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Local Formation of Antibody by the Skin.

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One of the basic problems of local tissue-immunity is the relationship of immune bodies to the reaction, Besredka[1] minimizes their importance and defines local immunity as "an immunity without the obligatory participation of antibodies". Gay[2] takes a broader view in defining the condition as "a locally superior mechanism for the disposal of a particular microorganism" and considers that it may be demonstrated either by the local presence of antibodies before their appearance elsewhere in the body, by their local presence in greater concentration than elsewhere, or by a superior method of direct disposal of bacteria in the particular area in question.

Cannon and Pacheco[3] have described the "fixation" of virulent staphylococci in the skin of guinea pigs which had been locally immunized by intradermal injections of a staphylococcal vaccine. This immunization caused thickening of the subcutis by a dense layer of newly formed histiocytic tissue. Living staphylococci, when introduced into such a tissue, remained localized in a small

[1] Besredka, A., Williams and Wilkins, Baltimore, Md., 1927.

[2] Gay, Frederick P., The Newer Knowledge of Bacteriology and Immunology, Jordan, E. O., and Falk, I. S., The University of Chicago Press, 1928, 881.

[3] Cannon, Paul R., and Pacheco, G. A., *Am. J. Path.*, 1930, **6**, 749.

area so that, in general, the animals suffered no serious effects. Similar injections into normal guinea pigs, however, led to a cellulitis and the death of many animals from generalized sepsis. Microscopical examinations showed the staphylococci concentrated in clumps and masses in the locally immunized tissues, whereas the microorganisms were diffusely disseminated through the subcutis of the normal animals. The assumption was made that an important element in the mechanism of fixation was an antigen-antibody reaction whereby the bacteria were opsonized and agglutinated by antibodies formed *in loco* by the histiocytes which had been mobilized during the period of local immunization. As a secondary reaction, leucocytes then infiltrated the area and kept the bacteria localized, thus preventing generalization with its serious consequences.

This assumption of agglutination *in vivo* was based solely upon morphological evidence. We have now demonstrated by a method of chemical extraction that antibodies may be present in relatively high concentration in tissues which have been locally immunized, thus confirming the assumption and furnishing a more definite basis for the recognition of local immunity as an important element in the general mechanism of defense.

Rabbits were injected intradermally in a local area of the abdominal wall with a suspension of *B. paratyphosum B* in 0.85% solution of sodium chloride to which 0.2% formalin had been added. This microorganism was chosen merely because of the ease with which its agglutinins can be demonstrated. The injections were made daily for from 5 to 12 days, during which period it was noted that the later injections were usually followed by an accelerated formation of nodules, frequently with ulceration, at the site of injection, thus suggesting an increasing tendency to localization of the vaccine injected. After the termination of the immunization the animals were kept in their cages for from 18 to 22 days and were then, with the exception of rabbits 5 and 6, reinoculated into the locally immunized area with 1 cc. of the original vaccine suspension, in order to cause more antibody to be liberated. Seven to 14 days later, the locally treated tissues and the corresponding untreated tissues of the opposite side, together with liver, spleen, and blood serum, were added to a solution of equal parts of sterile glycerol and 0.85% solution of sodium chloride in the proportion of one part of tissue to 15 parts of the extraction fluid, were ground aseptically with the exception of the serum, and kept under chloroform-toluol at 37°C. for from 6 to 9 days. The solutions were then titrated simultaneously against a suspension of living paratyphoid bacilli in order to

determine the relative concentrations of agglutinin in the various tissues under such comparable conditions. The glycerol-saline extractive was used because Cary[4] and Motohashi[5] found it the best of several tried in their study of the site of formation of antibodies. Their work furnished the first convincing evidence that antibodies are products of macrophages.

Results. Tables I and II show the comparative agglutinin titers for 10 animals. As is seen, the locally immunized skin, without exception, contained a higher concentration of agglutinins (from 2 to 4 times) than the corresponding skin of the opposite side (Rabbits 1, 2, 3, 4, 5, 7, and 9). Furthermore, the concentration was usually higher than that of the blood serum and always higher than that of comparable portions of liver and spleen. In one animal, not included in this report, the agglutinins appeared in high concentration within 48 hours after the intradermal injection of the antigen, at which time they were undetectable in the spleen, kidney and blood serum. Finally, rabbits immunized intravenously (rabbits 6, 8, and 10) with the same quantities of antigen introduced locally into Rabbits 5, 7, and 9, showed a higher concentration of agglutinin in the liver, spleen or both in 2 of the 3 pairs here reported and in 2 additional pairs not included in this paper because of lack of space.

These results indicate that the local injection of antigen into previously mobilized histiocytic tissue may lead to the local formation and retention of immune bodies in relatively high concentration, from which tissue they may diffuse and be recovered in lower concentration in organs elsewhere. These findings substantiate the view

TABLE I.

Comparative Agglutinin-Titer of Tissues from Rabbits Locally Immunized with *B. paratyphosum B.*

Rabbit	1		2		3		4			
Tissue*	u Sk	i Sk	u Sk	i Sk	u Sk	i Sk	u Sk	i Sk	S	Sp
3840					0	0	0	0	0	0
1920	tr	++	0	tr	0	tr	0	0	0	0
960	+	++	0	+	0	+	0	tr	tr	0
Titer 480	++	++	tr	++	tr	++	+	++	+	0
240	++	++	+	++	+	++	++	++	+	0
120	tr	++	+	+	+	++	+	++	++	tr
60	tr	+	tr	tr	tr	+	+	++	++	tr
30	tr	tr	0	0	tr	+	tr	tr	tr	tr

++ strong agglutination. + moderate agglutination. tr trace of agglutination. *u/Sk skin unimmunized side. i/Sk skin of locally immunized side. Sp spleen. S serum.

[4] Cary, William E., *J. Med. Res.*, 1922, **43**, 399.

[5] Motohashi, S., *J. Med. Res.*, 1922, **43**, 419.

TABLE II.

Comparative Agglutinin-Titer of Tissues from Rabbits Locally and Intravenously Immunized with Equal Quantities of *B. paratyphosum B.*

Rabbit	5				6			7				8			9				10		
Mode of Immunization*	Local				Intravenous			Local				Intravenous			Local				Intravenous		
Tissue	u Sk	i Sk	Sp	S	Sk	Sp	S	u Sk	i Sk	Sp	L	S	Sk	Sp	L	S	u Sk	i Sk	Sp	S	Sk	Sp	S
3840	0	0	0	0	0	0	0	0	tr	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1920	0	+	0	0	0	0	tr	0	+	0	0	tr	0	0	0	tr	0	+	0	0	0	0	tr
960	0	+	0	tr	0	0	+	tr	+	0	0	++	tr	0	0	+	+	+	0	tr	0	0	+
480	tr	+	0	+	0	tr	+	+	+	0	0	+	0	0	0	+	+	+	0	0	tr	tr	+
240	+	+	0	+	0	+	+	+	+	tr	tr	+	+	0	0	+	+	+	0	tr	tr	+	+
120	+	+	tr	+	tr	+	+	+	+	+	tr	+	+	0	tr	+	+	+	tr	+	+	+	+
60	tr	tr	tr	+	tr	+	+	+	+	+	+	+	tr	tr	+	+	+	+	+	tr	+	+	+
30	tr	+	tr	+	tr	+	+	+	tr	tr	tr	+	0	0	tr	+	+	+	+	0	0	+	+

Titer

++ strong agglutination. + moderate agglutination. tr trace of agglutination.

* u/Sk skin of unimmunized side. i/Sk skin of locally immunized side. Sp spleen. L liver. S blood serum.

that a locally increased resistance to infection may be due in part to a locally increased formation of antibodies. Therefore, local immunization of organs and tissues would seem to be indicated, whenever practically possible, as a supplement to the methods of general immunization as practiced at present.