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TISSUE RESISTANCE AND THE CAUSE OF PERMANENT ACQUIRED IMMUNITY.*

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It is well known that a single attack of certain infectious diseases protects an individual against subsequent infection, that is to say, there develops a permanent acquired immunity. To this group of diseases belong smallpox, scarlet fever, measles, typhoid fever, cholera, etc., while in such infectious diseases as gonorrhea, diphtheria, recurrent fever, influenza, pneumonia, etc., the patient acquires no lasting immunity. Immunity can be produced not only by natural infection, but also by artificial means. There are two kinds of immunity, one against infection, that is, antibacterial, and the other against toxin, that is, antitoxic.

According to the modern theory, acquired immunity, whether antibacterial or antitoxic, permanent or not, is due principally to the existence in the blood of specific antibodies, such as bacteriolysins, opsonins, bacteriotropins, antiaggressins, agglutinins, precipitins, and antitoxins. It is generally supposed that of these various antibodies bacteriolysins, opsonins, bacteriotropins, and antiaggressins are the most important in producing immunity against infection, the presence of agglutinins and precipitins being rather a phenomenon following upon the immunization, while antitoxins are considered as mere protective substances against toxins.

Whether or not the presence of antibodies in the blood has anything to do with immunity, it is at least generally admitted that there is no relationship between the degree of immunity and the quantity of

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specific antibodies in the blood. For instance, in typhoid fever, immunity persists throughout life, even after antibodies have disappeared from the blood.

The permanent acquired immunity which remains after antibodies have disappeared from the blood is usually explained by the supposition that at the second infection specific antibodies are more quickly produced than normally. Liebermann and Acél[1] observe, however, that following a second infection of typhoid-immune animals, from the blood of which specific antibodies have disappeared, antibodies appear in the blood only after several days, although the animals withstand such a virulent inoculation as would kill normal ones within 24 hours, a fact which cannot be accounted for by the theory of antibodies. The presence of antibodies in the blood, therefore, though an interesting serological phenomenon, does not in itself mean immunity, at least in the case of permanent acquired immunity against infection. This fact has led us to turn our attention to the tissues of typhoid-immune animals and to conduct researches with them. Our researches differ in intent from those upon acquired immunity against toxin, as recently reported by Gunn and Heathcote[2] and by Manwaring and Williams[3] with cobra venom.

We have made the following experiments in order to determine (1) whether the tissues of typhoid-immune animals acquire increased resistance against the toxic products of typhoid bacilli, (2) whether this resistance, if acquired, is specific or not, and (3) whether the increased resistance is caused by antitoxin or by a specific biological alteration of the tissue cells.

Methods.

The experiments were made on the hearts of rabbits. We found the heart most suitable for the purpose, because after isolation from the body it can with comparative ease be freed entirely of blood, and it beats automatically for a long time under suitable conditions.

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[1] von Liebermann, L., and Acél, D., *Deutsch. med. Woch.*, 1917, xliii, 867.

[2] Gunn, J. A., and Heathcote, R. S. A., *Proc. Roy. Soc. London, Series B*, 1921, xcii, 81.

[3] Manwaring, W. H., and Williams, T. B., *J. Immunol.*, 1923, viii, 75.

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(*a*) *Preparation of Animals.*—The rabbits were adult, of about 2,000 gm. weight, and mostly male. They were kept in cages under controlled conditions, and were all fed on the same diet. Care was taken to see that the quality and quantity of food were sufficient in every case to prevent diminution of the weight of animals.

The animals were immunized actively with vaccine which was in almost all cases injected into the marginal veins of the ears and in only a few cases subcutaneously giving a total of five injections at intervals of 5 to 7 days. The doses were progressively increased from $\frac{1}{4}$ to $\frac{1}{2}$, 1.0, 1.0, 1.0 mg. per kilo. All vaccines contained 1.0 mg. of bacteria per 1.0 cc. They were freshly prepared in the usual manner, and were sterilized by heating for 1 hour at 60°C.

Using the Langendorff-Locke apparatus, the isolated heart was perfused with Locke-Ringer solution for at least 10 minutes to remove all serum from the heart, and then the toxic solution was tested. The heart beats were registered on the smoked paper of a kymograph.

(*b*) *Preparation of a Solution Containing Toxic Products of Typhoid Bacilli.*—It is absolutely necessary that the toxic products do not lose the specificity of typhoid bacilli, yet they must be notably toxic at the same time. It is quite difficult to obtain a toxic solution, as the toxic substance in typhoid bacilli belongs to the so called endotoxin group. We tried several methods of preparation and finally found the following one most suitable for the experiments. Highly virulent typhoid bacilli were cultured in 2 per cent peptone water (20.0 gm. peptone + 9.2 gm. NaCl + 1,000.0 cc. aqua destillata) for 7 to 10 days in an incubator. The material was then heated for 1 hour at 60°C., again kept in an incubator for about 10 days, to permit the endotoxin to pass by digestion into the peptone water from the bacilli, and was finally stored in a dark cool place.

Prior to the experiments, after the toxic solution had been cleansed of sediment by filtration, salts were dissolved in it in the same proportion as in Locke-Ringer solution. Finally this toxic solution was diluted 200 to 400 per cent with Locke-Ringer solution freshly prepared.

The toxicity of the solution obtained in this manner is very variable. Therefore we have tested each lot of material upon the isolated heart of a normal rabbit. According to our experience, a toxic solution which stops the normal heart in several minutes, is most suitable for the experiment. If it is either too strong or too weak it is not suitable.

The total quantity required to stop the heart beat is naturally variable. In Experiment 6 (Table I) about 500 cc. were used in the course of 25 minutes, yet the heart still beat.

Each set of experiments has been carried out with the same toxic solution, and with an identical procedure and technique for the hearts.

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EXPERIMENTS AND RESULTS.

(*a*) *Comparison of the Resistance.*—We compared first the resistance of the hearts of typhoid-immune rabbits with that of normal non-immunized animals against toxic products of typhoid bacilli.

Table I shows clearly that the resistance of the hearts of typhoid-immune rabbits, examined between the 9th and 58th days after the

TABLE I.

No. of experiment.	Condition of rabbit.	No. of days between the last injection of vaccine and the experiment.	No. of min. during which the heart continued to beat.
1	Immune.	9	50
	Normal.		20
2	Immune.	9	45 (still beating).
	Normal.		4
3	Immune.	11	20
	Normal.		4
4	Immune.	11	15
	Normal (*a*).		4
	" (*b*).		14
5	Immune.	14	35
	Normal.		8
6	Immune (*a*).	14	25 (still beating).
	" (*b*).	58	25 (" ").
	Normal (*a*).		12
	" (*b*).		12
	" (*c*).		10

last injection of vaccine, is greater than that of the normal non-immunized rabbits as tested with the same solution of toxic products of typhoid bacilli.

(*b*) *Problem of Specificity.*—We next tried to compare the resistance to toxic products of typhoid bacilli of the hearts of typhoid-immune rabbits and those of animals immunized with bacteria other than typhoid bacilli.

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TABLE II.

No. of experiment.	Bacteria used for immunization.	No. of days between the last injection of vaccine and the experiment.	No. of min. during which the heart continued to beat.
7	*B. typhosus.*	11	25
	" *paratyphosus* B.	11	13
	" " A.	11	10
	Non-immunized.		20
8	*B. typhosus.*	10	4.5
	" *coli.*	10	3.5
	Non-immunized.		3.5
9	*B. typhosus.*	11	20 (still beating).
	" "	11	15 (" ").
	" *dysenteriæ.*	11	10
	Non-immunized.		10
10	*B. typhosus.*	11	18
	" *pneumoniæ.*	11	40 sec.
	Non-immunized.		15
	B. typhosus.	12	15
	" *pneumoniæ.*	12	10 sec.
	Non-immunized.		7
11	*B. typhosus.*	11	18
	" *influenzæ.*	11	15 sec.
	Non-immunized.		15
	B. typhosus.	12	15
	" *influenzæ.*	12	10 sec.
	Non-immunized.		7
12	*B. typhosus.*	11	18
	" *pertussis.*	11	15 sec.
	Non-immunized.		15
13	*Diplococcus pneumoniæ.*	11	30
	Non-immunized.		30
14	*B. typhosus.*	11	7
	Diplococcus gonorrhœæ	11	6
	" "	11	2
15	*B. typhosus.*	12	23
	" *pestis.*	12	16
	Non-immunized.		10
16	*B. typhosus.*	10	20
	" "	10	20
	Vibrio choleræ.	10	20
	Non-immunized.		16

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In Table II the resisting power of the hearts of typhoid-immune rabbits is shown to be greater than that of the hearts of rabbits immunized with bacteria other than typhoid bacilli. Therefore it is certain that the acquired increase of resisting power of the heart tissue of the typhoid-immune animals is specific.

(*c*) *The Cause of Increased Resistance.*—There are two possibilities: one, that the increased resistance is caused by antitoxin in the heart tissue, and the other that it is caused by a specific biological alteration of tissue cells. If the antitoxin cannot be demonstrated in the heart tissue, then the latter must be regarded as the cause.

The following experiments were performed to determine the presence or absence of antitoxin in the cardiac tissue of immunized animals.

TABLE III.

No. of experiment.	Condition of animal from the heart of which fluid was expressed.	No. of min. during which the perfused heart continued to beat.
17	Immune.	18
	Normal.	16
18	Immune.	12
	Normal.	12

In a first series of experiments the isolated hearts of normal rabbits were perfused with toxic solution which had previously been mixed with the fluids expressed from hearts, sometimes of immunized, sometimes of non-immunized animals. If antitoxin is contained in the heart tissue of typhoid-immune animals, the depressant action of a toxic solution mixed with the fluid from the heart tissue of typhoid-immune animals, ought to be less than that of the same toxic solution when mixed with the fluid from the hearts of normal animals.

There was, however, as Table III shows, no constant difference to be observed in the toxicity of solutions mixed with fluid from the hearts of immunized animals as compared with those mixed with fluid from the hearts of non-immunized animals. Thus we failed to detect antitoxin in the fluid from the heart tissue of typhoid-immune animals by means of this experiment.

We next conducted experiments to compare the antitoxic power of the fluid from the heart tissue of typhoid-immune animals with

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that of normal heart tissue, by means of subcutaneous injection into mice of these fluids mixed with emulsion of typhoid bacilli. The fluid was injected in dosage of 1.0 cc. for every mouse of 10 gm. body weight. The fluid injected contained in every 1.0 cc. the fluid procured from 1.0 gm. of heart tissue.

As Table IV shows, the existence of antitoxin in the fluid from heart tissue of the immunized rabbits was also negatived by this experiment.

Taking into consideration (1) the results of these two kinds of experiments and (2) the fact that even in the blood the occurrence of antitoxin against endotoxin has never been definitely shown, we feel justified in concluding that the increased resistance is not due to antitoxin, but is caused by a specific biological alteration of the tissue cells.

TABLE IV.

No. of mouse.	Condition of animal from the heart of which fluid was expressed.	No. of hrs. of survival.
1	Immune.	20
2	"	45
3	"	45
4	Normal.	45
5	"	45
6	"	Living.

DISCUSSION AND CONCLUSION.

In these experiments, we have demonstrated that the tissue cells of the hearts of typhoid-immune animals are altered in such a way that their resisting power is increased specifically against toxic products of typhoid bacilli. From this it may reasonably be inferred that other tissue cells the resisting power of which is low in the non-immunized state, simultaneously acquire an increased resisting power. Once the tissue cells acquire increased resistance against toxic products of bacilli, the bacilli lose their power of injuring the tissue cells, and become like saprophytes which do no injury to the host. Even under this condition bacteria may of course invade, but they do not cause symptoms. Even if they enter the blood, some of them will soon be carried out of the body, while others will be caught

by phagocytes or destroyed by the bactericidal strength of the blood. It need scarcely be pointed out that since the normal blood has natural non-specific bactericidal strength, specific antibodies are not required in order to destroy the bacilli. Moreover, as stated at the outset, it is plainly impossible to ascribe the cause of permanent acquired immunity to the existence of antibodies in the blood.

On the strength of the results of our experiments, we cannot but maintain that permanent acquired immunity, at least in typhoid, instead of being principally caused by antibodies in the blood, is due rather to a lasting specific increase of resisting power acquired by the tissue cells against the toxic products of bacteria. This seems to hold true not only with typhoid, but also with other infectious diseases, in which one attack causes lasting immunity. Many facts related to permanent acquired immunity which have hitherto been found difficult to explain through the theory of antibodies are rendered intelligible by this theory of tissue resistance.

For example, specific antibodies in the blood, even bacteriolysins and opsonins, are produced not only by bacteria which leave behind permanent immunity, but also by those which do not. This fact cannot be explained away on the theory of circulating antibodies. It might be that while bacteria which do not cause permanent immunity by infection, alter tissue cells only temporarily or in a slight degree, those which convey permanent immunity after infection, alter the tissue cells permanently and to a much higher extent. Such a supposition would at least explain satisfactorily why permanent immunity cannot be acquired through infection by all varieties of bacteria.

Furthermore, the preventive effect of vaccine has been demonstrated, save of smallpox, only in those of certain infectious diseases in which permanent immunity follows an attack, such as typhoid, cholera and plague. In these cases specific antibodies appear after vaccine injection just as upon spontaneous infection, yet complete immunity is not acquired as a result, for the individuals or animals receiving vaccine injection are not absolutely protected against infection. This fact seems also to argue against the conception that antibodies are the cause of permanent acquired immunity against infection. If, on the other hand, the tissue resistance is taken into

consideration it becomes easy to explain the observed phenomena. One need only suppose that whereas by spontaneous infection the resisting power of tissue cells is increased permanently, by vaccine injection the tissue cells are altered only incompletely.

Finally it may be mentioned that the so called "local immunity" usually means a production of antibodies by the tissue cells with which the antigen comes into direct contact. This view is founded on the experiments by Wassermann and Citron[4] among others. The term "tissue immunity" is often understood as a synonym for local immunity. For such reason this term seems to be unsuitable as applied to the increase of resisting power acquired by tissue cells, because this is, as stated before, not caused by antitoxin and has no direct relation to the production of antibodies.

SUMMARY.

1. The resistance against toxic products of typhoid bacilli of the heart tissues of typhoid-immune animals is greater than that of non-immunized animals.

2. The acquired increase of resisting power is specific.

3. The increased resistance is not caused by antitoxin, but by a specific biological alteration of tissue cells.

4. It is maintained that permanent acquired immunity, at least in typhoid, instead of being due principally to antibodies, is caused by a lasting specific increase of resisting power acquired by the tissue cells against the toxic products of bacteria.

5. This theory seems to hold good with other infectious diseases in which one attack conveys permanent immunity.

[4] Wassermann, A., and Citron, J., *Z. Hyg.*, 1905, l, 331; *Deutsch. med. Woch.*, 1905, xxxi, 573.