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ON THE ETIOLOGY OF CARCINOMA.

RESEARCHES ON THE MORPHOLOGY OF AN ORGANISM
ASSOCIATED WITH CARCINOMA MAMMAE, AND ON
THE ETIOLOGICAL SIGNIFICANCE OF THIS
ASSOCIATION.

(With Special Plate.)

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In a paper read before the Royal Society in 1899¹ I described the morphological characters of an organism isolated from carcinomata. During the last four years these researches have been continued, but have been confined to one clinical type of carcinoma—that affecting the female breast. These further researches have provided material out of which I have been able to construct the life-history of the organism, and have also afforded evidence of interest and importance relating to the question whether the organism is etiologically connected with the disease.

THE ISOLATION OF THE ORGANISM.

All the material which has been used in the attempts to isolate the organism has consisted of specimens of un-ulcerated carcinoma mammae removed by operation. These have been examined in the laboratory at periods varying from half an hour to four hours after removal, and with one exception I have been present at each operation, and have been personally responsible for the precautions taken to prevent contamination. These precautions I have described in a recent paper,² so that I need not here refer to them in detail.

The specimens thus treated have been twelve in number. From seven of these I have isolated the organism I am about to describe, one was contaminated with staphylococcus epidermidis albus, the remaining four yielded no cultures. A large variety of nutrient media have been tried, but the positive results have been obtained with ordinary nutrient broth in one case, with glucose broth (4 per cent.) and glucose agar (1 per cent.) in the other six. In all cases the method of cultivation has been that of immersing small pieces of the growth in the fluid media, and rubbing pieces over the surface of the solid medium, or leaving small slices on the surface of the medium. All tubes have been incubated at 37° C., and all the positive results have been obtained under aerobic conditions.

THE MORPHOLOGY AND LIFE CYCLE OF THE ORGANISM.

The organism is remarkably polymorphic. It exhibits two chief types, which differ to a marked degree (a) in morphology, (b) in behaviour in culture media, and (c) in the effects which they produce in the animal body. These two types I shall describe as Type A and Type C. I have been able to observe the changes which the organism undergoes in passing from one type to another, but not, hitherto, to determine exactly the conditions which govern and control these changes.

Type A is that form of the organism which is found in the primary cultures derived from the carcinomata; in one of the seven cases which I am here reporting, Type C was associated with Type A in these primary cultures, but, with this exception, Type A alone was present in these cultures.

In the form of Type A the organism is spherical; its size varies from that of a minute spherical granule to a diameter of 2 μ to 3 μ .

In the fluid media it is seen partly suspended, but for the most part as a sediment; on the solid medium it occurred as a scanty, greyish-white, very viscid and translucent streak. The viscosity is a marked characteristic. The amount of growth obtained has been very small in all cases; in no case was I able to obtain more than one generation of subcultures, and in most instances no subcultures at all developed.

The inoculation experiments also showed that the vitality of this type of the organism was of a very low order on the media employed.

In this form the organism is liable to be mistaken for a

coccus; the pronounced disparity in size of the different specimens is the chief peculiarity which distinguishes it from this class of organism.

In no case have I observed a development of this Type A into Type C *in vitro*. After injection into the animal body Type A may either (1) show no marked morphological change, or (2) exhibit changes in the course of which specimens develop of somewhat larger size than those in the culture, along with oval and club-shaped forms; or (3) pass on in development to what I shall now describe as Type C.

Type C is a spherical form, of a diameter varying from 5 μ to 10 μ . It possesses a capsule, a cell network, and one, a few, or many refractile granules in the interior. Romanowski's stain shows the presence of nuclear bodies, which may be single or multiple; as many as eight of these nuclear bodies have been observed within a single cell, each usually surrounded by a clear "halo," but this is not constant.

Type C multiplies readily on media; in particular on glucose-containing media, by budding. The details of this process, and the full description of the morphology of this type have been described by me in a recent paper.³

Type C has been found in the course of my observations (a) in the lesions in animals which have followed inoculation with Type A, and (b) in one case (excluding those reported in 1899) associated with Type A in the primary cultures obtained from the carcinomata.

The development of Type C from Type A has been followed in a considerable number of animals inoculated with the latter. In all these animals with one exception the number of organisms which showed the characteristic structure of Type C was small, and none showed the budding process peculiar to this type. In the one exception the number of specimens of Type C was large, and they showed active budding.

From these lesions which resulted from the inoculation of Type A, either no cultures at all were recovered, or cultures of Type A similar to that injected. The exceptional case just mentioned was also exceptional in this respect in that cultures of Type C were recovered from the lesions.

It has already been stated that Type A grew on media to a very slight degree only, and this difficulty of cultivation was further shown in that many of the animals, the lesions in which showed microscopically the presence of Type A, yielded no cultures. On the other hand, by direct inoculation from animal to animal by breaking up the nodules which had been experimentally produced, in normal saline, and injecting the material thus obtained, I was able to produce a series of lesions in which the organism showed no diminution of activity.

From these observations it follows that the organism in the form of Type A must be described as a facultative parasite, whereas in the form of Type C, which grows luxuriantly on different kinds of media and also shows a capacity for developing in the animal body, it must be described as a facultative saprophyte.

By combining the observations it is possible to construct a scheme of the life-history of the organism as follows:

Scheme of the life-history of the organism: I. Vegetative cycle.

A. Type A, the form in which the organism occurs in primary cultures from carcinomata mammae (facultative parasite). B. Intermediate (? involution) forms of A. C. The vegetative type (facultative saprophyte). D. Form of C with interior taking dense chromatin stain. S₁, S₂. Stages in the process of sporulation. T. Sporulation process showing extrusion. All specimens stained wet with methyl violet.

In addition to the method of reproduction by budding

which Type C exhibits, I have ascertained that this form of the organism proceeds to the development of a sporulating process. In spite of a large number of observations designed to discover the occurrence of this process *in vitro* I have not been able to produce it under these circumstances. The process was observed in animals which had been inoculated with cultures of the Type C form; it was also discovered in the one case of which I have made mention above in which the primary cultures from the carcinoma showed organisms of both types; in these cultures a few specimens of the C type showed the sporulation process in a very distinct manner.

The process consists in the separation of the interior protoplasm of the cell into small spherical bodies and the extrusion of these through a dehiscence in the capsule, which is denser than that of the ordinary specimen of Type C. These small spherical bodies when extruded give the staining reactions and present the same general appearances as the organisms which have been described as Type A.

To recapitulate the characters of the two main types. Type A is that form which is to be found in primary cultures from the carcinomata; in all the cases here recorded, with one exception, this was the only type present in these cultures; in the one exception it was associated with Type C, the latter being in part in a sporulating condition. Type A grows on ordinary media either not at all or to a very scanty extent only; after injection into animals it usually remains unchanged, but may show certain morphological alterations in the course of which a few specimens similar in all respects to Type C are produced. In a single animal the specimens of Type C were present in large numbers in the lesions following inoculation with Type A.

Type C was cultivated from the animal just mentioned, and in one case direct from a carcinoma. In this form the organism grows abundantly on ordinary media. On injection into animals it may remain unchanged or may develop a process of sporulation which results in the production of organisms which resemble Type A; this sporulation process was also observed in the single case in which Type C was found in the primary cultures from a carcinoma.

THE APPEARANCES PRESENTED BY THE ORGANISM AFTER INOCULATION INTO ANIMALS.

Both forms of the organism are readily demonstrated in the tissues after inoculation, by Gram's method. Both give rise to a certain degree of proliferation on the part of certain of the cells of the tissues with which they are brought in contact, but Type A presents an important difference from Type C in regard to its relation to these proliferating cells. Whereas Type C is not seen within cells but between them and in interstices in connective tissue, Type A is found characteristically as an intracellular parasite, usually extranuclear, occasionally intranuclear. Type A is demonstrable as an intracellular parasite by Gram's method (Fig. 4). Usually single specimens were seen within the cells, but from two to five have been observed in a single cell. Single specimens lie in a vacuole in the cell, which appears as a clear space or "halo" around the organism, and is well seen in Fig. 4. If more than one specimen is present in a cell each may lie in a separate vacuole, or the vacuole may be common. The organisms within the cells show the same marked variations in size as are to be seen in the cultures *in vitro*. They were seen within endothelial (peritoneal) and epithelial cells and in these alone. They were not numerous in any of the lesions, from one to three being usually visible in each microscopic field (Leitz $\frac{1}{2}$ in. objective; eyepiece IV). The organism in the form of Type A is therefore capable of infecting and entering epithelial and endothelial cells.

THE LESIONS PRODUCED IN ANIMALS BY INOCULATION OF THE ORGANISM.

Before describing the lesions which are produced by the inoculation of the organism in the form of Type A, which description is the second of the two chief objects of this paper, I shall first shortly refer to the effects which are produced by inoculation of the actively vegetating Type C. These I described at length in the paper⁴ to which reference has been made above, and the observations there recorded have been confirmed by later experiments, particularly by the examination of lesions in animals at varying periods after injection. When a culture of this type is inoculated intraperitoneally the early effect is the local stimulation of the endothelial cells of the peritoneum to proliferation, so that small nodules are formed composed of masses of these endothelial cells, with numbers of the organisms between them.

The organism in this type does not show a capacity for entering and inhabiting the endothelial cells; it continues its active multiplication by budding, with the result that the new-formed endothelial cells become compressed, and the nodules are vacuolated. Ultimately these nodules consist of masses of the organism, with a network of connective-tissue-like appearance around and between them. Analogous processes have been observed in other organs and tissues.

The effect which the organism produces when injected into the animal body in the form of Type A is of a quite different character.

The effects have been uniform with the exception of a certain number of negative results, which were apparently due to the rapid loss of vitality which this type displays *in vitro* in the media which I employed.

In stating that the results were uniform I do not intend to convey that the extent of the lesions produced has been equally great in all the animals used, but that the general character of the lesions was the same in the different animals.

A total of 23 guinea-pigs have been inoculated, and lesions were produced in 13 of these; 2 were inoculated subcutaneously without result; the rest were inoculated into the peritoneal cavity. Three dogs have been inoculated, 1 into the peritoneal cavity, 2 into the mammary gland; in all 3 cases lesions were produced. These numbers are of course exclusive of those animals which are still under observation. In illustration of the characters of the lesions, I shall describe those in 2 guinea-pigs and 2 dogs.

DESCRIPTION OF FIGURES.

Fig. 1.—Branching columns of parenchyma cells in a connective tissue stroma, from an experimental new formation in the omentum of the dog. $\frac{1}{2}$ in. obj.

Fig. 2.—New-formed tissue, consisting of cells arranged in alveolar-like groups, occupying a dilated bronchiole, and extending beyond this. From the guinea-pig. $\frac{1}{2}$ in. obj.

Fig. 3.—Carcinoma mammae in the human subject. Shows two specimens of the organism within parenchyma cells. Stained carmine and Gram's method. $\frac{1}{2}$ in. oil immersion.

Fig. 4.—Section from a nodule of new-formed tissue in the omentum of the guinea-pig. Shows two specimens of the organism occupying vacuoles in the cells. Stained carmine and Gram's method. $\frac{1}{2}$ in. oil immersion.

Fig. 5.—From the liver of the guinea-pig occupied by nodules of new-formed tissue. Shows the origin of this new-formed tissue by proliferation of the epithelium of biliary ducts, and its extrusion from these. $\frac{1}{2}$ in.

Fig. 6.—Section from a nodule of new-formed tissue in the peritoneum of the guinea-pig. Shows a structure composed of large cells derived from the peritoneal endothelium supported by a fine connective tissue reticulum.

GUINEA-PIG 1.—Inoculated into the peritoneal cavity, killed twenty-four days later. Nodules were found in the peritoneum, the abdominal glands, the liver, the lungs, and the spleen.

The peritoneal nodules consisted of masses of large oval, spherical, and polygonal cells arranged in part in parallel columns but also in part in an alveolar manner, in groups in the interstices of a stroma. The masses of cell growth were not defined at their margins, and in this situation the typical parenchyma cells were seen within perivascular lymphatic spaces; these cells could be definitely traced to have arisen by proliferation of the endothelium of the peritoneum.

The glands show areas which stand out when stained from the normal gland tissue and the differentiation can be seen with the naked eye. These areas are found to possess a structure which shows a definite resemblance to that of the peritoneal nodules both in the character of the cells and their arrangement.

The liver nodules are all situated in the portal canal regions; they consist of oval and spherical cells arranged in columns and masses bordered by connective tissue. It is seen (Fig. 5) that these columns and masses have originated by proliferation of the epithelium of the small bile ducts; they extend directly from these into the surrounding tissue in the portal canals and form masses of considerable size which separate and compress the hepatic lobules.

In the lung the nodules stand out as whitish patches of irregular shape; the majority can be traced to a connexion with small bronchioles, and from the epithelium of these they have arisen; in one or two instances the new-formed cell masses can be seen (Fig. 2) infiltrating along the lumina of bronchioles, but for the most part the direction of growth has been into the surrounding tissue; the cells of this new-formed tissue are arranged in an alveolar manner. In all the

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

nodules—peritoneal, glandular, hepatic, and pulmonary—organisms were demonstrated by Gram's method within the new-formed cells.

GUINEA-PIG II.—Inoculated into the peritoneal cavity; killed thirty-seven days later. The nodules in the peritoneum consist of oval and polygonal cells with a large cell body and large spherical and oval nuclei (Fig. 6). Lymphocytes are present throughout the nodule in fair numbers. The arrangement of the cells is similar to that described in Guinea-pig I. The mesenteric glands are occupied by masses of cell growth, which reproduces in the character of the cells and their arrangement the characteristics of the nodules in the peritoneum.

The liver nodules are numerous; the type of the cells composing these nodules and their arrangement are exactly similar to those of the peritoneal nodules. The new cell growth cannot, as in Guinea-pig I, be traced to the small bile ducts, and its structure is different from that found in Guinea-pig I.

I have recently had the opportunity of examining sections of a peritoneal carcinoma in the human subject kindly placed at my disposal by Dr. A. S. Grünbaum, and I find that the structure of this shows a marked resemblance to that of the nodules I have described in these guinea-pigs.

DOG I.—Killed forty-two days after intraperitoneal inoculation. Nodules were seen with the naked eye in the omentum, mesenteric glands, liver, and spleen. The omental nodules consist of a parenchyma and a stroma. The parenchyma consists of branched columns of epithelium-like cells irregularly arranged (Fig. 1). They can be traced from the surface endothelium, where their arrangement is in masses, downwards to the deeper parts, where they lie in a dense stroma. The mesenteric glands appear to be infiltrated with new tissue which can be seen with the naked eye. This is similar in structure to the omental nodules in regard to both the character and arrangement of the cells. Nodules in the liver are situated in relation to the portal canals, and between them the liver parenchyma is compressed; they consist of branching columns of cells and of fine tubules in a connective-tissue stroma; the cells composing the columns and tubules show characters which are identical with those of the epithelium of the bile ducts, but there are appearances also which seem to connect these cells with the parenchyma of the liver lobules, and the exact histogenesis of the nodules is somewhat uncertain. The spleen is studded with nodules; the structure of these is somewhat indistinct, but their general characters resemble those of nodules in the peritoneum.

DOG II.—Killed eighty-two days after inoculation into the mammary gland. A single nodule the size of a large hazelnut was present in the mamma, and there were numerous nodules in the spleen. The mammary nodule was composed of cells with large body and spherical or oval nuclei, supported by a fine connective tissue; lymphocytes were scattered throughout. The large cells are arranged for the most part in parallel columns within the stroma interstices, but the arrangement cannot be said to be regular or to have assumed a definite type. The nodules in the spleen are fibrocellular; they are not marked off from the splenic pulp by any fibrous boundary; the cells are arranged in groups within the connective-tissue stroma, but their histogenesis is uncertain. The stroma is denser than in the mammary nodule, and the nodule on the whole does not reproduce the characters of the latter, although it bears some resemblance to it.

These four examples illustrate the character of the new tissue formation which follows inoculation with the organism; in one animal alone were there nodules in the kidney; these consisted of groups of cells arranged in a distinctly alveolar manner in a fine stroma, and derived by proliferation from the epithelial cells of the renal tubules.

In all the lesions examined the characteristic organisms were demonstrated as intracellular parasites; occasionally intercellular specimens were seen.

SUMMARY OF THE INOCULATION RESULTS.

The organism showed a capacity for initiating active proliferation of endothelial and epithelial cells. The new-formed tissue resulting from this showed a parenchyma and a stroma, and at its edges a progressive growth without encapsulation. In neighbouring glands there was found in some instances a formation of new tissue whose structure resembled that of the primary nodules. The nodules in viscera appeared as a rule to arise from epithelium *in loco*, and showed a capacity for

active growth and infiltration similar to that observed in the nodules arising from endothelium. In one instance, at least, new-formed tissue in the liver reproduced exactly the characters of nodules in peritoneum and mesenteric glands, and, assuming that the observation that it did not arise from cells *in loco* was correct, must be considered a true visceral metastasis.

DEMONSTRATION OF THE ORGANISM IN HUMAN CARCINOMA.

Having found that the organism was demonstrated by Gram's method within the cells of the experimentally-produced nodules, the application of this method to carcinoma mammae naturally followed.

My observations on this point have up to the present been few in number and relate to three cases only. In these three carcinomata Gram's method shows the presence within the parenchyma cells of bodies which give the same staining reaction (Fig. 3) and have the same general appearance as the parasites in the cells of the experimentally-produced nodules; in the latter they were few in number in most cases, and in the carcinomata also they were not numerous. In the nodules they have been described as occupying vacuoles in the cells outside the nucleus, in the carcinomata they occupy the same position; in both cases they were also seen within nuclei forms which presented the same appearances, but this was an exceptional occurrence.

SUMMARY OF RESEARCHES.

1. From a considerable proportion (8.3 per cent.) of specimens of carcinoma mammae an organism presenting characteristic features was isolated.
2. This organism presents a life-history in which two cycles were traced—the one a vegetative budding cycle, the other a sporulating cycle.
3. The organism when injected into animals is capable of infecting and inhabiting endothelial and epithelial cells.
4. The organism initiates in endothelium and epithelium a process of proliferation, as a result of which masses of new-formed tissue are built up which consist of a parenchyma and a stroma, and grow and extend actively from their centres of origin.
5. This new cell-mass formation may be associated with growth of a similar character in neighbouring glands, and some evidence was also provided that visceral metastasis occurs.
6. Intracellular bodies are demonstrable in carcinomata mammae, which present the same features as the intracellular parasites of the experimentally produced nodules.
7. The evidence derived from these researches points to the conclusion that the organism described is an etiological factor in the morbid process known as carcinoma mammae.

[The expenses of these researches were in part defrayed by a grant from the British Medical Association.]

REFERENCES.

- 1 *Proc. Roy. Soc.*, December 14th, 1899. 2 *Thompson-Yates and Johnston Laboratories Report*, vol. v, part 1, 1903. 3 *Ibid.* 4 *Proc. Roy. Soc.*, December 14th, 1899.

CARCINOMA OF THE COLON AND ITS TREATMENT BY COLECTOMY.*

[WITH ABSTRACTS OF SEVEN SUCCESSFUL CASES.]

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GENTLEMEN.—When your President invited me to read a paper before this Society I was engaged in collecting and following up my cases of colectomy for carcinoma of the colon. I was going to publish a bare record of the cases, but, when your President assured me that it would be difficult to find a subject which, at the present time, would be of more interest to you than that of colectomy for malignant disease of the colon, I determined to recast my paper and give it a somewhat more general character in order to fit it for an address to this Society.

The subject of carcinoma of the colon is, I think, worthy of close attention, for it presents a very hopeful field for surgery if only the cases can be dealt with in an early stage. Given early cases, with the patients in good condition, not only should the immediate results be good, but a large percentage of permanent cures should be possible. Columnar-celled

* A paper read at a meeting of the East Sussex Medico-Chirurgical Society on November 17th, 1903.