ANTIBODY FORMATION IN THE OMENTUM.

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WITH the possible exception of the liver, tissues of the body containing a high proportion of reticulo-endothelial elements are able to produce antibody. The spleen is probably the main site of both the fixation of antigen and the production of antibody when moderate amounts of antigen are given intravenously, while the draining lymph nodes may be more important when antigen is given into the skin (Roberts, Adams and White, 1949 ; McMaster and Hudack, 1935). When particulate material is injected into the peritoneal cavity, it is removed partly by the draining lymphatics and partly by the macrophages of the omental " milk-spots ". Little is known of the rôle of the omentum in antibody formation after intraperitoneal injection. The present work describes the reaction of the omentum of the rabbit to intraperitoneal injections of formalin-killed *Salmonella typhi* organisms (H antigen).

EXPERIMENTAL METHODS.

Young adult rabbits of both sexes weighing from 1·5 to 2·5 kg. were used. The preparation of the antigen and the methods for estimating agglutinin titres were those described in a previous paper (Roberts, 1955). Intraperitoneal injections were made in the anaesthetised animal, the organisms for injection being suspended in up to 2 ml. of 0·9 per cent NaCl.

Estimation of tissue titres.—The animals were killed by exsanguination. Weighed portions of tissues were extracted into distilled water, either by grinding with sand or by homogenising in an ice-cold Potter tube for 1 min. The efficiency of extraction by these two methods was found to be similar ; a sample of omentum and another of spleen, treated in these two ways, gave consistent antibody titres. The extracted tissue was centrifuged and the antibody titre of the supernatant fluid assayed in the usual way. The method of expressing the tissue titres may best be shown by an example. Wt. of tissue : 3·7 g. : 6·3 ml. distilled water added and tissue homogenised. Titre of supernatant, 640. The tissue was " diluted " approximately 1/2·7 ; tissue titre is said to be 640 × 2·7 or 1728, which is given as 1720.

Tissue transfer methods.—Donor rabbits, previously immunised by intraperitoneal injection of *Salm. typhi*, H antigen, were killed by exsanguination. The omentum was removed and finely minced with sharp-pointed scissors. After a small quantity had been taken for antibody assay, the minced tissue was weighed and divided into two portions. One portion was placed in the peritoneal cavity of one of a pair of recipient rabbits through a small mid-line incision. The second portion of tissue was heated in a water-bath at 56° for 30 min. It was then inserted into the peritoneal cavity of the other recipient animal. In these experiments, the recipient pairs were albino, virgin female litter-mates of the same body weight.

Histological techniques.—Tissues were fixed in formol-saline and stained with haematoxylin and eosin. The omentum was also stained by the Unna-Pappenheim method, the section being taken to distilled water, left in methyl-green-pyronine (Carleton, 1938) for 20

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min., washed and then differentiated in equal parts of acetone and distilled water for a few seconds.

RESULTS.

Tissue titres of organs after intraperitoneal injection of antigen.

Killed *Salm. typhi* organisms were injected intraperitoneally into 7 rabbits on one or more occasions to give various serum titres of agglutinating antibody. The tissue titres were estimated and were compared with those from 3 other rabbits receiving antigen intravenously. The results are given in Table I.

TABLE I.—*Serum and Tissue Antibody Titres after Immunisation with Killed* Salm. typhi.

Rabbit No.	Number of organisms injected.	Days after last injection.	Serum titre	Tissue titres.				
				Liver.	Spleen.	Node.*	Omentum.	Kidney.
Intraperitoneal route—								
1	10^{11}	5	20	0	0	0	6	0
2	10^{10}	7	640	20	80	—	140	—
3	(2×10^{9}) twice	13	1,280	80	160	60	240	80
4	10^{10}	36	5,120	260	—	460	1,600	480
5	(2×10^{11}) twice	5	5,120	1,200	1,760	680	3,200	—
6	(2×10^{11}) thrice	5	40,960	960	1,200	1,600	3,560	—
7	10^{10} and, 200 days later, 2×10^{11}	8	81,920	960	1,040	1,600	3,360	—
Intravenous route—								
8	10^{11}	10	1,280	120	480	100	100	—
9	2×10^{10}	10	1,280	160	400	120	160	—
10	10^{11}	16	5,120	460	640	220	240	480

* Mesenteric lymph node.

These experiments show that a relatively high level of antibody may be found in the omentum after intraperitoneal, but not after intravenous, injections. The tissue titres of this organ are higher than those of the kidney, liver and lymph nodes, even some weeks after a single intraperitoneal injection when the acute inflammatory response may be assumed to have subsided (Rabbit No. 4).

In a series of 3 rabbits the technique first devised by McMaster and Hudack (1935) was used. An intraperitoneal injection containing 10^{10} killed *Salmonella paratyphi B* was given to a rabbit and, at the same time, 10^{10} killed *Salm. typhi*

TABLE II.—*Serum and Tissue Antibody Titres after Intraperitoneal Injection of Killed* Salm. paratyphi B, *and Simultaneous Injection of Killed* Salm. typhi *Intravenously.*

Rabbit No.		Serum titres.	Tissue titres.			
			Liver.	Spleen.	Node.	Omentum.
1	Against *Salm. paratyphi*	320	40	20	20	80
	„ *Salm. typhi*	640	140	400	60	80
2	Against *Salm. paratyphi*	640	40	60	—	180
	„ *Salm. typhi*	1,280	260	400	—	100
3	Against *Salm. paratyphi*	320	40	20	—	60
	„ *Salm. typhi*	320	60	120	—	40

organisms were given into the ear vein. The animal was killed 10 days later and the serum and tissue agglutination titres against the two antigens were estimated. These are shown in Table II. It can be seen that in each case the omentum has a higher tissue titre than the other organs to the intraperitoneally injected *Salm. paratyphi B* but a relatively low tissue titre to the intravenously injected *Salm. typhi*. This distribution is consistent with the suggestion that the omentum is itself involved in the production of antibody after an intraperitoneal injection of antigen.

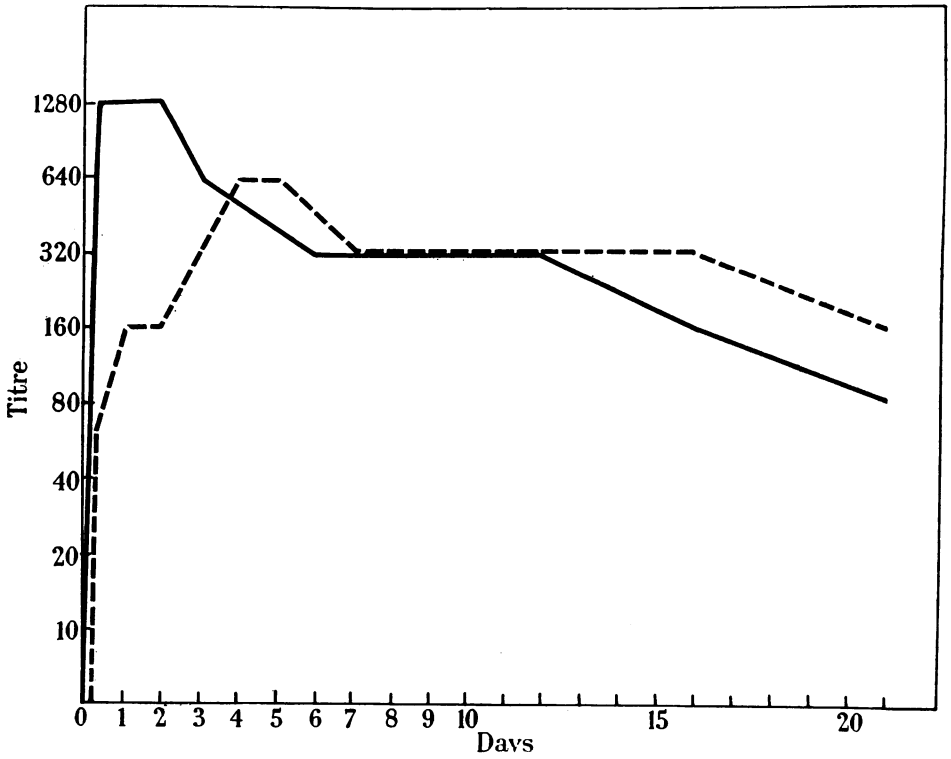

FIG. 1.—The serum agglutinin response in two recipient rabbits. — — — — — Received the donor's omentum. ———— Received the donor's serum.

Omental transfer experiments.

A rabbit was given 10^{11} organisms on alternate days on three occasions. It was killed 5 days after the last injection of antigen when the serum titre was 40,960. The omentum was removed, minced with scissors and 11·5 g. transplanted into the peritoneal cavity of a normal rabbit. Antibodies appeared in the serum of the recipient rabbit within 6 hr. and followed the course shown in Fig. 1. It may be compared with the antibody response, also shown in Fig. 1, of another normal rabbit which had received 11·5 ml. of the donor's serum with a titre of 40,960. The serum titres of these two animals are of the same order even though the 11·5 g. of transferred omentum was estimated to have a tissue titre of only 3360. This

could be explained by new antibody formation in the transplanted omentum, a transfer of antigen to give an active immune response in the recipient, an inefficient method for the extraction of antibody from the tissue, or by a combination of these factors. To overcome this difficulty the following experiment was done. Two intraperitoneal injections of 10^{11} killed organisms were given on consecutive days to a rabbit previously immunised 205 days before with 10^{10} organisms intraperitoneally. Eight days after the last injection, when the serum titre was 81,920, the animal was killed and the omentum was removed, minced and separated into two parts, each weighing 12·6 g. One part was transferred immediately, as described above, into the peritoneal cavity of one of a pair of recipient animals. The other

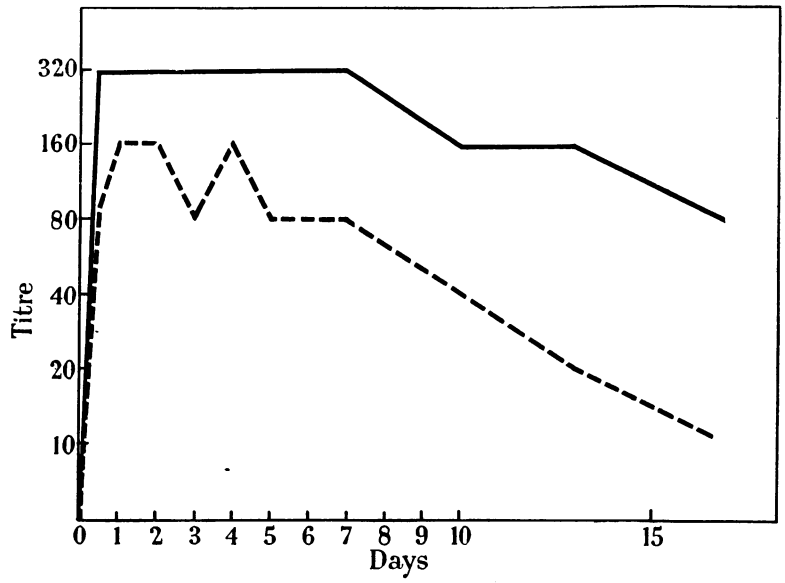

FIG. 2.—The serum agglutinin response in two recipient rabbits. ———— Received living omentum. — — — — — — Received heat-killed omentum.

part was heated to 56° for 30 min. before it was transferred to the second recipient animal. This treatment kills all the cells of the omentum without damaging any antigen or antibody that might be present in the tissue. The serum agglutinin responses in the two recipient animals are shown in Fig. 2. It is probable that the transferred living omentum continued for a while to produce antibody in the peritoneal cavity of the host for it may be seen that there was more antibody at all stages in the serum of the animal receiving living omentum. Two further experiments using this technique yielded similar results.

It may be of interest to note that the transferred omentum in each case contained large numbers of typical plasma cells. In one case the recipient animal was killed 8 days after transfer and the omental pieces examined histologically. The subserosal tissues contained large numbers of plasma cells, apparently normal in structure and in staining reactions, while all other cells had become necrotic. It is impossible to decide the origin of these plasma cells.

Histological observations.

Normal histology.—The structure of the omentum has been given by Portis (1924) and by Maximow (1928). The non-fatty parts of the omentum in the rabbit contain collections of reticulo-endothelial cells ; these are known as " milk-spots ". Elsewhere, except along the vessels, the tissue is relatively acellular.

The omentum from each of 6 normal rabbits was examined after staining with haematoxylin and eosin, and with methyl-green-pyronine. In 4 of these collections of lymphocytes were seen in the vicinity of the milk-spots. A small number of pyronine-staining plasma cells were also seen, but they did not form any considerable proportion of the " round cells " of the organ.

Immunised rabbits.—The changes in the omentum that follow the injection of organisms into the peritoneal cavity have been summarised by Wilson and Miles (1946). They were confirmed in this present work.

Seven rabbits were immunised intraperitoneally with 10^{10} organisms. The animals were killed 6 hr., 1, 5, 9, 13, 36 and 70 days after this injection. The omentum of those killed at 6 and 24 hr. showed a predominantly polymorphonuclear response which was followed later by one composed largely of macrophages. It was noticed, however, that at 5 days and subsequently there was, superimposed on this inflammatory response, a new formation of pyronine-staining cells. These appeared at first in the thickened subserosal tissues, especially in the hypertrophied milk-spots. A similar reaction was seen in 2 rabbits receiving 10^{11} organisms intraperitoneally, repeated on two and on three occasions, the last injection being given 6 and 5 days respectively before the animal was killed. In these animals, pyronine-staining cells had infiltrated throughout the greatly hypertrophied and fibrotic omentum. Cells similar to those described by Fagraeus (1948) as transitional cells, immature plasma cells and typical plasma cells were all present, but the majority of the pyronine-staining cells were of the last-named variety. The cells of lymphoid foci present in the omenta did not stain with pyronine. In the second of these two animals it could be seen that some portions of the omentum were composed entirely of plasma cells embedded in loose connective tissue.

The omentum from a rabbit immunised intravenously was similar in structure to that from a normal animal.

DISCUSSION AND CONCLUSIONS.

Portis (1924), among others, has shown that antigenic particles are taken up, when injected into the peritoneal cavity, by the macrophages of the omental milk-spots. The experiments reported here show that a relatively high level of antibody may be found in the omentum after intraperitoneal, but not after intravenous injection. From the experiments involving transfer of heat-killed omentum, it is probable that the tissue titre is much higher than that estimated from extraction *in vitro*. Experiments in which an injection of one antigen was made into the peritoneal cavity while another, but similar antigen was given intravenously, again suggest that antibody is being formed in the omentum and not merely localised in inflamed tissues.

The transfer experiments indicate that the omentum may continue to produce antibodies in the peritoneal cavity of another rabbit. The homograft cannot, of course, be expected to survive in this environment for more than a few days.

There is a marked plasma cell response in the omentum after an intraperitoneal

injection of antigen. This finding is in agreement with those of many others correlating the formation of plasma cells with antibody production (Fagraeus, 1948).

The work of Portis (1924) also suggests that the omentum may be a site of antibody formation, for he found that surgical removal of the omentum in rabbits lowered the immune response to intraperitoneal but not to intravenous injections of sheep erythrocytes.

SUMMARY.

When formalin-killed *Salm. typhi* organisms were injected into the peritoneal cavity of rabbits there was a higher level of antibody in the omentum than in the spleen, liver, kidney or mesenteric lymph node.

Injections of killed *Salm. paratyphi B* were made into the peritoneal cavity, while killed *Salm. typhi* were given intravenously. The resulting tissue antibody titres indicated an active formation of antibody by the omentum.

There is some indication that living omental tissue from rabbits which have been immunised intraperitoneally can continue to form antibody when transferred into the peritoneal cavity of normal recipient rabbits.

After an intraperitoneal injection of killed *Salm. typhi* organisms, there is an acute inflammatory response in the omentum. Superimposed on this is a new formation of pyronine-staining cells, many of which are typical plasma cells.

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