods of incubation in small and large animals. In the smaller animals, such as mice and guinea pigs, with their shorter nerves, the time of transport would be less and would account for the shorter period of incubation in these animals.

In laboratory experiments large quantities of the toxin, often amounting to many times the average fatal dose, were injected beneath the skin of the leg of an animal, and it was found that the nerves in close proximity to the point of injection absorbed some of it. If only two to five fatal doses were injected, only the more peripheral parts of the nerve were found to contain it, while the more centrally lying parts contained none. The proof of its presence in the nerves is given by injecting the crushed nerve or an extract of it into a much smaller and more susceptible animal, such as the mouse. In any case, however, even when very large doses of the toxin are injected, the amount of toxin present in the entire nerve amounts to only a very small fraction of what was injected. The entire sciatic nerve of a full-grown cat or rabbit of average size weighs about a gram. One of the upholders of the axon transport theory found indeed that after subcutaneous injection of one minimal lethal dose into the hind leg of a rabbit not enough toxin could be recovered from the sciatic nerve to kill a small mouse, that is to say, not even $\frac{1}{25{,}000}$ part of what was injected was present in the nerve. Now facts of this kind are not offered here in disproof of this variant of the transport theory now being discussed, as it may be assumed that the actual amount of toxin required by the central nervous system for the induction of convulsions must be very small indeed, and on theoretical grounds the assumed steady transportation to it of very small

amounts of toxin in a unit of time might well suffice to affect the central nervous system, if this mode of transportation could be proved to exist. The occurrence of a very interesting and puzzling phenomenon was early noted in laboratory experiments with various small animals that had received the toxin by subcutaneous, intramuscular or intraneural injection into one of the extremities, as the hind leg, let us say. After an interval of time varying from 17 hours to four days, the muscles of the injected leg become so rigid that all freedom of movement disappears, the leg is stiffly extended and so useless that the animal walks on three legs. This spastic paralytic condition is called a local tetanus, and it is certainly a striking occurrence in that it often persists for three or four days before the muscles of the trunk or the other extremities become involved. If the injected dose is small the local tetanus finally disappears and the animal regains its normal state. This local tetanus is the corner-stone of the foundation on which the theory is built. It was assumed that in the experiments just described the toxin travels along the axis cylinders of the sciatic nerve, in the peripheral parts of which it is generally found, to the spinal cord. The motor cells of the spinal cord receiving the toxin from the nerve are thrown into a condition of hyperexcitability, lasting for some days, and in this way the cause of local tetanus is supposed to be satisfactorily explained.

The theory of carriage by nerve fibrils rests on several unproved assumptions: (1) There is no experimental evidence that there exists anything in the nature of a slow "protoplasmic streaming" or current in the axis cylinders of nerves; (2) when the toxin is found to be present in an excised piece of a nerve, such as the sciatic, there is no trustworthy evidence that an appreciable amount of it is present in the axis cylinders and that the capillaries and the endo- and perineural lymph vessels of the nerve do not contain practically all of it. It has been repeatedly shown that the lymphatic capillaries readily absorb the toxin from an injected area, and there is no sound reason for believing that the lymphatics of any nerve in the immediate vicinity of the area do not also absorb it. That "the three types of peripheral neurones, the motor, the sensory and the sympathetic, are equally able to absorb the tetanus toxin," was conclusively proved by Morax and Marie thirty years ago, who, however, nevertheless assumed that the toxin is carried upward by motor fibrils only. The findings of these authors were criticized on the ground that absorption of toxin by the neural lymphatics could not be excluded. In our day, however, carriage by the neural lymphatic seems to be the prevailing theory. In any event, how can the theory of the exclusive transport by the neurons of motor nerves be any longer upheld, in view of the observations of the French authors just cited? (3) The theory was devised to explain local tetanus and was then applied to all cases of generalized tetanus in which no local tetanus appears. Now neither this theory nor either of its two associates can be reconciled with the following observations which were made in my laboratory by myself and my assistants a number of years ago. That lower vertebrate, the frog, is susceptible to the tetanus toxin and reacts to it with convulsions. There is no reason to believe that the proposed axis cylinder transport theory will not hold for this animal as well as for the higher vertebrates and man. Numerous experiments were made on frogs with alkaloidal convulsants and with convulsant dye stuffs. In the case of the latter convulsants their presence can be detected in the brain and spinal cord aftey they have exerted their action. It was very conclusively shown that all these convulsants can reach the reactive cells of the brain and cord by one pathway only, namely, by the circulating blood. Ever since those experiments were made I have entertained serious doubts in respect to the validity of the current nerve transport theory, which goes so far as to deny the possibility that the cells of the central nervous system can abstract the toxin from the circulating blood. Pochhammer has shown that local tetanus appears in the muscles of the hind leg of a rabbit that has received a subcutaneous injection of a lethal dose of toxin into the lower leg, *before* toxin can be detected in the sciatic nerve, and his findings have been fully substantiated by Sawamura, who, however, minimizes their significance, on quite inadequate grounds in our opinion. We have not yet repeated this experiment, but there can be no doubt of its correctness, and it certainly gives little support to the theory that local tetanus is due solely and entirely to an early invasion of the spinal cord by toxin by way of motor nerve fibrils. It is surely difficult to bring the fact that the peripheral parts of a nerve contain no demonstrable amount of toxin until *after* local tetanus appears, into agreement with the theory. The adherents of the theory will perhaps maintain that a small amount of the toxin was carried to the cord even though it could not be detected in the nerve.

We will not offer here a theory in respect to the true nature of local tetanus or inquire to what extent a myogenous factor is more largely involved in that form of tetanus than in the spasms of descending tetanus. I would remark, however, that the rigid, unremitting, long-continued closure of the jaws that we have observed in our experiments with horses and sheep, and that is so frequently found in man suffering from the natural disease, can hardly be distinguished from the local tetanus of a hind limb of

a dog. In our opinion no such significance attaches to local tetanus as to warrant making it the basis of a theory of carriage of the toxin to the central nervous system, either by way of the axis cylinders, the lymphatics or the tissue spaces of nerve trunks. Had not this form of tetanus been so generally observed in laboratory experiments with small animals after the injection of lethal or sub-lethal doses of toxin into them, it is very improbable that a neural transport theory would ever have been proposed. It has long been known, not only to the few opponents of the theory, but also to its numerous adherents, that local tetanus can be made the antecedent phenomenon to descending tetanus or not, as desired. In the dog or rabbit one can produce a most striking and lasting local tetanus which is later followed by spasms in other muscles, or one can so choose the site of injection and so regulate the dose of toxin that generalized descending tetanus occurs without the appearance of a definitely recognizable local tetanus. Zupnik was the first to show that even in the small animals used in laboratory experiments, local tetanus does not occur if the toxin is injected in the neighborhood of certain joints or into the tip of the toe or the dorsum of the foot. After the usual period of incubation in such experiments only *tetanus descendens* appears. Here again we have evidence that proves conclusively that local tetanus can only appear when the muscles in question are exposed to a sufficient *concentration* of the toxin and for a sufficient period of *time*. May I lay emphasis on these two conditions as indispensable to the appearance of local tetanus? There are other very striking experiments, which can not be detailed here, that indicate that local tetanus may be entirely or almost entirely averted in the dog, if the animal is made to perform hard work on a treadmill for six or more hours a day. The control animal that had received an equal dose of the toxin and was confined to its cage developed local tetanus in its most perfect form. We can not for a moment agree with Ponomarew in his interpretation of this experiment, which was first performed by him. We have repeated the experiment with great success by exercising our dogs vigorously and for longer periods of time, and in our opinion absence of local tetanus in the working dog is best explained by the assumption that the poison is rapidly removed by the capillaries anl lymphatics of the injected limb in consequence of the increased circulation and the metabolic events associated with activity.

We may now consider briefly the second variant of the nerve transport theory, which states that the toxin is carried to the central nervous system by way of the endo- and perineural lymphatics. Professor Lee and his assistants have kindly performed, after consultation with us, a considerable number of experiments of different types on large dogs to test this theory. In one type of the experiments one sciatic nerve of a dog was sectioned high up in the thigh at a convenient distance below the trochanter major, and the severed parts of the nerve were then very carefully sutured so that the continuity of the perineural sheath was restored. Naturally, all these operations were done in an aseptic manner. After the lapse of a month or more, the nerve was again exposed and one or more lethal doses of tetanus toxin, either in the form of a toxic filtrate or in the form of a solution of the dry toxin, were injected into the nerve several centimeters distal to the point of suture. At the time of the injection it was assumed that the lymph passages across the line of transection and suture had been regenerated and were capable of functioning normally. Aside from the symptoms that appeared in the injected leg, which we can not pause to describe here, it was found that generalized tetanus of the usual type occurred in the animal exactly as would have been the case had the toxin been injected into an intact sciatic nerve. It is evident that the poison could not have reached the cord by way of the degenerated axis cylinders of the sciatic, nor do we stress this point, as the advocates of the theory have always admitted that degenerated axis cylinders can not convey the poison. The advocates of the lymph transport theory will, however, find strong support for their belief in the results of this experiment. They will very naturally assert that the toxin was first transported to those centers of the spinal cord that have a functional connection with the muscles of the infected leg, and they will also hold to their belief that the large amount of the toxin that escaped from the nerve and found its way into the circulating blood was in its turn absorbed by the lymphatics of the other motor nerves of the body and transported by them to other parts of the central nervous system than those of the lower cord. We have here a fine example of the difficulties so often encountered in physiological and pharmacological experiments, namely, that the results can apparently be interpreted in at least two ways. It is a singular fact that the advocates of this theory have apparently never asked themselves the question, Where does the lymph of a nerve-trunk such as the sciatic go? The theory that we are now discussing implies that the lymph of all our peripheral nerves regularly, together with such substances as it may have taken up from the environment, finds its way to the spinal cord and the cerebrospinal fluid. Unfortunately for the advocates of this theory, a number of anatomists have finally answered the question. They have traced the course of the lymphatics of the nerve-trunks of both the fore and hind limbs. As in the case of the fore limbs, the lymphatics of the nerves of the leg accom-

pany the arteries of the nerve and throw their contents into more deeply-lying "collectors." In all cases the lymph finally reaches lymphatic glands of the general lymphatic system. Thus, those of the upper part of the sciatic reach deeply lying inguinal glands, and those of its lumbo-sacral trunk finally empty into the hypogastric lymph glands. In a word, we derive no evidence from the study of anatomists that the lymph of any of the peripheral nerve-trunks finds its way into the spinal cord or the cerebrospinal fluid. It may be recalled at this point that the nerve roots within the spinal cord have no perineural sheath and are covered only by the thin neurilemma. It is gratifying, indeed, to have at our disposal these anatomical findings of the last few years in regard to the disposal of the lymph of nerve-trunks. It would be a remarkable fact if the central nervous system in all its parts—a system in which, according to neurologists and anatomists, there are interposed "barriers" that protect its cells from a too ready entrance into them of a great number of soluble substances—should be so accessible to all the numerous substances that can enter the lymphatics of the nerve-trunks at so many points in the body. An experiment that was made by me on frogs many years ago proves how difficult it is to introduce a powerful convulsant, such as strychnine, into the spinal canal in the case of the frog after excision of its heart and complete destruction of its four lymph hearts. A frog that has been treated in this way, and all of whose abdominal organs have been carefully removed without opening any one of the calcareous saccules that lie close to the spinal column, can be immersed in a quarter per cent. solution of nitrate of strychnine in Locke's fluid, kept oxygenated at a temperature of 12° C. for half an hour or more, without the appearance of any convulsions whatever. After removal of this "reflex-frog" from the strychnine bath and carefully washing away every trace of strychnine with cold water, it is found that it responds at once to the injection of a small amount of strychnine into the exposed spinal canal with typical strychnine convulsions. How many hours it would take for the strychnine of the bath to penetrate into the spinal canal by the slow process of diffusion, I have never determined. The experiment, however, shows very conclusively how difficult it is to introduce a rapidly acting and powerful drug into the central nervous system by any other path than by the arterial route. I may conclude this part of my address by stating my conviction that in the natural disease, tetanus, there is no carriage of the toxin to the central nervous system either by way of nerve fibrils or by way of neural lymphatics, and that the only route by which toxin can be carried to the cells of that system is the arterial pathway.

Let us now consider again the more recently proposed third variant of the neural transport theory, stating that the toxin is conveyed to the central nervous system by way of the tissue spaces of nerve trunks. The proponers of this theory, Speransky, Ponomarew and Wischnewsky, rest their claims on certain anatomical beliefs and on the supposed validity of deductions drawn from the results of intraneural and intramuscular injections of the toxin. We find that our experimental and critical analysis of the experiments of these investigators (which will be published in a later paper) does not support their theory in the slightest degree. Some anatomists have expressed the opinion that the tissue spaces (*Lymphräume*) of nerve-trunks, which must be differentiated from lymphatic capillaries, stand in connection with the subarachnoid and subdural spaces of the brain and spinal cord (*"im Zusammehnang stehen," Shdanow*). The tissue pressure is not of sufficient magnitude to move solutions through such narrow spaces, frequently interrupted as they are by barriers. It is freely admitted that diffusion goes on in tissue spaces of organs, as witness the lens, whose low metabolic requirements are met solely by this slow process. Diffusion can be effective only in a short space of time when it operates in structures of minute dimensions, as in the pulmonary alveolar capillaries whose wall thickness is only one thousandth of a millimeter. It would be impossible for the slow process of diffusion to do the work necessary for the maintenance of life in the four seconds of each respiratory act, were not the interposed barriers extremely thin. The minute tissue space mechanisms are energized by molecular forces only (surface energy and diffusion), and it remains to be proved that such forces can compass the astonishing feat of transporting a poison over long distances and through intervening cell barriers to the central nervous system. My associates and I have shown that, in the frog, even the foramina through which the nerves and blood vessels enter or leave the bony encasement of the spinal cord are guarded against the entrance of solutions by any other than the vascular route. Injections of suitable solutions or masses in large amounts for the purpose of outlining the entire course of arteries, veins or lymphatics are legitimate operations, since preformed pathways are utilized in experiments of this nature. But when a solution of toxin (0.1–0.3 cc and more) is injected into a living nerve-trunk, such as the sciatic of a cat or rabbit, for example, the needle of the syringe rarely if ever enters one of the veins or lymphatics of the nerve. The non-rigid, soft and distensible nerve is greatly dilated at the point of injection and the resulting internal pressure forces the solution both downward and upward in artificially formed paths. It is not permissible to believe, on the basis of such experiments, that anything of this

kind occurs in the *natural* disease, even when the terminal portions of the nerve have absorbed some of the poison from the infected area. We have often examined toxin-containing nerves, after *subcutaneous* injections of the poison, and have never observed that they differ in appearance from corresponding non-poisoned nerves. There is little likelihood of an increase in the normal tissue pressure of such nerves and little possibility here of a forcible transportation of the toxin to the central nervous system in mechanically produced pathways in the connective tissue spaces of the nerve, as is the case in nerves that have been distended by an injection. Furthermore, is it not more than likely that such of the toxin as may find its way into the tissue spaces of a nerve-trunk in proximity to the infected area, in the *natural* disease, if indeed assumption of toxin by the peripheral nerves occurs in that case, could not be carried far centrally but would be removed from these spaces by the capillaries and lymphatics? We have seen that the lymphatics of nerve-trunks finally discharge their contents into more deeply lying collectors and lymph glands that communicate with larger lymphatic trunks. Support for this opinion is found in the well-known fact that the middle and upper portions of the sciatic nerve do not contain a demonstrable amount of toxin after this has been injected subcutaneously in the region of the gastrocnemius muscle in such considerable amounts as from two to five lethal doses.

Distribution of Tetanus Toxin in the Body

During the past forty years and more, many investigators have shown that the toxin may be present in the spleen and liver for a short time after injection into the animal and that it persists for a longer time in the blood, lymph, lymph glands and in the peripheral parts of mixed nerves and that it also appears occasionally in the urine. It has never been found in the muscles. All this early work was of a qualitative character only, that is to say, no attempt was made to determine with any degree of accuracy how much toxin was injected and how much of it could be recovered from the blood and lymph and other parts of the body. A few quantitative experiments were made by Bieling and Gottschalk, who injected known amounts of the toxin directly into the heart of guinea pigs and then assayed the toxin content of the blood and of extracts of various organs within an hour after the injection. The results of these authors are often cited as showing that a large amount of the toxin soon disappears from the blood and that this part is either irreversibly bound or rendered inactive in various tissues and organs of the body. More than thirty years ago, Ransom, alone among early investigators, attacked the problem of the distribution of both tetanus toxin and antitoxin between the blood and lymph in a more serious manner in many experiments on dogs. After injection into the blood stream of known and large amounts of the toxin it was found that about 26 per cent. of it had disappeared from both the lymph and blood in three hours, and after the lapse of 26 hours somewhat more than 70 per cent. of what had been injected had disappeared from the two fluids. Ransom also showed how rapidly the toxin passes into the lymph after intravenous injection and that the two fluids contain an equal amount of the toxin in equal volumes after the lapse of 26 hours. The fact that in the relatively insensitive dog the toxin disappears so rapidly from the blood and lymph is in striking contrast with our own experiments on a much more sensitive animal, the sheep.

In repeating and amplifying the earlier work of this character we have aimed primarily at the establishment of values that are easily reproducible and as accurate as our methods would permit. The quantitative data of experiments of the kind here offered are inevitably burdened with the fluctuations due to variations in the response of animals to the action of a given poison and it is therefore necessary to assemble enough data to satisfy the requirements of statistical theory and thus reduce the disturbing action of variations of unknown cause to such a degree that the conclusions thus arrived at will be valid within certain limits. We have attempted to interpret our data in accordance with this principle, but can not here give the details of our methods of assaying the amount of toxin injected by various pathways or that found to be present in the blood and lymph on subsequent days. The animals used for assaying the injected toxin and that present in the blood of the infected sheep were guinea pigs of standard age and weight, and more than a hundred of these testing animals were used for each sheep. The variation in the values obtained in these experiments is probably on the average not more than 10 per cent. and we believe that this figure represents a degree of precision far exceeding that of earlier estimations.

We have found sheep to be the most satisfactory experimental animals for these studies and have investigated the toxin content of the blood and lymph of a series of nine of these animals of approximately the same weight and after the administration of comparable doses of tetanus toxin by several routes. At the time the toxin was injected into the animals the toxicity of an aliquot portion was again carefully determined in the usual manner. Thereafter daily estimations of the toxin content of the blood were made. In this manner, a complete record of the daily variations of the toxin content of the blood of an experimental animal was obtained.

After an intravenous injection of 35.6 guinea pig minimum lethal doses of toxin per kilogram of body weight into a sheep, the toxic content of the blood fell to 60 per cent. of the injected dose at the end of 24 hours. Forty-eight hours after the injection the blood and lymph each contained, volume for volume, equal amounts of the toxin which in the blood was still maintained at the level of 50 per cent. of the injected dose, if it can be assumed that the total amount of blood in the sheep was 6.6 per cent. of its body weight. In this animal, whose lymph had been collected on one day, the blood content in toxin dropped to less than 30 per cent. of the injected dose at the time of death. In a second animal that had not been subjected to the long operation involved in the collection of the lymph from the thoracic duct, the blood content in toxin, after intravenous injection of 53.3 guinea pig minimum lethal doses of tetanus toxin per kilogram of body weight was also estimated. One hour after the injection the blood contained 90 per cent. of the injected toxin; seven hours after the injection 60 per cent.; after 24 hours again 60 per cent. This high level was maintained up to the first appearance of trismus on the third day after the injection. Thereafter the toxin content of the blood dropped, being somewhat less than 40 per cent. at the time of death on the sixth day after injection.

When a standardized quantity of toxin amounting to from five to six lethal doses is injected at one time into a sheep, the blood and lymph content of toxin finally reach much the same level, irrespective of the site of injection, whether this be the tip of the tail, the subcutaneous tissue of a leg, the interior of a muscle or a vein. We give here two instances in which intramuscular injections were made. One of the two sheep received, by this route, 49.6 guinea pig minimum lethal doses per kilogram of body weight, while the second received 54.1 guinea pig minimum lethal doses per kilogram. The toxin content of the blood of these animals varied from 40 to 50 per cent. of the amount of toxin injected and was maintained at this high level during the three to five days that preceded the onset of symptoms. It is indeed extraordinary that the highly toxic blood can penetrate every region of the body during the several days of the incubation period without apparently manifesting any evidence of its poisonous nature. An analogous instance is that of the action of certain convulsant dyestuffs on frogs when the blood, surcharged with dyestuff, also circulates for a certain time without exhibiting any signs of toxicity. And many other examples of this kind are known, but I can not be sure at the moment that the blood content of poison during the period of incubation has actually been determined for any of them. In the experiments under consideration the toxin content of the blood dropped somewhat after the appearance of general symptoms but was still present at a concentration of 20 to 30 per cent. of the injected dose at the time of death. With the assistance of Professor Lee we collected the lymph from the thoracic duct of one of our sheep, 36 hours after it had received an intramuscular injection of 40.7 guinea pig minimum lethal doses of tetanus toxin per kilogram. Just before the operation for the collection of lymph, the toxin content of the venous blood of the sheep was 40 per cent. of the amount injected. At this time, the lymph contains, volume for volume, an amount of toxin equal to that in the blood. Unfortunately we have no reliable data in regard to the total volume of lymph in the higher animals, and for this reason an estimate of the actual percentage of the injected toxin present in the lymph can not be made. However, it is apparent that after intramuscular injection of the tetanus toxin, as after intravenous injection, an equilibrium exists, if we may term it so, between the blood and lymph in the sense that equal volumes of both fluids contain equal amounts of the toxin. Could we assume, as was done by Ransom in his experiments on dogs, that the total amount of lymph in the entire lymphatic system, inclusive of the lymph glands, can be conservatively estimated as equal to that of the blood, we would be in a position to affirm that fully 90 per cent. of the injected toxin is present in the blood and lymph of sheep under the conditions of the experiments here described.

We have also made an experiment in which a total of 1,600 guinea pig minimum lethal doses (45.7 guinea pig lethal doses per kilogram of body weight) were injected intramuscularly into a sheep. This quantity was not injected at one time but was divided into sixteen portions, and these were injected into a limited area of a shoulder muscle at regular intervals over a period of thirty-two hours. On comparing the toxin content of the blood of this animal with that of a sheep receiving a markedly smaller dose of toxin (40.7 guinea pig minimum lethal doses per kilo) in a single intramuscular injection, we find that only 30 per cent. of the injected toxin was present in the blood during the incubation period as compared with 40 to 60 per cent. in the blood of the second animal at the same time. It is evident that the amount of toxin bound by the tissues is relatively greater when it is introduced into the body in small amounts over a period of time. The situation is somewhat more comparable to that observed in the natural disease than when a large dose is injected at one time.

Perhaps the most striking fact that has been established by these studies is the persistence of the toxin, in high concentration and over a long period, in the vascular and lymphatic systems of the animal. That such surprisingly large amounts of the toxin can be

shown to circulate in the blood and lymph up to the time of the death of an animal is indeed surprising when we recall the unanimous opinion of other workers in this field that the toxin is rapidly, and, to a very considerable degree, detoxified or bound by the animal tissues.

It will be seen from the above account of our investigations that my associates and I have come to the conclusion that the theory of carriage of tetanus toxin to the central nervous system by way of the peripheral nerves is no longer tenable. In so far as this poison reaches the central nervous system it can do so only by being brought to it by the arterial blood. The various aspects of the disease have been the subject of intensive investigation by clinicians, bacteriologists, immunologists, physiologists and pharmacologists during the past fifty years. A great body of frequently conflicting facts has been brought to light, but there is little agreement in respect to their explanation. Four different theories have been proposed to explain local tetanus and four more have been devised to show the pathways by which the toxin reaches the central nervous system. We have discussed three of the second quartet as variants of the neural transport theory. The fourth, which receives our hearty support, states that the toxin can reach the central nervous system only by way of the blood stream. In regard to the exact nature of the poison or poisons concerned, their possible alteration in the animal body and the manner in which they induce the formation of an anti-body, we are without definite knowledge. All these questions are of great significance and have an important bearing on the mode of action of bacterial toxins as a class. It will no doubt require the united efforts of many investigators for many years to find an adequate solution of these and other fundamental problems of this disease. We are continuing our work in the hope of throwing some light on one or another of these difficult questions.

ORGANIZED INDUSTRIAL RESEARCH[1]

By Dr. W. D. COOLIDGE

DIRECTOR OF RESEARCH LABORATORY, GENERAL ELECTRIC COMPANY, SCHENECTADY, N. Y.

THIRTY years ago an industrial research laboratory was still a novelty. Professor Elihu Thomson, Mr. Edison and Dr. Steinmetz had made many of their great individual contributions, and it was because of the traditions built up by them that Dr. E. W. Rice, then technical director of the company, had decided to embark on the new experiment—*organized* industrial research. The value of science to industry had not yet come to be generally recognized, nor had industry yet convinced science that a union of the two could be anything but a misalliance. Many scientists in that day felt, and some openly proclaimed, that to apply to industrial problems scientific brains and scientific methods was to debase them.

Let me say as emphatically as I can that I yield to none in my appreciation and admiration of those scientists who, in academic surroundings, with little thought of financial reward but with a passion for knowledge and an eye single to the truth, are pushing their researches even farther into the unknown, and broadening and deepening the foundation of science on which our civilization rests. For our civilization is an engineering civilization which could endure scarcely a day if all the products of engineering were suddenly swept away, and it is science which serves as the basis of engineering. So the most cloistered scientist, however remote from the marts of trade and however innocent of the least thought tinged with utilitarianism, is perhaps, all unknown to him, preparing the way for some new appliance for the service of mankind, some appliance perhaps that will bring new industries into being. When Professor J. J. Thomson, in his university laboratory, by a beautiful experiment first determined the charge of the electron, he certainly had no thought of any interest but that of pure science, of extending our insight into the fundamentals of things. He surely did not foresee the great new industry of radio broadcasting which was to bring employment to tens of thousands and entertainment and instruction to millions. But Professor Thomson's work helped to lay the foundations of that industry, just as truly as did the other beautiful experiments and keen analysis of Langmuir, when in our own laboratory, he discovered the space charge effect, the effect produced in vacuum by those very charges which Professor Thomson had measured, the effect which, when understood, made possible for the first time the design of high power vacuum tubes suitable for radio broadcasting.

And I venture to say this—and this is the point to which I have wished to lead—that Langmuir in his work, like Professor Thomson, was absorbed wholly in the pursuit of truth, and the attainment of new, fundamental facts was for him an all-sufficient goal.

[1] An address given at a meeting of the American Institute of the City of New York on February 1, 1934, when a gold medal was presented by the institute to the General Electric Company "for pioneering in industrial research."