"THE PROTECTIVE FUNCTION OF THE GREATER OMENTUM"

BY

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PREFACE

The first experiments in the work of this Thesis were performed in the Department of Pathology, The Royal Infirmary, Glasgow, in 1957. I wish to acknowledge the advice and facilities which were offered to me by Professor T. Symington and Dr. J. Ives.

Subsequently, with the great help of Professor W.A. Mackey, St. Mungo Professor of Surgery, The University of Glasgow, and Professor M.F.A. Woodruff, Department of Surgical Sciences, The University of Edinburgh, and the generosity of Professor J. Squire, Leith Professor of Experimental Pathology, The University of Birmingham, I was awarded a Clinical Research Fellowship by the Medical Research Council in 1958 in the Department of Experimental Pathology, The University of Birmingham. In this department I met Dr. P. Gell, Reader in Immunology, and benefited greatly from his encouragement and advice.

Chapter I

1. ANATOMY OF THE GREATER OMENTUM.

The greater omentum is the largest peritoneal fold occurring in the abdomen. It consists of a double sheet folded upon itself, so that it is made up of four layers. The two layers which descend from the stomach, and the commencement of the duodenum, pass downwards in front of the small intestine for a variable distance; they then turn upon themselves and ascend again as far as the transverse colon. At this point, they appear to separate and enclose this reach of the large bowel although, in fact, the outer of the two layers has become fused with the upper layer of the transverse mesocolon and the superior aspect of the transverse colon. In a young subject, these layers can nearly always be demonstrated, but in the adult, they are usually inseparably blended.

The left border of the greater omentum is continuous, above, with the gastro-splenic ligament, which passes between the stomach, anteriorly, and the hilum of the spleen, posteriorly. The right border of the structure extends as far as the commencement of the duodenum, and a prolongation of this may reach the cystic duct.

The greater omentum is usually thin, and presents a cribriform appearance, but it always contains some adipose tissue which, in fat people, is present in considerable quantity.

Between its anterior two layers, about a finger's breadth below the greater curvature of the stomach, the right and left gastro-epiploic arteries and veins anastomose with each other. The arteries and the veins are but loosely attached to the omentum.

In the so-called normal position it extends from the stomach and transverse colon to the symphysis pubis below, and to or over the colon on either side. Seldom, however, even in abdomens showing no evidence of disease, is it found occupying this position. Its peripheral portions are usually folded, reduplicated or "crumpled", so that they do not reach to much beyond the umbilicus below and the inner edges of the colon from side to side. It therefore occupies a smaller area than that of which it is capable of occupying when fully extended. Not infrequently, the structure has some attachment to the gall-bladder; an attachment, which at one time, was considered pathological, a belief which is now more or less relinquished.

2. HISTOLOGY OF THE GREATER OMENTUM.

The greater omentum contains numerous connective tissue cells, some of which can be mobilised into free macrophages. These cells

may accumulate, in places, into dense, oval or round patches, which are visible to the naked eye and are termed the "milk-spots" (taches laiteuses) of the structure. The omentum is, therefore, a network of blood vessels, along the main branches of which, varying amounts of fat are deposited. Supporting the blood vessels is a thin, transparent and somewhat elastic membrane formed by the union of the two peritoneal plates and containing a delicate meshwork of connective tissue, bearing minute blood vessels. These latter vessels are, in the resting omentum, practically empty. Only in the reactive omentum do they actually take on the function of blood vessels. They have been called "potential vessels" on this account. The arterial blood supply arises from six or eight fair sized branches of the gastro-epiploic arteries. Near their origin they give off very few branches and run a parallel and more or less straight course. As they approach the periphery of the omentum their course becomes more tortuous and they give off many small anastomosing branches which divide into the "potential vessels", which have already been described.

3. EMBRYOLOGY OF THE GREATER OMENTUM.

The "anlage" of the greater omentum is found in the lateral plate mesoderm, which first becomes evident between the twenty first and thirty first days of human development. By the development of the intra-embryonic coelome this lateral plate mesoderm becomes divided into the splanchnopleuric and somatotrophic membranes, and it is from the

latter that the greater omentum eventually develops. The adult configuration of the structure arises by the complicated series of developments and alterations in position, consequent upon the rotation of the gut, after its re-entry from the extra-embryonic coelome.

The first stage occurs when the persisting right pneumato-enteric recess enlarges and burrows behind the, as yet, unrotated stomach. This burrowing continues in a posterior and cranial direction on the right side of the oesophagus to form the lesser sac. While the stomach is rotating and being drawn to the left from its midline position the duodenal loop "falls" to the right and the mesoduodenum fuses with the posterior abdominal wall peritoneum and becomes retro-peritoneal. The dorsal mesogastrium becomes elongated and is carried, as a reduplicated membrane, hanging from the greater curvature of the stomach, in front of the transverse colon and mesocolon. The latter arises from the posterior abdominal wall.

During this process the spleen makes its appearance in embryos at the ten millimetre stage. It forms a localised condensation of mesodermal cells in the left leaf of the dorsal mesogastrium. At about the fourteen millimetre stage this condensation forms a mass which projects from the left leaf of the dorsal mesogastrium, in the general peritoneal cavity. The mesogastrium can now be sub-divided into a portion between the posterior abdominal wall and the spleen, and a portion between the spleen and the greater curvature of the stomach. At the fifty

millimetre stage the lesser sac enlarges, as a result of growth in a caudal direction of that part of the dorsal mesogastrium which lies caudal to the spleen and the gastro-splenic ligament. Meanwhile, the dorsal pancreatic bud extends cranially in the mesoduodenum and grows into the dorsal part of the mesogastrium as far as the spleen. When this dorsal bud meets the ventral pancreatic bud and fuses with it the pancreas lies within the mesogastrium (Fig. 1). Later the organ falls against the posterior abdominal wall and, with the absorption of the right layer of peritoneum, becomes a retro-peritoneal organ. This fusion extends caudally to the transverse colon (Fig. 1) so that in adult life the greater omentum appears to be attached to, or arise from, the transverse colon. The peritoneum of the greater sac which lies to the left of the mesogastrium, and behind it, becomes obliterated and the mesogastrium, falling against, and fusing with this area gives rise to the definitive lieno-renal ligament, attaching the spleen to the anterior surface of the left kidney. Thus the original artery (a branch of the coeliac), which originally passed directly forward in the dorsal mesogastrium, now pursues a circuitous course and gives rise to the splenic and gastro-epiploic arteries.

It is worthy of note that the development of the greater omentum and spleen is so intimate.

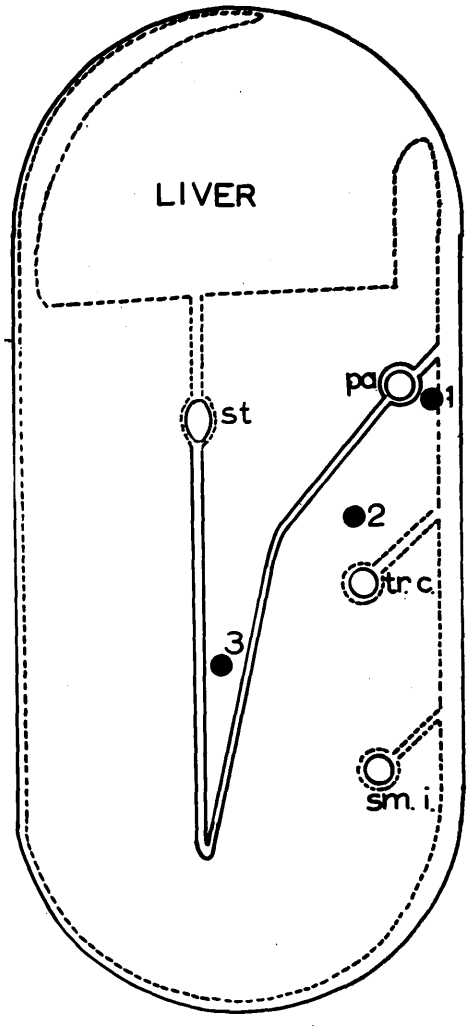

Fig.1. A sagittal section in diagramatic form to illustrate the extent of the greater omentum. The omentum is shown in full line while the remaining peritoneum is in interrupted line. The potential spaces marked with a filled-in circle become obliterated later, and in the order in which they are numbered. It is thus seen how the definitive omentum gains its attachments.

st = stomach. pa = pancreas. tr.c = transverse colon. s.i. = Small intestine.

4. COMPARATIVE ANATOMY OF THE GREATER OMENTUM.

Some points of the comparative anatomy of the greater omentum are of interest. It is completely absent in fish, and is first met with in vertebrates in the Giant Salamander. Most birds have a rudimentary omentum but, on the other hand, it is well formed in the birds of prey, and it was suggested that it formed a protective pad of fat against the piercing of the intestines by swallowed spicules of bone. Dogs, cats and carniverous animals in general have a remarkably well developed structure.

5. GENETICAL CONSIDERATIONS.

The attachment and size of the greater omentum is inherited as a Mendelian dominant. The structure is sometimes congenitally absent.

Chapter II

A REVIEW OF THE LITERATURE.

The physiological function of the greater omentum has excited interest from the very earliest time. It has been the subject of research for more than three thousand six hundred years and yet, in the present era of scientific enlightenment, it is still an organ of much mystery. The word omentum has its origin in the practice of the Egyptian priests examining the abdominal viscera while embalming the human body. As this organ, in particular, was a mystery to them they used it for gain and would tell fortunes by it and, according to the purse that the relatives of the deceased were able to pay, by examining the omentum would give good or evil omens as to the hereafter of the dead. Thus the first part of the name (omen) and, by adding the suffix (um) of the language, it came to be called the omentum.

At the dawn of medical history, Hippocrates (460 - 388 B.C.) considered that the greater omentum was concerned in some way with the secretion and absorption of free fluid in the general peritoneal cavity. These views he drew from observations of patients dying of conditions associated with ascites. He also noted that if the omentum protruded from a wound it necessarily mortified and dropped off. After Hippocrates we find that we owe to Aristotle (384 - 322 B.C.) the commonly accepted opinion, that the omentum constitutes a fatty apron covering the intestines to preserve the innate heat of the body.

Eristratus, however, could attribute no useful function to the greater omentum and declared that it was a redundant fold. However, Galen accepted and expanded the view of Aristotle. He cites the instance of a gladiator who, having lost his omentum through a wound recovered from the injury but thereafter felt cold in his abdomen. Vesalius (1514 - 1564 A.D.), from the dissections of many cadavers but with no knowledge of the embryology of the structure, described it as a supporting ligament or a ligament of fixation of the transverse colon and associated its anomalies of size and insertion with many different maladies. Verhagen considered that it existed to prevent injury to the underlying viscera from jars, jolts and friction by adapting itself to the alterations in configuration of the abdominal cavity and interposing itself between the parieties and the intestines and cushioning the latter. Malphigii (1629 - 1694) considered that the omentum was both the cause of ascites and also functioned as a storehouse for fat. Fabricus "ab aqua pendentii", well known for his discovery of the circulation, held that it was the seat of a sort of "emergency" fold which, by widening when the stomach assumed a more anterior position, enabled this organ to accommodate itself to its environment. Cordeus taught that it was a second stomach or food store from which the stomach again received the food when, by him, man was declared to ruminate "like unto brute beasts". Curvier held the same view.

For many centuries nothing further was added to the knowledge of the greater omentum, although many fanciful writings occurred. Indeed,

this organ has remained the object of much controversial speculation. This is not surprising when it is remembered that the medicine of the ages, of necessity, relied on observation and had no ready access to the abdominal cavity. The majority of their references relate to observations which they made upon the dead. The microscope had not yet been conceived and there was no knowledge of the cellular structure of the tissues.

Subsequent to the development of the microscope, and following the detailed examinations of the different tissues, much was written of the omentum but little of value was contributed until von Recklinghausen (1854) published the results of his examination of the histology of serous membranes. He described the presence of intercellular clefts or spaces (stomata) existing in these membranes and suggested that particulate material or cells of the underlying connective tissue was able to enter the integument through these naturally occurring passages. This observation was followed by many contributions, particularly in relation to the capacity of the greater omentum to absorb particulate material lying within the greater sac. Later, Muscatello produced evidence to suggest that these stomata were consequences of the staining or fixing techniques used and, finally, McCallum (1903) established that the clefts did not exist and were histological artefacts. In spite of this false conception of natural portals, the original publication, however, stimulated much fresh work and heralded the experimental era of the elucidation of the function of the hitherto mysterious membrane which appeared so inert.

This experimental work was most intense in the last decade of the 19th century and in the early part of the 20th century. By this time the work of Louis Pasteur and Lord Lister had been published and acclaimed. The existence of micro-organisms had been established and because of the discovery of anaesthesia, observations were being recorded of phenomena occurring in the living from various surgical centres, and fresh views and processes were evolving from the pathological departments of various research centres. From the many papers which were written there emerged the conception of the greater omentum as having some sort of protective function against the bacterial invasion of the peritoneal cavity. The first reference to this protective function is that of Ross in 1893. He considered that the principal function of the greater omentum was that of providing a protective cushion for the underlying viscera and that, being freely mobile, it adapted itself to the changes in the configuration of the abdominal cavity and thus assisted the intestines to move away from the point of greatest pressure. However, he also noted the manner in which the omentum seemed to hasten to prevent the bacterial invasion of the peritoneal cavity. He described the manner in which the structure glued itself to the site of inflammation and thus prevented an extension of the lesion, and likened the function of the greater omentum, in this respect, to a "man-of-war ready to sail to any port in which there was impending trouble". But perhaps the best known work on omental function is the now classical contribution of Sir Rutherford Morrison (1906). He wrote - "The greater omentum travels about in the abdomen with

considerable rapidity and is attracted by some sort of information to neighbourhoods in which mischief is brewing. It may effect radical cure of hernia by blocking the hernial orifice with an omental plug. It surrounds and adheres to a recently reduced and strangulated loop of intestine and may keep it alive and prevent a leak. It is generally found in the neighbourhood of a diseased or inflamed appendix, and by wrapping it up when gangrenous or blocking up the pus from an appendiceal abscess it may prevent general peritonitis. In a similar manner it may prevent the perforation of an ulcerating malignant growth, or a gastric ulcer, or the death of a damaged portion of bowel, or the perforation of a suppurating gall-bladder. Its effective mobility is shown by the fact that whether the lesion be in the diaphragmatic roof of the abdomen or in the floor of the pelvis, the omentum can, and does, find its way."

The first major experimental advance was that of the work of Rubin (1911). He carried out specific observations upon the cadavers of fresh mortem subjects and established that without an intra-abdominal pathological lesion the position of the omentum is a variable one. Its length varied from a few inches to a comparatively enormous length. He pointed out that it may be longer and wider on one side than the other, and often it possessed two or more tongue-like processes. In emaciated individuals, as a rule, the omentum was found to be thin and devoid of fat and sometimes shrivelled to a mere thread along the lower border of the transverse colon. In fat individuals it could attain an enormous size and weight, and he recorded one instance of the omentum weighing as

much as one kilogram. He then examined a further series of patients dying of an intra-peritoneal lesion. He established that in pelvic suppurative processes the edge of the omentum played the essential role in the walling off of the inflammatory processes, so common in the female. When the omentum was involved in an inflammatory process in the pelvis it would form, as a rule, the roof of the abscess cavity, extending sometimes from one inguinal region to the other. He also noted that the omentum was capable of participating in prostatic and peri-prostatic abscesses, in peri-cystic inflammations and in infected supra-pubic cystotomy wounds. In intestinal anastomoses the omentum, usually, was attached to the circumference of the sutures. He observed that new growths, as a rule, were not attacked by the omentum. If the omentum was found adherent to an intra-abdominal mass, the probability was that the tumour was of an inflammatory character rather than a neoplasm. If the tumour proved to be a true neoplasm, an adherent omentum nearly always denoted an inflammatory change. He considered this a diagnostic sign which would be helpful to the surgeon. The capacity of this structure to move about the abdomen and take part in any inflammatory focus and thus wall it off, as pointed out by Sir Rutherford Morrison, stimulated much work as to the mechanism of the migration of the greater omentum. This aspect is discussed in a later chapter.

Clairmont and Haberer in their work on the absorbative capacity of the peritoneum showed what Muscatello, von Recklinghausen, Schnitzler and others had already demonstrated, namely the difference in resorption

from the diaphragmatic peritoneum and that of the rest of the abdomen. They made use of the ingenious method of painting collodion over the diaphragmatic peritoneum for the purpose of blocking the "stomata". Absorption was delayed. In the same way, when a portion of parietal peritoneum was resected there was a delay in absorption. Potassium iodine was used in solution. A test was made of the urine excreted every few minutes for forty eight hours in order to see how soon the iodine would be discovered. Further tests were carried out to determine the time required for the total elimination of potassium iodine. They also induced artificial peritonitis in other experiments with a view to ascertaining its influence on absorption as measured by the potassium iodine excretion. Unfortunately, in the last experiments the omentum was scarcely considered. Rubin, however, noted that in as much as the omentum was composed of a wide expansion of peritoneum, it should serve as an important factor in the absorption of material from the greater sac. Accordingly, he resected the omentum in a series of cats and used an equal number of normal intact cats as controls. Indigo carmine, instead of potassium iodine, was injected intra-peritoneally. He used the same method of injection as that of Clairmont and Haberer. He confirmed their observations that anaesthesia had no appreciable effect on the rate of absorption. In five cats, in whom the omentum had been resected, a bluish colouration of the urine was observed forty to sixty minutes after the injection. In one cat, blue urine occurred after twenty four minutes; this cat had been permitted to run about immediately after the injection while the

others were kept on their backs. In the control animals, subcutaneous and intra-peritoneal injections showed an excretion of the indigo carmine within twelve to fifteen minutes. This represented a four-fold excretion in the intact animal. In these animals the omentum was then resected and seven to eight days later the injection was repeated. The same relative delay in the indigo carmine occurred as in the first series.

In the same year Sir David Wilkie performed a similar type of experiment. He had observed that ascites did not follow the complete removal of the omentum in animals, which made him believe that the absorptive function of the structure was not all important. The method he employed was to remove completely the omentum in an animal and three weeks later to inject into its peritoneal cavity a certain quantity of saline solution, then to kill the animal after a certain number of hours and to carefully collect all the fluid left in the abdomen. A control animal with its omentum intact was injected with the same quantity of saline solution and was killed and examined after the same interval of time. Cats were used for these experiments as they possessed the largest omentum of all the available experimental animals. The removal of the omentum did cause an appreciable, but not a marked, delay in the absorption of saline from the peritoneal cavity and, on the average, it was found that the rate of absorption in animals with intact omenta was to that of animals after the excision of the omentum, in the ratio of three to two. It was, therefore, unjustifiable to maintain that the omentum was a specialised absorptive organ. These results confirmed those of Rubin.

THE ABSORPTION OF SOLID PARTICLES BY THE GREATER OMENTUM.

Heger used lamp black, cinnabar, sulphurate of mercury, vermillion pigment and bismuth subnitrate and injected these substances into the peritoneal cavity. In the case of the lamp black, he observed black strands along the omentum; while the mesentery and peritoneum showed no similar infiltration. While Muscatello believed that these particles were ingested and carried by wandering leukocytes and absorbed by the abundant lymph channels of the omentum, Heger held that the body of the omentum accomplished the attachment of the particles by a sort of "balayage" or sweeping movement, which the action of the diaphragm facilitated. For his experiments Heger used:-

(1) Adult animals - guinea pigs and rabbits.

(2) The same animals in which the omentum was resected.

(3) Very young guinea pigs and rabbits in which the omentum was very small.

(4) Frogs and fish, in which no omentum could be found.

In those animals in which the omentum was absent, particles injected intra-peritoneally were disseminated in the abdomen, and they remained on the surface of all the organs. At the end of forty eight hours the apices of the lungs showed an infiltration by the particles of pigment. This same phenomenon was seen in the young rabbits in whom

the omentum was rudimentary. He introduced fifty glass beads, varying from 1 - 3 millimeters in diameter, into the peritoneal cavity of a dog. At the end of four days all the beads were found to be attached to the greater omentum. Wilkie noted that when at an abdominal operation some foreign bodies, such as a piece of gauze, a silk thread or an instrument were inadvertently left in the peritoneal cavity, and it was necessary at a later date to make a further search, it was usual to find it wrapped up and encapsulated in the omentum. Small solid particles, such as bacteria and particles of organic debris of any sort, when they escaped into the free peritoneal cavity were caught up by the omental net, in a very striking manner. He clearly demonstrated this by the following experiment:-

The abdomen of a cat, under ether anaesthesia, was opened and five grams of sterilised powdered animal charcoal was introduced into the peritoneal cavity and the abdomen was closed. In a second cat, the omentum was removed and the same quantity of charcoal introduced before closing the wound. The latter animal died two days later and all the abdominal viscera were found irregularly coated with the black powder. The first cat was killed on the second day after operation and, on opening the abdomen, it was found that the whole of the charcoal had been taken up by the omentum and that the other abdominal viscera had a normal glistening appearance.

That this scavenging function of the omentum was practiced in a

similar manner on bacteria, was later pointed out by Durham. Shipley and Cunningham showed that decerebrate animals with exteriorised omenta immersed in solutions and pseudo-solutions of high molecular dye stuffs, like trypan blue, or in colloidal metals and India ink, absorbed these high molecular substances. In other experiments in which the omentum was immersed in a solution of potassium ferrocyanide and iron ammonium citrate, and the omentum was later fixed in acid formalin, the absorptive function of the omentum was visually demonstrated by the distribution of precipitates of Prussian blue.

In 1941, Baillif examined the reaction of the greater omentum to the injection of particulate material. He used trypan blue, lithium-carmine and India ink. He described the marked histiocytic response occurring within eighteen hours of the injection of particulate material and demonstrated the reaction which occurred in the "milk spots", culminating in the production of many phagocytic cells which emerge from the "outer zone" of the activated spot. These cells then engulfed the foreign material.

THE PROTECTIVE FUNCTION OF THE GREATER OMENTUM.

The first experimental evidence of the protective capacity of the greater omentum was, however, the outcome of the work of Durham in 1897, who injected emulsions of staphylococcus aureus into the peritoneal cavities of a group of animals possessing an omentum and in a control group of animals in whom the omentum had been removed. He found that those animals deprived of an omentum died in the first day or two following the injection, while those with an omentum survived. Roger, in 1898, resected the omenta of rabbits and guinea pigs. In from fourteen days to two months after the resection, he injected a few drops of cultures of staphylococcus aureus intra-peritoneally. While control animals survived, the former succumbed in two to three days. When less virulent or smaller quantities of the same cultures were injected the animals in which the omentum had been resected, emaciated and died. The control animals, however, survived.

Sir David Wilkie also examined this problem in 1911. A series of seven experiments were carried out to determine whether the removal of the omentum in fact lowered the degree of the animals resistance to peritoneal infection. In seven rabbits, the omentum was removed and three weeks later the animals, all being in excellent condition, were

given a slightly sublethal dose of an emulsion of staphylococcus aureus which was injected in the peritoneal cavity of each. Seven other rabbits of equal size and weight acted as controls. Five of the rabbits, without omenta, died of peritonitis, whereas but two of the control animals died and these two survived for a considerably longer period than the fatal cases in the animals without omenta. To further elucidate this function Wilkie then injected a certain dose of an emulsion of staphylococcus aureus into the peritoneal cavity of a series of animals, in half of whom the omentum had previously been excised, and then by drawing off the peritoneal fluid by means of a capillary pipette at intervals of an hour he was able to study the cellular changes in the peritoneal exudate in each case. It was found that the cellular reaction in the fluid was quite as rapid and even more intense in the animals with no omentum as in those with an omentum. It was noteworthy, however, that whereas in the latter, very few bacteria were visible in the fluid within two or three hours after the injection, in the former, cocci were found in large quantities both free in the fluid and within the polymorphonuclear-leucocytes. By killing one of the animals with an intact omentum at this stage he could readily demonstrate that the omentum was densely covered by the cocci, very many of which were being phagocyted by polymorphonuclear-leucocytes. The omentum seemed to act to bacteria "as a spider's web to a fly" and "the battle of the bacteria and the leucocytes was transferred from the free fluid to the surface of this vascular peritoneal fold where the phagocytes were in close touch with their lines of transport and their

reinforcements". When smaller sublethal doses were injected and the peritoneal fluid examined daily for ten days or so, he was interested to note that the non-nucleated phagocytes had appeared almost as quickly and in as large numbers in the exudate of the animals without, as in those with, an omentum. Restitutio ad integrum was invariably much more rapid in an animal with an omentum than in one that had lost that organ, and fluid containing flecks of lymph and debris in a fair number of cells, was frequently obtained from the abdomen of one of the latter class of animal, even one to two weeks after the peritoneum of the control animal had returned to normal. Pirone demonstrated further the importance of a scavenging action of the omentum.

In 1916, Charles Mayo, addressing the Minnesota State Medical Society in Minneapolis, very prophetically stated - "It (the greater omentum) holds a high percentage of phagocytes of the defensive repair type and, unlike the other areas, did not require the presence of structural infection to slowly develop or gather the phagocytes. The omentum is always ready with its protective army. In time of need there is an exudate of lymph and adhesions formed by the omentum". He pointed out that Pirone and Heger had shown that with the loss of the omentum the main phagocytic power of the peritoneal cavity was lost. The work to which he referred was that in which Pirone and Heger had ligated the main splenic vessels in a series of animals and thereafter had found that the omentum had come to encapsulate this tissue and, in fact, to give rise to formed blood vessels which penetrated the splenic capsule, and by a process of phagocytosis had removed the ischaemic

splenic remnants. Pirone concluded that the omentum had a protective function and he claimed that this was shown, firstly, by the formation of blood vessels contained in the connective tissue capsule, and secondly, by the phagocytic efficiency of its transformed endothelial cells and of the emigrated leukocytes. In a further series of experiments Pirone ligated and extirpated the spleen. The animals were killed at the end of twenty, thirty and sixty days. In some cases the omentum showed a pus focus at the site of ligature. In all instances the omentum was fixed to the cicatrix of the peritoneal wound. The remaining peritoneum, intestines and other abdominal organs were always normal. In other words, he believed his experiments confirmed those of DeRenzi and Boeri, and besides an "intelligent motility", as Cornell called it, the omentum under certain conditions possessed a true defensive function. Scheiffendecker, quoting Lewis's observations on haemolymph glands in which the endothelial cells became transformed into phagocytes, concluded that phagocytic cell changes could take place from the endothelium of the omentum. Whether this is true of the omentum is still debated. Von Recklinghausen, Bizzozero, Golgi, Muscatello and others have shown a similarity in the structure of the omentum to that of the lymph system. On the basis of this analogy, Scheiffendecker argued against Heusner's statement that "there exists no special organ for pathological conditions". From the fact that the spleen in the rabbit, cat and dog is of intra-omental origin, receiving its blood supply by blood vessels that are carried in the substance of the omentum, it seemed to Rubin that the ligation of the

splenic vessels could not be readily accomplished without, at the same time, injuring the omentum and thereby vitiating the purpose of the experiment. He argued that in the first experiment of DeRenzi and Boeri the conditions were not valid because the blood supply was maintained by the untied splenic omental vessels. The spleen would not suffer because only "the main splenic vessels" were tied off; enough blood circulated to the spleen from the remaining vessels which the omentum normally carried to the spleen. He pointed out that the omentum could only, in this specific case, "restore the circulation of a contiguous organ. It manifestly could not serve this purpose for any other organ because of a lack of a similar intimate vascular connection". Instead of ligating isolated vessels, Rubin ligated the entire omental splenic ligament in cats, dogs and rabbits, and the same results were seen as those obtained by DeRenzi and Boeri. The animals survived, apparently unharmed. The spleen became encapsulated as in the second experiment of DeRenzi and Boeri, in which total ligation of the splenic vessels alone was made. To still further investigate the so-called protective role of the omentum, particularly in relation to the spleen, Rubin resorted to two series of experiments. First, the greater part of the omentum was resected. When this was done the spleen was found, up to from eight to fourteen days, to be much smaller, showing that the blood supply had been diminished. Secondly, he resected the spleen completely and he replaced the resected spleen within the abdomen. According to DeRenzi and Boeri death of the animal should have occurred from "auto-digestion", in fact, the animals

survived for weeks and in some instances, months. In order to note the behaviour of the omentum towards a foreign spleen Rubin operated on two dogs simultaneously - the spleen of the one was placed in the abdomen of the other, in each case. Two weeks later the abdomen of the dog containing a foreign spleen was examined. The greater portion of the omentum was seen to be rolled up into a rather large, firm, solid mass. The spleen was nowhere visible in the abdomen. On resecting the omental mass it became evident that the spleen had been completely surrounded by omentum. The spleen was doubled up, reduced to about two thirds its original size, and was closely encapsulated by omentum. This section formed a white, glistening, more or less homogeneous capsule: the central portion of the spleen was pultaceous and on microscopic examination showed various stages of necrosis, which was less marked towards the periphery.

THE MIGRATING CAPACITY OF THE OMENTUM.

As had been mentioned previously, the advent of anaesthesia allowed observations of inflammatory phenomena to be made increasingly by surgeons. Much appeared in the literature of observations in which it had been noted that the omentum was, in a great many instances, to be found in the vicinity of some inflammatory focus. As will be recalled, in 1906 Sir Rutherford Morrison described this structure as "the policeman of the abdomen", and in fact considered that it was a diagnostic pointer to the surgeon. The surgeon had merely to follow

the greater omentum to find the inflammatory lesion within the abdomen. He claimed that it could move to any focus between the pelvic floor and the diaphragmatic roof of the peritoneal cavity. The mechanism of the migration was the subject of great controversy. Finding the omentum frequently at the site of inflammation, at the operating table and in the post mortem room, had led surgeons to speculate concerning the motion of the omentum. Many theories of motion had been evolved.

Ross in 1893 wrote that the omentum was endowed with some vermicular-like action and that it was able to move itself from place to place. As has been mentioned before, he also noted that it was capable of glueing itself to the point of a peritoneal inflammation, and this limited the extension of the lesion. Durham in 1897 noted that the omenta of animals, dead of peritoneal infections, became rolled and folded up because of the peristalsis of the intestines, he thought. Adami in 1898 thought that the omentum was endowed with the power of active locomotion. He thought of it as rolling up and down, to the right and left and, in effect, covering the entire abdominal cavity. Milian spoke of the omentum as reacting to positive or negative chemotaxis. He attempted to demonstrate this by producing an area of irritation upon a part of the intestine of a guinea pig, but failed to note any response on the part of the omentum. His observations on a cadaver, however, led him to believe that "in certain forms of virulent peritonitis the omentum has actually been repelled from the poisonous process" and lay coiled up along the lower border of the transverse colon. Rubin in 1911 studied this point in post mortem examinations

but he could not confirm it. In the majority of cases of peritonitis the omentum was found firmly adherent to the anterior parieties or the intestines. To throw further light on the question of motility Rubin performed the following experiments upon rabbits, cats and dogs:-

Firstly, a rabbit was killed by the anaesthetic. The abdomen was opened in order to inspect the relations of the omentum to the viscera: very active peristalsis was observed. The omentum was then spread over the intestines and it was soon seen to move back to its original position. When the large bowel or a portion of the intestine which showed sluggish or no peristalsis was placed beneath it, the omentum retained its newly acquired position. A rather energetic peristalsis was required in order to displace the omentum. The intestines rose to various heights and caused the omentum to glide back to its original position. Secondly, the peritoneum was layed bare by separating the abdominal muscles, but was not opened. Saline solution and irritants were then poured over the intact peritoneum. Active peristalsis could be observed through the intact peritoneum, the omentum, however, did not change its position laterally or vertically. His conclusions were, firstly, that the omentum had no spontaneous motility and that the displacement of the omentum could be explained by:-

(a) Intestinal peristalsis.

(b) Intra-abdominal tension.

(c) The static conditions of the stomach, colon and small intestine.

(d) The anatomical relationship of the omentum to the gall-bladder

and spleen.

Secondly, that the omentum had no demonstrable chemotaxis. The amount of peritoneal fluid plus the amount of gas contained in the large intestine could be held to account for this apparent intelligent retreat of the omentum from virulent and infective processes. In addition, the suction action of the diaphragm under changed conditions of intra-abdominal tension explained the upward "chemotaxis" of the omentum in inflammatory lesions of the upper abdomen. To Rubin the omentum had no intelligence or spontaneous protective role; such protection that it apparently displayed was simply due to its properties as a piece of peritoneum and not as a superior organ with definite functions. Sir Rutherford Morrison also believed that the omentum was attracted to the point of injury by some sort of information - that is, some form of chemotaxis as postulated by Milian, and he likened its movements to that of a jelly-fish. Theodor Fisher in 1906, Norris in 1908, Wilkie in 1911 and Adams in 1913 believed that the movement of the omentum was dependent upon peristalsis of the intestines. Fisher assumed that if the peristalsis affected all areas of the intestines the omentum was spread out over the intestines; however, if there was paresis of one segment of the intestine the active moving segments propelled the omentum to the area of stasis. In his paper of 1911 Wilkie specifically sets out to answer the question, "is the omentum capable of intelligent movement"? He observed that inflammatory processes which were situated outside the normal range of the omental excursion did not attract the organ. For example, an inflammatory

process at the bottom of the pelvis was usually entirely ignored by the omentum unless the latter happened to be unusually long and pendulous. He considered that it was only by the transmission of irritation from one peritoneal surface to another and on account of its peculiar adhesive properties that the omentum came to adhere more firmly to the focus of greatest irritation. He performed an experiment which settled the question completely to his mind. Under ether anaesthesia the abdomen of a rabbit was opened; its omentum was lifted up and laid over the liver. The left hand corner of the omentum was fixed with one catgut stitch to the parietal peritoneum in front of the liver and an emulsion of staphylococcus aureus was then injected into the submucous and sub-peritoneal coats of the appendix. The animal died five days later from an appendicitis with localised peritonitis. At the post mortem it was demonstrated that the omentum had not made the slightest movement in the direction of the inflamed appendix, although its right hand border could readily be lifted down to the seat of trouble. He considered that this was no case of "negative chemotaxis" but was merely the inability for combined action between those two physical factors which determined the position taken up by the omentum - namely, respiratory and peristaltic movements and peritoneal irritation and roughening. Hertzler in 1935 stated, if there were no leukocytic cellular infiltration into the omentum there would be no migration. He explained the motion as similar to the influence that draws any leukocyte to an area of infection or inflammation. He did not believe that the position of a normal omentum was influenced by the change of

position of the individual or animal or that peristalsis influenced the advance of the omentum to the site of injury. He stated, "if we are to speak of the 'intelligence' of the omentum we may safely add that an individual's omentum is as smart as his leukocytes". Schutz in 1930 approached the mechanism controlling the migration of the omentum from an entirely different point of view and one not previously presented. He likened the motion of the stretching out of the omentum to the uncoiling of a curled up garden hose when an increased pressure of water is caused to flow through it. Similarly, in the omentum he postulated that the arteries, which were tortuous, became hyperaemic, the intra-arterial blood supply was increased, the arteries straightened out and the omentum, which was closely attached to these, was pulled by them to their advanced position. He noted a resemblance between the omentum and erectile tissue.

Cantacuzene and Soru in 1931 stated that the migration of the omentum to lesions of the intestines was strictly a function of a difference of electric potential that existed between these two points. They determined that the electrical potential between the omentum and the intestines was usually eight or less millivolts. After an injury to the peritoneal surface the electrical potential between the two areas was immediately elevated to fifty three millivolts. The omentum, which was electro-negative under normal conditions, became electro-positive - thus the omentum was attracted to the injured area.

Rothenberg and Rosenblatt in 1942, by means of omental marking agents and fluoroscopic studies, concluded that omental migration

depended upon the motion of the underlying bowel. Their experiments are of some interest. They used radiopaque materials, such as silver brain clips and Michele clips and leaded glass thread, buried in the free edge of the mid portion of the most dependent part of the omentum. They first noted the relationship of movement to the differences in posture, in intestinal movement and also in vomiting and defaecation. Subsequently, two to three months later 1½ ccs of a fresh virulent culture of B. coli containing about 5,000,000,000 organisms was injected into the peritoneal cavity and fluoroscopic examinations later showed no change in position of the leaded thread or other radiopaque fluoroscopic agents. Exploratory laparotomy performed six weeks after the implantation of bacteria showed that the omentum was completely free of adhesions. The leaded thread was encapsulated by the omentum which was only slightly thickened. These experiments were repeated and they always noted that there was some slight thickening of the omentum, although there were no adhesions and no evidence of active movement or migration towards a particular side. However, no histology was performed in this series. Thomas, Green and Rhoads in 1950 studied a process of adhesion formation through abdominal windows, made of methyl methacrylate or lucite, in animal experiments. In the course of their observations they stated that in respect to an area of injury the omentum seemed to be pushed or attracted to the sticky coagulum to which it adhered. Allen in 1954 made an exhaustive study, again using a fluoroscopic technique. His conclusions were that his fluoroscopic agent, tantalum wire, was an excellent non-irritating stable marking

agent for the omentum. Secondly, the normal omentum pattern shown in the x-ray films after its edge had been marked with tantalum underwent variations of a considerable degree. Thirdly, these variations were shown to be dependent, in varying degrees, upon:-

(a) Peristalsis.

(b) Gravity.

(c) Diaphragmatic movement.

Fourthly, migration of the omentum had no relationship to:-

(a) Hyperaemia of the omentum.

(b) Elevation of omental arterial pressure.

(c) Increase in omental volume flow.

(d) Any combination of these factors.

Fifthly, that the omentum was involved only in those intra-abdominal inflammatory processes which were situated within its normal range of excursion and with which, therefore, it might, by chance, come into contact. Six, that the omentum reacted as other peritoneal surfaces when in contact with intra-abdominal inflammations. This confirmed the earlier impressions of Florey, Walker and Carleton (1926) and Siciliani (1832).

The greatest reported series of examinations of post mortem subjects is that of Rothenberg and Rosenblatt in 1943 when they examined one hundred and sixty consecutive autopsied cases. These cases were unselected and forty six of them at one time or another showed evidence of intra-peritoneal infection. Omental response in the form of adhesions occurred in eighteen of these cases, or thirty-nine point one

per cent. This appeared to be a poor response if the greater omentum were actually the active protective force it was supposed to be, they claimed. An analysis of lack of omental response showed that it could not be explained on the basis of insufficient time, insufficient severity of the infection, previous adhesions limiting the mobility of the omentum or because the omentum was anatomically unable to reach the infected area due to inadequate length. They concluded that these findings supported their previous impression that the greater omentum did not respond to intra-peritoneum insult by actively moving to the site and forming adhesions in the involved area. They were not prepared to state that the omentum played no part in intra-peritoneal infection, because numerous studies had shown that it did throw out an exudate and that it did often become involved in delimiting adhesive processes. However, in those instances in which adhesions were formed, as in thirty-nine point one per cent of their cases, the adhesions appeared to be the result of organisation of such an exudate upon the surface of the omentum. It was their opinion that adhesions were formed only if the omentum happened to be at or near the site of infection, and that the omentum took part in the local inflammatory process in a passive manner.

These various researches into the protective function of the greater omentum and into the mechanism of its migration were not, however, apparently considered conclusive because many centres continued to work upon this subject.

By the early part of the present century, therefore, by a combination of clinical and pathological observation and experimental investigation in animals, it seemed that the greater omentum in some way exercised a protective function in the general peritoneal cavity. The structure had certainly been shown to be capable of absorbing fluids and of taking up particulate materials. The great controversy was whether the omentum was capable of active or "intelligent" movement towards the focus of trouble or whether it was carried thither purely by chance or in some passive manner. At this stage it seemed only natural that a more subtle role would be searched for which would be capable of reconciliation with the opposing views that have already been described.

The first indication of a search for a function more subtle than a capacity for localising an inflammatory lesion appears to be the work of Portis in 1919. He investigated the capacity of certain tissues to produce antibody when he injected the peritoneal cavity with a suspension of bacteria. He found that the omentum had a tissue-titre which exceeded that of the spleen, liver and bone marrow. This work was not, however, pursued. At the Mayo Clinic, Bargen and Rankin in 1928 were following up a clinical impression that patients having had their intestines opened at operation and requiring a further procedure

seemed to have acquired some form of immunity towards their own intestinal flora in the interval between the operative procedures. These workers selected a group of patients who were diagnosed to have operable carcinomata of the colon and divided them into two sections. The first section were then "vaccinated" with virulent cultures of streptococci and coliform organisms by intra-peritoneal injection. An examination of the temperature charts of these patients revealed that they reacted rather stormily to this treatment but, nevertheless, their convalescence appeared to be smoother than that of the control group. The authors were most enthusiastic about the response but again this work was not pursued or taken up elsewhere.

One of the most significant papers, from the point of this thesis, is that of Jens Bing (1946). He examined the histological changes occurring in the greater omentum after injections of proteins and protein hydrolysates. He performed a series of experiments in rats and reported that after an intra-peritoneal injection of glucose, glycocoll, "hepsol", broth or an amino-acid solution "Aminosol", there was, in a great majority of his experiments, a considerable increase in plasma cells. He recalled that attention had been drawn to the existence of a hyperglobulinaemia in cases associated with accumulations of plasma cells and suggested the association of these cells with antibody production. This subject was again investigated by Roberts in 1955, who confirmed the findings of Portis and suggested that the greater omentum was able to produce antibody. However, this work did not exclude the possibility of the antibody being elaborated

elsewhere and being accumulated locally by the structure. Neither of these authors attempted to identify the type of cell responsible for the production of antibody.

The greater omentum has, as has been described, areas which have been called "Milk Spots". These areas occur in the non-fatty parts of the structure and are constituted of groups of reticular cells which, when particulate material is taken up, proliferate and give rise to populations of macrophages. With these macrophages are associated many lymphocytes and plasma cells. These two types of cell have been the subject of much controversy in the field of immunology and there is much evidence in support of each that it is antibody producer. Because these cells have been encountered in much of the present work (particularly the plasma cell) it is relevant to review some of the evidence in support of this conception.

Much of the evidence for the association of a particular cell with the production of specific antibody has been gained within the last twenty years. Prior to this period, however, attention had been focused on the members of the reticulo-endothelial system. This was because of the specific capacity of cells of this system to engulf foreign material and it was postulated that they might be capable of elaborating specific antibody to the material they ingested. This supposition was supported by early investigators who "blockaded" the system by massive injections of India ink or other particulate materials and thereafter injected antigen. It was found that these animals produced substantially less antibody than the normal controls.

The lymphocyte, in particular, was suspected of being a site of antibody production. Hektoen in 1915 irradiated rats with doses of x-rays which were sufficient to produce a shrinkage of lymphoid tissue, and he thereafter found that this resulted in a diminution of antibody production in the response to the injection of antigen. Murphy and Sturm in 1925 developed this method and employed a dosage of irradiation which was sufficient to diminish the volume of lymphoid tissue without producing changes in the bone marrow. Again the animals were demonstrated to produce less antibody than their non-irradiated controls. These workers also demonstrated that the application of dry heat was responsible for an increase of lymphoid tissue in animals and that this was accompanied by an increased production of antibody in response to injected antigen.

The incrimination of plasma cells was suggested by Heubschmann in 1913 as a result of his histological observations of accumulations of plasma cells in the spleens of patients dying of acute and chronic infections. Great controversy reigned as to the derivation of plasma cells and it was considered by Klein (1914) and Arneth (1920) that they represented a phase of the lymphocyte which, because of local conditions, was associated with the immunological response of the patient. It had been observed that patients with chronic infections, chronic liver disease and myeloma had increased numbers of plasma cells and that this was associated with a rise in the serum globulins. This fact led Bing (1937) to postulate that plasma cells may be associated with the elaboration of the serum globulins.

McMaster et al in 1953 injected cellular antigens into the ears of mice and at various intervals thereafter the regional lymph nodes were dissected out, pooled and extracts prepared by a process of homogenisation. These extracts were found to have antibodies towards the cellular antigens and the extracts of the lymph nodes removed early after the immunisation of the animal had titres which exceeded that of the blood serum.

Mice which were infected with influenza virus by inhalation were found to have extracts of mediastinal lymph nodes with titres in excess of the blood serum at the same time, by Burnet and Lush in 1938.

Further experiments supporting the lymphatic system as responsible for antibody production was obtained by the injection of antigen into the foot pads of rabbits and thereafter excising and extracting the popliteal group of lymph nodes. At the same time, lymph was obtained by cannulating both the afferent and efferent lymph channels to this group of nodes. In the early part of the experiment the amount of antibody was often found to be higher in these extracts and in the efferent lymph flow than in the blood serum at the same time. No antibody production was demonstrated at the site of injection of the antigen or in the afferent channel to the popliteal nodes until after a substantial titre had developed in the serum and, even then, the titre remained of a low order.

However, other investigators of the lymphatic system were not always able to reproduce the findings which have been cited. Habel et al in 1949 failed to find substantial differences between the amount of

antibody he recovered from extracts of popliteal lymph nodes of the injected leg and of the opposite leg. Soloviev in 1946 injected influenza virus into the foot pads of rabbits but failed to demonstrate antibody in extracts of the inguinal lymph nodes. The disparity in some of these findings is, however, accountable by the difference in anatomical site of the injection or the difference in time at which attempts were made to demonstrate antibody in the extracts of the draining lymph nodes, or in the returning lymph. Such standardisation is essential because of the volume and distribution of lymphatic tissue in the body. More recent studies of this aspect of immunology have consistently demonstrated homologous antibody in the regional lymph nodes of the part injected with the antigen.

The spleen has for a long period been associated with the production of antibody. The classical work of Pfieffer and Marx in 1898, and later of Topley in 1930, have substantiated this function. The researches which have supported this as one of the most important sites of antibody synthesis have been reviewed by McMaster in 1953 and have included procedures involving splenectomy, assays of tissue extracts of the organ and of its response to irradiation before or shortly after the injection of a course of immunising antigen. The spleen has been found to perform its greatest immunological role following the injection of antigen by the intravenous route.

The observations that lymphatic tissue could be the site of formation of antibody have led to many investigations into the type of cell which occurs in such tissue and which might be involved in the

synthesis of homologous antibody. These researches have been directed primarily towards the lymphocyte or the plasma cell, or to cells which are considered to be their precursors. Several of the experiments which are relevant in support of either of these cells being thus responsible are described:-

In the lymphatic tissue of guinea pigs reared in a sterile environment Glimstedt in 1936 found no lymphatic germinal centres. These structures, however, developed rapidly after exposure of the animals to bacteria of different kinds.

Ehrich and Harris in 1942 injected suspensions of Salmonella typhi into the foot pads of rabbits and demonstrated that extracts of the popliteal lymph nodes had developed an antibody titre from the third day after the injection and reached its highest concentration on the fifth or sixth day. Histologically they described the changes in the cortices of the popliteal lymph nodes and state that two days after the injection of the antigen the cortex had become tremendously enlarged and consisted of a diffuse lymphoid tissue which contained mainly large lymphocytes and mitotic figures. A few days subsequently this diffuse proliferation had become divided into secondary nodules. The efferent lymph which they collected exhibited a rise in the cell count from the normal level (before injection) of from 16,000 to 18,000 cells/cu.mm. to values of between 40,000 to 150,000 cells/cu.mm. on the fourth to the sixth day after injection. Ninety-nine per cent of the cells visualised were lymphocytes and this corresponded to the period at which

the antibody titre of the efferent lymph was at its maximum.

Oesterlind in 1938 found that lymph nodes draining sites of injections of diphtheria toxoid into rabbits developed an increase in the number of germinal centres, the number of which was in direct proportion to the level of serum antitoxin concentration.

Rich et al in 1939 was able to reproduce the "acute splenic tumour", so well known in infectious diseases, by the injection of foreign protein into experimental animals.

A further series of experiments were, however, concerned with investigations supporting the plasma cell as an antibody producing cell type. Kolouch in 1938 injected rabbits with suspensions of streptococcus viridans and examined bone marrow biopsies. Within a few hours there was a reticular cell proliferation and a transformation of a maturation of these cells into plasma cells, until finally, small plasma cells were predominant in the bone marrow on the fifth day.

Bjorneboe and Gormsen in 1943 studied the phenomena associated with the hyper-immune state in rabbits. They highly immunised these animals with injections of different strains of pneumococcus and demonstrated the increasing population of different tissues with plasma cells. The animals developed a hyperglobulinaemia which could be correlated with the increase of plasma cells, and these authors suggested that the hyperglobulinaemia was due to the increase of the fraction of the protein which was concerned with antibody towards the injected bacteria. They demonstrated that the fat of the renal sinus

became infiltrated with large groups of plasma cells with only a few scattered lymphocytes and, by extracting this fatty tissue, they demonstrated a high titre against the bacteria with which they had immunised the animals.

Fagreus in 1948 injected groups of rabbits with bacteria and protein antigens and studied the cellular histology of the spleen in relationship to the development of the serum level of antibody. It was seen that the cellular proliferation occurred before any serum antibody was detectable and that many small round cells ("transitional cells") became recognisable as early plasma cells on the second or third day, which corresponded with the demonstration of serum antibody. There then followed a gradual transition into mature plasma cells.

Marshall and White in 1950 demonstrated that following the intravenous injection of antigen in rabbits, undifferentiated reticulo-endothelial cells of the spleen, lungs, liver and bone marrow proliferated and formed recognisable plasma cells.

The cellular source of antibody has also been investigated by techniques involving antibody assays of the medium when the tissue, or groups of cells, have been maintained in tissue-culture. Up to the present time, this invitro technique has been restricted to the examination of tissue removed from animals which have been immunised previously. Fagreus in 1948 immunised rabbits with suspensions of Salmonella typhi and later removed the spleens of these animals and separated the white from the red pulp. Plasma cells are relatively

more numberable in the latter than are lymphocytes, while the reverse holds true for the white pulp. Portions of these specimens were explanted into tissue-culture and here maintained at thirty-seven degrees centigrade. It was found that the portion of red pulp had produced specific anti-typhoid antibody while the white pulp had not, and neither had the controls in which cellular activity was inhibited. Fagreus concluded, therefore, that plasma cells were primarily concerned with the production of the antibody which had been demonstrated. These findings were confirmed by other investigators, among whom were Keuning and van der Slikke (1950) who, however, found some evidence of antibody production in the white pulp. Histological examination of this tissue demonstrated the presence of many "lymphoblasts" and these authors suggested that these cells may, when stimulated by an antigen, give rise to either of the cells which were considered to be antibody-producing, i.e. the plasma cell or the lymphocyte.

A further technique, utilised to identify the cell type, was that practiced by Reiss, Mertens and Ehrich in 1950, who injected Salmonella typhi into the foot pads of rabbits and dissected out the popliteal lymph nodes. By a micro-dissection technique they prepared cell-suspensions which they washed and thus cleared of preformed antibody. When these washed cells were mixed with the suspension of Salmonella typhi the organisms were observed to collect on the surface of some of the cells. Some of the cells which induced this aggregation were identified as plasma cells but the phenomenon

occurred, however, most commonly in relation to an undifferentiated precursor which could not be identified as either a plasma cell or a lymphocyte.

For many years attempts have been made to label antibody protein with a dye which would facilitate its visual identification. In 1942 Coons developed a technique of conjugating either an antigen or an antibody with a fluorochrome which fluoresced in ultra-violet light. The principle of his method was to immerse sections of the tissue which were being investigated in solutions of the antibody towards the particular antigen which was being used. This antibody had been previously coupled with a fluorochrome and at the point at which the concentration of the antigen was sufficient to combine with the labelled antibody, the latter became "fixed". When the "unfixed" and superfluous antibody was washed off, the site of the original antigen could be visualised by examining the section through an ultra-violet microscope. This method was later developed to be used for the identification of antibody in tissue and cells. Here the section of tissue under examination was immersed in a solution of the homologous antigen which "satisfied" the antibody presenting on the surface of the section. Where the concentration of antibody was sufficient to allow an antigen-antibody complex to be formed, the antigen became fixed to the tissue and this could be identified by treating the section with its homologous antibody which had been coupled with the fluorochrome. The cellular situation of the original antibody can thus be identified.

It will be seen that the process is one of immunological "sandwiching". This method is inexact in that the sections which have been treated with the fluorochrome cannot be treated with the routine histological stains and contiguous sections require to be seen for the identification of cell-types involved. Using this method, Coons identified the plasma cells of the red pulp of the spleen (after intravenous injection of the antigen) and the popliteal lymph nodes (after foot pad injections of the antigen) as containing antibody. He concluded that the plasma cell was responsible for the elaboration of specific antibody but could not exclude the lymphocyte entirely because of the staining of some unidentified cells.

Much attention has been paid to a method which has been practiced extensively in recent years to elucidate the problem of the cellular source of antibody. This method involves the homografting of a tissue which is being investigated as antibody-producing. This method usually consists of transferring a tissue from an animal immunised by a particular route into the body of an animal of the same species which has had no previous experience of the antigen. The serum titre of the recipient animal is then measured and if this rises is accepted as evidence of the capacity of the tissue to produce antibody.

Harris et al in 1951 and 1954 immunised a group of rabbits with dysentery organisms by injecting these organisms into their foot pads. The popliteal group of lymph nodes of the limb were then removed and suspensions were then prepared of the cells of the nodes and

transferred to non-immune rabbits. The recipients developed antibody titres in their serum while cells which had been killed failed to do so. A similar type of procedure was practiced by Fagreus and Grabar in 1953, who transplanted portions of the spleen of an immunised donor into the peritoneal cavity of non-immune recipients and demonstrated the development of antibody in the latter's serum.

Examinations of the cell-types responsible for the continued production of antibody in these homotransplantation experiments have suggested that cells of the lymphocyte series are involved. An examination of the relevant literature confirms it to contain many references to these cells and particularly to the small round cells which in most cases have been called small lymphocytes. Probably many of the disparities in cell identification are reflections of different cytological interpretations by other workers. As has been pointed out by Harris in 1956, probably the "acute splenic tumour" cell, which was called a lymphoblast by Rich et al (1939), is the one called a "transitional cell" by Fagreus in 1948, and an immature plasma cell by Kolouch et al (1938).

Chapter III

GENERAL PLAN OF THE WORK.

It seemed possible that the apparently divergent view of many of the writers in the previous chapter could be reconciled if a protective function, other than a simple capacity to circumscribe an inflammatory lesion in a mechanical fashion, could be uncovered for the greater omentum. It is a clinical impression that patients are able to develop some degree of immunity towards the organisms of their own gastro-intestinal tract, a fact that is suggested by the comparatively smoother convalescence they experience following a second surgical operation if it is required, e.g. the resection of a segment of bowel after colostomy. Indeed, it was the surprising degree of apparent immunity in such conditions which prompted this search for a tissue which was capable of elaborating antibody to organisms which might enter the peritoneal cavity. It seemed reasonable to suspect most strongly the structure which had already been called "the policeman of the abdomen" and the "man-of-war, ready to sail to any port in which trouble threatened", and which was encountered so commonly in the vicinity of an intra-peritoneal inflammatory process.

It will have been seen from the investigations which have been conducted to establish the capacity of a tissue to synthesise antibody,

that the evidence has been obtained in the main by three different methods:-

(1) The first used (e.g. Pfeiffer and Marx, 1898) was to show that antibody could be found in particular tissues in higher concentrations than in the blood plasma at the same time. The theoretical objections to this method, that high concentration of antibody might occur by some process of local accumulation, have been met by Oakley's comparison of the responses to the injections of two different antigens.

(2) The second method has been to show that tissues from an immunised animal can produce antibodies either in vitro (e.g. Fagreus, 1948) or after transplantation to another host (e.g. Topley, 1930).

(3) The third has been by the use of fluorescent-staining technique of Coons, whereby antibody containing cells can be directly demonstrated in histological preparations.

Very little work has previously been performed upon the greater omentum in this way and it was decided to investigate the capacity, if any, of the structure to elaborate antibody with each of the three methods which have been described.

The experimental plan of the work, therefore, falls into three parts and a chapter is devoted to each. In addition, a very recent technique has been applied and an attempt has been made to demonstrate that the structure can function in this way towards organisms which

occurred naturally within the bowel of the animal.

In summary, therefore, the plan of work is:-

(a) The comparison of greater omental tissue-titres with those of the spleen, liver, peritoneum, lymph nodes and bone marrow (most of them known to be capable of producing antibody) and an attempt to show that antibody occurs in greater concentration within the omentum than in the blood plasma at the same time in animals immunised by the intra-peritoneal injection of antigen.

(b) To show that the omentum of an animal immunised by intra-peritoneal injection of an antigen will, when homotransplanted, continue to produce specific antibody and that such a continued activity will also occur under conditions of tissue-culture.

(c) To attempt to identify the cellular source of the antigen, using a modification of Coons' fluorescent-staining technique.

(d) To attempt to verify the results by the application of the

Passive Cutaneous Anaphylactic Phenomenon in guinea pigs, and to demonstrate that antibody synthesis can occur in the omentum against organisms of the gastro-intestinal tract of the animal.

Chapter IV

MATERIALS AND METHODS.

1. The Animals.

Adult, white, black or sandylop rabbits were employed for the experiments. They weighed 2.0 - 2.8 kilograms at the time of use. The sandylop rabbits were bred either in the Department of Experimental Pathology of the University of Birmingham, or at the National Institute for Medical Research, Millhill. Their larger ear greatly facilitated the taking of the many blood specimens as was required by the nature of the experiments. The animals were maintained on pelleted Diet number 18 (Bruce and Parkes, 1940) and this was supplemented with hay.

The rabbit was selected for the investigation because much of the previous research in immunology has been carried out in this animal and the results of the study would be, therefore, in many respects comparable. Additionally, as has been stated, the large ear with the readily accessible marginal vein made the taking of many blood specimens at frequent intervals easier. The animal possesses a well formed greater omentum, which allows operative procedures involving it possible without being too friable. It approaches the relative

dimensions of the human structure.

2. The Antigen.

In order to stimulate the production of antibodies it was decided to use a bacterial body as the antigen. Since it was desired to assess the capacity of the greater omentum to produce antibody in inflammatory conditions it was considered that the antigen would be selected appropriately from the organisms of the gastro-intestinal tract, which were capable of becoming pathogenic.

A swab was taken of free pus occurring in a human patient suffering from acute, diffuse peritonitis, the result of an obstructive appendicitis. This swab was plated out on to McConky's medium and incubated at 37 degrees centigrade over night. A "coliform" type of organism was isolated and this was subsequently used in the experimental procedures. The antigen was prepared by culturing the gram-negative rod in nutrient agar and harvesting the colonies which were then suspended in normal saline (0.85% sodium chloride) solution. These were standardised by titrating to number 7 of Brown's Opacity tubes.

These suspensions were then used for the intra-peritoneal immunisation of the animals and were, in the early cases, successful in stimulating circulating antibodies. However, a difficulty arose when the initial supply of bacterial suspension was exhausted. The original

colonies which had been sub-cultured had undergone a smooth to rough variation. This variation was first described by Arkwright in 1920 when working with organisms of the typhoid-paratyphoid-dysentery group. In this variation the normal parent form gives smooth colonies on a solid medium and a diffuse growth in broth. The variant form gives rough or granular colonies on solid medium and a granular growth in broth and is auto-agglutinable in normal saline, though a stable suspension can usually be prepared in distilled water, or in a saline solution with a greatly decreased salt content. These differences in colonial form, growth and salt sensitiveness are associated with a profound change in antigenic structure which is a consequence of a loss of virulence of the organism. It was found that it was a vicarious and difficult procedure to secure the reversal of this variation and, in consequence, the first series of experiments were considered to be unreliable without a constant and stable antigen. A further criticism of this antigen was that it was not in pure form and, besides a predominance of Escherichia coli, some colonies of streptococcus faecalis and Proteus were identified.

The experiments were then repeated, using as the antigen a pure culture of Proteus OX19, which was obtained from the National Collection of Type Cultures. The results of the experiments strongly suggested that the greater omentum was capable of producing antibody.

Because much of the research in immunity has been carried out in

rabbits using Salmonella typhi, it was then decided to carry out the experiments using this organism in its somatic (O antigen) phase. Standard suspensions of this organism, which had been treated with alcohol, were obtained from the National Collection of Type Cultures, Colindale. Each millilitre of the suspension contained about 6,500,000 organisms.

3. The Immunisation of Animals.

The immunising course of antigen consisted of six separate injections, each of one millilitre, of the suspension of Salmonella typhi. The injections were made at three day intervals and the immune response of each animal was followed by measurement of its serum titre on the first, second, third, sometimes fourth, fifth, tenth, fifteenth and twentieth days. The animals were sacrificed four days after the final injection.

Before the commencement of the immunising course of injections each animal was tested to verify that it was non-immune to the antigen which was being used.

The antigen was administered at as nearly a constant site as possible in the different experiments. These were:-

(a) In the group of rabbits with an intact omentum the site was two inches above the symphysis pubis and in the midline.

(b) In the group of rabbits without omenta the site was two inches above the symphysis pubis and in the midline.

(c) In the animals with the omenta withdrawn into a subcutaneous "pouch" the site was the central prominence of the "pouch".

(d) In animals in which the omentum had been withdrawn into a subcutaneous "pouch" but were controls for (c) the injections were made to the left of the prominence of the "pouch", into the general peritoneal cavity.

(e) In animals used as intravenous controls the site was the marginal vein of the ear.

(f) In the rabbits in which the spleen was removed the site was two inches above the symphysis pubis in the midline.

(g) In the homotransplantation and tissue-culture experiments the omenta of animals immunised as in (a) were used.

4. Method of Sacrifice of Experimental Animals.

The animals were sacrificed four days after the final injection of antigen, that is, on the twentieth day.

The animals were lightly anaesthetised with two millilitres of Veterinary Nembutal, injected intravenously into the marginal vein of the ear. The throat was then shaved and the main vessels of the neck were divided with a scalpel. The thorax was immediately opened and the right auricle was identified and was removed with a pair of scissors. A cannula was then thrust through the inter-auricular septum into the left auricle and the remnant of the right auricle was

tied about the body of the cannula. This cannula was attached to a gravity perfusion arrangement and through it the animal was perfused with two litres of normal saline solution. The heart was used as a manual pump for this system. This method resulted in a very efficient removal of blood from the various organs which were later examined. The blood from the neck wound was collected in a number of Universal containers which were clean and protein-free. After being allowed to clot the serum was removed by pipette and transferred to a further set of Universal containers. These were either used immediately or stored until used.

The abdomen (and "pouches", where these were present) of the animals were opened and a naked eye examination of the abdominal and "pouch" contents was made and recorded. Portions of the greater omentum, spleen, liver, axillary lymph nodes, peritoneum and the bone marrow were removed and transferred to clean and protein-free Universal containers until used for tissue titre assay, histological examination or histo-chemical study. Specimens of bone marrow were obtained from the femoral shafts. These were dissected out and disarticulated at the hip and the knee and the marrow was scraped out, after the bone had been longitudinally split.

Where the greater omentum of an immunised animal was being used for homotransplantation or tissue-culture studies, the transfer of the

tissue was made immediately. In the former case, the recipient animal was anaesthetised with its abdomen open at the time of removal of the omental homotransplant.

5. The Estimation of Serum and Tissue Titres.

The tissues were prepared for tissue titre estimation by washing in tap water, blotting and weighing. To this known weight of tissue was added that volume of normal saline solution which would give a total weight of ten grammes. Each specimen was then minced with curved scissors in a watch-glass. At the conclusion of this mincing process the tissue was placed in a blender and homogenised for a period of five minutes. Care was taken during this process to ensure that the specimens could not become overheated, and this was prevented by placing the Universal container in a beaker of cool water. At the end of this period a turbid fluid was obtained which was quickly frozen and thawed in order to rupture the cellular membranes of any intact cells, and thus to liberate all formed antibody which might be contained within the cytoplasm. The solution was then centrifuged at 3,000 revolutions per minute (centrifuge radius 22.5 cm) for thirty minutes, after which the supernatent fluid was removed and the antibody in it assayed by tube-agglutination. The reading was then corrected by a factor which depended upon the dilution of the original weight of tissue in saline,

thus standardising the original weight of tissue. The method of tube-agglutination was that described originally by Widal. The dilution of antigen (Salmonella typhi) was that of a number 7 Brown's Opacity tube. The assays were carried out using a dropping pipette and for each specimen twelve Dreyer's tubes were used. After the addition of the antigen to doubling dilutions of the extract, the rack was transferred to a water-bath at thirty seven degrees centigrade and readings were made at four hours and twenty four hours. Initially, the rack was left in the water-bath for this period, but later, as recommended in the "Handbook of Practical Bacteriology" by Mackey and McCartney, the rack was transferred after the fourth hour into a refrigerator and a further reading was made at twenty four hours. The end point was when agglutination was seen as a small and granular clumping of bacteria, and the visualisation of these was aided by the use of a hand lens and a strong illuminant. As controls suspensions of the organism were added to normal saline and it was ascertained that no auto-agglutination occurred. Where there was any doubt the process was repeated with the use of fresh antigen.

6. <u>The Soxhlet Estimation of Fat of the Greater Omentum</u>.

The greater omentum is a structure which contains a variable amount of fat, which is presumably inert in respect of antibody

synthesis. Since the tissue titre of the omentum was being compared with organs which are relatively non-fatty it was considered desirable to estimate the percentage of the total wet weight of the structure which was composed of this fat. The active tissue of the omentum could be considered to be diluted in its own fat, unless a correction were made. Estimates were accordingly made of the percentage of the total wet weight of the rabbit omentum which was composed of fat. The estimation was also made on the omentum of animals which had been immunised by the intra-peritoneal route.

The procedure which was adopted was that using the Soxhlet Extractor. The apparatus is illustrated in Fig. 2. It consists of a dry flask to which is attached the Soxhlet Extractor. To this system is added a reflux water condenser. The Soxhlet Extractor consists of a tube in which there is placed a porous thimble made of toughened filter paper. Into this receptacle is placed a measured weight of tissue, of which the fat is to be estimated. The extractor is associated with two external tubes. One is for the passage upwards of evaporated fat-solvent to reach the water condenser. Being condensed, the solvent enters the porous thimble. When this thimble is filled with the solvent the remaining external tube is utilised, since this is

a siphon for the return of the solvent to the flask.

A measured weight of the omentum was placed in the porous thimble. The flask was then filled with fat solvent. The solvent which was used was either petroleum ether or ether. The flask was then immersed in a water-bath which was heated. The petroleum ether evaporated and passed up the first external tube into the condenser and from thence into the porous thimble. It was repeatedly siphoned back into the original flask and this circuit was maintained on each of four days. At the expiration of this period the omentum was reduced to its organic remnants and appeared as a finely powdered substance. This was removed and accurately weighed. The flask was then evaporated of the solvent and the residual fat was measured.

This measurement was performed upon five omenta from animals which had had a course of intra-peritoneal antigen injections. It was found that the average percentage of the total wet weight of the structure which was composed of fat was ninety-three per cent. In the case of the normal omentum, the percentage was ninety-five per cent.

The implication of this estimate is that the titres which are shown in the figures for each animal represent the activity of only

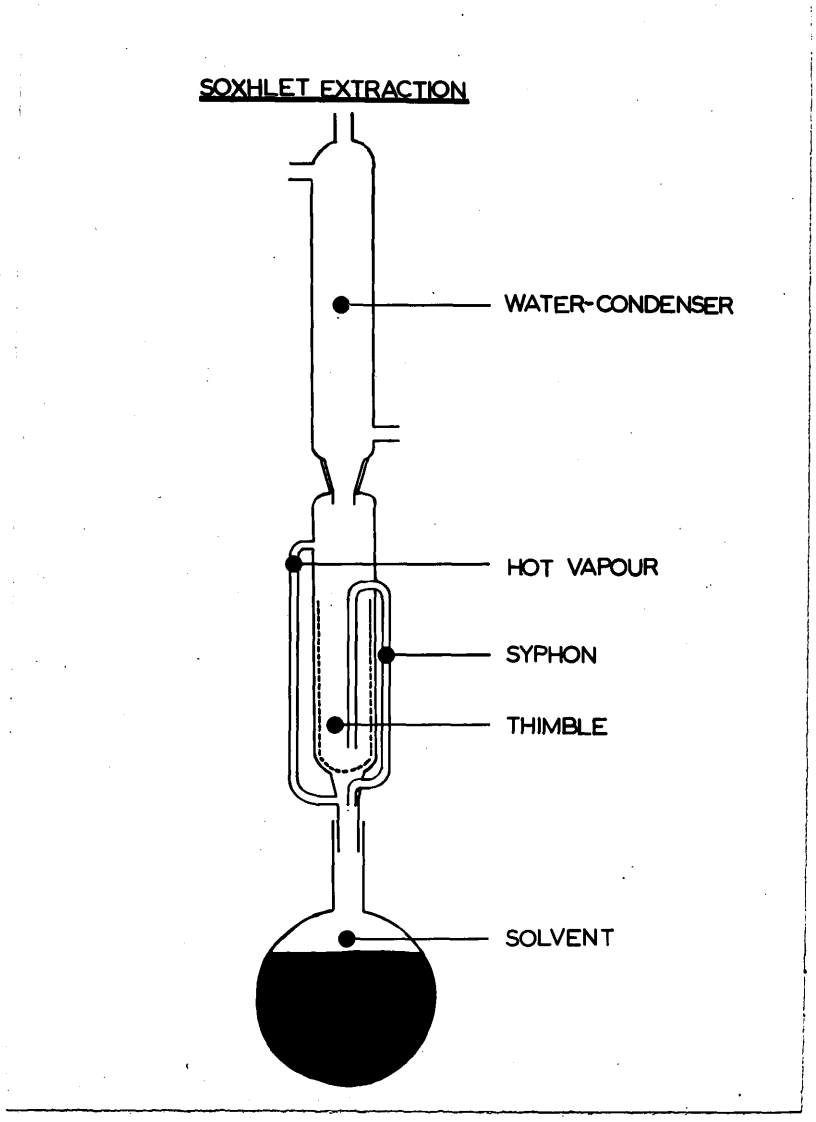

Fig.2. The apparatus used for the extraction of fat from the greater omentum.

seven per cent of the original weight of greater omentum removed for extraction. If this correction is made it will be seen that the greater omentum is capable of presenting a titre which exceeds that of the serum, at the same time.

Chapter V

THE COMPARISON OF GREATER OMENTAL TISSUE TITRE WITH THOSE OF THE SERUM, SPLEEN, LIVER, PERITONEUM, LYMPH NODES AND BONE MARROW.

1. Normal Rabbits.

Immunisation was carried out in a group of rabbits, as has been described in a previous section. The injections of antigen were made into the peritoneal cavity of each animal at the same anatomical site on each occasion. This site was two inches above the pubic symphysis, in the midline.

The animals accepted the course of antigen without any apparent ill effects and were able to take their food and fluid. There were no fatalities.

Blood was withdrawn from the marginal vein of the right ear at regular intervals and this blood was allowed to clot in a clean, protein-free Universal container. The serum was then removed and was utilised for the assay of serum titres against a suspension of Salmonella typhi. The method used was that of Widal, using a dropping pipette and setting up the serum in doubling dilutions in Dreyer's agglutination tubes. A constant volume of a suspension of Salmonella typhi, standardised to a number 7 Brown's Opacity tube, was then added

and the mixture transferred to a water-bath at thirty seven degrees centigrade. Readings were made at four hours and thereafter the rack was transferred to a refrigerator, and a further reading was made at twenty four hours. Each specimen of serum was controlled by a tube containing normal saline (0.85 per cent sodium chloride) to eliminate a faulty reading being made due to auto-agglutination of the organism.

Estimates of serum titres were made more frequently in the first two experimental animals so that the general pattern of the immune response could be assessed, and for the purpose of checking that the immunising course of antigen was correct in respect of the concentration of bacteria in the suspension and in frequency and number of doses. When the general pattern of response had become apparent it was the habit to take specimens of serum on each day following the initial dose to ascertain the first day on which agglutination occurred. Thereafter, serum titre readings were made on the fifth, tenth, fifteenth and twentieth days. The antigen was injected at three day intervals, therefore the last serum titre reading was made four days after the completion of the course of six injections of the antigenic suspension.

On the twentieth day the animals were sacrificed in the manner which has already been described. This method was found to result in a very efficient removal of blood from the tissues. However, it is appreciated that even this method will not succeed in removing all the blood or serum.

Specimens of serum, greater omentum, spleen, liver, peritoneum, axillary lymph nodes and bone marrow were then removed and prepared for the assay of tissue titre. This included mincing, homogenisation and freezing and thawing, as has been described. The results which were obtained are demonstrated in ten rabbits which were employed in this section of the research. At this stage no assessment of the presumably inert fat contained in the specimen of the greater omentum which was examined had been made. This was later evaluated using the Soxhlet Extraction apparatus, and this has already been described. It will be recalled that, using petroleum ether and ether as the fat solvent and with this apparatus, the weight of inert fat in the specimens of omenta, which occurred in these researches, was ninety-three per cent of the total weight of omentum under the condition of the experiments. It will be appreciated that if the appropriate correction were to be made (since the titres recorded in the figures represent only seven per cent of active omental tissue) the true titre of the omentum would be considerably higher than shown.

In the figures the immune response of each animal is shown as it was followed by regular serum titre assays. The symbols which are seen recorded on the twentieth day are the tissue titres of the extracts of the tissues against which a comparison is being made. The symbol for the greater omentum is an open circle, the spleen is depicted by a black, filled-in triangle and the liver is indicated by an open

triangle. On no occasion was an agglutinating titre obtained in respect of the peritoneum, the axillary lymph nodes or the bone marrow.

It was found that the identification of the mesenteric lymph nodes was most difficult, even with the use of the dye Pontamine Sky Blue which was tried. Because of this difficulty the axillary group of lymph nodes was selected for examination. These were easily identified and were simple to recover. It was considered that they were representative of the lymphatic tissue of the body, albeit that they were comparatively far removed from the general peritoneal cavity. Because of this failure to obtain an identical group of mesenteric lymph nodes in each rabbit, control experiments were carried out in which the omentum was removed from the animals by operative excision or "functionally" removed by a procedure in which the structure was exteriorised in a subcutaneous "pouch". These experiments are described in a later section of this chapter. If the mesenteric lymph nodes were the principal contributors to the immunity of an animal against an antigen introduced into the peritoneal cavity, it would be anticipated that the animals response would be little changed by the removal or exteriorisation of the greater omentum. Incidentally, the failure to obtain a tissue titre from extracts of such distantly placed nodes can be considered a measure of the efficiency of the process of exsanguination and perfusion of the tissues. It will be noted that

a substantial titre against the antigen is reflected in the serum values. If the exsanguination technique were inefficient then the extracts of the axillary lymph nodes would be expected to have an agglutinating titre, in virtue of transferred antibody carried over from the animal in the interstices of the tissue. That this "carry-over" does occur is confirmed in a later chapter of the research, where controls for this aspect of the problem are applied to the technique of homotransplantation.

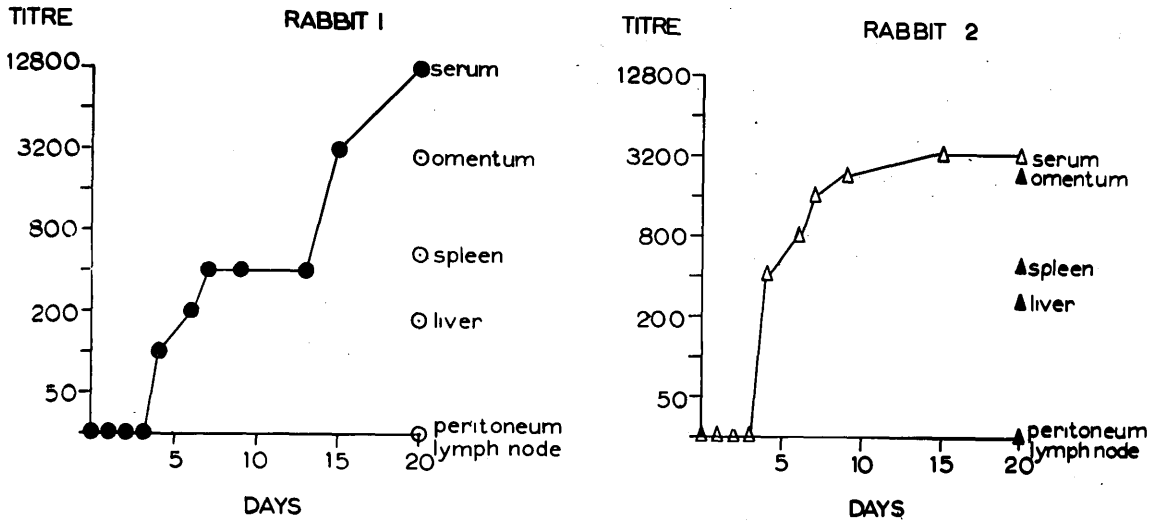

Fig.3. This diagram records the immune response in Rabbit 1 and in Rabbit 2. Prior to the first injection, the titres of the animals were tested and confirmed that they were not immune to Salmonella typhi. It will be seen that antibody was first detected on the fourth day after the commencement of the course of antigen. The serum titre rose steadily to reach a maximum on the twentieth day in the case of Rabbit 1, while this maximum was reached rather earlier in the case of Rabbit 2.

At post mortem examination, the omentum was found to be freely mobile but both structures exhibited a slight thickening of the free peritoneal edge. No other intraperitoneal abnormality was apparent and there was no evidence of peritonitis.

It will be seen that in both instances, the omental tissue titre exceeds that of the other tissues. The ratio of omentum: spleen: liver: is 16:3:1 and 8:1.3:1.

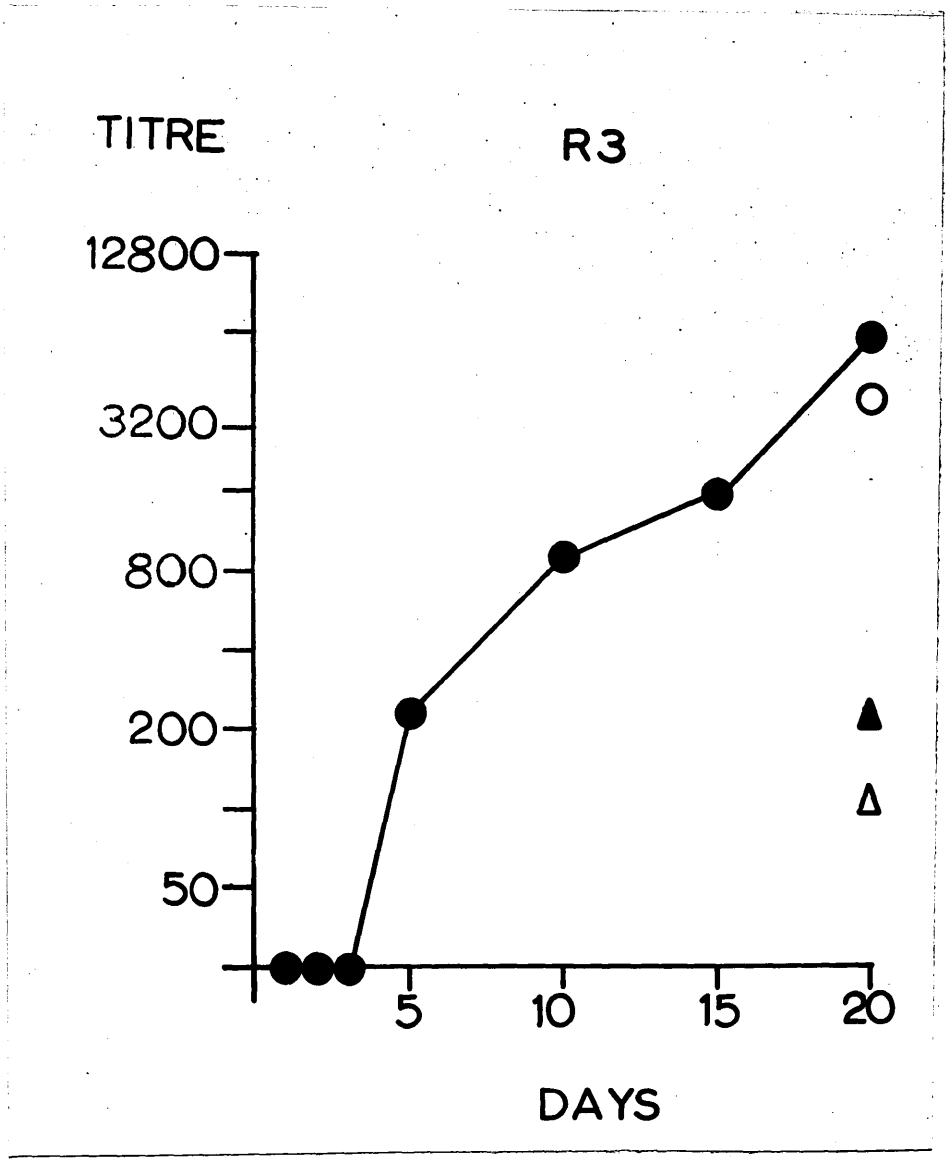

Fig. 4. No serum titre has been detected on the first three days after the initial injection. On the fifth day, however, the titre has started to rise and this is continued until the twentieth day.

At postmortem, the omentum, at first sight appeared normal, but closer scrutiny indicated that the peripheral edge of the structure was slightly thickened. The omentum was free and mobile.

The omental titre is seen to greatly exceed that of the spleen and the liver. The ratio of titres is 32 : 2 : 1.

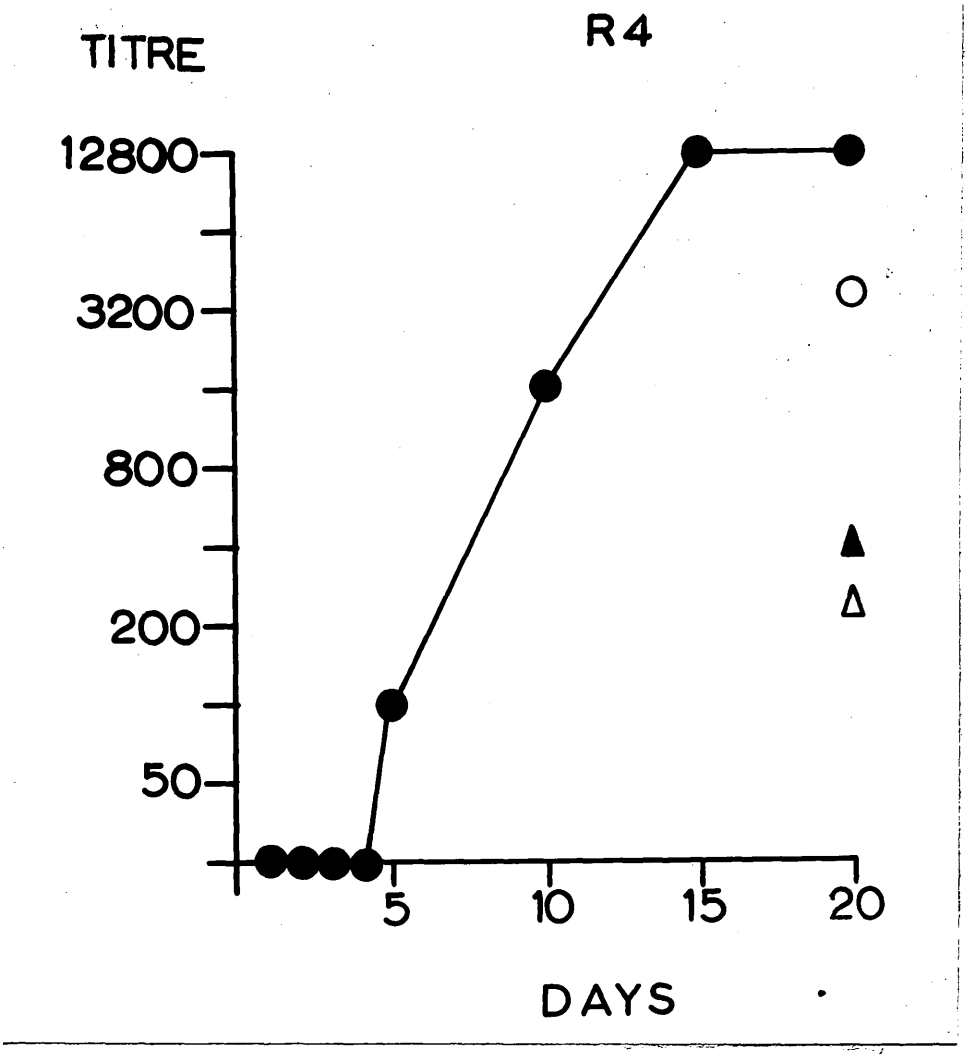

Fig. 5. No demonstrable circulating antibody until the fifth day after the initial injection. There followed a steady rise in titre which reached a maximum on the fifteenth day.

At post mortem, there was no visible abnormality in the peritoneal cavity except for some thickening of the peripheral edge of the omentum. This structure was free and mobile.

The omental titre is seen to exceed that of the spleen and the liver. The ratio of the titres is 16 : 2 : 1.

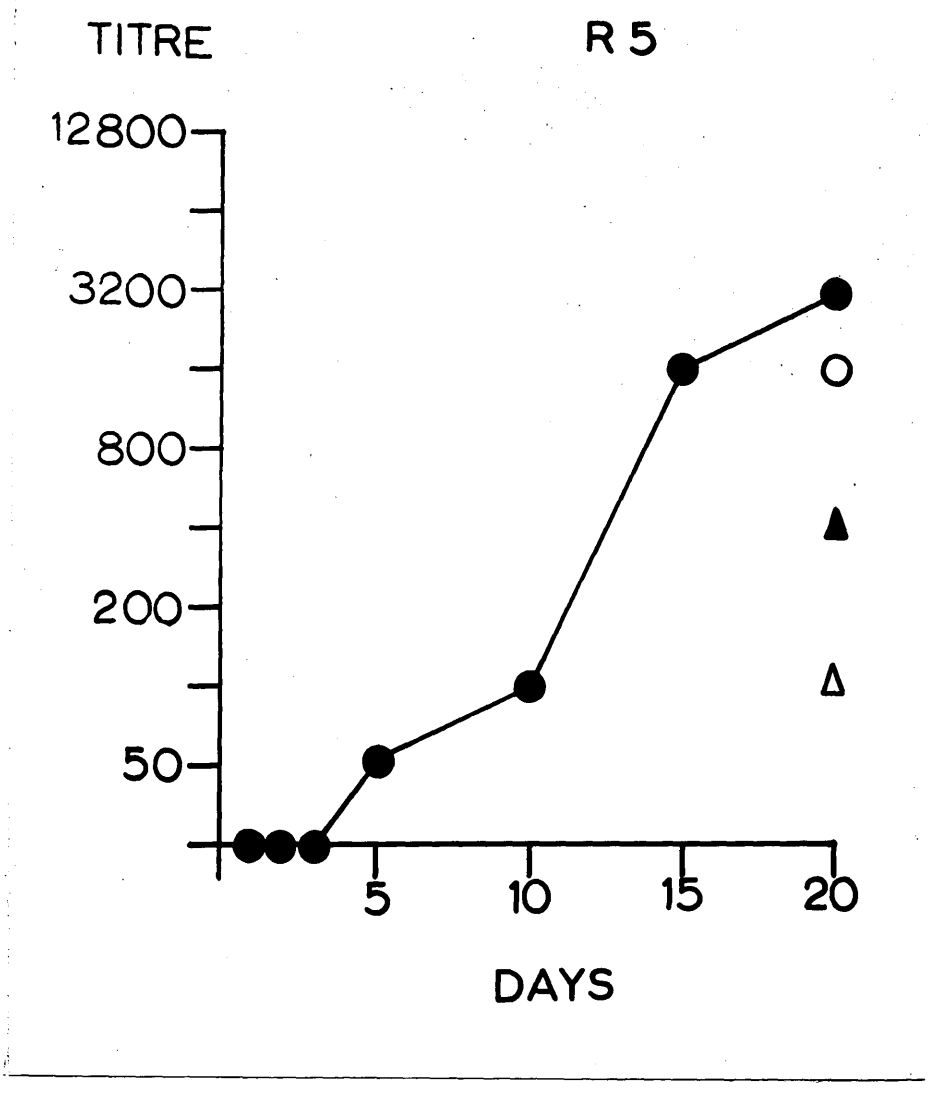

Fig.6. The serum titre is seen to climb steadily from the fifth day after the initial injection.

At post mortem examination a slight thickening of the free edge of the greater omentum was visible. The structure was, however, free and mobile. There was no evidence of peritoneal inflammation or irritation.

The omental titre is seen to exceed that of the spleen or the liver. The ratio of these titres is 32 : 4 : 1.

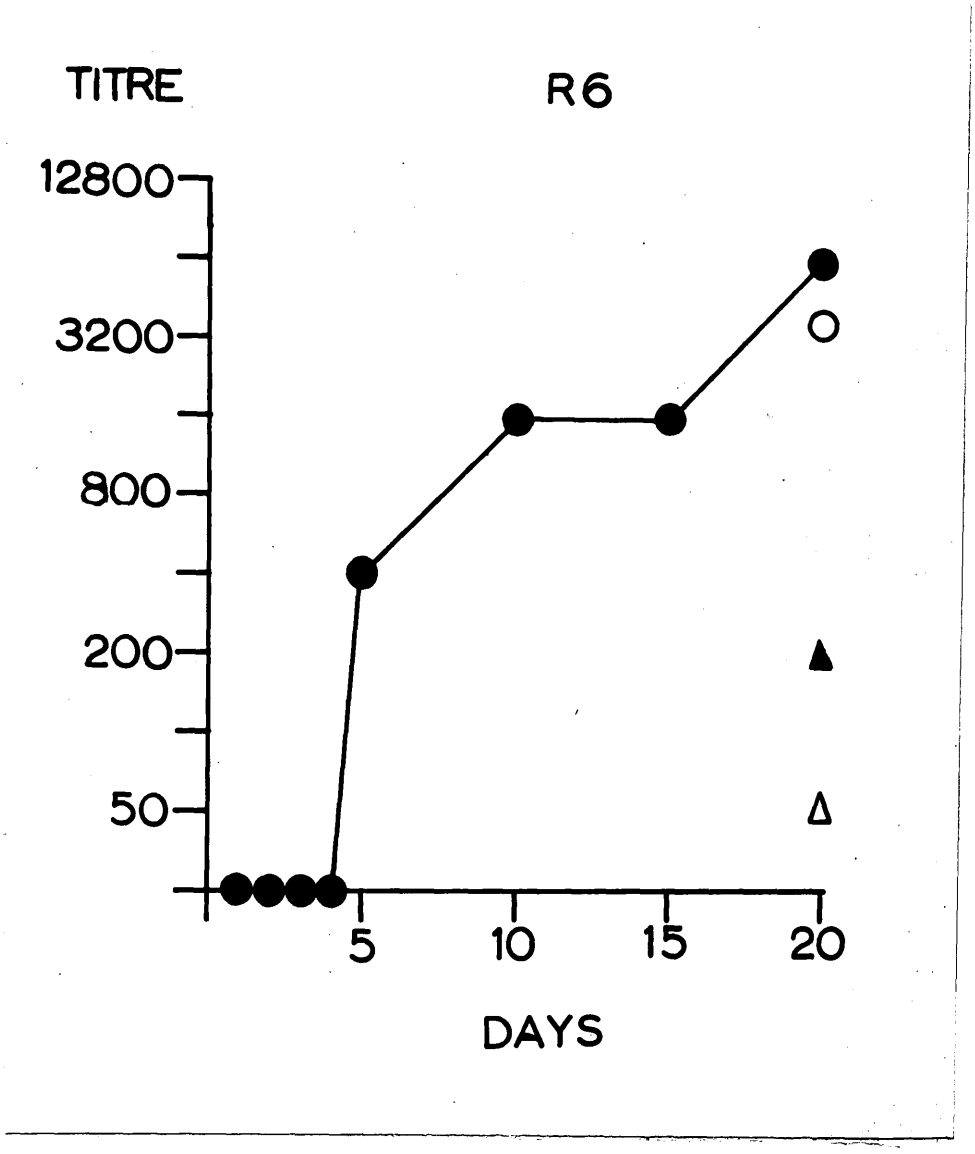

Fig.7. No circulating antibody is detectable until the fifth day after the commencement of the immunising process. There follows a steady rise which reaches a maximum on the twentieth day.

At post mortem there was only a suggestion of thickening of the free edge of the greater omentum. The structure appeared normal in all other respects. There was no peritonitis.

It will be seen that the omental titre greatly exceeds that of the spleen or the liver and the ratio of titres is 64 : 4 : 1.

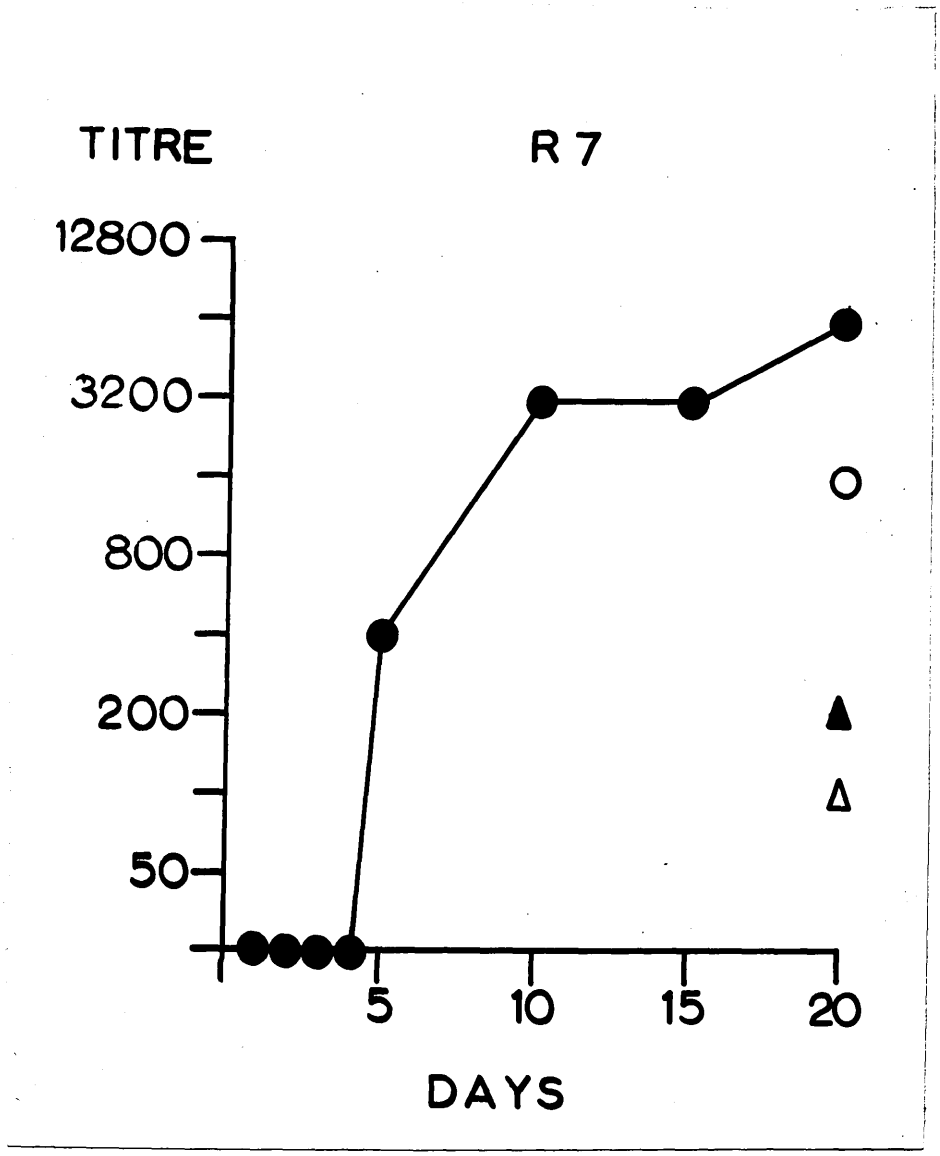

Fig. 8. No circulating antibody is detected until the fifth day after the commencement of the immunising course of antigen. There is a sharp rise of titre which later climbs to a maximum on the twentieth day.

At post mortem examination there was no visible evidence of inflammation of the greater omentum which was found to be freely mobile.

The tissue titre of the structure is seen to greatly exceed that of the spleen or the liver. The ratio of the titres is 16:2:1.

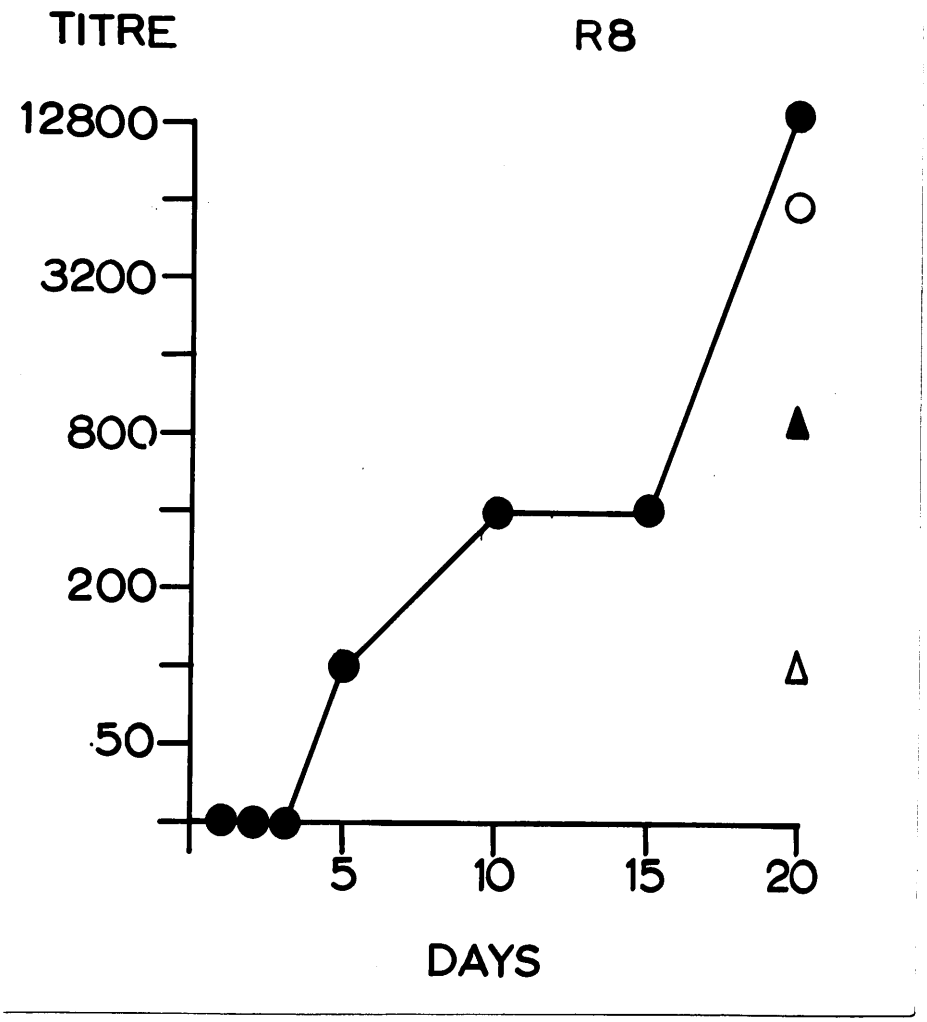

Fig.9. No circulating antibody has been demonstrated on the first three days after the initial injection of antigen but, thereafter, the titre climbs to reach a maximum on the twentieth day.

At post mortem examination a visible thickening of the free edge of the greater omentum was present. There was no evidence of peritonitis.

The omental titre greatly exceeds that of the spleen or the liver. The ratio of the titres is 64 : 8 : 1.

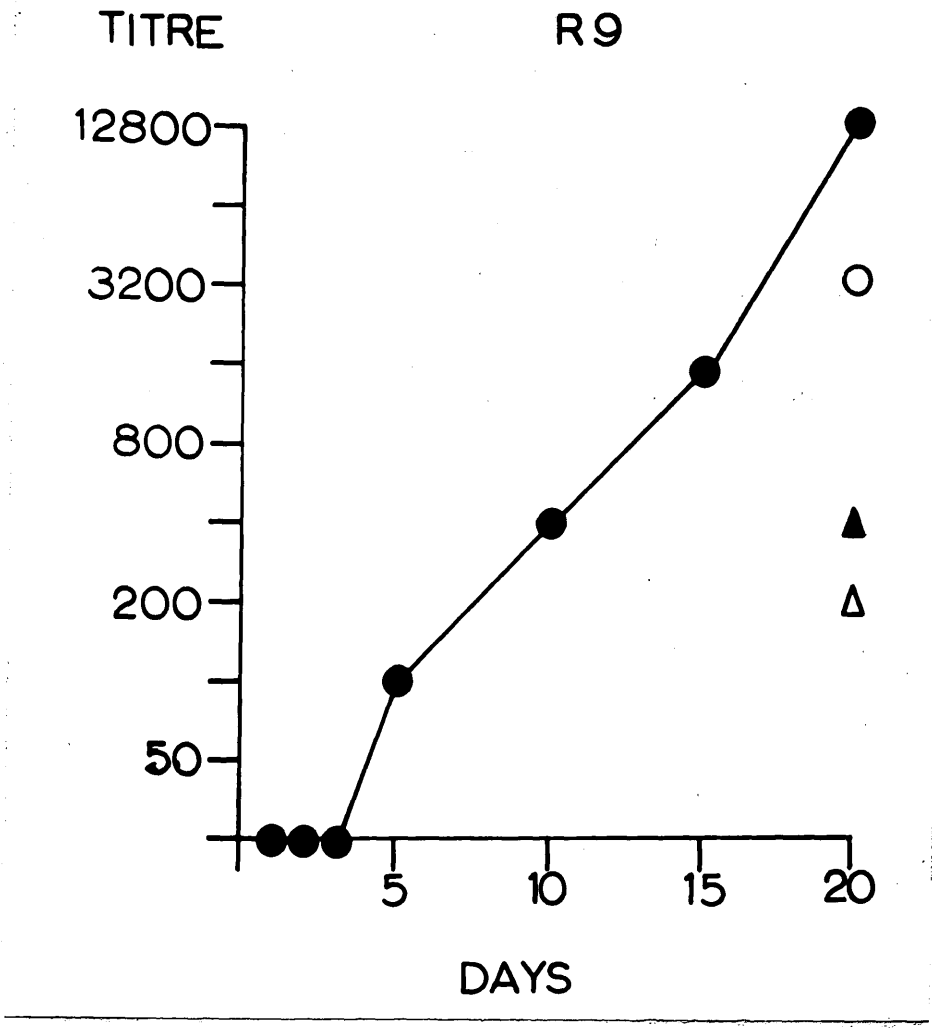

Fig.10. No circulating antibody has been detected on the first three days after the initial injection of antigen. There is a steady rise of serum antibody which reaches a maximum on the twentieth day.

At post mortem examination there was a visible thickening of the free edge of the greater omentum. The structure was otherwise normal and was freely mobile.

The tissue-titre of the omentum is seen to exceed that of the spleen or the liver. The ratio of titres is 16 : 2 : 1.

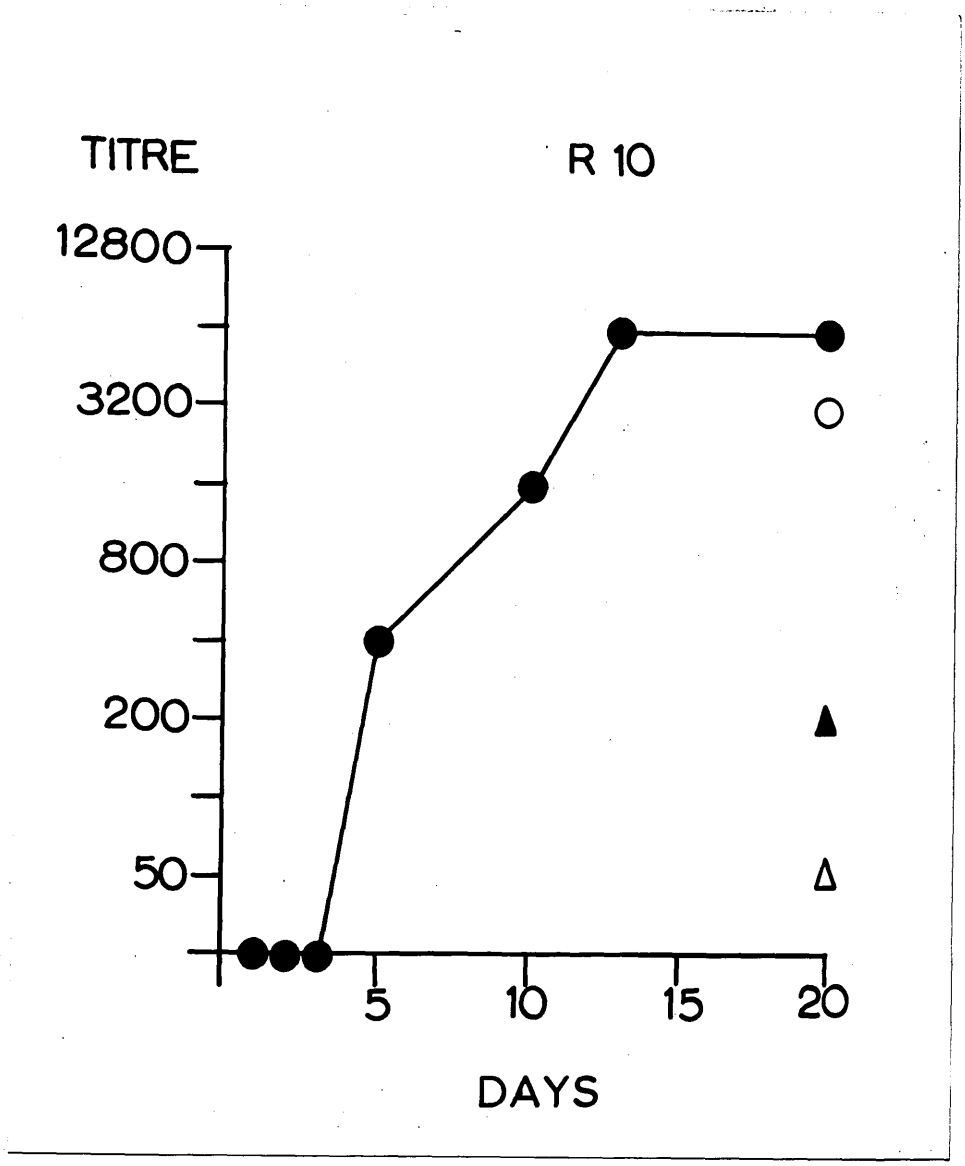

Fig.11. No circulating antibody has been detected in the first three days after the commencement of the immunising course of antigen. There follows a sharp rise of serum titre which reaches a maximum on the fifteenthday.

At post mortem examination there was a slight thickening of the peripheral, free edge of the omentum.

The omental titre greatly exceeds that of the spleen or the liver. The ratio of the titres is 64 : 4 : 1.

The immune response of the intact experimental animals to the intra-peritoneal injection of antigen is comparatively rapidly and actively acquired. The circulating antibody appears in demonstrable amounts between the third and fifth days and reaches a value of between 1 in 1600 and 1 in 12,800.

The response shows some individual variation and some animals are seen to reach the maximum titres by the fifteenth day after the initial injection, while others do not attain this until the twentieth day. The measurements which have been made apply only to the twenty day experiment. Had the animals been allowed to survive the serum titre would be higher in value.

When the tissue titres are examined it will be seen that the greater omentum appears to play the greatest part in the elaboration of antibody towards the antigen when the latter is injected into the peritoneal cavity. The ratios of the titres of the omentum : spleen : liver vary from 64 : 4 : 1 to 6 : 1.3 : 1. The most commonly encountered ratio has been 15 : 2 : 1. This is demonstrated in Fig. 12 which is the diagrammatic representation of the tissue immune response when the antigen is injected intra-peritoneally. It will be seen that no record is made of tissue titres of the bone marrow or the axillary lymph nodes. This is because no agglutinating titre was ever detected in these tissues and it will be seen that the peritoneum appears to make no contribution towards the elaboration of antibody.

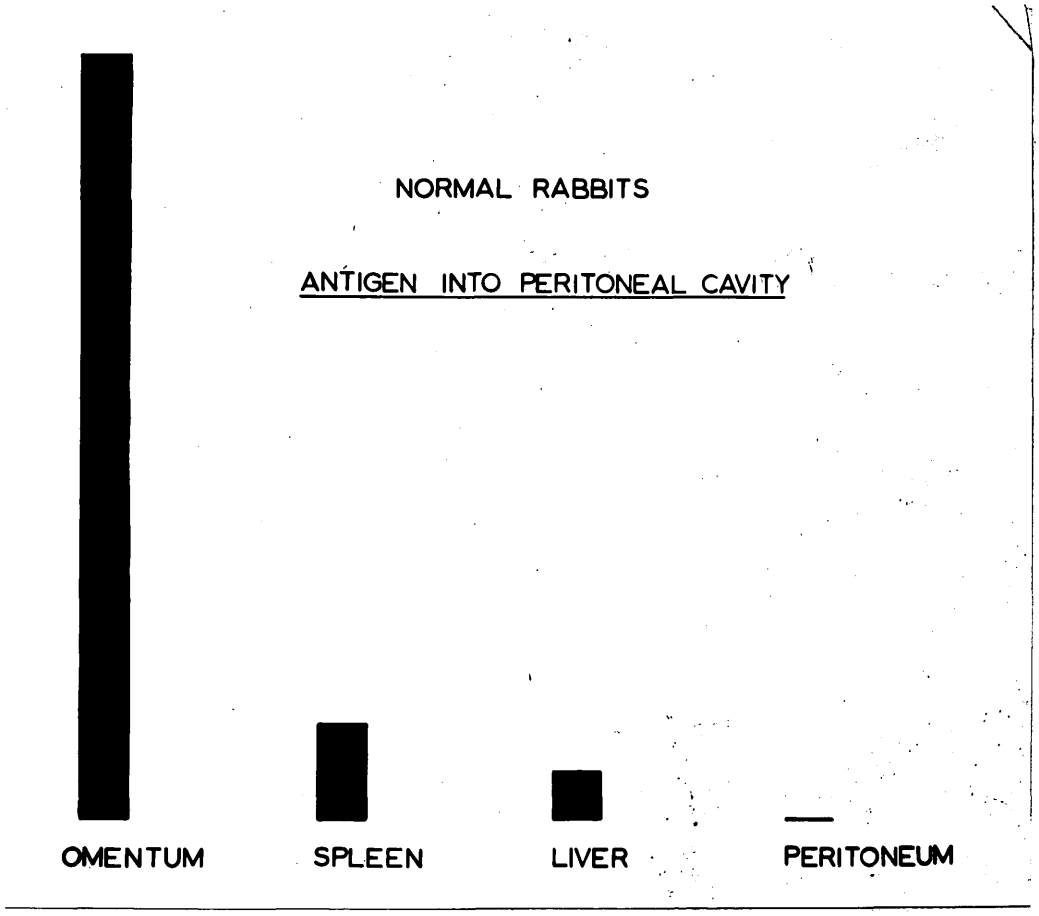

Fig.12. This is the diagrammatic representation of the tissue - titres which have been found in a group of normal rabbits following the intraperitoneal injection of a course of antigen. It will be seen that the greatest titre is seen in extracts of the greater omentum and that this is followed by the spleen. The liver makes only a small contribution while the peritoneum exhibits no agglutinating titre. The ratio of the titres of omentum : spleen : liver is 15 : 2 : 1, and this was the most common pattern encountered.

After a course of immunising antigen has been injected intraperitoneally a visible thickening of the greater omentum has been seen at the post mortem examination, which was carried out on the twentieth day. In many cases, at first sight the greater omentum appeared to be quite normal and it was only on closer scrutiny that the thickening was apparent. In all cases the structure was freely mobile and had not contracted adhesions to the parietes or to other viscera. A search of the peritoneal surfaces failed to reveal any overt evidence of reaction. In no cases was the spleen seen to be noticeably enlarged. There was no apparent mesenteric lymphadenopathy.

From these experiments it was considered that the greater omentum was capable of either:-

(a) elaborating specific agglutinating antibody,

or (b) functioning as a local reservoir for antibody which might have been synthesised in some other site.

The structure was considered likely, either to actively concentrate circulating antibody or to absorb antibody from the peritoneal cavity if it were to fulfil the second alternative.

In order to investigate this problem further a group of rabbits were subjected to the same immunising procedure after the removal of their greater omenta.

2. Rabbits in which the Greater Omentum has been Excised.

A group of rabbits were selected to act as a control in the elucidation of the possible capacity of the greater omentum to elaborate antibody.

These animals were subjected to laparotomy through a small midline incision and the greater omentum was identified and removed in its entirety. This structure is very friable in the rabbit. The bleeding points were tied with fine arterial silk and the wound was also closed with the same material.

The animals appeared to accept this treatment well and no untoward effects were observed and there were no fatalities, except for the occasional anaesthetic death. The rabbits ate and drank normally.

Fourteen days after excision of the greater omentum, when the animals were in all respects well, they received a course of immunising antigen suspension identical to that injected into the peritoneal cavities of the normal, intact animals. The injections were made two inches above the pubic symphysis in the midline.

The immune responses of these animals are described and illustrated in the following figures.

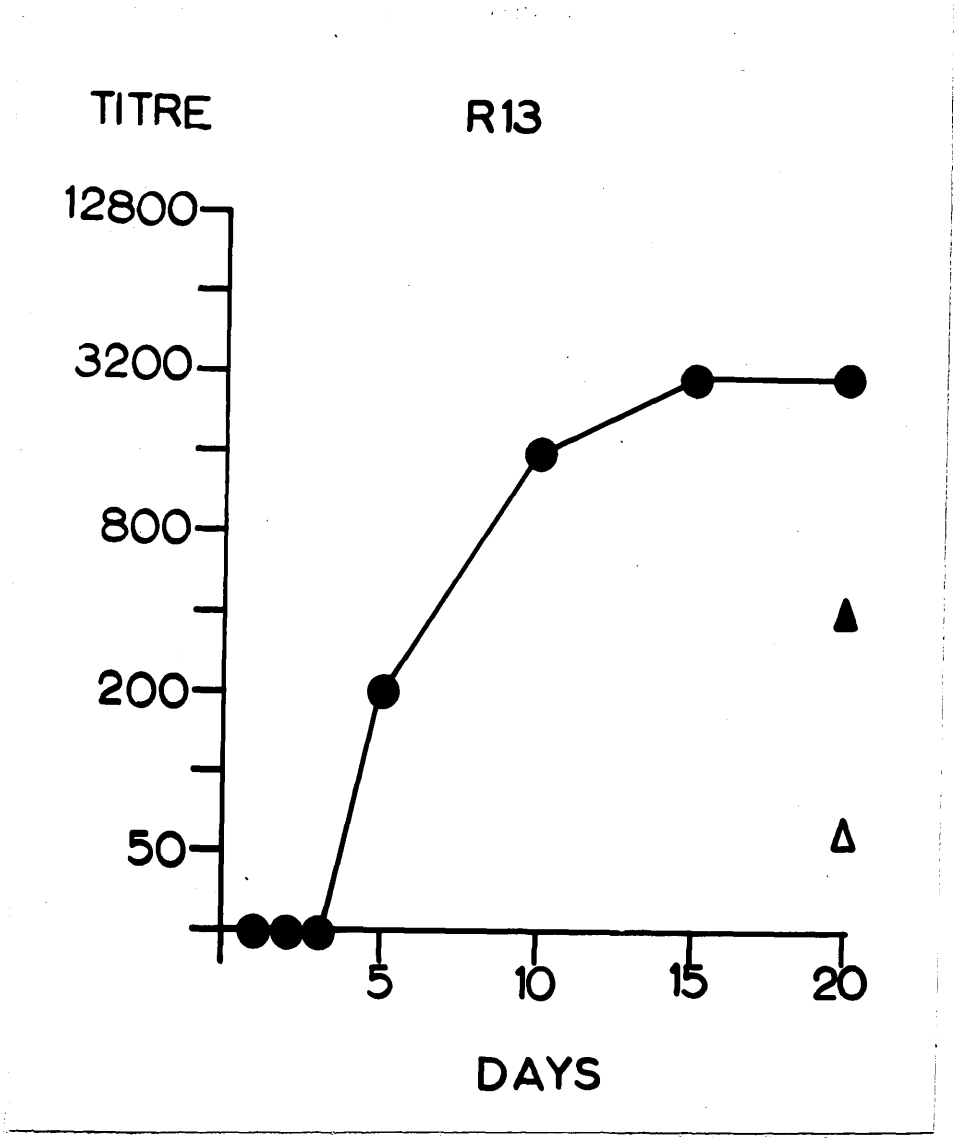

Fig.13. The omentum of this animal has been removed fourteen days prior to the commencement of the immunising course of antigen.

The serum titre ascends steadily from the fifth day after the initial injection of antigen. The pattern of the response is similar to that in the intact animal.

At post mortem examination it was found that the right edge of the greater omentum had been overlooked and had been left in situ. This residual tissue amounted to about two grams and was visibly thickened and was related to the gastro-duodenal junction.

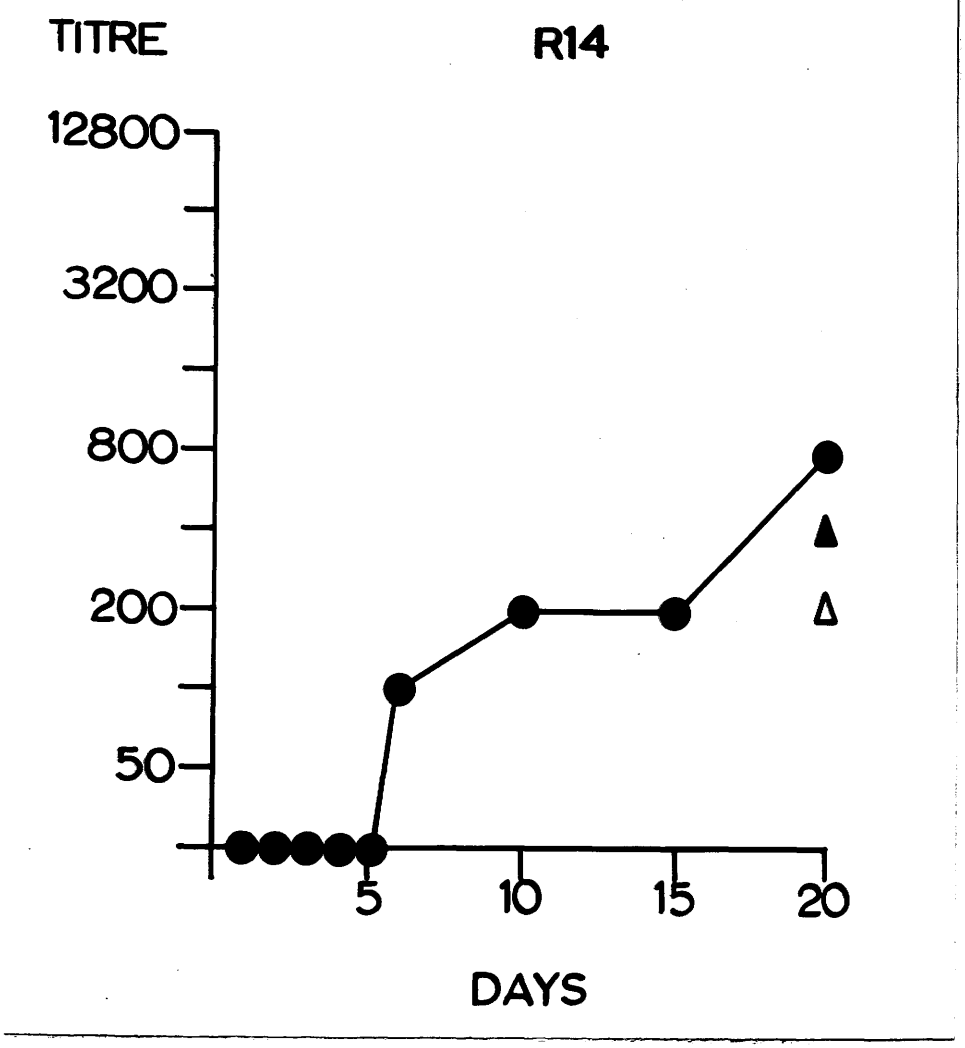

Fig.14. The greater omentum has been removed from this rabbit fourteen days prior to the commencement of the immunising course of antigen.

The rise of serum titre does not occur until the sixth day after the initial injection. Thereafter, there is a steady rise which, however, remains low.

At post mortem examination the complete removal of the omentum was confirmed.

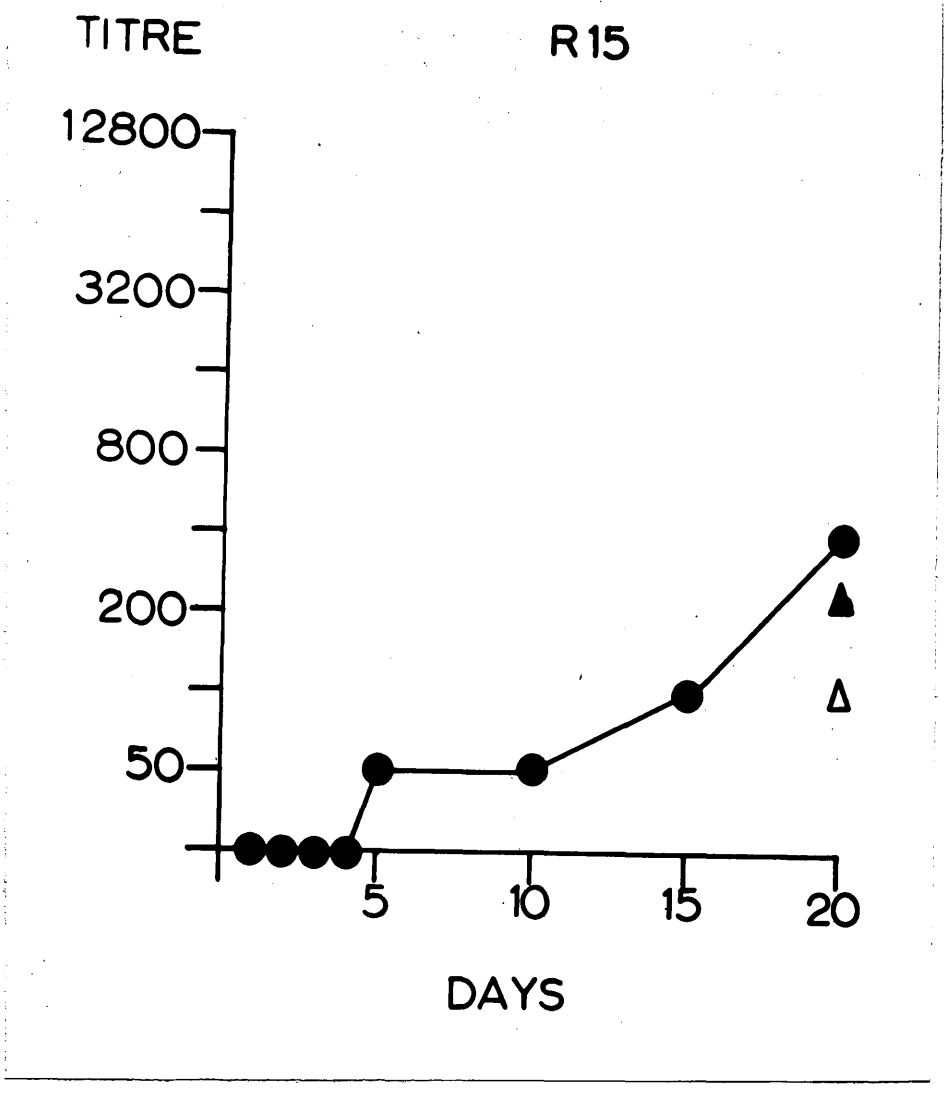

Fig.15. The greater omentum of this animal was removed fourteen days prior to the commencement of the course of immunising antigen.

The serum titre is seen to commence to rise on the fifth day after the initial dose of antigen. The level of circulating antibody, however, remains low.

At post mortem examination, the complete removal of the omentum was confirmed.

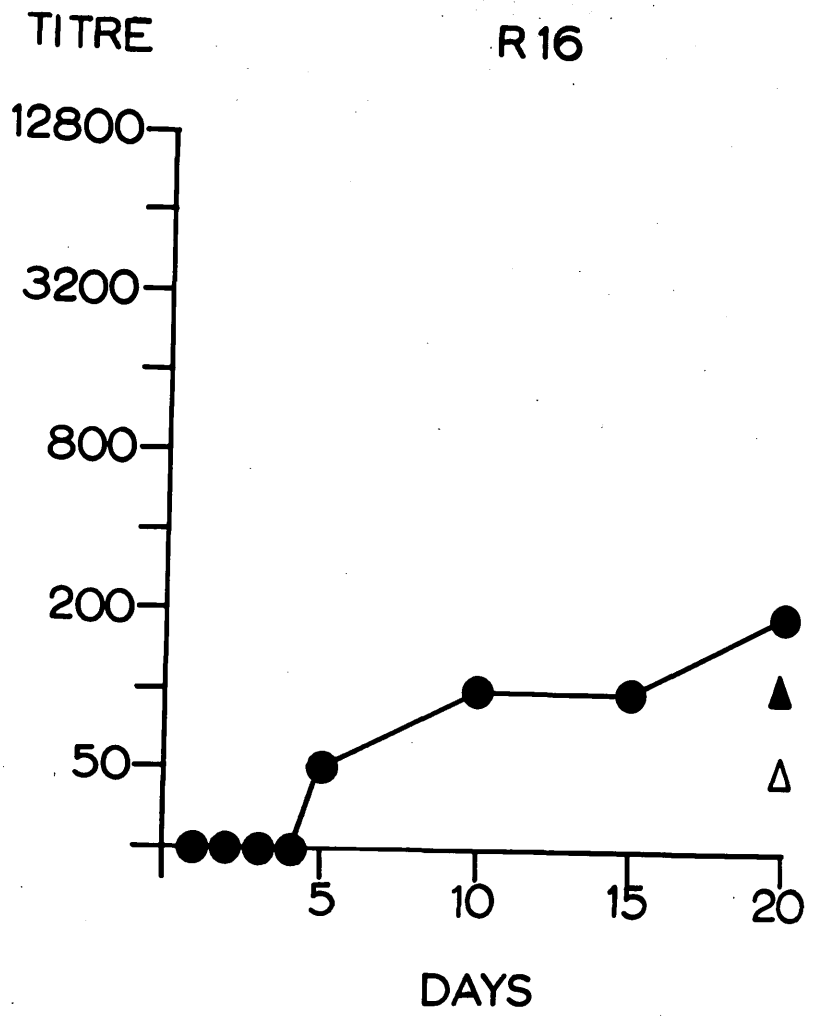

Fig.16. The greater omentum of this animal was removed fourteen days prior to the commencement of the course of immunising antigen.

The serum titre rises on the fifth day after the initial injection of antigen. The level of circulating antibody remains low, however.

At post mortem the complete removal of the greater omentum was confirmed.

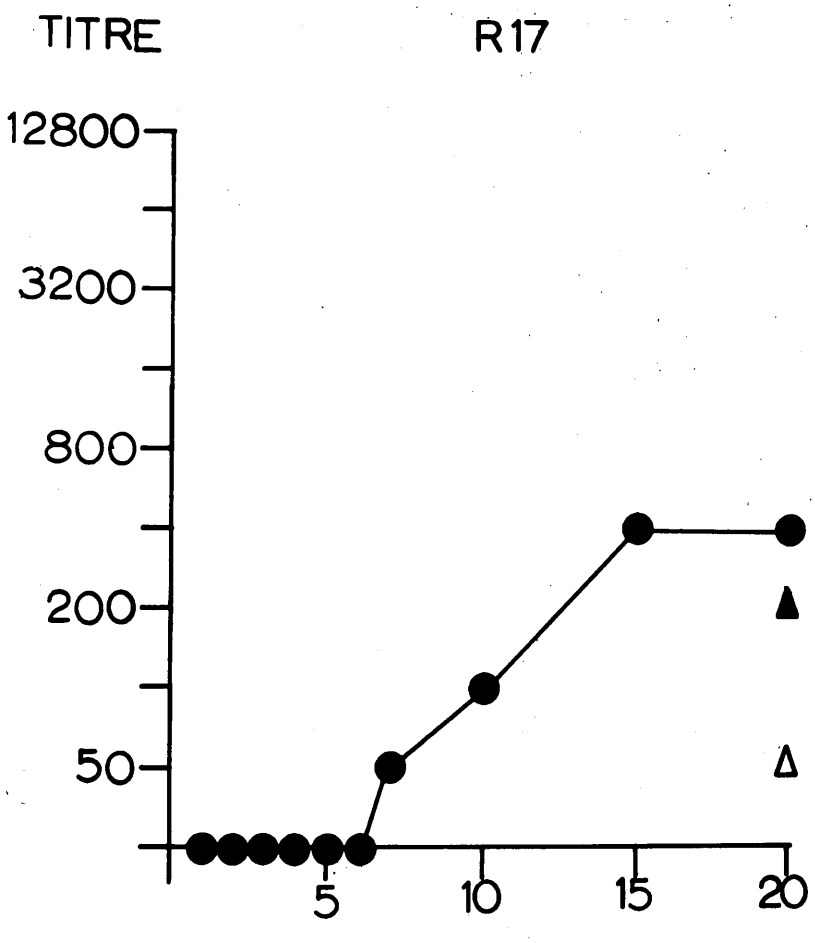

Fig.17. The greater omentum of this animal was removed fourteen days prior to the commencement of the course of immunising antigen.

The serum titre does not develop until the seventh day after the initial injection of the antigen. The level of circulating antibody remains low.

At post mortem the complete removal of the omentum was confirmed.

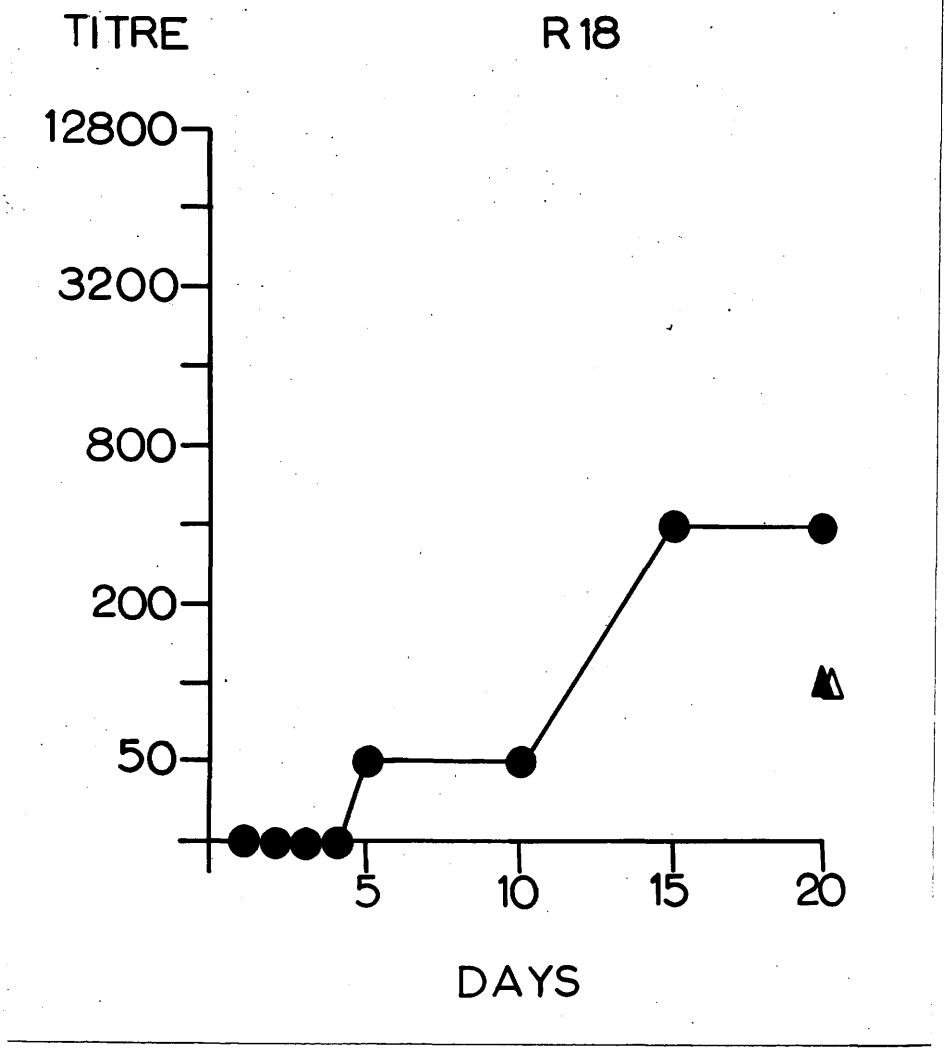

Fig. 18. The omentum of this rabbit was removed fourteen days prior to the commencement of the immunising course of antigen.

Circulating antibody was not detected until the fifth day after the initial injection of the antigen. There follows a slow rise in the serum titre which, however, remains low.

At post mortem the complete removal of the omentum was confirmed.

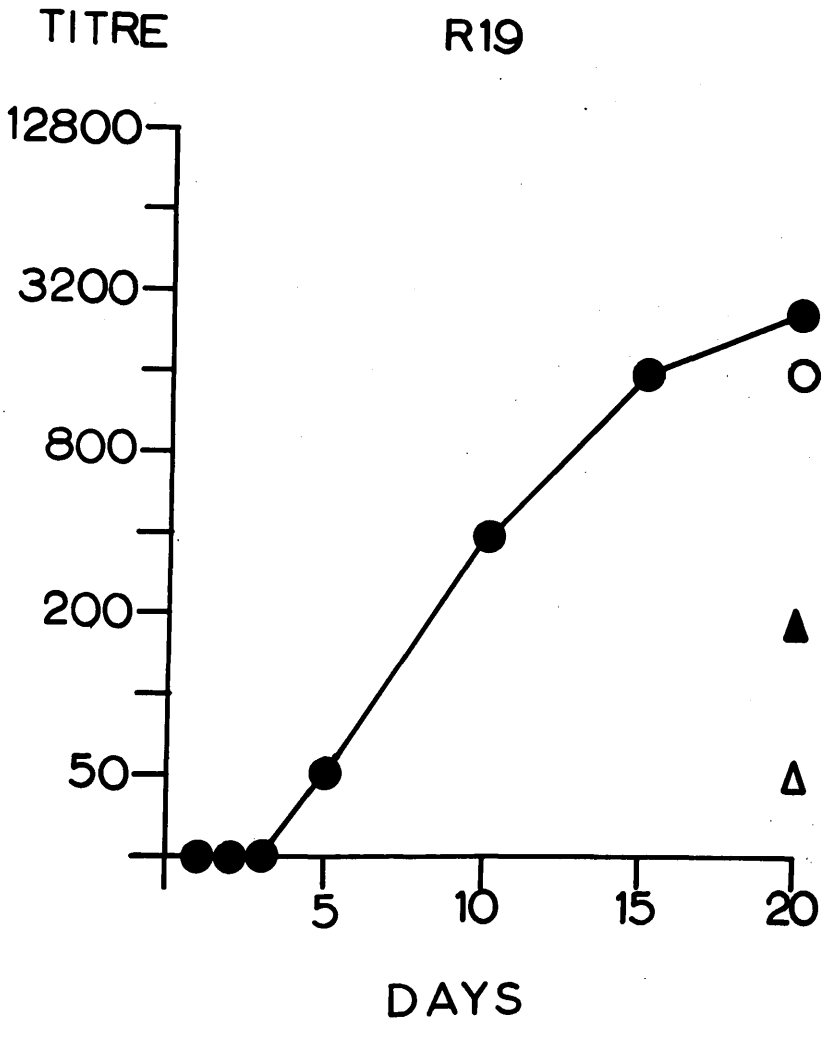

Fig. 19. Because of the immune response exhibited by Rabbit 13 (Fig.13) was associated with an overlooked residuum of omentum, this animal (R 19) had all but a fringe of greater omentum excised fourteen days prior to the course of immunising antigen. The amount of tissue left was two grams. The serum titres are those of a normal intact animal.

At post mortem there was a thickening of the fringe of omentum which had been left. The tissue-titres were 30 : 4 : 1, of omentum : spleen : liver.

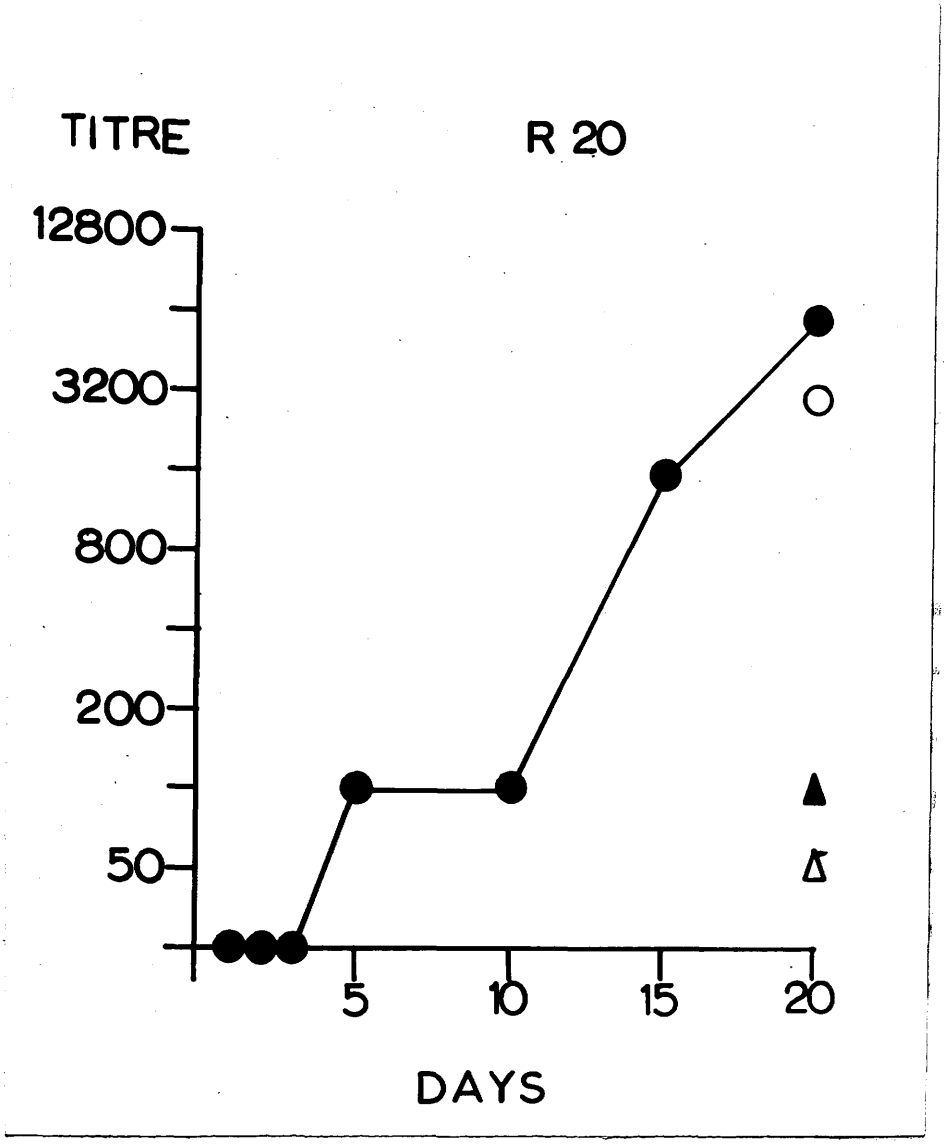

Fig.20. In order to confirm the results which were obtained in Rabbit 19, this animal (R 20) was treated in the same way. The greater omentum was excised, except for a residual fringe, fourteen days prior to the injection of antigen. The amount of omental residuum was about two grams.

It will be seen that the response is similar to that of a normal, intact animal.

At post mortem the omental fringe was visibly thickened. The ration of omental : splenic : hepatic titres was 64 : 2 : 1.

The results of these experiments suggest that the rabbit omentum contributes a substantial part of the immune response of the animal to the intra-peritoneal injection of antigen.

Whereas the normal, intact animal rapidly develops a serum titre against the antigen of the order of between 1 in 12,800 and 1 in 1600, the animal which is deprived of its omentum responds to this stimulation with a low serum antibody titre which does not rise above 1 in 800. Moreover, the initial rise of antibody may be delayed until the seventh day after the initial injection of the antigen.

A comparison of the serum titres between the group of intact, normal rabbits and that group which had had their omenta excised revealed a wide disparity. It was found that with the same course of immunising antigen the intact animals developed a serum titre which was, on the average, fifteen times that of the titre attained by the omentectomised animals.

This comparison of the serum titres of the two groups of animals is depicted diagrammatically in Fig. 21.

A further point of interest is the small volume of the omentum which appears able to confer an immune response which is comparable to the response seen in a normal animal. The results suggest that as little as two grams of the structure confer a response to intra-peritoneal antigen under the conditions of these experiments.

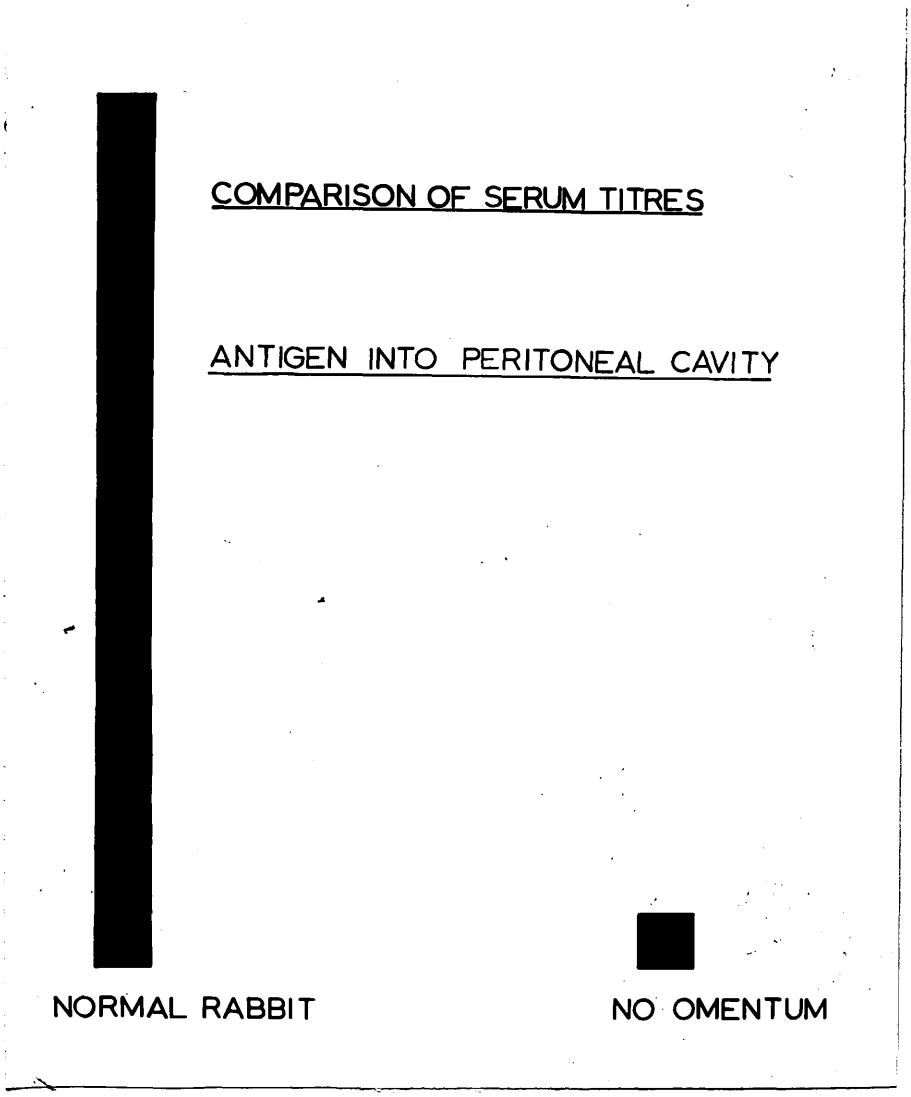

Fig. 21. A comparison of the serum titres which occur in normal rabbits and rabbits deprived of their omenta when the animals receive a course of intraperitoneal antigen.

The normal rabbits develop a titre which is fifteen times that of the omentectomised animals.

The residual fringes of greater omentum which were left, either by default or design in these experiments, invariably exhibited a visible thickening. Portions of these thickened omenta were removed for histological examination and the results are discussed in a later chapter of this study.

It will also be observed that the tissue titres of the spleen and the liver appear to more nearly approach the serum titre in the animals which have been deprived of their omenta. This is probably a consequence of the lower level of serum titre occurring in this group of animals; the titres of spleen and liver remaining comparatively constant.

It was considered that the results of these experiments supported the hypothesis that the omentum was capable of producing antibody. It was thought possible, however, that the tissue titres demonstrated in omental extracts could have been the result of the absorption of antibody by the structure from the peritoneal cavity. It was difficult to imagine which tissue could then have elaborated the antibody, because the peritoneum exhibited no titre and the other members of the reticulo-endothelial system had been examined and determined to be of small antibody content.

3. Rabbits with exteriorised greater omenta.

In order to preclude the possibility of the greater omentum absorbing antibody which had been secreted into the general peritoneal cavity by some other tissue the situation of which was unknown, the greater omenta of a group of animals were exteriorised.

The abdomen of the animal was opened by an upper, midline incision. The greater omentum was then identified and drawn through the abdominal wall into a subcutaneous "pouch" which had been created for it in the subcutaneous tissues. The rectus muscle was split in the line of its fibres to allow the transit of the structure. The opening was made sufficiently wide to prevent the strangulation of the omentum. The laparotomy wound was then closed with silk. At the conclusion of the operation the animal presented a small swelling to the right of the midline in which was contained the greater omentum. It was found possible to exteriorise the entire structure.

The animals survived this operation without apparent ill-effect and fed and drank normally.

Fourteen days after the performance of this procedure the animals were immunised with a course of Salmonella typhi, identical to that used in the other groups of animals. They differed, however, in one respect; the antigen injections were made into the subcutaneous sac and not into the peritoneal cavity.

Figure 22 is the record of the immune response of a member of this group of animals. It will be seen that there is detectable circulating antibody on the fifth day after the initial injection of antigen. There followed a steep ascent of the serum titre, which reached a high maximum on the fifteenth day, and this was maintained until the twentieth day after the commencement of the immunising course.

At post mortem the omentum which had been exteriorised was found to be clearly viable and was a little thickened and was adherent to the walls of the cavity which had been created for it in the subcutaneous tissues. At some points on the surface of the structure were small, white, ovoid spots and these lesions were excised and examined histologically. The general peritoneal cavity was normal in appearance and there was no evidence of lymphadenopathy. There was no free fluid in the cavity.

It will be observed that the omentum has a tissue titre which exceeds that of the spleen or the liver, and the ratio of the titres is 16 : 2 : 1. This ratio and pattern of the immune response of the animal is similar to that which has been seen in the normal intact animal after a similar course of antigen injected into the general peritoneal cavity. The implication of this experiment is that it is the greater omentum which elaborates the antibody. If some other

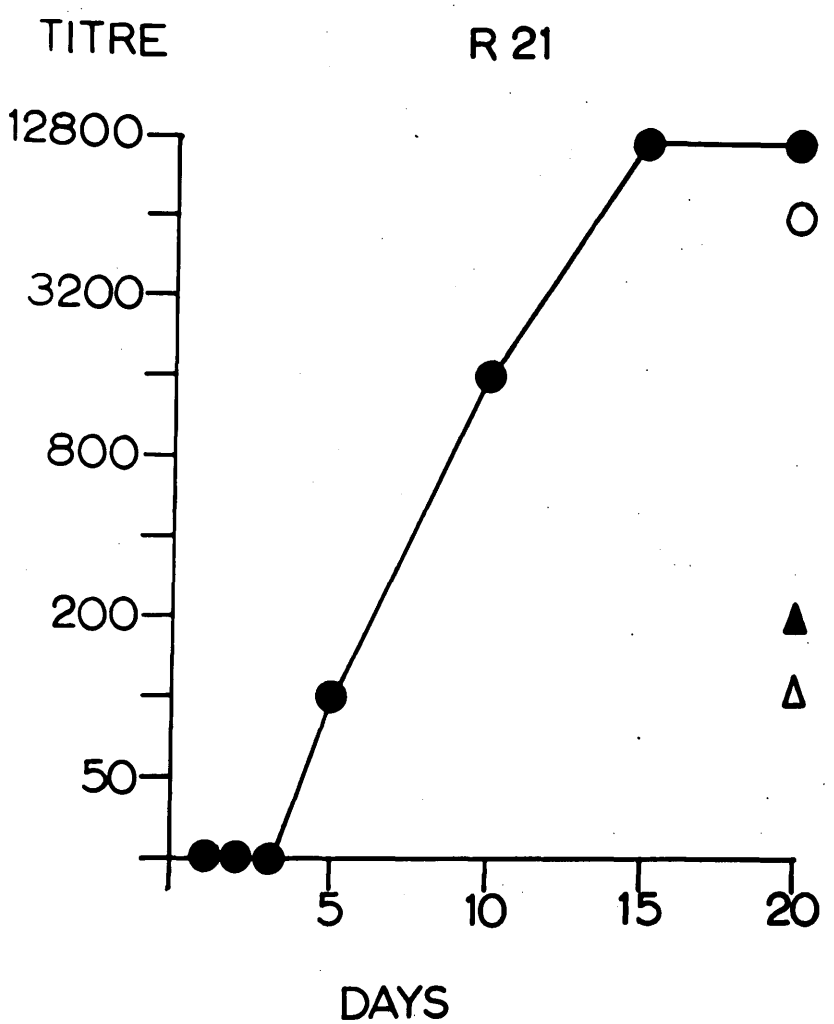

Fig.22. The greater omentum of this animal was exteriorised in a subcutaneous "pouch" fourteen days prior to the commencement of the immunising course of antigen. This antigen was injected into the "pouch" and not into the general peritoneal cavity.

The serum titre will be seen to have developed by the fifth day after the first injection. It ascends sharply to reach a maximum on the fifteenth day. At post mortem examination the omentum was viable and it was observed that the omental titre exceeded that of the spleen and liver. The ratio is 16:2:1. White spots were visible on its surface.

source had done so and the omentum had failed to absorb it, two features would be present:-

(a) The serum titre would not have developed.

(b) Free fluid would be present in the peritoneal cavity.

In order to control the group of animals in which the omentum had been exteriorised in a subcutaneous "pouch", two animals in which the greater omentum had been exteriorised in the same way were subjected to the same course of antigen. The antigen here, however, was injected into the general peritoneal cavity and not into the "pouch".

An examination of the immune response in these control animals (Fig. 23) reveals that the serum titre of the animals developed on the fifth day after the injection of the antigen, and thereafter the level of circulating antibody remained low, reaching a maximum on the twentieth day. This response is similar to that which has been seen in the group of animals in which the greater omentum was excised. In effect, this exteriorising procedure has "functionally excluded" the structure from the peritoneal cavity.

At post mortem no abnormality was apparent in the abdominal cavity.

This group of experiments substantiated the view that the greater omentum was responsible for the synthesis of antibody, or that it concentrated antibody which had been produced by some other tissue, the site of which was unknown.

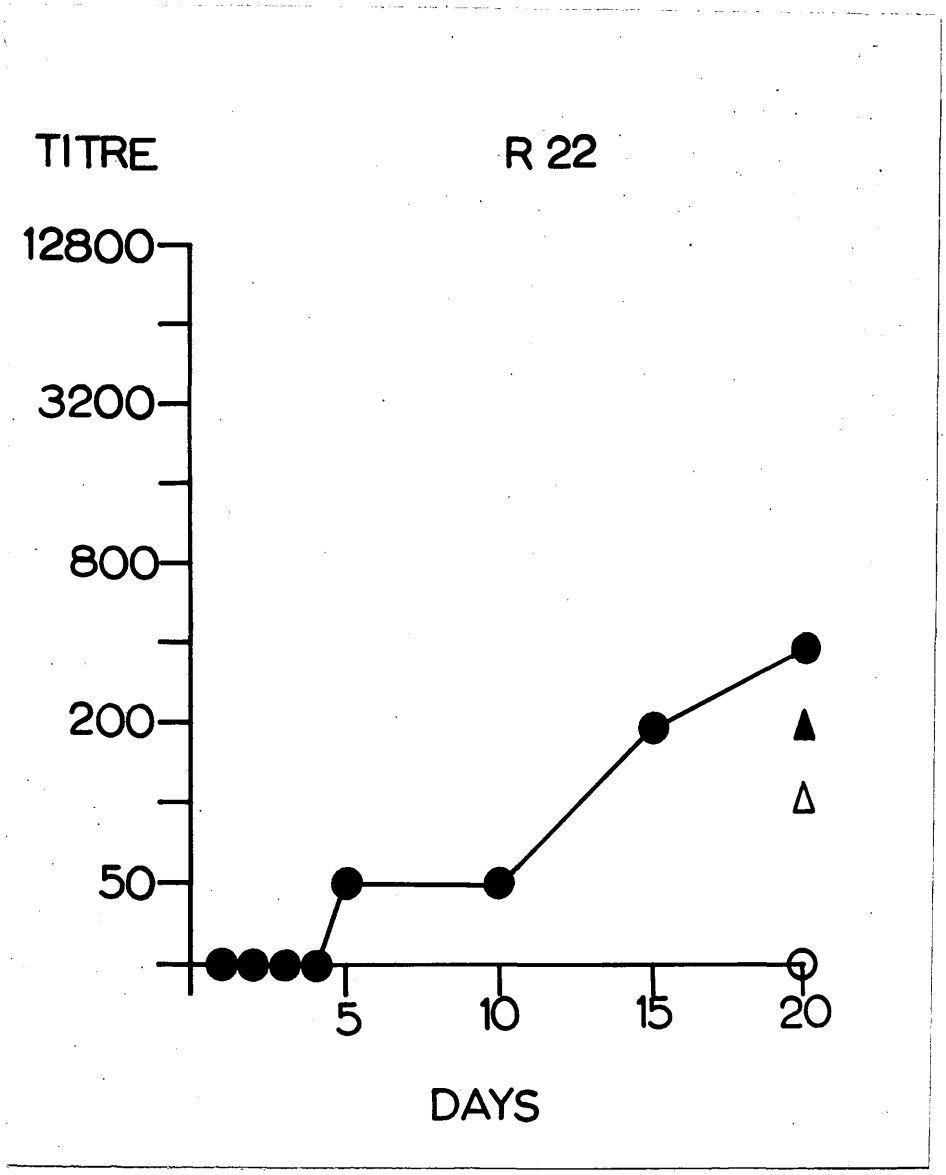

Fig. 23. The greater omentum was withdrawn into a subcutaneous "pouch" fourteen days prior to the commencement of the course of immunising antigen. This antigen was injected into the general peritoneal cavity and not into the "pouch".

The response is seen to be similar to that of animals which have been deprived of their omenta.

No pathological features were evident in the peritoneal cavity.

4. Rabbits after splenectomy.

A further control experiment was performed upon two animals. It has been seen that those animals which had had their greater omenta removed responded to the immunising course of antigen with a serum titre which was considerably lower than that of the intact animals. It was considered that this phenomenon might have been because the animals had recently been operated upon. Although this was unlikely, it was necessary to exclude that an operation per se was capable of "damping down" the immune response of an animal in some mysterious way.

Two animals were subjected to splenectomy and fourteen days after the performance of this operation they received the course of immunising antigen by injection into the general peritoneal cavity.

The results of this experiment are set out in Figure 24. It will be seen that there is a steady rise in circulating antibody, which reaches a maximum on the twentieth day. At post mortem examination a visible thickening of the free peripheral edge of the greater omentum was present. It is evident that the diminution in immune response of animals deprived of their greater omenta is not a consequence of an operation as such. An incidental control was served by the last experiment for the spleen as the source of immunity

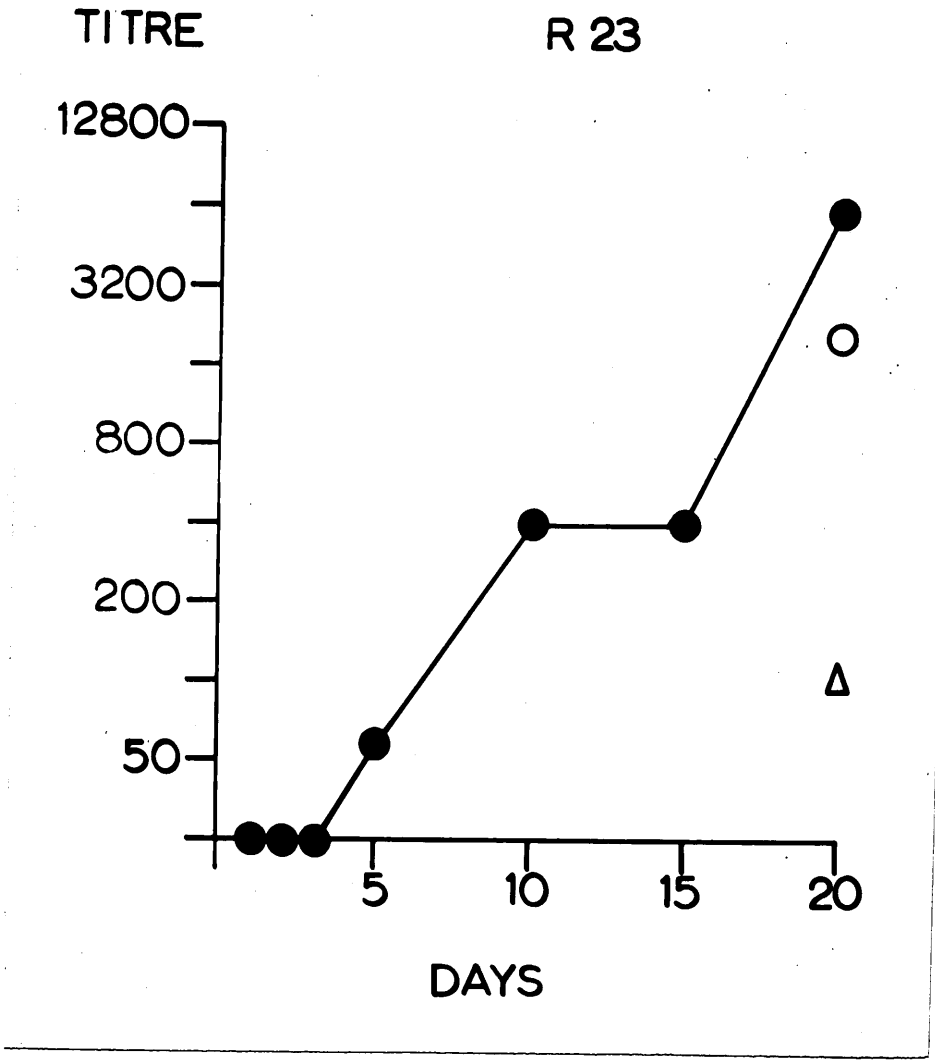

Fig.24. The spleen on this animal was removed fourteen days prior to the commencement of the immunising course of antigen. This antigen was injected into the general peritoneal cavity.

The response is seen to be similar to that of the intact animals.

The omental tissue-titre is high and greatly exceeds that of the liver. The ratio is 16 : 1.

following intra-peritoneal injection. Had this structure been a significant contributor to the animals immunity, under these conditions, the serum titre would be anticipated to be low. In fact, the animal has made a response which is comparable to that of an intact animal. It will also be noted that the omental tissue titre is high and exceeds that of the liver. The ratio of the titres is 16 : 1.

5. Rabbits receiving the antigen intravenously.

As has been remarked previously, little work has been performed on the protective function of the greater omentum in respect of its contribution to the humeral response of an animal to infection. The spleen is generally considered to be the principal tissue in which specific antibody is elaborated and released in response to infection. Much of the experimental work which has led to this belief has followed the intravenous immunisation of the animal and little account has been taken of any omental response under such conditions. It was felt desirable to elucidate this matter, since it was possible that the greater omentum functioned as an antibody-producer in the same way as has been shown, when the immunisation is carried out by the intra-peritoneal injection of the antigen.

A further group of animals were employed for this investigation. The antigen was injected into the marginal vein of the ear, in a course identical to that which was given to the other groups of animals.

All the injections were made into the same marginal vein and blood for serum titre assay was removed from the other ear. This was done to avoid the theoretical likelihood of adsorption of circulating antibody on any organismal residues which may have remained at the site of entry of the needle into the vein during the immunising process.

The immune response which was elicited under these conditions is represented in Figures 25 and 26. It will be seen that there is a rapid and early production of circulating antibody, which attains an agglutinating titre of 1 in 3,200 in both instances by the twentieth day. In this respect the development of serum antibody is similar in rate and in elevation to the serum values occurring after the intraperitoneal injection of the antigen. Of greater significance are the tissue titres of extracts of the spleen, liver and the omentum and their relation to the serum titre. It will be seen that the highest tissue titre in each case occurs in splenic extracts and that this exceeds the titre of the serum. This is followed by the liver and the omentum has only a small tissue titre. The ratio of the different tissue titres is quite different from those which are seen after intraperitoneal immunisation. Following intravenous immunisation it is apparent that the titres of spleen : liver : omentum, are:-

(a) Rabbit 25 64 : 16 : 1.

(b) Rabbit 26 32 : 16 : 1.

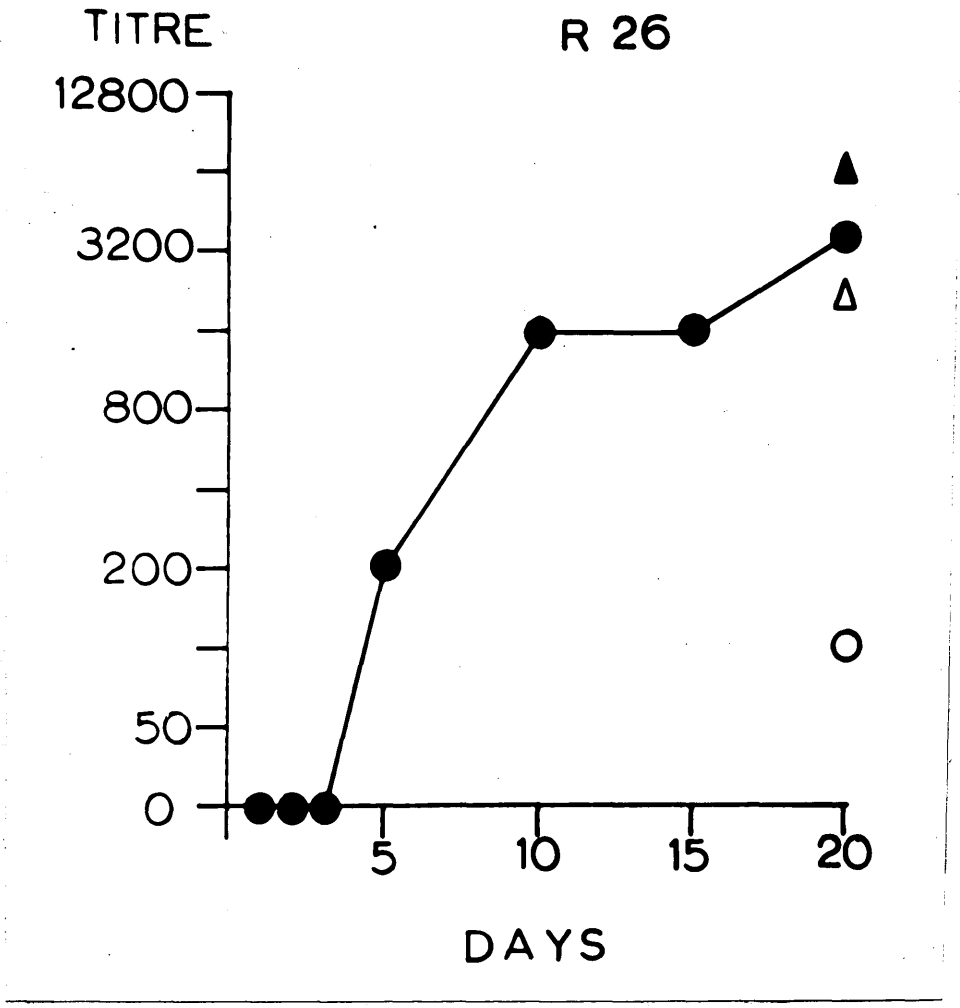

Fig. 25. Showing the response of a rabbit to the intravenous injection of the course of antigen. It will be seen that here, the spleen has the greatest titre and the omentum only a small one. The ratio of the contributions is almost the reverse of that following the intraperitoneal immunisation of an animal.

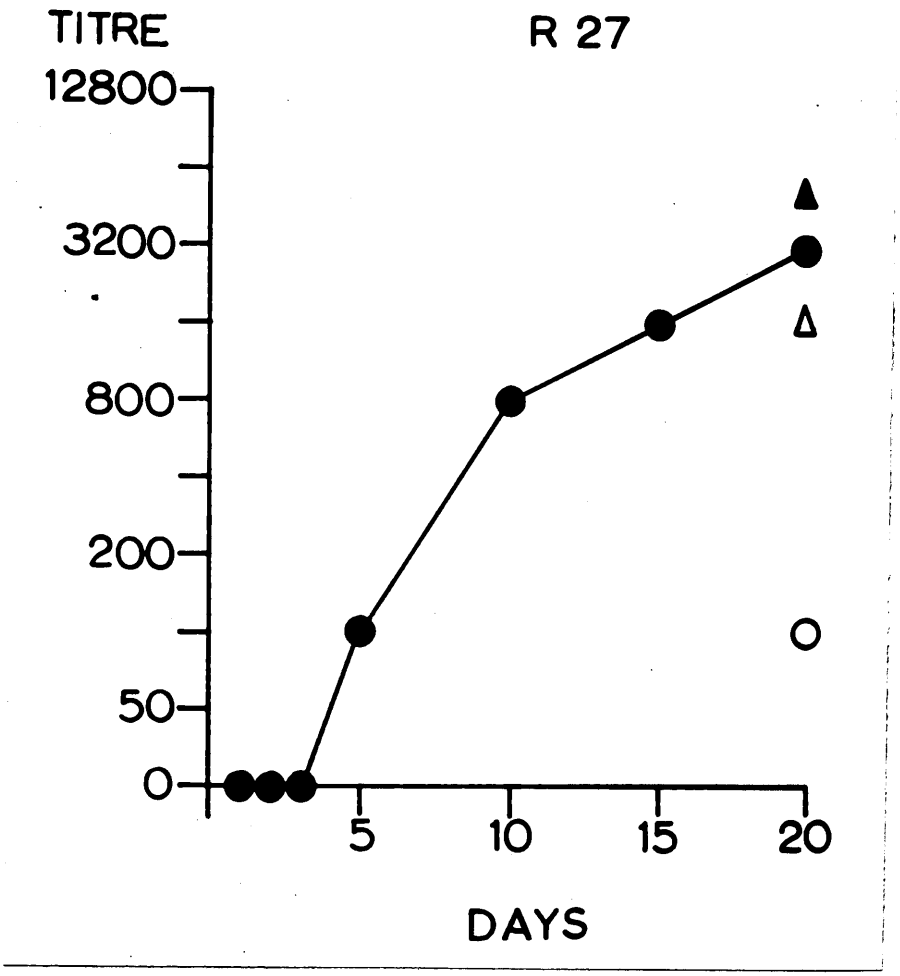

Fig. 26. Showing the response of a rabbit to the intravenous injection of the antigen. It will be seen that, under these conditions, the spleen makes the greatest contribution.

This is almost a reversal of the ratios which have been demonstrated in the intra-peritoneally immunised animals.

In these intravenous control animals, titres were obtainable for both the axillary lymph nodes and the bone marrow. The latter was 1 in 100 on both occasions, while the former was 1 in 50 and 1 in 100 respectively. It will be recalled that neither of these tissues exhibited an agglutinating titre when the antigen was injected intra-peritoneally.

The pattern of the immune response in the animal which has been immunised by the intravenous route is depicted diagrammatically in Fig. 27, where the relative contributions of omentum, spleen, liver and peritoneum are shown. These results are in accord with the work of other investigators in this field, and demonstrate that the greater omentum plays an insignificant part in the animals immune response under these conditions.

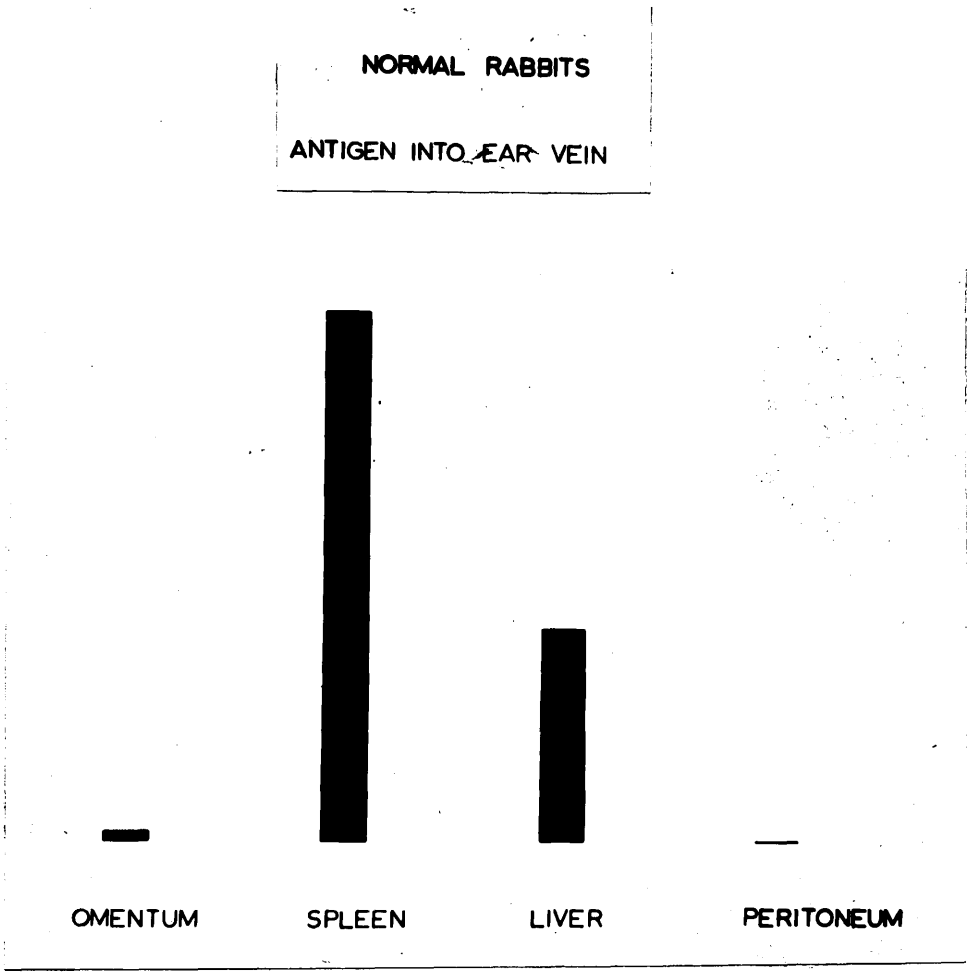

Fig. 27. This is the diagramatic representation of the tissue-titre of antibody which occur after the intravenous immunisation of rabbits. It demonstrates that, under these conditions, the greater omentum plays an insignificant part. The principal contributor is the spleen.

Chapter VI

HOMOTRANSPLANTATION AND TISSUE-CULTURE EXPERIMENTS.

1. Homotransplantation Experiments.

The technique of transplantation of tissues for the elucidation of immune phenomena is a comparatively recent approach to the problem. It has, thus far, required that the transplanted tissue be from an animal of the same species as the recipient, and that the donor has been previously immunised against the specific antigen which is being used in the investigation. It is good evidence of the capacity of a tissue to actively produce antibody if that tissue is able to be shown to confer a degree of immunity upon a recipient animal when transplanted into its tissues.

It was decided to attempt the demonstration of such a function in the greater omentum by transplanting portions of this structure from animals immunised by a course of intra-peritoneal injections of the antigen. However, since even homografts are rejected by the recipient it was first decided to establish, as accurately as possible, the stage at which this phenomenon occurred, in order to facilitate the interpretation of the results.

A group of rabbits were selected for this study. An area two

centimetres in breadth and six centimetres in length of whole skin was excised from the dorsal surface of one ear of each of the rabbits and grafted into the excised area of a different member of the group. The grafts were sutured with silk and "took" in every instance. These grafts were examined at regular intervals for evidence of rejection. Rejection was seen to occur between the seventh and the tenth day after the transference of the graft. These observations refer to the first visible evidence of the rejection of the graft.

The omentum of each of Rabbit 6, Rabbit 7 and Rabbit 8 was then transplanted into the peritoneal cavity of Rabbits 11, 12 and 25, respectively. The donor rabbits were members of the group which received a course of intra-peritoneal injections of the antigen, and all three rabbits were demonstrated to have developed a high omental titre. The transference was performed on the twentieth day after the commencement of the immunising course of antigen. The recipients were all confirmed to be non-immune prior to the transplantation.

The recipient animals were anaesthetised and their abdominal cavities were opened before the removal of the portion of omentum from the donor. The omentum from the donor was removed with an aseptic technique after the animal had been sacrificed by exsanguination and had been perfused. It was considered essential that the viability of the transplant should not be jeopardised by bacterial infection.

The results of the homotransplantation experiments are shown in Figures 28, 29 and 30. It was found that a positive titre occurred in the serum of the recipient within six hours of the homotransplantation. There was then a continued rise of the serum titre which was maintained until between the seventh and the ninth day, thereafter the serum titre fell rather sharply to between 1 in 800 and 1 in 50, and this was then followed by a period in which a flattening out of the serum levels occurred. The curves will be seen to terminate in a gradual fall until finally no titre could be demonstrated in the recipients. The figures demonstrate levels of circulating antibody which are proportional to the weights of omentum removed from the donors for transplantation. Thus, Rabbit 6 donated 1.5 grams into Rabbit 11: Rabbit 7 donated 5.5 grams into Rabbit 12 and Rabbit 8 donated 11.5 grams into Rabbit 25.

The very early and rapid development of a serum titre in the recipient animals would seem to exclude the phenomenon from being one of acquired primary or secondary immunity because the patterns of such responses are different in time.

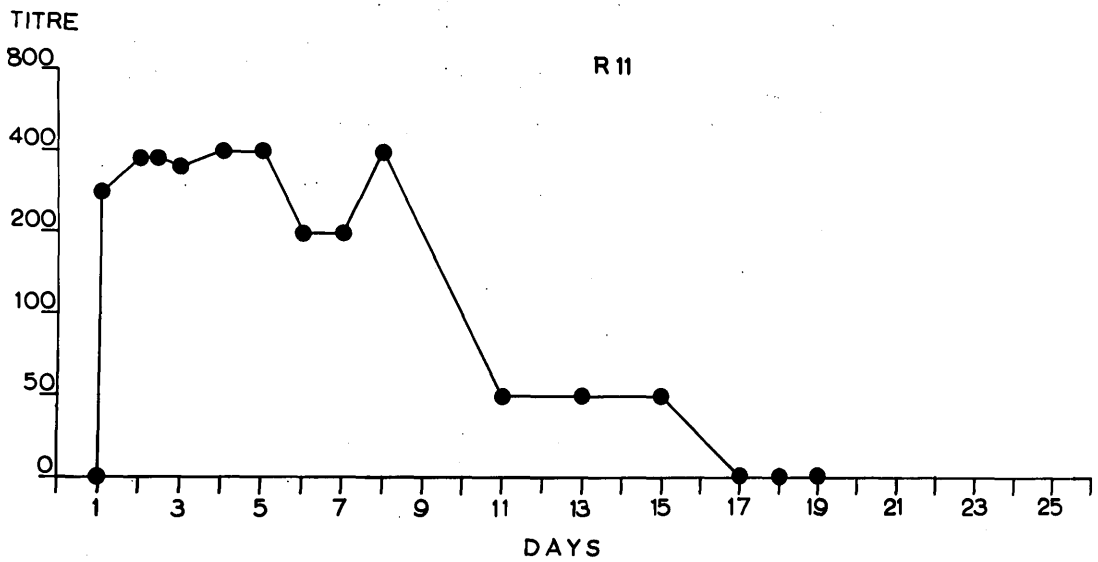

Fig. 28. Showing the serum-titre conferred on a non-immune recipient by the homotransplantation of 1.5 grams of the omentum of a rabbit immunised with a course of intraperitoneal antigen. The early appearance of the titre will be noticed and this occurred within six hours of the transplant. The titre is then seen to climb and continue until about the time of rejection of the transplant.

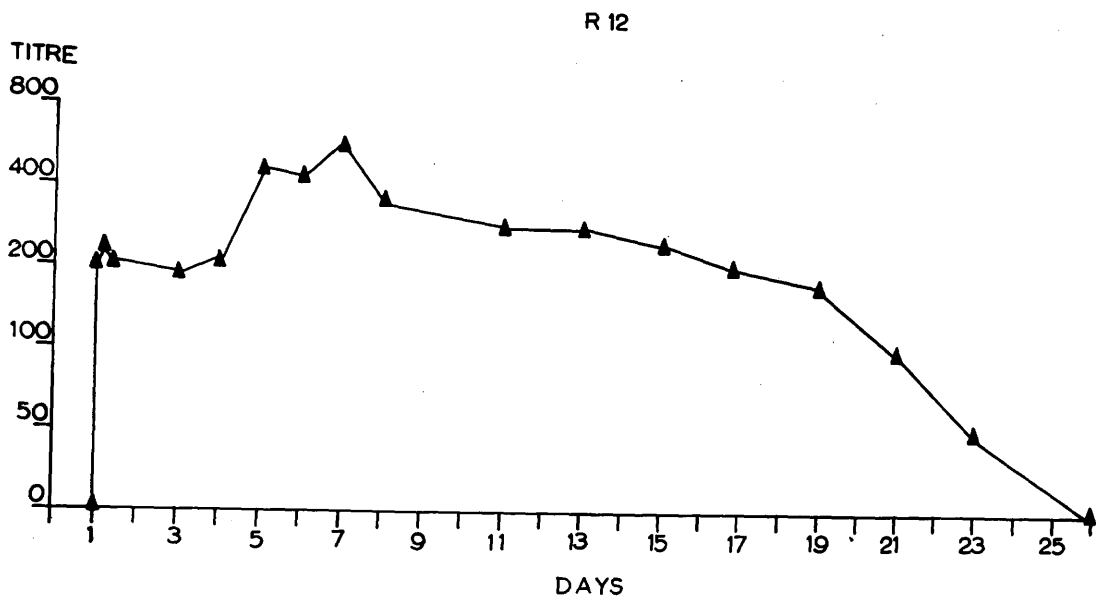

Fig. 29. Showing the serum titre conferred by the homotransplantation of the omentum from an immunised rabbit. A positive serum titre was demonstrated within six hours and it will be seen to rise steadily until about the time it would be anticipated that the transplant would be rejected.

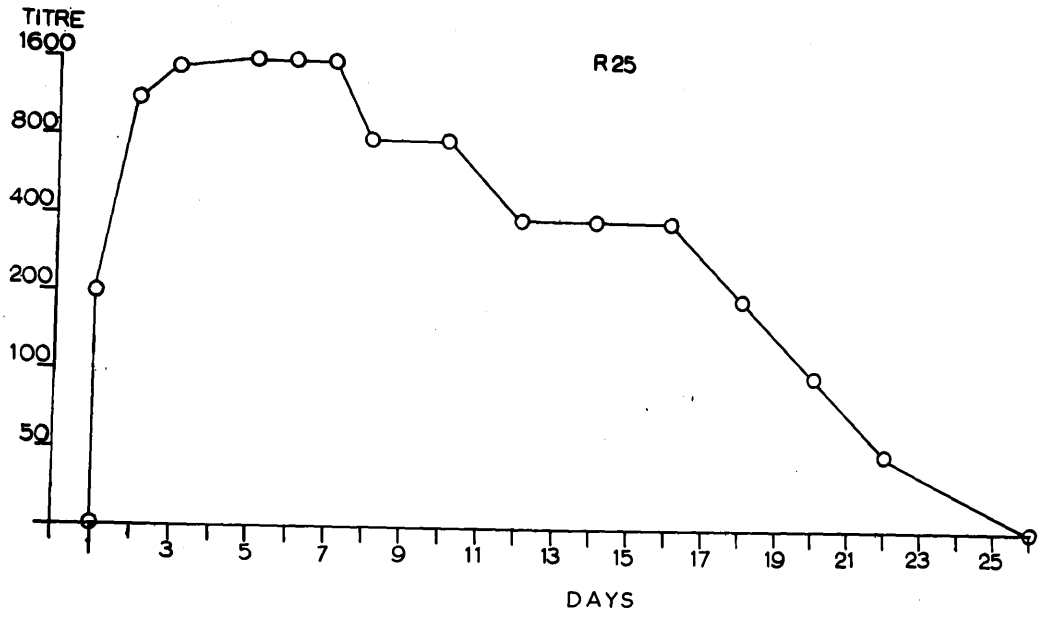

Fig.30. Showing the serum titre conferred upon a non-immune recipient by the homotransplantation of 11.5 grams of the omentum of an animal immunised by the intraperitoneal route. It will be noted that the titre is demonstrated early and that it climbs and continues at a high level until about the time that it would be anticipated to be rejected by the host.

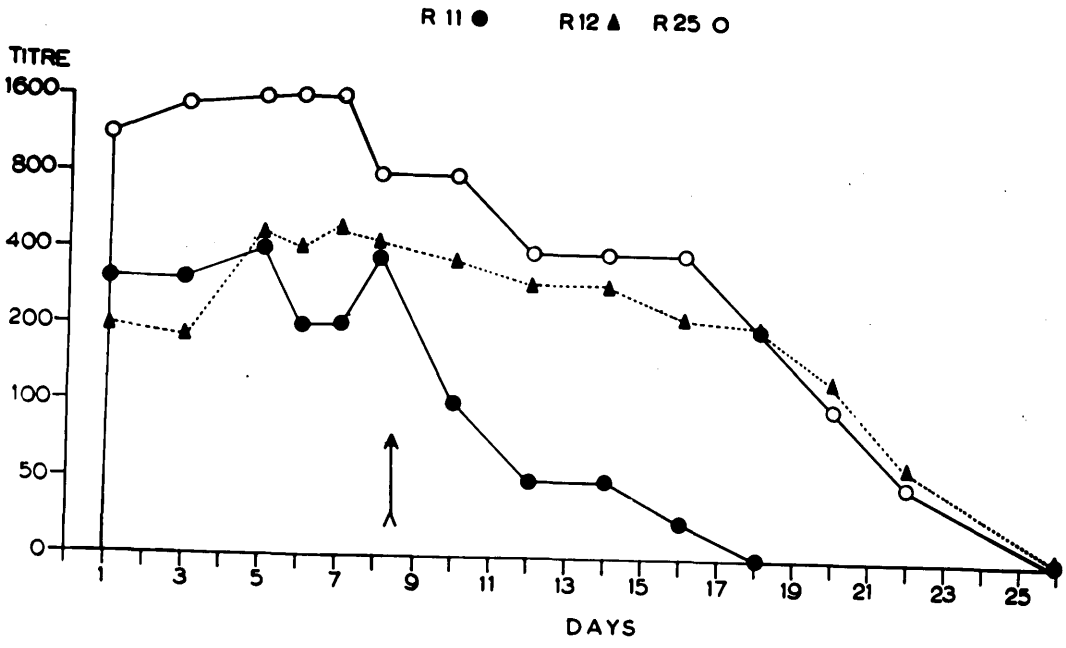

Fig. 31. Showing the titres conferred by homotransplanted omenta from rabbits immunised by the intraperitoneal route. There is a uniform fall in the recipient's serum titre at the period indicated by the arrow, which probably corresponds to the period at which the transplant is being rejected by the host. The recipients were confirmed to be non-immune prior to the transplant.

It will be seen from Figure 31, where all three curves are shown together, that the rather sharp fall in titre of the recipient's serum occurs at about the same time in each of the three animals. Because this occurs between the seventh and the ninth day it is probable that the homotransplant is being rejected at about this time. This would conform to the time of rejection of the skin homografts which were used as a control for the experiments. That the rejection does not occur in one single reaction is suggested by the slow fall of serum titre which occurs in the recipients.

When a portion of omentum is transplanted in this way it is unavoidable that free, preformed antibody lying within the tissue interstices is carried over to the recipient. This antibody will subsequently be absorbed by the recipient and it is therefore necessary to have some indication of the amount of such antibody which is carried over when interpreting the result in the host. In order to clarify this problem, three control animals were employed.

These animals were confirmed to have no titre to the antigen employed. Thereafter, the omentum of a rabbit which had been immunised by a course of intra-peritoneal antigen in the usual way was divided into two equal portions. Each portion weighed 3.5 grams. One of these portions was immediately transferred into the peritoneal cavity of one of the recipients. The remaining portion of omentum was then

placed in a water-bath at fifty six degrees centigrade for a period of thirty minutes (using a sterile bag as a receptacle). This treatment was designed to be lethal to the cells of the tissue and yet not sufficient to denature the antibody protein molecule. This omentum was then transplanted into the abdominal cavity of the second recipient.

As has been mentioned earlier, note has been taken of the slow fall of circulating antibody in the serum of the recipient animals following the homotransplantation of "immune" omenta. It was felt desirable to have a third control which received a passive intravenous injection of antibody so that an indication of the rate of fall of such a passive donation could be gained. If the live omental homotransplants were, in fact, actively producing antibody there should be a sharp difference in the pattern of fall of serum titre from the last control animal. Accordingly, the third recipient animal received an intravenous injection of 3.5 millilitres of the donor's serum.

The results of these controls are seen in Figure 32. It will be observed that the live homotransplant gives rise to demonstrable circulating antibody in the recipients serum within six hours of the transplantation. This is followed by a continual and steady rise of serum titre which reached a high maximum about the fourth day. This level is maintained until the seventh day and is followed by a sharp drop, probably corresponding to the period during which rejection is occurring.

The heat-killed portion of the omentum gives rise to a comparatively mild and transient degree of immunity in the recipient. This amounts to a serum titre of less than 1 in 200 on the second day, and this falls and disappears by the third day after transplantation. This must be antibody which has been carried over in the transplant, since the cells have been killed prior to transplantation.

The passive intravenous injection of serum from the immune donor is comparatively rapidly eliminated and it will be seen that at the seventh day the recipient with the live omentum has a serum titre of 1 in 800 while the recipient of the serum has a titre of only 1 in 50.

The pattern of immunity conferred on the recipient by the living transplanted omentum differs markedly from either of the controls to such a degree that it seems only explicable by a capacity of the transferred omentum to actively synthesise specific antibody until finally the transplant is rejected by its host. As has been mentioned previously, and is borne out by the group of animals injected by intraperitoneal antigen, the phenomena of homotransplantation cannot be due to acquired primary or secondary immunity to any transferred antigen in the transplant because antibody appears too early for this.

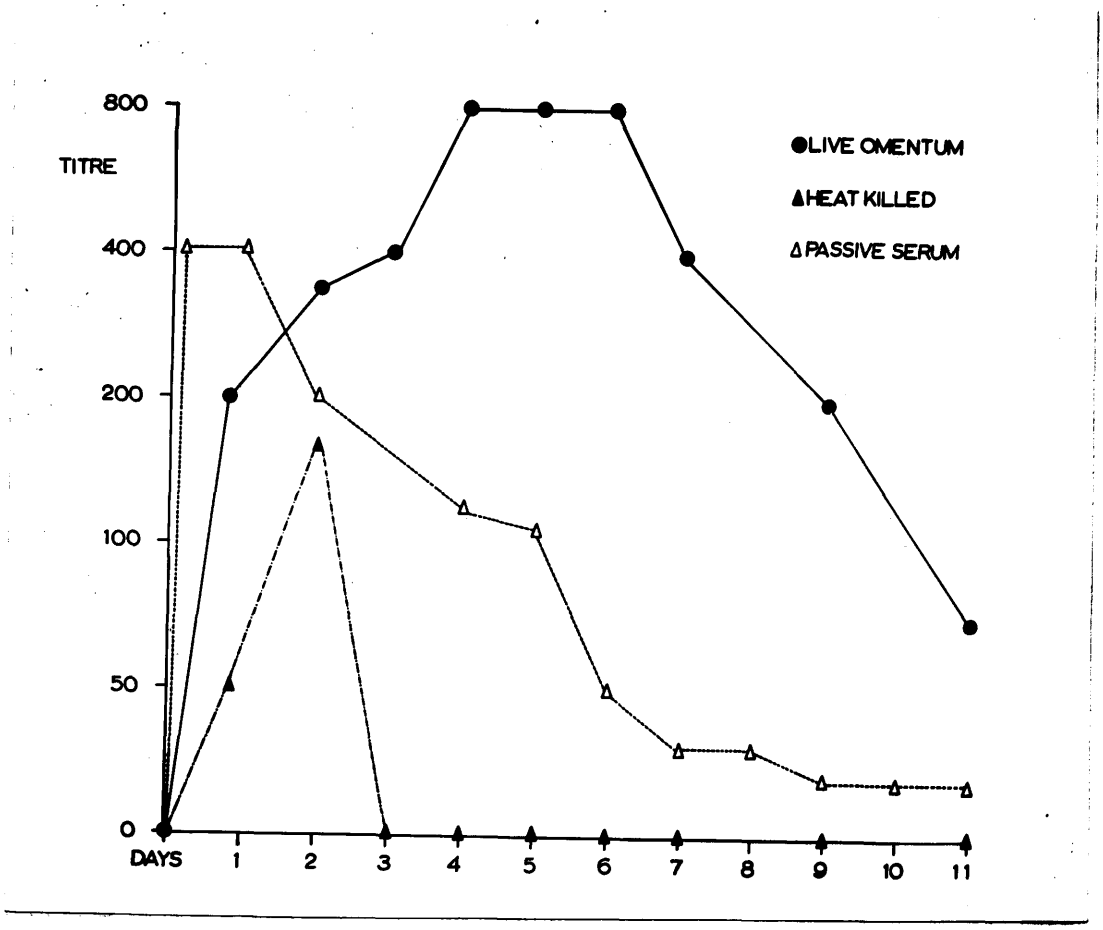

Fig. 32. Showing the results of the homotransplantation of 3.5 grams of (a) living (b) heat-killed omentum, and (c) the injection of 3.5 millilitres of serum from a rabbit immunised by the intraperitoneal injection of antigen, into non-immune recipient rabbits. It will be seen that there is a wide divergence of the curves and that the viable omentum gives rise to a much higher serum-titre which continues until the time at which it would be anticipated to be rejected by the host.

In order to investigate the state of a homotransplant at about the time of rejection, several recipients were sacrificed at different times and the abdomens inspected. The transplant was found to be always wrapped up in the recipient's greater omentum. Sections of the transplants were taken for histological study and are described in a later chapter. As far as could be assessed histologically, there were still viable cells present and these cells were predominantly plasma cells or their precursors.

2. Tissue-culture Experiments.

Weighed portions of the omenta of rabbits which had received the full course of intra-peritoneal injections of antigen were transferred to a watch-glass and finely teased with forceps. The tissue was then transferred to a tissue-culture chamber which contained 0.2 millilitres of medium, consisting of forty per cent horse serum and sixty per cent of Hank's solution. The medium was then aerated with fifty per cent of oxygen in air and the chamber was placed in an incubator at thirty seven degrees centigrade. Infection was controlled by performing the manoeuvres with aseptic precautions, and by the addition of penicillin and streptomycin to the medium. The specimens were examined daily under the microscope and were observed to be actively growing out from the edges of the cultured tissue. A sample of the medium was confirmed

to have no titre against the antigen used for immunising the omental donor. At various intervals the medium was removed and replaced with a fresh volume of medium of the same composition. The titre of the medium which was withdrawn was measured for its antibody content.

As a control for this experiment, an equivalent weight of omentum from an immunised donor was placed in a water-bath at fifty six degrees centigrade for a period of thirty minutes. Again this was designed to kill the cells but preserve the pre-formed antibody, and thus give some indication of the amount of the latter which was being "leeched" out from the explant. This specimen was then transferred to a tissue-culture chamber in the same way as the living omentum and examined daily by microscope. That the tissue was inactivated was confirmed by the lack of growth at the edges of the control. The medium was withdrawn and replaced as in the case of the live omentum.

The results of these experiments are shown in Figure 33. It will be seen that the heat killed omentum confers a titre upon the medium which is, at all stages, lower than that of the living omentum. This is most marked on the fifth day after explant. It is difficult to explain this result other than by postulating a capacity of the living omentum to actively produce antibody.

It was considered that it should be possible to contain and measure the quantity of antibody formed by a living homotransplant

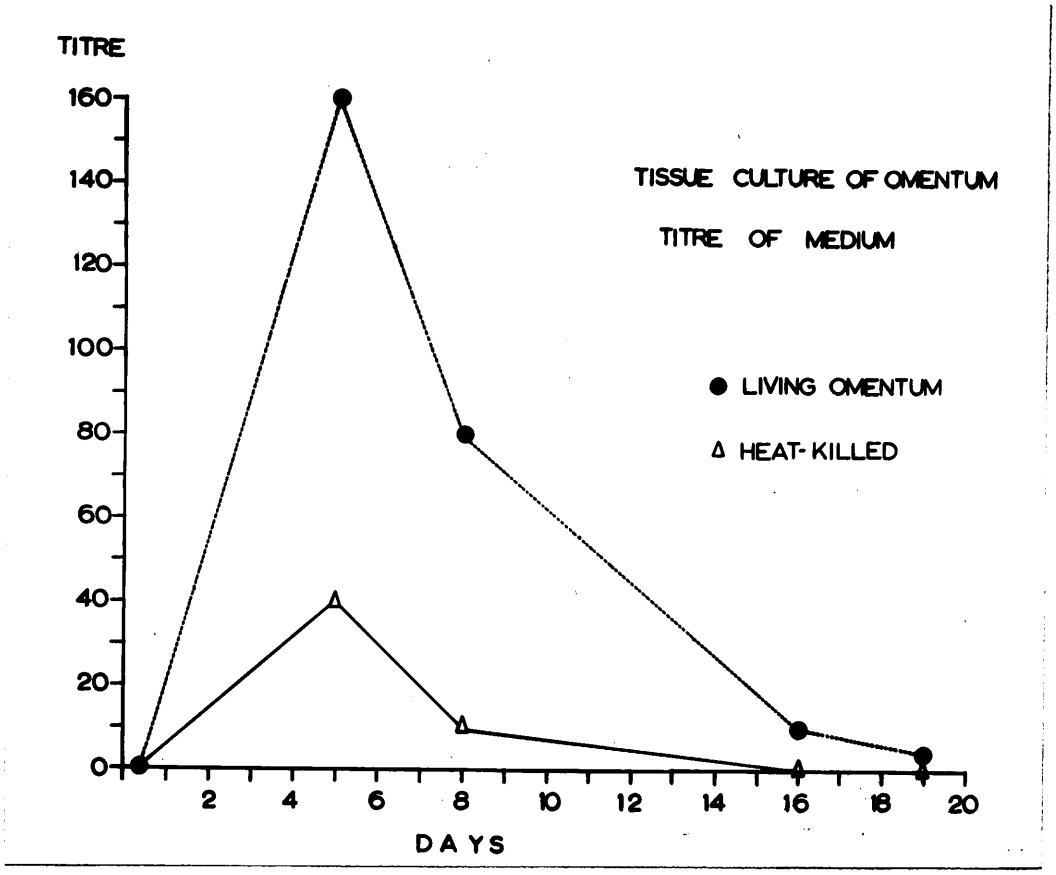

Fig. 33. Showing the titres of the media when a portion of viable omentum and heat-killed omentum are placed in tissue-culture. The omenta were each from animals immunised by a course of intraperitoneal injections. It will be seen that the titre of the viable omentum is higher at all stages.

of omentum by enclosing it in a semi-permeable sac of some material such as cellophane. This should allow the transplant to survive in a medium of the recipient's tissue fluid but, in virtue of the microporous nature of the membrane, the large antibody molecule would be retained. This antibody would be expected to reach a high concentration within the sac.

To test this procedure guinea pigs were used as experimental animals. A group of three donors and six recipients were selected and the donors were immunised with six separate injections, each of 0.5 millilitres of the suspension of Salmonella typhi, in the same manner as the rabbits of previous experiments. The donors were killed by exsanguination on the twentieth day. The omentum of each of the donors was then divided into two equal parts. One part from each donor was immediately placed in the peritoneal cavity of one of the recipients, having been enclosed in a sterile dialysis sac (of cellophane). The remaining portion was then heat-killed in the way previously described and transferred in a dialysis sac into the peritoneal cavity of the other recipient. Thus, there were three recipients of living omentum and three of heat-killed omentum.

The recipient animals were killed on the sixth day after transplantation when it was considered that significant differences of titre of antibody would have occurred. In all instances it was found that the cellophane dialysis sac had excited a most intense

inflammatory reaction by the recipient, and all the transplants were found to be necrotic and to be decomposing. The fluid from within the sacs failed to exhibit any titre against the antigen. This procedure was, therefore, abandoned.

Chapter VII

THE HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION OF THE GREATER OMENTUM IN IMMUNISED RABBITS.

1. Histological Examination.

The microscopical appearances of the normal omentum are familiar to all, and indeed, it is one of the sections frequently demonstrated to medical students as an example of fatty and areolar tissue. In sections stained with haematoxylin and eosin it is seen to consist of many wide, empty spaces which have previously been occupied by fat cells but which have been destroyed by solvent. These spaces are bounded by delicate strands of connective tissue which form a supporting structure in which only occasional cells of the reticular or lymphoid series are seen. A high-power section is illustrated in Figure 34.

It will be recalled that at the post mortem examinations of the group of normal, intact rabbits which were immunised by a course of intra-peritoneal antigen, a visible thickening of the free, peripheral edge of the greater omentum was evident. The structure was always quite freely mobile and presented none of the appearances of being acutely inflamed. This thickening was also present in that group of

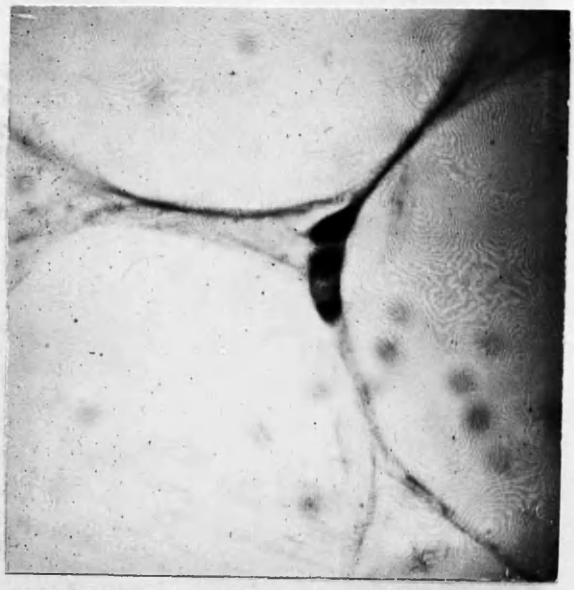

Fig. 34. A high-power photomicrograph of the normal omentum. The large "windows" have recently been occupied with fat. This has been dissolved out. The section has been stained with haematoxylin and eosin. The areolar septa contain few cells.

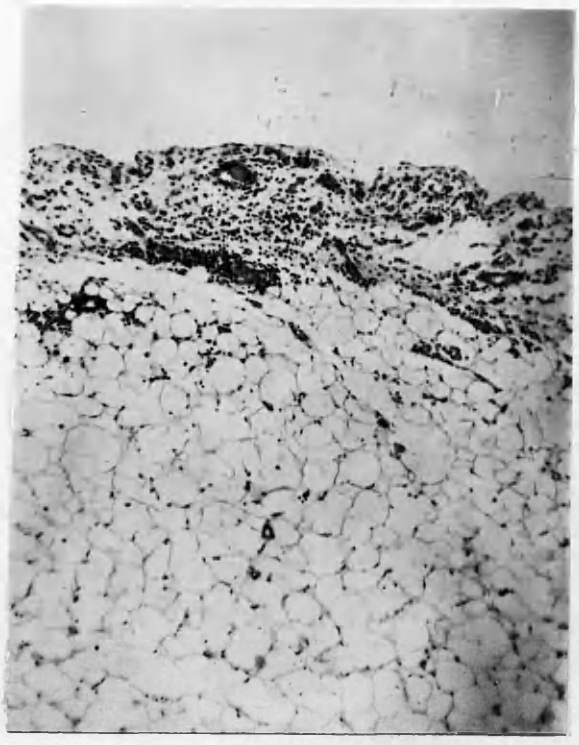

Fig. 35. A low-power photomicrograph of a section stained with H and E. The section shows the peripheral thickened edge of the omentum from a rabbit immunised by intraperitoneal injections. The edge is seen to be thickly populated with cells. There are many perivascular aggregations.

rabbits which had had the greater part of their omenta excised, except for a fringe, which was left by accident or by design. Sections of the thickened portions of these omenta were prepared by the routine histological technique and stained with haematoxylin and eosin. Figure 35 is a low-power photomicrograph of such a section. It will be observed that the periphery of the structure has become thickly populated with cells. The points of greatest density of the cells occur in relation to the minute "potential" vessels which could be described as forming perivascular cuffs to the vessels.

Figure 36 is a high-power photomicrograph of the same field and the features of perivascular aggregation are more clearly seen. The most common type of cell appears to be a small round cell, although closer scrutiny will establish many of these to be plasma cells.

Figure 37 is a section from a different "immune" omentum which is seen, however, with greater magnification. From this photomicrograph it is clear that the predominant type of cell is the plasma cell. The eccentric location of the nucleus is seen and closer scrutiny reveals the "cart-wheel" distribution of the chromatin in the cell nuclei. Associated with these plasma cells are a few small, round cells which may be small lymphocytes or some precursor of either of these types of cell.

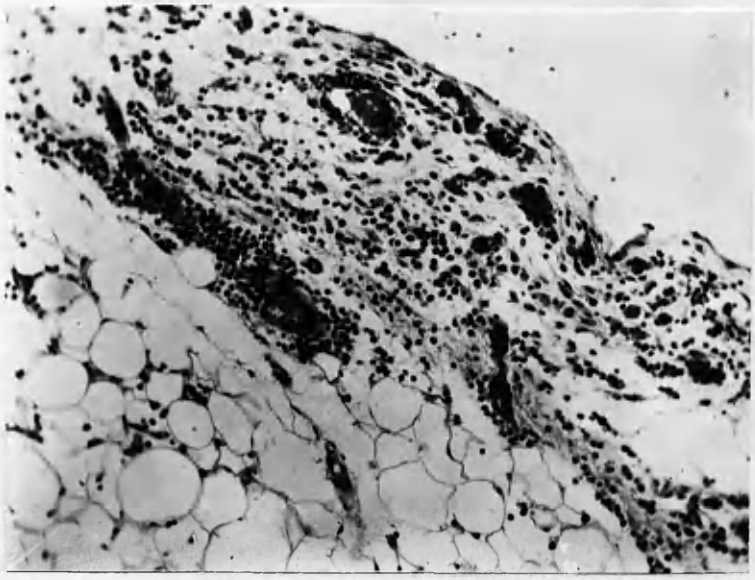

Fig. 36. A high-power photomicrograph of a section stained with haematoxylin and eosin. This is the same field as shown in Fig. 35. The perivascular and peripheral grouping of the cells is evident, Many of the cells are plasma cells.

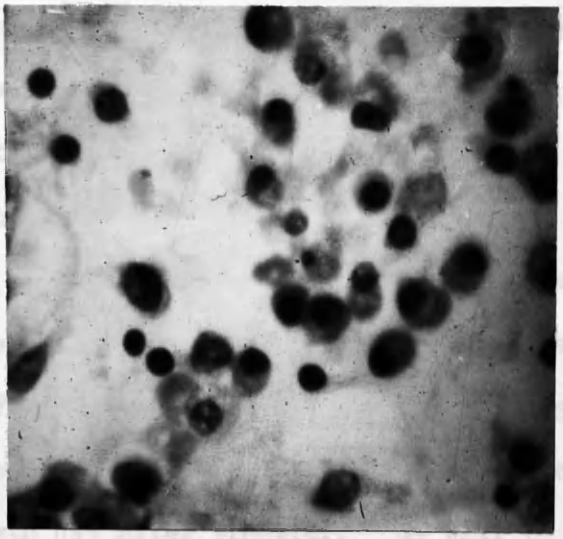

Fig. 37. A high-power photomicrograph of a section of omentum, stained with haematoxylin and eosin, from an animal immunised by a course of intraperitoneal injections of antigen. The field is seen to contain many plasma cells.

It is interesting to recall the various experiments in the immunological field which associate these type of cells with the elaboration of antibody and to appreciate that the situation of these cells would suggest that they were concerned with something occurring within the abdominal cavity and, because of the perivascular collections, the vascular response to it. Sections of the parietal and visceral peritoneum failed to reveal any evidence of these histological features.

Histological sections were also prepared from omental homotransplants removed at various times from the abdominal cavity of the recipient. Figure 38 is a high-power photomicrograph of a section of a transplanted omentum from an immunised donor which was removed on the sixth day after transfer. It will be seen that the omentum contains many plasma cells which give the histological impression of having been viable at the time of removal. It is difficult to establish, beyond dispute, that these cells actually belong to the transplant and have not infiltrated from the recipient because the identification by "sexing", using chromosome patterns, is not practicable in the rabbit. It is most probable, however, that they belong to the transplant because the omentum of the recipient which surrounded it did not show the same cellular picture. Indeed, there was a remarkable paucity of cells.

Figure 39 is a photomicrograph of an omental homotransplant

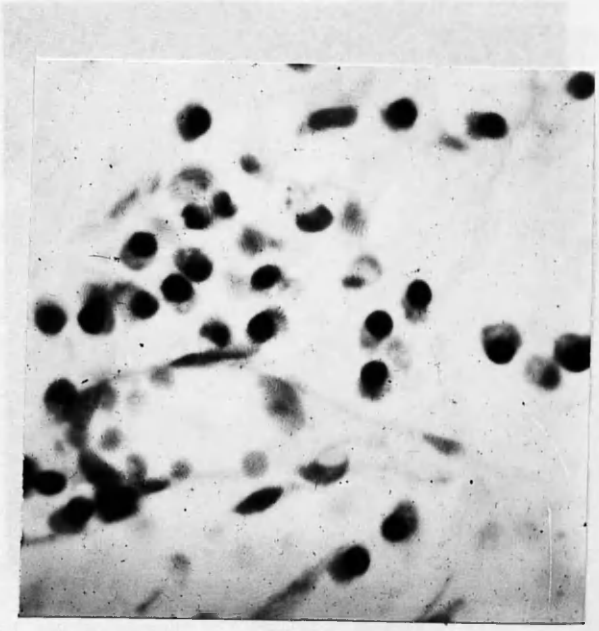

Fig.38. A high-power photomicrograph of a section of transplanted omentum, stained with haematoxylin and eosin. This transplant was recovered from the abdomen of the recipient on the sixth day. Many plasma cells are seen.

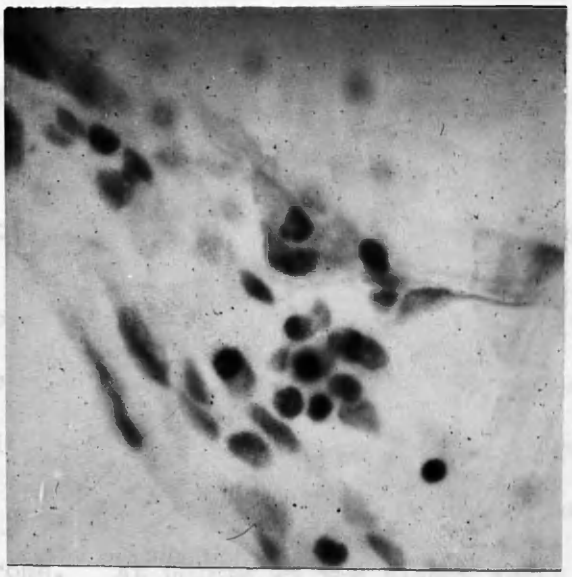

Fig. 39. High-power photomicrograph of a section of transplanted omentum, stained with haematoxylin and eosin. This omentum was from an animal immunised intraperitoneally and transferred into the abdomen of a non-immune recipient. It was recovered on the eleventh day. Plasma cells are still to be seen.

removed from the recipient on the eleventh day after transplantation. Plasma cells are still in evidence but are less numerous than in the previous section.

It is difficult to avoid the association of the plasma cell with the tissue titre of the omentum and with the serum titre of the animal from an examination of these histological sections.

2. Fluorochrome-staining of Antibody-containing Cells of the Greater Omentum.

In 1942, Coons described a method which he had developed for the identification of antigen in tissues. In this method sections of tissues from animals immunised with a specific antigen are treated with a solution of the homologous antibody, which has been conjugated with a fluorochrome. At points in the tissue where the concentration of the antigen is sufficient to cause combination with the coupled antibody, bright fluorescence is visible when the section is examined by ultra-violet microscopy. Subsequently, Coons developed this technique so that it could be employed to detect the situation of antibody in tissues.

For the identification of antibody a modification of the technique is necessary. The section of tissue which is thought to contain antibody is immersed in a solution of the homologous antigen, allowing an antigen-antibody precipitate to be formed at those points where the

antibody is sufficiently concentrated in the tissue. The section is then treated with a solution of fluorochrome-coupled antibody when the antigen, which has been precipitated on the tissue antibody, can combine also with this coupled antibody, thus marking the original site of antibody in the tissue.

The Coons technique, using antibody labelled with the fluorochrome "fluorescein", has been established for about seventeen years. Protein conjugates have also been tried with other fluorochromes from time to time, e.g. anthryl isocyanate, 1-dimethylaminonaphthalene-5sulphonyl chloride and nuclear fast red and rhodamine B isocyanate. None of these fluorochromes has been extensively used because the products compared unfavourably with fluorescein conjugates.

The method of conjugation of fluorescein is a laborious procedure, and also there is a lack of sharp contrast between the apple-green colour of its fluorescence and the blue-green of tissue auto-fluorescence. The recent work of Chadwick, McEntegart and Nairn (1958) has demonstrated the use of a further fluorochrome called Lissamine Rhodamine B 200 (R.B. 200). This dye has a brilliant red fluorescence in aqueous solution; in the form of its sulphonyl chloride it can be easily combined with serum proteins without denaturing the protein to yield stable conjugates which have a brilliant orange fluorescence.

Because of the facility with which Lissamine Rhodamine may be used, it was selected for the investigation of the capacity of the cells of the greater omentum to produce antibody in response to intraperitoneal immunisation.

A high-titre specific antiserum against Salmonella typhi was prepared in a rabbit and this was conjugated with Lissamine Rhodamine (R.B. 200), as recommended by the authors, and described below:-

One gram of R.B. 200 and two grams of phosphorous pentachloride were ground together in a mortar for five minutes in a fume cupboard. Ten millilitres of dry acetone were then added and the mixture was then allowed to stand for a further five minutes with occasional stirring, after which it was filtered. The acetone solution of the sulphonyl chloride of R.B. 200 was then immediately coupled with the serum antibody, since its stability is not certain.

Conjugation with the antibody-containing serum was carried out at 0 - 2 degrees centigrade. Each millilitre of serum was diluted and buffered with one millilitre of physiological saline solution and one millilitre of carbonate-bicarbonate buffer (0.5M pH 9.0). Then 0.1 millilitre of the acetone solution of R.B. 200 was added drop by drop using an automatic stirring device which continued stirring for 12 - 18 hours. The product was dialysed against regular changes of buffered saline until the dialysate appeared clear. The final volume of the conjugate was about three times that of the original volume of the

serum and this was reconcentrated by exposing the dialysis sac to the draught of a fan. Coupled sera were stored at -15 - -20 degrees centigrade. When the sera were required for staining they were absorbed with activated charcoal which reduced the non-specific staining.

Fresh frozen sections of the omenta of rabbits immunised by a course of injections of a solution of Salmonella typhi were cut to four μ in a cryostat. These sections were then treated with an extract of the homologous antigen, which had been prepared in a supersonic bacterial disintegrator. After washing in buffered saline the sections were then immersed in a solution of the Rhodamine-coupled antibody. These sections were examined by fluorescent microscopy using a Zeiss H.B. 200 high-pressure mercury vapour bulb as a light source. The specificity of the results were controlled by ensuring firstly, no staining occurred in normal tissues treated with conjugated immune serum, secondly, no staining occurred in non-immune omenta treated with conjugated normal serum and thirdly, the sections failed to show fluorescent staining when pre-treated with unconjugated immune serum.

Since the sections of tissue which are stained with the fluorochrome cannot be stained with the routine histological stains such as haematoxylin and eosin, contiguous sections must be cut and stained for the identification of the precise cellular source of the fluorescence.

Figure 40 is a high-power photomicrograph of a section of the omentum from an animal which has received a course of immunising injections of antigen intra-peritoneally stained with Rhodamine-coupled antibody. Bright points of fluorescence occur at points corresponding to the situation of cells containing antibody. Figure 41 is the high-power photomicrograph of the immediately contiguous section which is stained with haematoxylin and eosin. The cellular course of the fluorescent points can be related by a comparison of the sections. There are, however, areas in which no cell is seen in one or other of the sections and yet is represented in the other section. This is because the sections are thin and because they are contiguous and not identical. Figures 42 and 43 are the fluorescent and haematoxylin and eosin contiguous sections of another "immune" omentum. Here again the antibody containing cells are easily identified. The nature of these cells has been demonstrated in the preceding section.

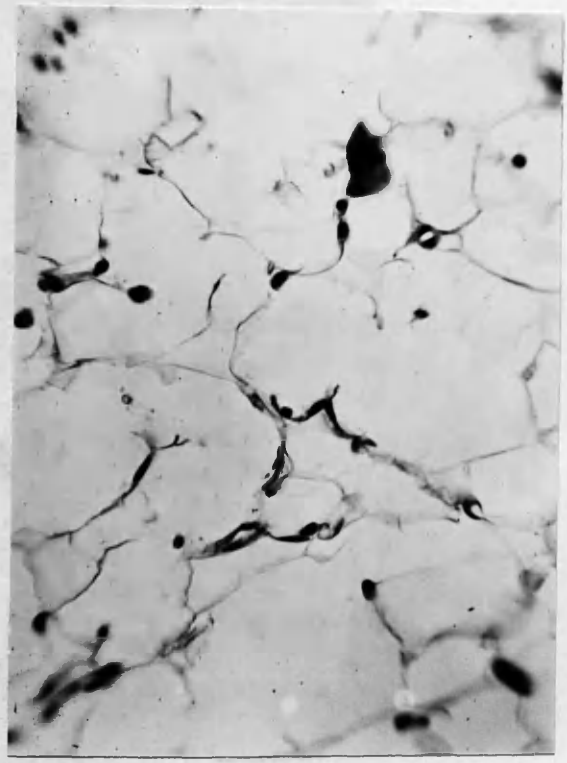

Fig. 40.

Fig. 41.

These figures show, on the left, the high-power photomicrograph of a section of "immune" omentum, stained with Rhodamine-coupled antibody. The section on the right is the immediately contiguous section stained with haematoxylin and eosin. The fluorescent points of light are seen to correspond to the sites of cells which are seen in Fig. 41.

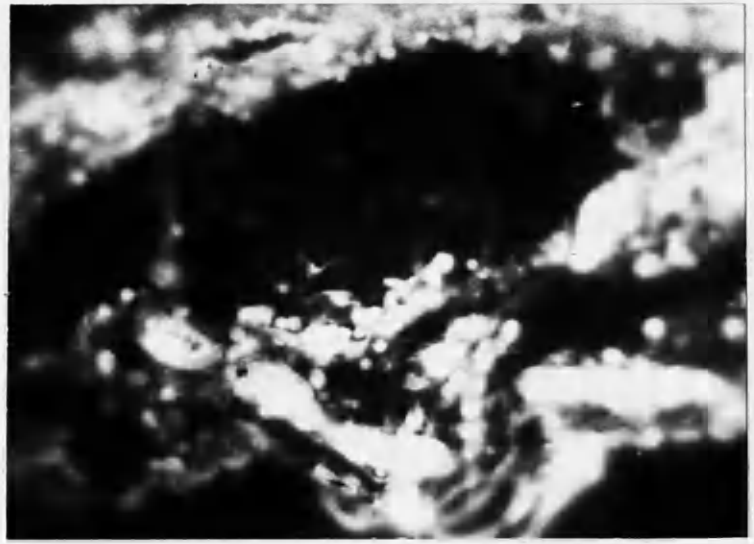

Fig. 42

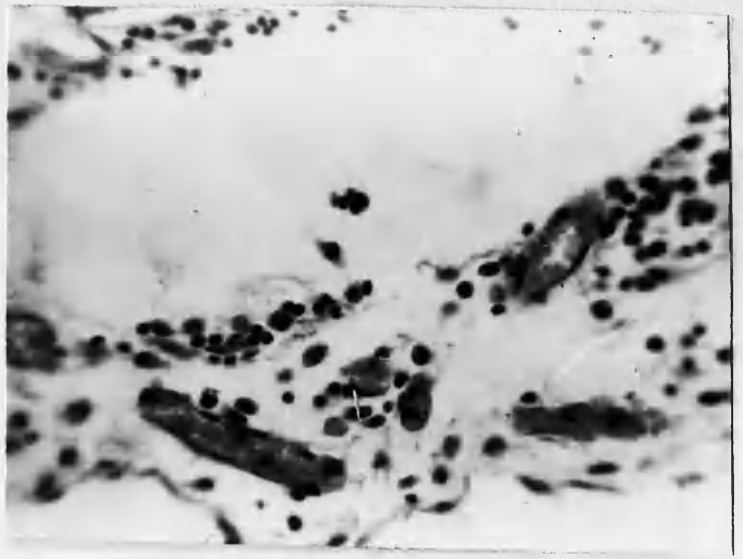

Fig. 43

Fig. 42 is a high-power photomicrograph of a section of omentum from an animal immunised by the intraperitoneal injection of antigen. The section has been stained by Rhodamine-coupled antibody and the bright, fluorescent points of light correspond to the situation of antibody in cells. The location of these cells is seen from the contiguous section (Fig.43) which is stained with haematoxylin and eosin.

Chapter VIII

FURTHER EXPERIMENTAL PROCEDURES.

1. The study of antibody production by cells has been facilitated by the technique of cell transfer. It has been shown that when a guinea pig is immunised against a specific antigen, and the cells of a tissue which produces antibody towards it are removed and injected intra-cutaneously into a non-immune recipient, and this recipient is challenged with an excess of antigen, a phenomenon termed Passive Cutaneous Anaphylaxis occurs where the antigen meets the antibody produced by the transplanted cells. The development of the use of this method is quite recent (Rosenberg, Chandler, Gordon and Fischel, in 1958) and it appeared to offer a means of confirming the capacity of the greater omentum to produce antibody. In the experiments it is necessary to use a marker dye which leaks from the excessively permeable vessels at the site of local anaphylaxis, thus making a visual measurement of the antibody produced possible, since the extravasation has been demonstrated to be directly proportional to the concentration of antibody.

For the experiment guinea pigs weighing about 400 grams were immunised by the intra-peritoneal route with suspensions of Salmonella typhi. Each animal received a course of six injections, each of 0.5 millilitres of a suspension containing 3,250,000 organisms per millilitre. The animals were killed on the twentieth day. Cell suspensions of the spleen, omentum, liver and a specimen of the serum were transferred to the non-immune recipient.

The cell suspensions were prepared by gently homogenising the donor's tissue in a micro-tissue grinder. Chilled Hank's balanced salt solution was used as a suspending medium, both for homogenising and for washing the cells. The cell suspensions were washed three times in chilled Hank's solution (ten volumes) by centrifugation. Counts of the nucleated cells in the final suspensions were performed by diluting the cell suspensions with dilute acetic acid.

Volumes of 0.1 millilitre of the cell suspensions were then injected intra-cutaneously into the shaven flank of the recipient animal. Two days later the animal was intravenously injected with an excess of the antigen mixed with 0.5 millilitres of 0.5 per cent aqueous Evan's Blue dye. The recipient was then killed by a blow on the head and the flank skin was dissected off. The extravasations of dye were visible on the deep surface of the skin. Figure 43 is the photograph of the reaction where A represents the extravasation at the site of 5,000,000 cells of the spleen; B is of 1,500,000 cells of the omentum, C is 3,000,000 cells of the liver, D is a saline control and

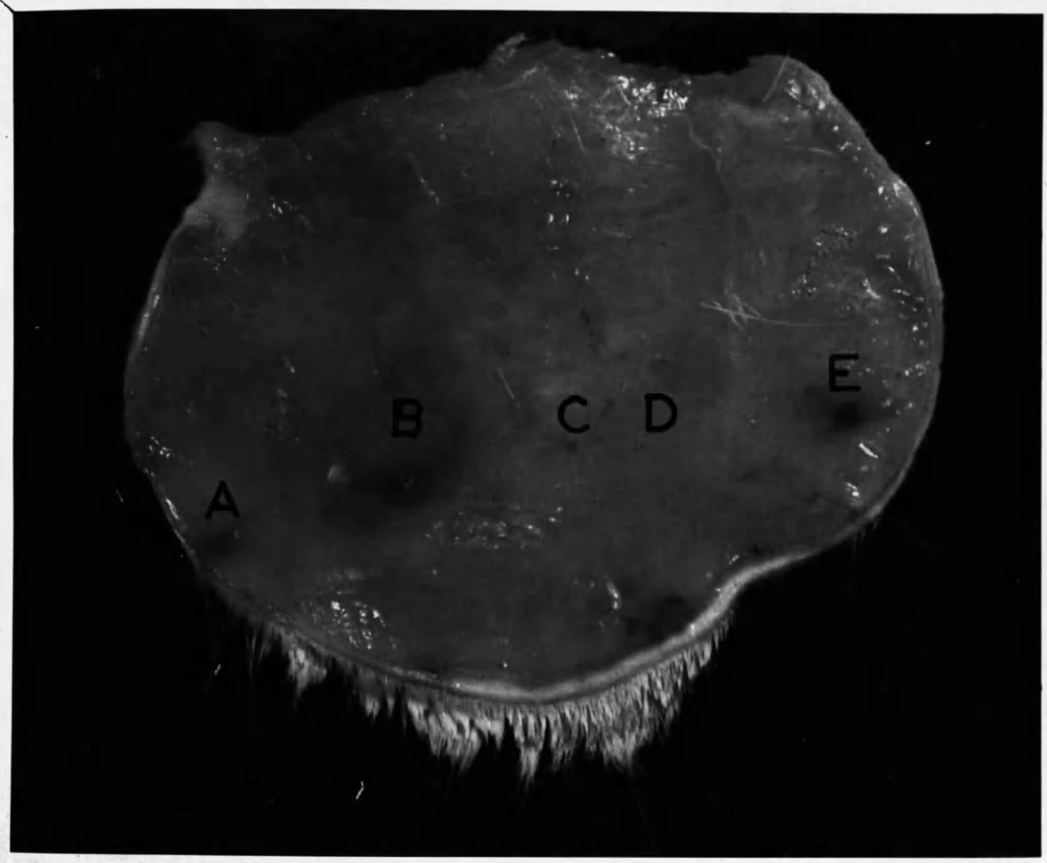

Fig.44. Showing the extravasations of dye occurring at the sites of transfer of cell suspensions into the skin of a non-immune guinea pig, from an animal immunised intraperitoneally.
A = splenic cells. B = omental cells. C = hepatic cells. D = saline control. E = serum from donor. It will be seen that the greater extravasation occurs at the site of transfer of omental cells.

E is 0.1 millilitre of the donor's serum. It will be seen that the omental extravasation is of greater dimension than either the splenic or hepatic and yet the cellular suspension is more dilute. It was considered that this reaction tended to confirm the impression that the greater omentum was capable of producing antibody.

2. A further experiment was carried out using three guinea pigs in an attempt to demonstrate that the greater omentum was capable of protecting the animal from its own bowel flora.

Specimens of blood were obtained from each of the three animals by heart puncture. Thereafter, laparotomy was performed and the large bowel in each was opened. Swabs were then taken from the bowel and its content, and the intact omentum was then sutured to the margins of the wound in such a way that the greater omentum presented into the lumen of the bowel. The swabs were plated out on McConky's medium and incubated overnight. An antigen was prepared by suspending the developing colonies in saline and the pre-operative serum titres of the animals were assayed by tube agglutination. The animals were demonstrated to have pre-operative serum titres of 1 in 40, 1 in 40 and 1 in 20, against their own flora. The reaction to the omental exposure was then followed by heart puncture removal of blood on alternate days, the animals being sacrificed on the sixth day. The results are demonstrated below:-

Animal	First day	Third day	Fifth day	Sixth day
A	1/40	1/80	1/320	1/320
B	1/40	1/40	1/80	1/160
C	1/20	1/80	1/160	1/160

Histological sections were prepared of the site of suture of the greater omentum to the opening made in the bowel wall. Figure 45 is a photomicrograph of one of the sections. It will be seen that there are many plasma cells at this site which, in association with the developing titre of the animals, is suggestive of a capacity of the greater omentum to produce antibody.

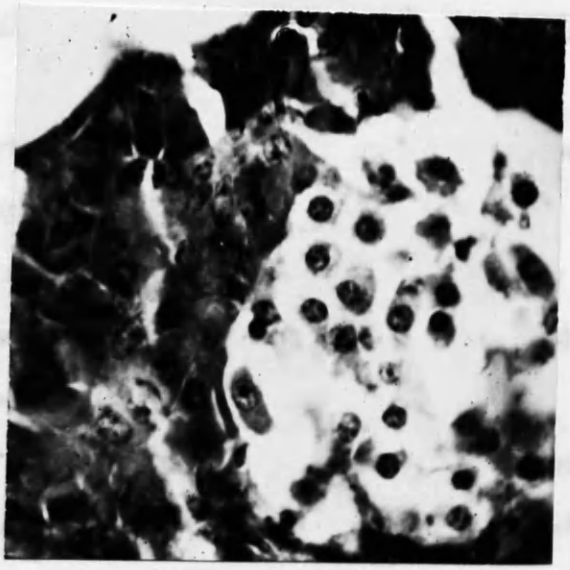

Fig.45. High-power photomicrograph of section of the junction at which the omentum was sutured to the wound in the large bowel of a guinea pig. The section is stained with haematoxylin and eosin. Many plasma cells will be seen.

Chapter IX

DISCUSSION OF THE RESULTS OF THE EXPERIMENTS

The concept of the greater omentum as a structure capable of active physiological response is not a recent one. As has been related in the review of the literature controversy on its function has raged for many centuries and several roles, both active and passive, have been ascribed to it.

It is difficult to understand why, if the structure was truly inert, it should have been retained through the continuous adaptation of evolution. However, the functions which through the ages have been related to the omentum were, until the histological examination of the structure, based only upon conjecture, some of it fanciful, and observations of the position of the structure in the cadavers of patients dying of many different conditions.

The experimental findings are of interest, but in many instances they did not appear to properly relate to activities which from clinical impression seemed more likely. For example, the absorption of free fluid by the structure did not appear to be its principal function since excision of the organ was not followed by ascites.

It is apparently to Ross (1893) that we owe the first reference to the protective function of the omentum. That there was indeed some protective function was confirmed by the first controlled experiments which were carried out to clarify this problem. The early experiments of this era were carried out by Durham, Roger, Wilkie and Rubin, as has already been related.

The mode of protection which received most attention at this time was the capacity of the omentum to find its way to an inflammatory site and circumscribe the lesion. It will be recalled that many investigations were carried out to determine the influences which attracted or drew the omentum to the pathological focus. The structure was endowed with the most fanciful of attributes and was even considered to have an "inner intelligence". Milian at about this time introduced the hypothesis that there was some "chemotactic force" which attracted the structure to the point of danger. This attractive force did not appear to function consistently and many reports were made of instances in which the omentum was found some considerable distance away from the inflammatory focus. This necessitated the postulation of an influence which could repel the structure and was referred to as "negative chemotaxis."

Over the course of the years, this aspect of the protective function of the organ has been less emphasised and the "migrations" of the omentum have come to be considered as the resultant of a

combination of factors which include intestinal peristalsis, intra-abdominal tension and respiration and, not least, the fortuitous presence of the greater omentum in the vicinity of the pathological process.

In spite of the divergent views and arguments which were expressed, it remained a clinical impression that the organ was in some way protective. It was noticed that patients requiring the opening of their gastro-intestinal tracts at operation, and thus exposing them to contamination by their own intestinal flora, seemed in some way to acquire some degree of tolerance or immunity towards these organisms. If a subsequent operative procedure was required, it was unusual to see the development of the dangerous septic sequelae that were liable to attend the initial intervention. That this was the impression is confirmed by the experiments of Bargen and Rankin of the Mayo Clinic who, in 1928, deliberately attempted the pre-operative immunisation of their patients who were later to undergo intestinal resection for carcinoma. The results of their work tended to suggest that some degree of immunity could be acquired and that this was followed by a smoother convalescence than was experienced by the untreated patients at that time.

It is still not rare to witness the apparently uneventful and smooth recovery of a patient convalescing from a second intestinal operative procedure interrupted by the discharge of a considerable quantity of pus from the wound. The collection of pus is often not

suspected because the temperature and the pulse charts have given no indication of systemic reaction. Indeed, an examination of the abdomen of such patients can be misleading. It was the witnessing of this phenomenon that was initially responsible for the author embarking upon a search for a tissue which could be capable of conferring this apparent immunity.

It was considered that the observations of many of the writers of the early part of the century could be reconciled by the conception of the greater omentum as part of the reticulo-endothelial system and able to produce antibody towards those noxious materials which its constituent cells were able to ingest. In this way its function would be more subtle than its capacity to mechanically localise an inflammatory focus. It was considered that if this function was shown to exist, the metaphorical description of the organ as "the policeman of the abdomen", by Sir Rutherford Morrison, would be more accurately expressed as "the SECRET policeman of the abdomen". This view seems not unreasonable when the embryological derivation of the spleen is considered. This organ is derived from the left leaf of the dorsal mesogastrium which also gives rise to the greater omentum. The spleen is an established member of the reticulo-endothelial system and it seems reasonable to suppose that the tissues in its immediate vicinity could become endowed with at least some of its properties in this respect. This seems even more likely when cognisance is taken of the abundance of macrophage cells which occur in the normal and reactive omentum. It will be recalled that the

progress of the identification of the cellular source of antibody by the immunologists progressed in a similar way. This search narrowed the field to two perhaps related systems of cells - the plasma cell and the lymphocyte, and these cells are common in the cellular populace of the greater omentum. It seemed of fundamental interest that this capacity, if any, should be elucidated in view of the many surgical uses to which the omentum is put. In many instances the capacity to produce antibodies would provide a rational basis for such uses. For many years this structure has been employed to reinforce suture lines and it may be that this immune capacity plays a part, since it is known that the pedicled omentum is more effective than the free graft.

The first group of experiments, in which comparison is made of the tissue titre of extracts of the omentum with extracts of organs which are well documented in respect of antibody production, suggests that the greater omentum is able to elaborate specific antibody. This structure consistently presented a tissue titre which greatly exceeded that of the organs against which it was compared. The tissue titres which are shown to have been obtained for the structure are, however, very much higher because the greater part of the specimen from which the extract was prepared consisted of fat. Adipose tissue per se is presumably inert. There is no experimental evidence to show that a fat cell is capable in any site of elaborating antibody. It seemed of interest to ascertain the proportion of the specimen which was being employed, which was composed of this inert fat. As will be

recalled, this was ascertained by the extraction of the tissue with fat solvents. The organic residue and the fat were thus separated and were measured. These omenta were found to be composed of ninety three per cent of fat and the, presumably, active tissue formed only seven per cent of the total weight of the tissue. This means that the measurements which were made record values for the omentum when this specimen is in greater dilution (with fat) than are the organs against which a comparison is made. Even ignoring this dilution it is evident that the omentum is more active than the spleen or the liver when an animal is immunised by the peritoneal route. Of greater significance is that if the appropriate correction is made the titre of the omental extract exceeds that of the blood serum. This fulfills the earliest criterion, which was used to establish the capacity of an organ to synthesis antibody (Pfeiffer and Marx, 1898).

That there is a diminution in the magnitude of the immune response in animals which lack an omentum is suggested from the second group of experiments which were performed. The serum titre of animals deprived of their omenta is considerably lower than that which is attained in the group of intact animals. This observation would seem to confirm the earlier observations of Sir David Wilkie and other workers who injected doses of staphylococcus in doses which were sublethal to normal animals, but which were lethal when injected into omentectomised animals. The demonstration that the animals immune response was diminished after the excision of the greater omentum suggested several possibilities:-

(a) The animal had had a recent operation and therefore had been "stressed".

(b) Antibody which could have been secreted into the peritoneal cavity from some remote tissue was not now being absorbed by the omentum.

(c) The greater omentum actively elaborated specific antibody.

The first of these possibilities was tested by immunising animals fourteen days after splenectomy. The serum titre of the animals conformed to the pattern of the intact animals similarly immunised. If the operative procedure, as such, was responsible for the diminution of serum titre this would not have occurred. Incidentally, this experiment also functioned as a control for the splenic contribution towards the immune response.

The second possibility was examined by exteriorising the greater omentum in a subcutaneous "pouch". When the animal was immunised by injecting the antigen into this pouch it presented the immune response which was typical of the intact, normal animal.

The most likely explanation was, therefore, that the greater omentum was capable of elaborating antibody.

It will have been observed that the omentum only presents a high titre suggestive of antibody synthesis when the animal is immunised intra-peritoneally. The contribution of the structure is insignificant when the animal is immunised by intravenous injections.

This finding is in keeping with the work of others in this field. Under the last condition, the spleen appears to be the principal contributor of antibody.

The homotransplantation experiments have demonstrated that free grafts of omentum confer some degree of immunity upon the recipient animal. When the omentum of an immunised animal is homotransplanted into the peritoneal cavity of another animal, it is clear that some pre-formed antibody will be carried over with it. This antibody presumably lies within the interstices of the transplant and will gradually be "leeched" out and become absorbed by the recipient. It is, therefore, necessary to take this "passive" donation of antibody into account in the interpretation of the results. It will be seen that in these homotransplantation experiments this passive transfer of antibody was measured by the transplantation of a heat-killed portion of the omentum of an animal which had been immunised in the same way as those donating the viable graft. The titre conferred by such a control is of a low and transient nature and is quite different from the titre which is seen to develop in the recipient in the transfer of the viable graft. The fact that the titre steadily rises in the recipient of a viable graft can only be explained by assuming that the cells of the graft are continuing to form specific antibody. This is supported by the fall, which occurs in the titre at about the same period when the rejection of the homotransplant would be anticipated.

The rise in the recipient's serum titre occurs so early that it cannot be a primary or secondary immunity against any "carried over" antigen.

The experiments with the transfer of omenta from immunised animals into tissue-culture media confirm the likelihood of the explant continuing to produce antibody.

The histological examination of the omenta of animals immunised by intra-peritoneal injection were of particular interest because they confirmed that the thickening which was visible in the free edge of the structure was due to the dense population of this area of the omentum with cells which are associated with the elaboration of antibody. It will be remembered that reference was made in an earlier chapter to the substantial body of evidence which has identified the plasma cell and the lymphocyte with the immune response of an animal. That these cells were, in fact, associated with the production of antibody was then demonstrated by a modification of the technique developed by Coons. This actually indicated the antibody within the greater omentum and also identified the cells, in the cytoplasm of which the antibody occurred. It has, therefore, been possible to correlate the immune response of the animals with the histological and histochemical appearances of the tissue which was suspected of being able to actively produce specific antibody.

By the use of the recently developed phenomenon of Passive Cutaneous Anaphylaxis, it has been possible to demonstrate the immune

function of the greater omentum and to establish that this function occurs in another species of animals.

That this immune function of the structure can occur without the use of an artificial antigen is confirmed by the correlation of a rise in serum titre and the local aggregation of plasma cells, which occurs when the intact omentum is sutured over a defect in the large bowel of guinea pigs. The plasma cells are seen at the junctional zone between omentum and bowel.

In conclusion, it appears that the greater omentum is capable of exercising its protective function in two ways. The first is the, sometimes fortuitous, capacity of mechanically localising an intra-peritoneal inflammatory focus by walling it off, or by envelopment, while the second is the more complex attribute of being capable of mobilising aggregates of cells which actively elaborate specific antibody against foreign invaders of the peritoneal cavity. It thus places a humeral barrier about the invader and diminishes the likelihood of a successful invasion.

Because the greater omentum is endowed with the beneficial functions which have been described, it would seem to justify its preservation when possible; as much of the organ as is expedient should be allowed to remain in situ during the various operative procedures.

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