CELLULAR ACTIVITY IN HEALTH AND DISEASE:

BIOCHEMICAL STUDIES BASED UPON NEW METHODS OF INTRA-VITAM STAINING.

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THE new histological methods introduced by Altmann, Ehrlich, Arnold, and others, have enabled us to establish closer relations between the morphological appearances of the cell and its specific physiological activity. It is now undisputed that where a granular condition exists in the protoplasm of a cell we may assume that the granules are associated with some form of secretion, either internal or external, according to the anatomical relations of the cell to its surroundings. No wonder, therefore, that histologists should endeavour to search for new methods to enable us to demonstrate internal secretion, under conditions where the ordinary methods of physiological research fail.

What modern histology owes to Ehrlich, who first demonstrated the neuro-, lipo-, and polytropic properties of various aniline stains, needs no comment, since these investigations on the chemical affinity of certain aniline colours to animal tissues have resulted in chemotherapy and the discovery of salvarsan. The great gulf which separates anatomy, the study of the dead, and physiology, the study of the living cell, has now been bridged by the introduction of intra-vitam staining, which though in its infancy has already yielded results hitherto unapproached by other means. Through the liberality of Professor Ehrlich I became acquainted with various aniline colours which after injection produce a more or less rapid and universal colouring of the skin and various internal organs in mice, rats, guinea-pigs, and rabbits.

The best results in intra-vitam staining of rats are achieved by subcutaneous injections of a 1 per cent. solution of isamine blue, 1 grm. per 20 grm. of the animal's weight. On the beginning of the third day a faint blue appears on the animal's ears, and then the blue colour gradually spreads over the whole surface of its body. Repeated injections at intervals of 8 to 10 days increase the intensity of the colouring without impairing the animal's health. But the stain is not restricted to the surface; we find it microscopically throughout the whole of the body, always embodied in granules of specific cells. Thus in the skin the stain is found in the granules of the fixed connective tissue cells of the cutis, but chiefly in free round cells belonging to the lower layer of the subcutis. Here the cells aggregate in great numbers, and especially in spots where an irritation or lesion of the skin is produced by artificial means or by pathological processes. These cells, which belong to the type of the "histogenic migratory cell," are by no means confined to the skin; they appear in every internal organ (with the one exception of the nervous system) and always in connexion with interstitial fibrous tissue. We find them in muscles and tendons, in glands, but especially in serous membranes—e.g., in the peritoneum—whose *taches laiteuses* seem to form a matrix for their production.

By means of the intra-vitam isamine blue stain we can differentiate the "Kupffer" star cell in the liver, the reticulum cell of the lymph glands, the spleen and bone marrow, the interstitial cell of the testicle, the follicle cell in the maturing Graafian follicle, cortex cells of the suprarenal, the epithelial cells of the choroid plexus, and the epithelial lining of the convoluted tubes of the kidney.

Most striking is the appearance of the placenta and its behaviour in relation to the rest of body. When pregnancy occurs in the vitally stained animal the blue colour disappears from its skin and is concentrated in the uterus, the latter forming a centre of attraction for the vital stain, and actually dispossessing the remaining tissues of their blue. In the uterus we find the blue chiefly in the free cells of the decidua serotina, but also in the cells of the reflexa during the period of its existence. In an early stage of pregnancy (sixth to eighth day) these granular cells in the decidua of peritoneal descent cast off vitally stained granules which are eagerly snatched up by the fœtal components of the developing placenta. Once the placenta has attained its maturity, we discover the vital stain in the "giant cells" forming the boundary line between the maternal and fœtal part of the placenta. We also find it in those fœtal cells which constitute the only barrier between the maternal blood spaces and the endothelial-lined capillaries of the fœtus. Finally, the vital stain effects a most striking specific differentiation in the granular cells of the vitelline membrane. Notwithstanding the fact that the yolk membrane is deeply stained throughout its whole extent and that the amniotic fluid shows a faint bluish colour, the embryo remains perfectly colourless, the placenta and its appendages thus forming a protective barrier against the passage of the stain from the maternal into the fœtal organs. Although the stain circulates in the blood no blood cell accepts it, nor has it any effect on the cells of the vascular coats.

After many futile experiments I finally found ways and means of fixing the vital stains, so that we can now prepare permanent histological specimens which recall the conditions of the living cells in all detail and accuracy. As a rule, we inject the anæsthetised and deeply blue-stained animal from the beating heart with formalin solution, open the body cavities by a median incision, and immerse the mouse or rat in formalin. In 48 hours the vital stain has become sufficiently fixed in the cell to allow of the preparation of sections. I have specimens which have not faded in the least within the last three years. As the vital strain is strictly confined to the protoplasm counter stains for the nucleus, &c., are necessary.

Ovum and Placenta.

My studies have been confined so far to the rat and mouse, since these animals still form unrivalled material for cancer research. I will first take up the remarkable conditions revealed in the ovary and placenta. As the primordial egg cell grows into the Graafian follicle the inner layer of follicle cells display granules in their protoplasm which show a marked affinity for the blue of the vital stain, whereas the ovum itself shows no trace of blue. The question arose as to whether these follicular cells are the bearers of any special nutritive material which the ovum receives before it bursts the follicular membrane and starts on its downward journey.

I first applied the ordinary fat stains, such as osmic acid, Sudan and scarlet red, only to corroborate a fact frequently mentioned by various authors, that the inner follicular layer of cells gradually succumbs to "fatty degeneration." Here, again, we discovered that although the cells immediately contiguous to the ovum were highly charged with fat no fat or lipoid substance could be found in the ovum itself. It seemed, therefore, the more remarkable that on application of glycogen tests the result in the maturing ovum was positive, whereas in all the remaining cells of the follicle and the ovary the test failed. On full development the protoplasm of the ovum is rich in glycogen. In sections stained by Best's carmine method the ova show a brilliant red in their protoplasm and a rich red in their nucleolus. In order to avoid decomposition of the relatively small amount of glycogen found in the minute ova of mouse and rat, it is essential to precipitate the glycogen rapidly by means of alcohol injection from the beating heart of the anæsthetised animal according to the method mentioned before. Without these precautions glycogen in the ova becomes invisible. Hence the fact that so many have failed to trace it in the ova by the ordinary methods.

Oddly enough, I have found in the ovaries of rats and mice, and especially in such as had been tumour bearers, ova in various stages of division. In one specimen within the zona pellucida of the ovum no less than five cells with well defined nucleus and nucleolus were discovered. The ovum lay either in the centre of the follicle or in an eccentric position apparently dislocated thither by the abnormally widened follicular cavity. The membrana granulosa had disappeared almost completely. Such ova had all the appearances of a morula. The cells constituting the morula were richly filled with glycogen granules.

I am well aware that after what Bonnet has contributed to our knowledge on the division of ova within the ovaries of mammals, it would be a mistake to talk of parthenogenesis in our cases, since such ova after division differentiate no further, but rapidly degenerate. Yet the fact remains that division occurred and that the segments of the ovum contained glycogen. Was it possible that such abnormal

division of ova, within the ovary, and without the impetus of a male cell, could be induced by the malignant growth or its biochemical action? Such an inducement does not seem at all improbable, since Loeb has shown that under normal conditions the egg-cell may be caused to divide by agents which precipitate lecithin in its protoplasm. Similar observations have been described by Wassermann and his pupils, who showed that by adding serum of syphilitic patients to the eggs of cœlenterates these begin to divide, the cause of the division being the power of the syphilitic serum to precipitate lecithin.

I hope on a future occasion to be able to throw more light on the question of disturbance in the normal development of ova by the influence of bodies derived from malignant growths. In any case we have proved that both in the ripe egg and in its first segmentations glycogen is a stable ingredient of its protoplasm. The importance of this fact will appear from what I now have to report on glycogen in the placenta and embryo. As in the case of the ovum, my studies on the placenta have been much influenced by what the vital stain has revealed in the placenta—namely, that the placenta forms a powerful centre of attraction for the blue colour, extracting it from the circulating blood as well as from stained tissues in order to deposit it finally in specific cell elements of fœtal origin. Can similar facts be established for the passage of glycogen, fat, iron, and hæmoglobin from the maternal system into the embryo, through the medium of the placenta?

Embryonic development produces in rodents (as several authors have demonstrated in the human being) a functional hyperactivity in the production of glycogen, chiefly in the maternal liver. In the pregnant animal a high percentage of glycogen is found in the blood. On submitting the uterine vessels to the Best or iodine test it can easily be shown that glycogen passes in great quantities into the uterus and placenta through the uterine arteries. When, as in early stages of embryonic development, hæmorrhages form an important element of nutrition for the embryo, glycogen appears in the extravasated blood patches which surround the growing embryo, and form a line of cleavage between maternal and fœtal placenta. In mice and rats Sobotta, Duval, and others have shown that the parietal layer of the vitelline membrane very soon disappears, whereupon the visceral layer is brought into immediate contact with the extravasated blood that originally surrounded the yolk sac. The granular cells of this yolk membrane are a strong absorbent of the vital stain. Glycogen is likewise hoarded up in their granules, and especially in the cells lining the villi which form such a characteristic feature in the yolk-sac membrane near its connexion with the placenta. The vitelline cells receive their glycogen from the blood pools in which the villi are steeped, and then pass it into the fœtal vessels underlying the villi. But a more important absorption of glycogen takes place in the placenta itself, when the allantois has brought the fœtal blood-vessels into the growing placenta.

It is well to classify the full-grown placenta into three separate zones. The outer zone bordering on the uterine wall is chiefly composed of decidual structures and therefore deserves the name of decidual layer. In the central zone maternal and fœtal elements coalesce, but in varying abundance, according to the stage of embryonic development. Towards the final stage of the embryo the maternal cells are almost wholly substituted by fœtal tissues. The name (which Grober suggested) "Umlagerungs-Zone" best expresses the constant morphological changes of this central layer. Finally, the innermost layer of the placenta is almost completely occupied by fœtal capillaries with their endothelial lining and by the irregular maternal blood spaces, whose walls are solely constituted by big fœtal cells, probably of the same origin as the "giant cells" that separate maternal and fœtal placenta in the central zone.

As soon as the placenta begins to absorb glycogen a peculiar structural change occurs in the coats of the uterine vessels. In the place of flattened endothelial cells we find swollen polygonal cells, which bulge into the vessel's cavity, multiply and segregate themselves from the wall of the vessel. They start on a pilgrimage into the decidual layer of the placenta, where they form whole bands of cells, following the branching of the maternal vessels. These cells are filled with glycogen, for which reason I have named them "glycogen carrier cells." As they pass into the central layer of the placenta they excrete their glycogenic material. This is eagerly taken up by the fœtal cells, which constitute the only barrier between the fœtal capillaries and the maternal blood spaces. Specimens stained by Best's method show such glycogen in the shape of a fine red dust, lining the fœtal capillaries and stretching through the whole length of the placenta's labyrinth right up to its hilus. Here it disappears, and on its transition into the fœtal blood-vessels it becomes invisible.

We can therefore distinguish two different types of glycogenic absorption in the placenta. We have a "direct" type characterised by the absorption of glycogen from the extravasated blood. Here the absorption is carried out by the granular cells of the vitelline membrane, the villi of which float in the uncoagulated blood. The "indirect" type of glycogenic absorption is marked by the formation of special glycogen carrier cells, which originate from elements of the uterine blood-vessels, wander into the substance of the placenta, and give off their glycogen to fœtal cells, that form, as it were, an inner coat to the maternal blood spaces and an outer one to the fœtal capillaries. In both cases maternal glycogen undergoes some modification in the fœtal elements of the placenta before it enters the fœtal circulation.

As to the absorption of fat in the placenta, I may mention at the very outset that it is not produced by the placenta but borne thither by the uterine vessels. Sudan stains of frozen sections reveal fat in these uterine channels as an orange-coloured homogeneous mass, which occupies a lateral position in the vessel cavity, whilst the blood corpuscles remain in the central stream. On entering the middle layer of the placenta the uterine vessels undergo a complete disintegration of their walls. Fat diffuses from its cavity into the fœtal cells, which take the place of the atrophied vascular walls. Now we observe a most characteristic metamorphosis of fat, found throughout the whole of the placenta and fœtus. As soon as fat is absorbed by fœtal cells its intracellular condition is marked by the appearance of fat droplets which take up both the Sudan and osmic acid stain. Whereas Sudan will stain fat both in solution and in emulsion, osmic acid only differentiates the latter. Hence, in the case of fat we can follow by means of the Sudan stain its migration into the fœtal capillaries. Especially in the inner layer of the placenta we find fat droplets like strings of beads, lining the fœtal capillaries and encased in the same fœtal cells, which are all-important in the absorption of the vital stain as well as of glycogen. We can determine the passage of fat from these cells into the fœtal capillaries by the fact that, as in the uterine vessels, the intravascular dissolved fat stains with Sudan, and a homogeneous substance shows in the lateral stream of the vessel. As the fœtal capillaries collect to form the branches of the umbilical vein a change in the condition of the vascular fat becomes apparent. It is no longer homogeneous, but is broken up into minute droplets (emulsion).

Another proof that fat is not produced in the placenta, but carried thither by the blood stream, is the fact that wherever hæmorrhages occur fat is a component of the extravasated blood. It is easily demonstrated by Sudan, osmic acid, and other fat stains. Now these hæmorrhages in the placenta are "physiological" and not "pathological." They always occur in the same regions of the placenta, either on the boundary line between maternal and fœtal placenta or in the vitelline cavity. In the yolk sac fat is absorbed from the extravasated blood by the same granular cells of the yolk membrane which take such an active part in the absorption of glycogen and the vital stain. The hæmorrhages which occur on the border between maternal and fœtal placenta serve as a fat reservoir for the "giant cells." These "giant cells" are undoubtedly also of fœtal origin, although Sobotta and Burkhard have recently tried to prove that they are descendants of decidual cells. The "giant cells" likewise eagerly retain the vital stain in their granular protoplasm. Hence, we have shown that, exactly as with glycogen, maternal fat, before being accepted by the fœtal capillaries, is temporarily retained in fœtal cells of the placenta, in whose protoplasm it certainly suffers some modification, which deprives it of properties alien to the fœtus and facilitates its assimilation by the fœtus.

As regards iron, my investigations have brought to light only one important fact. I have been able to prove that minute iron granules appear in nucleated red blood corpuscles wherever hæmoglobin begins to form. In the

placenta this condition is only found in fœtal capillaries of the inner and central layer adjacent to extravasated blood or maternal blood spaces. My attention was thus directed to the important question: Whence does the embryo draw its supply of hæmoglobin?

Fœtal hæmoglobin is a derivative of the maternal, and is manufactured in the placenta. I base my statement on most interesting observations, gained by a special method—viz., an intra-vitam injection of 1 per cent. osmic acid solution through the beating heart of the anæsthetised and pregnant animal. After injection the placenta remains in osmic acid or Flemming's solution for a short time, and is then treated in the ordinary manner for microscopical purposes. The intra-vitam osmic acid fixation differentiates hæmoglobin more effectively than any other method known to me. On examining specimens of the placenta and embryo subjected to this method I noticed the remarkable fact that whereas the red blood corpuscles in the embryo and the umbilical artery were poor in hæmoglobin, those in the venous capillaries and smaller veins of the placenta show a deep hæmoglobin stain. The concourse of hæmoglobin-bearing erythrocytes from the placenta is best demonstrated at the entrance of fœtal venous capillaries into the umbilical veins. The hæmoglobin cell is most distinct from its pale relation. Whilst the hæmoglobin-bearing cell circulates in the embryo, it loses its hæmoglobin and returns to the placenta, through the umbilical artery, in a pale condition, in order to receive a fresh supply of hæmoglobin. As yet I have not been able to ascertain when the nucleated red blood corpuscle loses its nucleus. It does not seem improbable that this loss happens as soon as the embryo is able to produce hæmoglobin in its own organs and needs the assistance of the placenta no longer.

The foregoing facts prove conclusively that the fertilised egg exercises a formative, nutritive, and functional effect on the whole maternal organism, consisting not only in the new formation of cells, such as the glycogen-carrier cell, the decidual cell, and others, but also in the hyperactivity of organs (liver, hypophysis, and others) producing nutrient material such as glycogen, fat, &c., for the growing embryo. Here already we detect an important resemblance between the influence of the fertilised egg and the malignant growth cell.

These facts show that the placenta is not, as Claude Bernard maintained, an organ which produces the material needed for the nutrition of the embryo. The nutrient material proceeds from the maternal organism, and is attracted by the placenta in great quantities. Here it is temporarily incorporated in fœtal cells before it enters the fœtal circulation.

I next proceeded to determine the order and sequence in which the substances are distributed in the various organs of the growing animal. No better example than the pregnant uterus of mouse and rat could be selected for this purpose, since, through its smallness, we can control in one single microscopical section the conditions obtaining in both placenta and embryo. Thus I have been able to determine that, according to the stage of their development, the various organs of the embryo evince varied and distinct powers of selection for specific nutrients. No sooner do we see the first traces of the fœtal heart and respiratory system than glycogen is largely attracted by, and deposited in, the muscular cell of the heart and in the epithelial cells of primitive respiratory alveoli. Almost the whole system of striped muscle is provided with glycogen before the liver begins to take it, although the fœtal vessels and vena cava are charged with glycogen. When we bear in mind that the primitive function of the liver is a hæmopoietic one, and, further, that all hæmopoietic tissue, such as spleen, bone, marrow, and lymphatic glands, never include glycogen in their cell contents, we can well understand that glycogen makes its appearance in the liver only after the specific liver cell is differentiated in a later period of embryonic growth.

In comparing the successive stages of embryonic growth we become familiar with the fact that glycogen remains a staple ingredient in the protoplasm of certain tissues, such as ossifying cartilage, whereas it has only a transitory existence in others, such as the lung and spinal cord. It is solely on the strength of studies on embryonic tissue that we understand why under pathological conditions—tuberculosis, for instance—the epithelial cell of the bronchus re-assumes its fœtal power of forming glycogen. It is of extraordinary interest to compare when and whither fat and glycogen migrate respectively into the various units of the fœtal system. With but few exceptions their distribution is a similar one.

If I have succeeded in convincing my reader to what an extent biochemical study on the ovum and placenta has been stimulated by the new method of intra-vitam staining, I feel confident of retaining his attention during my description of diseased conditions viewed from a similar standpoint. Among the great number of pathological conditions which have engaged my attention since I have learnt to use the vital stain I intentionally select those affecting the abdominal organs, as they in particular afford striking evidence of the usefulness of the method.

Peritoneum.

In the vitally stained animal the peritoneum is of a deep blue colour, varying in intensity in certain areas of the omentum and mesentery, also in the peritoneal coats of the stomach and bowel. Thus, for instance, the cardiac portion of the mouse's stomach only stains light blue, whereas the glandular pyloric half has a dark shade. On closer examination this difference is traceable to the presence of larger or smaller numbers of vitally stained free peritoneal cells in the respective parts. The difference in colour corresponds exactly to a difference in physiological function, inasmuch as the cardiac section of the mouse's stomach serves a more mechanical, the pyloric a purely digestive, purpose.

Again, in the omentum, mesentery, and certain ligaments, such as the ligamentum gastro-lienale, we find the membranes studded with dark blue spots, which microscopical analysis reveals to be crowded aggregations of blue-stained peritoneal cells. Nothing could be of greater interest than to determine how these spots vary in size and number according to the age of the animal, the phase of digestion, and according to certain pathological conditions produced by poisons (phosphorus, cumarin, &c.), or by pathogenic germs, such as tubercle bacilli. These blue spots in the omentum correspond to the *taches laiteuses* first described by Ranvier. The cells are similar in appearance to endothelial cells, but their nucleus is smaller. They, moreover, show biochemical qualities wholly distinct from those of the endothelial cell, since in contradistinction to it their protoplasm is granular and shows a strong attraction for the vital blue.

These granular cells are widely distributed through the whole peritoneum, but seem chiefly to follow the perilymphatic spaces of the large vessels which divide the omentum into vascular and non-vascular fields. Groups of these cells, the *taches laiteuses*, are found both in the vascular and non-vascular parts of the omentum, hence their subdivision into primary and secondary *taches laiteuses*. In an early stage of development they are invariably connected with the rich plexus of fœtal capillaries. In later stages of growth the vessels atrophy to a large extent; thus the secondary *taches laiteuses* appear in non-vascularised tissue.

The variety in their size is not only due to the greater or smaller number of cells which constitute them, but is largely due to certain morphological conditions in the size of the individual cell and its granular protoplasm; as in the germ centre of the lymphatic gland we may discriminate between young and full-grown cells. The latter show the characteristic vital blue stain in their granules, which colour distinguishes them from every other cell either temporarily or permanently situated in the peritoneum. Besides their affinity to the vital stain these cells show other highly characteristic qualities. They are wandering cells and migrate not only into the most remote corners of the peritoneal cavity, but also into organs, as, for instance, the liver and the spleen, the pregnant uterus, the mesenteric glands, and even into the lung.

As my investigations on the healing of wounds have shown, these cells play an important part in the formation of scar tissue. They become transformed into fixed fibrous cells, gradually losing all visible traces of granules and the power of attracting the vital stain. But in the first place I must mention that these cells constitute the bulk of so-called free serous cells found in the peritoneal cavity, where they exercise phagocytosis in a marked manner. On injecting finely powdered substances such as carmine, we find the carmine granules greedily seized by these cells and carried into the omentum. In vitally stained animals the blue granules of the serous cells show no diminution in their

power of phagocytosis. I may add that the same applies to the granules of Kupffer cells in the liver. These are the main recipients of Chinese ink injected into the peritoneal cavity, and so form an impassable barrier for small doses of that poison.

As the vital stain does not inhibit the phagocytic activity of the serous cell, the intra-vitam method enables us to study most accurately the distribution of bodies injected into the peritoneal cavity. Thus I found that part of the carmine dust injected remains in the substance of the omentum, where it is incorporated in serous cells which, when fully charged with carmine, ultimately succumb and form the pseudo-tubercles, first mentioned by Hippolyte Martin. But a large quantity of the injected carmine powder is carried away from the peritoneal cavity into the liver, spleen, and mesenteric glands, through the agency of the serous cells, which, for brevity's sake, I will now call macrophages. In these organs the macrophages follow the perivascular lymphatics, become stationary, and form similar tubercles to those in the omentum. But for the application of the vital stain these macrophages in the liver and spleen, and, above all, their peritoneal origin, could never have been demonstrated. I may add that the size, position, and form of the macrophage readily distinguish it from Kupffer's star cell, the granules of which likewise take up the vital stain.

My work in relation to inflammatory conditions of the peritoneum may be briefly summarised by mentioning that in acute inflammation the macrophagic cell of the peritoneum is merely called into action after the storm of leucocytic invasion has abated, and regeneration is succeeding the ravage of inflammation. On the other hand, chronic inflammation of the peritoneum is characterised by the extraordinary activity of the macrophage.

Avian and Bovine Tuberculosis in the Mouse.

Here I must emphasise the fundamental difference which I established with regard to the distribution of avian and bovine bacilli of tuberculosis when grafted into the peritoneal cavity of the mouse. Hitherto in all cases of *spontaneous* tuberculosis in the mouse the "avian" bacillus has been found. Koch had already drawn attention to the chronic course of tuberculosis in the mouse. And yet, when the mouse is subjected to an injection with bovine or human tuberculosis, either through the blood-vessels or the peritoneal cavity, the disease runs a comparatively rapid course, in many cases assuming a form of bacillary septicæmia or miliary tuberculosis of the lung.

In accordance with these facts I was able to show that after peritoneal injection of bovine material, besides rapidly caseating tuberculosis of the peritoneum, the chief seat of trouble was the lung, whither the bacilli had been carried by the blood stream after penetrating the portal vein and causing extensive tubercular thrombi throughout its larger branches. The liver and spleen contained merely microscopic lesions when compared with the large areas of tubercular necrosis in the lung. The macrophages of the peritoneum took no active part in the acute form of experimental tuberculosis.

An entirely different result followed the intraperitoneal injection of the avian bacillus. On macroscopical examination of the vitally stained animal several weeks after inoculation of the virus the peritoneum and intraperitoneal organs hardly showed any trace of disease. All the more remarkable were the lesions revealed by the microscope. The omentum was full of blue patches, which to the naked eye wore the appearance of *taches laiteuses*. By means of the specific stain for bacilli I was able to prove that these blue patches consisted entirely of macrophages, whose blue protoplasm was choked with myriads of bacilli. No trace of inflammation could be found in their immediate or more distant surroundings Such aggregations of macrophages laden with bacilli were also discovered in the liver, spleen, mesenteric glands, and, in a smaller number, in the lung. In all these organs the tubercles had the vital stain and were thus easily distinguished by a low magnifying power. They lay in lymphatic spaces, in the liver surrounding the portal vessels, in the spleen arranged round the Malpighian bodies. The blood-vessels were, with few exceptions in the liver, intact. No caseation occurred, nor could small cell infiltration or giant cells be anywhere found in connexion with these vitally stained tubercles of peritoneal origin.

A key to the whole process was afforded by the examination of animals at short intervals after the injection of the avian bacilli. The latter are quickly conveyed to the liver, where they are destroyed in great numbers by the Kupffer cells. Such as remain in the peritoneal cavity are ingested by the vitally stained macrophage. They multiply in those cells, which wander into the omentum, liver, spleen, and mesenteric glands, and eventually into the lung. As the bacilli increase, a most characteristic morphological change occurs in the cell. The granules of the protoplasm gradually disintegrate; the vital stain, which had originally been confined to the granule, now effects a diffuse stain of the whole cell. Eventually it may disappear entirely. As this metamorphosis of the cell protoplasm proceeds its biochemical reaction alters, inasmuch as in the place of a specific attraction for the vital stain the protoplasm now shows an increased affinity for the fat stains. Fat first appears in the shape of tiny droplets. In the final stage these droplets increase in size and eventually usurp the place of the cell body. And yet the cell continues to live, for even in this stage of excessive fat infiltration the nucleus takes up the nuclear stain and shows no signs of degeneration. Naturally in the end the cell succumbs. After death the fat also disappears from its necrosed body, whose shape still remains visible as a ghost in the tubercles undergoing necrosis.

We have thus established a fundamental difference in the distribution of bovine and avian tuberculosis when injected into the peritoneal cavity of the mouse. In the bovine variety metastasis occurs along the blood stream through the thrombosed portal veins; in the avian variety the dissemination is effected by the lymphatics. The "avian" tubercles, which appear in the omentum, liver, spleen, mesenteric glands, and lung, consist of peritoneal macrophages, which are vitally stained. Through the intracellular multiplication of the bacilli the granular protoplasm of the macrophage gradually disappears. Consecutively the vital stain becomes indistinct and diffuse. Fat takes the place of the protoplasmic granule.

The localisation of the avian tubercles in the liver is highly characteristic. They are chiefly arranged in the peri-lymphatic spaces which surround the portal vessels. As a rule they contain, besides the vitally stained macrophages, a ring of plasma cells. Both of these cells are strangers to the adult liver. It is interesting to note that both these strangers migrate into the liver along the same channels used by fœtal blood-cells which form in the embryonic liver and join the lymphatic circulation. My observations on the peritoneal injection of lifeless particles of carmine, as well as of avian tubercle bacilli, prove that an open lymphatic connexion exists between the peritoneal cavity and the lymph spaces in the liver. The same holds good for the spleen.

No less interesting is the general reaction in the liver and the spleen after peritoneal injection of *bovine* material. Along the perivascular lymphatics of the portal vessels the liver exhibits a dense infiltration of lymphocytes, plasma cells, and even of megakaryocytes, an arrangement so typical of that period in the fœtal liver which is associated with the genesis of blood cells. Exaggerated hæmopoiesis may also be inferred from the enormous increase of plasma cells in the spleen, which occupy the pulp as well as the Malpighian bodies. Similar phenomena make their appearance in the liver and spleen when diffused peritoneal cancer is experimentally produced or when complete cancer immunity has been established.

As to the local reaction in peritoneal cancer I wish to add that exactly as in avian tuberculosis the peritoneal nodules are invaded by vitally stained macrophages. In cases of peritoneal cancer in which I injected carmine powder into the peritoneal cavity I found the carmine-bearing macrophages exclusively in and around the cancer nodules, none in the liver or spleen, so strong was the avidity of the cancerous growth for the macrophages.

Lesions of the Liver and Skin.

I conclude my remarks on intraperitoneal disturbances with a short reference to experimental necrosis, produced in the liver by various poisons, such as phosphorus, cumarin, cocaine, and above all things by a substance first prepared by Ehrlich called icterogen. The latter is an arsenic compound of Ehrlich's "606" series. On injection of a centigramme of a 1 in 5000 solution the mouse develops severe jaundice, followed by miliary bland necrosis of the liver cells Similar to cumarin, icterogen induces thrombosis in

the smaller interlobular portal vessels, which explains the consecutive necrosis of liver cells. I have included these experiments in this review to show that, wherever non-inflammatory necrosis in the liver occurs, vitally stained macrophages leave the peritoneal cavity, migrate along the liver lymphatics towards the seat of trouble, and eventually assist in the repair of the damage. No less important is the part played by these macrophages where the liver is wounded or becomes the seat of parasites.

I have given ample proof of the extreme chemotactic sensibility peculiar to the peritoneal macrophage. This cell is in no wise to be distinguished from the histogenic migratory cell of interstitial tissue, but corresponds in every detail to that cell which on vital staining gives rise to the blue colour in the skin. Careful injections of isamine blue in newly born animals have revealed the interesting fact that an enormous depôt of such cells exists under their skin. They migrate from the subcutis into the upper and deeper layer of muscles, and eventually join hands, so to say, with the macrophage of the serous cavities. All these cells are granular in structure. Have we reasons for doubting Renaut when he argues that the interstitial tissue is one large mass of cells unrivalled in the body for their powers of internal secretion?

All lesions of the skin, but most especially such as show a minimum of inflammatory sequelæ, attract the blue-stained macrophage in great quantities. Its activity is effectively displayed in the healing of aseptic skin incisions. Stage by stage biochemical reactions enable us to follow the physiological effects produced by the various cells which combine in perfecting the healing process. No sooner does the leucocyte emigrate from the dilated blood-vessel into the injured area than glycogen appears in its protoplasm. The macrophage is the next on the scene. It absorbs the exuded glycogen by means of its vitally stained granules. Then the transformation of the macrophage sets in, the granules and, in their train, the vital stain disappear, and fat in tiny droplets takes their place. Finally, these also disappear as the rounded cell stretches into the spindle cell of the young scar tissue.

Malignant Growths.

But in no case, to my knowledge, does the aggregation of blue-stained macrophages assume such extraordinary dimensions as in the instance of malignant growths placed under the skin. They swarm around the growing tumour and penetrate it along the endless blood channels which furrow its lobules. In the interior of the growth most of these cells succumb. I am still engaged in an inquiry into the relations existing between the growing tumours and these cells. Not wishing to forsake the sure ground of established facts I merely state that the appearance of these blue-stained cells on the field of tumour grafts may be regarded as a specific local reaction, induced by the tumour cell. When exempt from inflammatory agents the tumour attracts no other migratory cell. Are they the bearers of nutritive material? Hitherto I have failed to discover glycogen, fat, or iron in the surroundings or interior of an experimental growth in appreciable quantity. One cannot, therefore, assume that the growths attract nutrients as the fertilised egg attracts them through the medium of the placenta. And yet the growing tumour has a distinct general effect on internal organs.

The liver and spleen, as I said, evince signs of increased hæmopoiesis under the influence of growths. In cases of rat sarcoma exceptionally large quantities of glycogen are hoarded up in the liver cells. More striking is the appearance of diffuse glycogen infiltration in the fatty tissue which surrounds the kidney and suprarenal. Similar but less extensive and regular are these appearances in tumour-bearing mice.

Here we have a number of points which bear a striking analogy to those I have dealt with at such length in connexion with the placenta and the nutrition of the fœtus. A great step forward in our knowledge of malignant growths would be achieved were we able to determine which bodies are needed for the growth and which for the nutrition only of the tumour cell. As yet we only know that bodies similar to those needed for the growth of the embryo serve the same purpose for the malignant cell. In pregnant tumour-bearing animals a race for these bodies actually takes place between the embryo and the tumour cell. In most cases the embryo is victorious—it thrives, and the tumour either atrophies or remains stationary in size, until its competition with the embryo is concluded by the birth of the latter. We also know that under certain conditions, when the number of embryos is a smaller one, the foodstuffs are produced in excess, so that besides the embryo the tumour gets its fill and increases rapidly in size.

I have shown of what great importance fat, glycogen, hæmoglobin, and iron are in the building up of the fœtal organism, how their production is increased in the maternal tissues, how, finally, after having suffered a temporary delay and after having been transformed in that great fœtal food depôt, the placenta, they reach the embryo. Hence, it seemed to me of the greatest biological and practical value to inquire as to whether the body of the adult tumour-bearing animal was in possession of any organ analogous in function to the placenta of the pregnant animal, and to determine how far it is in our power to inhibit or delay the transmission of substances needful for the growth of the tumour cell.

It is now many years ago that Claude Bernard, on the strength of his glycogen studies, first drew attention to the functional analogy existing between the placenta and liver. He actually claimed to have discovered that the placenta took on the functions of the fœtal liver until the latter had, so to say, come of age. What a number of new facts have been ascertained in recent years which seem to give fresh colour to Claude Bernard's hypothesis! We know that under certain conditions the liver, like the placenta, may become a great storehouse for glycogen and fat, we have learnt to appreciate the liver's powers of forming blood, and we have above all things ascertained to what extent the liver acts as a poison filter to the body, retaining substances of a deleterious nature or rendering them innocuous.

From this point of view I have started an experimental inquiry into the possibility of arresting malignant growth by producing lesions in the liver of such a nature that its functions, though temporarily impaired, are not entirely and permanently suspended. Such an inquiry seemed the more justified since all local and general methods of treatment, even sero-therapy, have failed in the cure of experimental tumours.

My inquiry has been much facilitated by the knowledge of Ehrlich's "icterogen," the arsenic preparation to which I have already alluded, in connexion with my studies on degeneration in the liver produced by specific poisons. My mode of procedure has been the following. The injection of 1 centigramme of a 1 in 5000 solution of icterogen was followed up in the mouse by an inoculation of the tumour graft. It is essential to time the latter, so that the first stage of growth in the tumour graft synchronises with the maximum effect of the poison—that is to say, with the appearance of jaundice. After the ill-effects of the first icterogen injection have subsided a second one of the same or diminished (1 in 7500) strength may be applied.

The effect of the icterogen treatment is not the same in all cases and in all varieties of tumours. As far as I am able to judge at present, it appears to me that the primary stage of growth is not inhibited; although in a number of my experiments the tumour began to grow later in the icterogen mouse than in the normal mouse. But there can be no doubt that the growth is eventually retarded under the influence of icterogen. At first I was inclined to believe that in tumours of naturally slow-growing tendencies, for instance in Ehrlich's chondroma, the inhibitory effect of icterogen was more pronounced than in tumours of greater virulence. But more recent experiences have convinced me that even in carcinoma and sarcoma similar results to those achieved in chondroma may result from a larger dose of icterogen. When in later stages, from two to three weeks after inoculation, we compare the size of growths in normal and icterogen mice, in the normal animal the growth may be the size of a walnut, whereas in the icterogen mouse it is hardly as big as a pea.

Experiments on rat sarcoma have hitherto failed, but as yet I have not succeeded in producing any specific effect on the rat through icterogen injections. In the injected rats jaundice did not appear, nor could I find any trace of necrosis in the liver after the application of the drug. These negative results seem to form an additional proof that, if at all, icterogen acts on tumours through the medium of the liver.

From what I have seen in the growth itself, after the successful application of icterogen, it appears that the tumour cell undergoes more rapid degeneration than usual.

Frequently ulceration is an early symptom; it may result in complete disappearance of the neoplasm. Icterogen makes no difference in the appearance of the macrophage in the tumour's surroundings. The macrophage invariably shows a distinct bile stain, easily preserved in microscopic specimens by rapid fixation and early histological preparation.

I have not lost sight of the fact that icterogen is a powerful toxic agent producing widely extended circulatory trouble and damage in the liver. Under such conditions, it may well be argued, it is not astonishing to find in the animal, as a sequel of general malnutrition, an inhibition or even degeneration of a tumour graft. But it has not been my intention to advocate an icterogen cure of malignant growths. I merely endeavoured to prove that by damage to the liver the tumour also suffers, probably through the want of material needful for its growth. If so, then we have a new field of therapeutical cancer research thrown open to us. It would, indeed, be of greatest interest were we able to establish a scientific basis for the experience gathered by our medical forefathers, that amongst all inorganic agents used in the treatment of malignant growths none has ever equalled arsenic, whose specific effects on the liver have been so frequently and widely proved, whose curative influence on cancer has but recently again been advocated.

A CASE OF LIGNEOUS THYROIDITIS.[1]

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LIGNEOUS thyroiditis, or Riedel's disease, is an uncommon malady, and therefore we consider it worth while to give a short account of a case which has recently come under our observation. The disease was originally described by Riedel[2] in 1896 as "Eisenharte Strumitis," but we have not seen his original paper. Three cases are described by Mr. James Berry[3] in his well-known book as examples of "primary chronic inflammation of the thyroid gland." Recently a full account of the subject has been given by X. Delore and H. Alamartine,[4] in which short histories of 13 published cases are given, in addition to the details of one case observed by the authors.

The patient, a male, aged 23 years, was brought to us by Dr. E. Perkins on July 11th, 1911, with the following history. For about 18 months he had noticed that his neck was gradually becoming somewhat swollen, and during the last two or three months the enlargement had increased more rapidly, his voice and breathing also becoming affected. Latterly the pressure symptoms had become much exaggerated, respiration at times being attended with great difficulty, so that at night he was often unable to lie down, relief being only obtained by sitting up in bed and bending the head forwards. The dyspnœa was paroxysmal and increased on any exertion, respiration being attended by stridor, at times so loud as to be heard in an adjoining room.

On examining the neck a well-marked uniform enlargement of both lobes of the thyroid gland was found to be present. The swelling was unusually firm in consistence, with a smooth surface, and somewhat fixed, so that it moved very slightly on deglutition. Its nature was rather obscure, as it did not present the symptoms of an ordinary goitre, the fixity and density suggesting the possibility of the early stage of malignant disease. As the symptoms were rapidly becoming urgent, and it was evident that relief would soon be required by surgical interference, it was resolved to explore the swelling without delay, and, if possible, remove the isthmus and one lateral lobe; if, however, this was found to be impracticable, then to perform tracheotomy.

The patient accordingly went into a nursing home, and the operation was performed on July 26th, our colleague, Mr. J. Howson Ray, very kindly assisting, and the anæsthetic being administered by Mr. Alexander Wilson. As there was no difference in the size of the two lobes, it was decided, if possible, to remove the right lateral lobe and isthmus, which were exposed by the ordinary "collar" incision, commencing on the right side of the neck and continued beyond the middle line. After dividing the integument, it was found that the overlying muscles and fascia were adherent to the outer surface of the thyroid, the glandular tissue, capsule, and adjacent structures being converted into dense, apparently fibrous tissue, of a whitish colour, and of such hard consistence that it was not easy to cut it with a scalpel. On making an exploratory incision into the exposed lobe it presented the same structure throughout, all trace of glandular tissue having completely disappeared, and a similar condition was found in the isthmus. A wedge-shaped piece of the gland, consisting of the inner portion of the right lobe, together with the isthmus were first excised, very little hæmorrhage attending the process. Then successive layers were removed from the remaining portion of the same lobe until its periphery was approached, when the bleeding became more profuse, and somewhat difficult to arrest, in consequence of the dense tissue in which the vessels lay. As the trachea was extremely narrowed laterally, presenting the appearance described as "scabbard" trachea—in order completely to remove the lateral pressure on both sides—it was thought advisable also to excise the greater part of the left lobe, and this was dealt with in a similar manner.

Though the trachea was now completely exposed and relieved of all lateral pressure, there was still marked obstruction to the respiration. This was found to be due to a definite band of dense tissue, extending from the tip of one lateral lobe to the other, and continuous with the lower border of the isthmus, which ran in front of the trachea, compressing it antero-posteriorly. This was cautiously cut through upon a director passed beneath it, its division relieving the trachea of all pressure, and being almost immediately followed by a distinct improvement in the breathing. All bleeding having been carefully arrested, the wound was closed, free drainage being provided by the insertion of two tubes—one on either side of the neck.

The after-history was uneventful, the patient making a rapid recovery from the operation: the pulse never rose above 84, and the highest temperature was 99° F. After the removal of the tubes on the second day the wound quickly healed, and the patient left the nursing home at the end of three weeks. The relief afforded by the operation was immediate and very marked, the dyspnœa at once ceasing and the voice rapidly improving.

The patient was seen again on Oct. 4th, when he complained of feeling cold. The face had changed in appearance owing to the development of some solid œdema of the subcutaneous tissues of the face and lips. There was also a red flush in each cheek, so that he had the characteristic facial appearance of an early stage of myxœdema. The skin generally was dry and rather rough, and he only perspired in the axillæ. The hair was dry, the voice was husky, and the tongue was slightly swollen. The memory had become rather defective. These symptoms clearly showed that he was suffering from secondary myxœdema which had developed after the partial thyroidectomy. He was treated with thyroid tablets, and when last heard of was in good health and doing his ordinary work.

Examination of microscopical sections prepared by Dr. W. Mair from the portions of the thyroid gland which had been removed showed that they contained no normal thyroid tissue. For the most part they consisted of dense fibrous tissue, some parts of which contained but few nuclei, while others were more richly nucleated and appeared to be more recent in origin. A few small collections of epithelial cells were found. In one case they were arranged in a row, partially inclosing a space which contained a small group of epithelial cells. At one part some areas—mostly oval in shape—were found, lined by fibrous tissue, and containing a homogeneous material which was probably colloid. No normal epithelial lining to these spaces could be distinguished, and they were separated from each other by dense strands of

[1] A paper read at a meeting of the Pathological Society of Manchester on April 24th, 1912.
[2] Die chronische zur Bildung eisenharter Tumoren führende Entzündung der Schilddrüse, Verhandlungen der Deutschen Gesellschaft für Chirurgie, Berlin, 1896, Band i., p. 101.
[3] Diseases of the Thyroid Gland, p. 137.
[4] La Thyroidite Ligneuse (Maladie de Riedel), Revue de Chirurgie, tome xliv., p. 1, July, 1911.