"A Preliminary Note upon certain Organisms isolated from Cancer, and their Pathogenic Effects upon Animals." By H. G. PLIMMER, M.R.C.S., F.L.S., Pathologist, and Lecturer on Pathology and Bacteriology, St. Mary's Hospital, London. Communicated by Professor J. ROSE BRADFORD, F.R.S. Received February 22,—Read March 9, 1899.

(The following specimens were exhibited at the reading of the paper:—

1. Sections of the cancer from which the cultures were made.
2. The cultures on various media.
3. Preparations of the cultures.
4. Sections of the organs of the animals in which tumours have been produced.
5. Animals, or portions of them, in which tumours have been produced.)

During the past six years I have been studying the cell-inclusions found in cancer, and their relation both to the origin and course of the disease; and for this work I have had to examine 1278 cancers taken from various organs and parts, and of all possible varieties. Out of this large number of cases there have been a few—nine in all—in which the cell-inclusions have been extremely numerous; so that at the growing edge, and even far into the tumour, scarcely a cell could be found without an inclusion, sometimes with as many as thirty-six even of these inclusions in one cell; and these bodies have been similar to those which Metschnikoff, Ruffer, and others, as well as myself, have regarded and described as parasites, standing in causal relationship to the disease. In two out of the nine cases mentioned, these bodies have been present in enormous numbers; and I have succeeded in isolating from the last of these remarkable cases, an organism, which is pathogenic, in a peculiar manner, to certain animals, and whose virulence I have been able to keep unimpaired for some months.

Previous Work on the Experimental Production of Tumours in Animals.

The only work, I think, that needs mention here in connection with this heading is that of Sanfelice, in Cagliari, and of Roncali, in Rome. Sanfelice has produced tumours in animals with organisms which he isolated from infusions of various fruits; and they both have isolated organisms from cancers, which, I believe, from their descriptions—I have not seen their cultures—are morphologically somewhat similar to those I am about to bring before you. But Sanfelice's organism appears to have been very difficult to isolate in a virulent form from human cancer, and to keep virulent; so that in his last paper,* he

* 'Zeitschrift für Hygiene,' 1899.

treats only of the organisms derived from fruit infusions, and of their effects upon animals. Most of their statements are doubted by the German pathologists, including such a good observer as Baumgarten. But, from my own experience, I do not find any reason to doubt any of Sanfelice's statements; and I think that he deserves the greatest credit for removing the study of the ætiology of cancer from the histological to the experimental region of work.

On the Method of Isolation adopted.

The cancer, from which the organisms I describe were isolated, and with which my experiments have been made, was taken from the breast of a woman, aged thirty-five years; it had a history of only two months' duration, and it was growing rapidly at the time of the operation. Immediately after removal, I examined a fresh scraping, and, finding such an extraordinary number of the bodies I have mentioned in the cells, I cut, with all possible precautions against contamination, with a carefully sterilised knife, very thin slices from the growth, which I placed with a little of the juice scraped from the cut surface in a flask containing the following liquid, which was of course carefully sterilised. This medium consisted of an infusion made from cancer, just as the ordinary beef infusion is made, to which was added, after careful neutralisation, 2 per cent. of glucose and 1 per cent. of tartaric acid. This medium was the outcome of many trials with all kinds of mixtures, and I tried it in this case as I had already got similar organisms to grow in it from two previous cases; but they had no pathogenic properties, and this, I think, was due to the omission of the next step. This medium, too, is particularly useful, as hardly any bacteria, however hardy, will grow upon it.

Then, remembering that in the body these organisms were under anaërobic conditions, I exhausted the air from my flasks, and passed hydrogen into them, finally sealing them up. This I have found is of great importance as regards the maintenance of the virulence; and I find, consequently, that there is no falling off in the virulence of my cultures, which are as active now as they were four months ago. Five flasks were made in this way, but, in spite of precautions, two became contaminated with moulds; in the other three, however, I got, after from three to five days, a pure culture of the organism I describe, and which has been kept growing in this and various other media ever since.

Morphology and Relation to Media.

The organism is apparently a saccharomyces; but I am informed that, according to some authorities (such as De Bary, Cuboni, and

Duclaux), the Saccharomycetes are nothing but the developmental stages of fungi which really belong to either the Phyco-, Asco-, or Basidio-mycetes. Moreover, they state that in some species of mycelium-forming fungi, single parts, especially conidia, can grow in the saccharomyces form on certain nutrient media: so I will not attempt to locate this organism at present. Sanfelice and Roncali, however, definitely state that the organisms they have isolated are Blastomycetes.

When grown in the medium described, these organisms produce a cloudiness which becomes visible in about forty-eight hours, and increases till about the sixth day, when the growth sinks to the bottom, the medium then becoming clear; no scum or pellicle is formed.

When grown on this medium solidified with agar, the organisms form small round colonies which remain separate; after some weeks the colour, which was originally white, becomes yellow; the colonies do not attain a much greater size than those here shown.

Gelatin is not liquefied, but the growth on this medium is never luxuriant. On potato, a thick white layer is formed, which in about two weeks will cover the entire surface, changing then to a yellowish-brown colour.

They will grow aërobically, but not so well, at any rate at first; and they lose their virulence in a short time if continuously grown in this way.

Microscopically, they are round bodies, frequently growing in clumps, with a nucleus which stains deeply, and, in most cases, with a thin, strongly refractile capsule, which sometimes shows a double contour; but some young forms can be seen which are apparently without a capsule. The size varies from 0·004 mm. to 0·04 mm.

Their reproduction appears to be by budding, but I have fancied that I have also seen, in a few instances, endogenous budding; of this, however, I am not certain.

These bodies correspond morphologically with those found in the original tumour, and also with those described by Ruffer and myself, and by some others of those who have worked at the microscopical appearances of cancer.

Experimental Results.

I have selected, from the experimental work which I have done with these organisms, those experiments which seemed to me to be the most important. Up to the present, I have not been able to make any experiments upon such animals as would allow of the easy bringing of these organisms into contact with a likely epithelial surface, with the exception of the cornea (*vide* Experiment 4 below); but, through

the kindness of Dr. Bradford, I have been enabled now, at the Brown Institution, to inoculate a bitch in the mammæ, but the time is as yet too short to enable me to make any statement as to the result.

The cultures used in the following experiments were made in the medium previously described.

(1) *Rabbit.*—Intravenous injection of 5 c.c. of an eight days old culture.

No obvious result.

The rabbit was killed thirteen weeks after and found apparently normal.

(2) *Rabbit.*—Intraperitoneal injection of 10 c.c. of a twenty-one days old culture.

No obvious result.

The rabbit was killed eight weeks after and found apparently normal.

(3) *Rabbit.*—Subcutaneous injection of 5 c.c. of a three days old culture.

No obvious result.

This rabbit was used later for experiment No. 4, and when killed, fourteen weeks after this experiment, was found normal; nothing was found at the seat of injection.

(4) *Rabbit.*—Both corneæ were scraped, and the sediment of a ten days old culture rubbed over them.

The rabbit was killed in forty-eight hours.

There was considerable proliferation of the corneal epithelium, which had forced its way into the subjacent tissue.

The organisms were found in the epithelial cells, after fixing and staining, with appearances similar to those of the inclusions in cancer cells, as described by Ruffer and myself.

(5) *Rabbit.*—Trephined and inoculated beneath the dura mater with 5 c.c. of a seven days old culture.

The rabbit died in nine and a half days; wound healed.

The brain and cord contained the organisms in large numbers. Pure cultures were obtained from brain, liver, and kidney. Nothing obtained from blood, spleen, or peritoneal fluid.

(6) *Rabbit.*—Trephined and inoculated beneath the dura mater with 5 c.c. of a three days old culture, made from brain of No. 5.

The rabbit died in eight days; wound healed.

Organisms found in heart, blood, brain, and cord. Pure cultures made from brain, cord, kidney, and liver.

(7) *Guinea-pig.*—Subcutaneous injection of 5 c.c. of a ten days old culture.

No obvious result.

The guinea-pig was killed in fifteen days, and was found apparently normal; nothing was found at seat of injection.

(8) *Guinea-pig.*—Intraperitoneal injection of 10 c.c. of a three weeks old culture.

Died in twenty days.

Liver, lungs, and peritoneum studded with new growths of a white colour; lymphatic glands in abdomen enlarged. Pure cultures made from liver, lungs, and abdominal glands; nothing obtained from blood.

Sections of the above mentioned parts showed new growths of an endothelial nature, with the organisms within the cells, and free in the tissues.

(9) *Guinea-pig.*—Intraperitoneal injection of 10 c.c. of an eight days old culture, made from abdominal glands of No. 8.

The guinea-pig died in seventeen and a half days, and showed the same appearances as No. 8.

Cultures were made as before, and also from the blood. In this case the omentum was also studded with new growths.

I have given here some of the failures and successes which have been constant; and I should like to add that Professor Wright, of Netley, has repeated some of the experiments I have made, and that his results coincide with mine.

The important point, of course, of all this is—the experimental production of malignant tumours in animals by an organism isolated from a malignant tumour in man. That these experimental tumours are, so far, of endothelial origin is due to the fact that until I was enabled to inoculate a dog, I found it very difficult to get the organism in contact with likely epithelium; all the above methods of inoculation, save one, could only bring them into contact with endothelial surfaces. No. 4 (the corneal experiment) is the only one in which an epithelial surface was tried; and in this case the great proliferation of the epithelium, the appearances of the organisms in the cells, and the irritation produced, are very striking. But the fact of being able to excite a malignant growth with an organism isolated from cancer is, I think, a point of some importance in the ætiology of cancer.

I am at present experimenting with the view of observing the effects produced by these organisms when brought into contact with epithelia.

The deductions which I think may fairly be made from these observations and experiments are as follows:—

(1) That there are certain cancers, which occur very rarely, in which there are, in enormous numbers, intracellular bodies of the kind described by Ruffer, myself, and others, as parasitic Protozoa. (From the rarity of these cases and their comparatively acute course, one is tempted to think that they are not due to the same cause as ordinary cancers; but there is really no more difference between them and ordinary cancers than between acute and chronic tubercle.)

(2) That by the use of appropriate means these intracellular bodies can be isolated and cultivated outside the body.

(3) That these cultures, when introduced into certain animals, can cause death, with the production of tumours, so far of endothelial origin; and that pure cultures can be made from these growths which, when inoculated into suitable animals, will produce similar tumours.

"On the Gastric Gland of Mollusca and Decapod Crustacea: its Structure and Functions." By C. A. MacMunn, M.A., M.D. Communicated by Dr. M. Foster, Sec. R.S. Received February 23,—Read March 9, 1899.

(Abstract.)

In 1883 I communicated a paper* to the Royal Society, in which I described the occurrence of a pigment closely resembling vegetable chlorophyll in the so-called liver of Invertebrates, and in 1885 a further contribution in continuation of the same subject, which was published in the 'Philosophical Transactions' (Part I, 1886).

I named this colouring matter "enterochlorophyll," because after comparing it with all the animal pigments and plant pigments known to me, it seemed to resemble, both in its chemical and spectroscopic characters, the chlorophyll of plants.

In the latter paper, I endeavoured to describe the microscopic characters of this pigment, as it was found in the digestive gland, and I applied all the tests then considered to be distinctive of chlorophyll to the solutions of the pigment. I found that whereas enterochlorophyll appeared to be a chlorophyll, or a modified chlorophyll, it yet differed in some respects from chlorophyll, as it is obtained *directly* from fresh green leaves. Some recent writers have called in question the right to call this pigment by the above name, so I have reinvestigated the whole subject.

It was, however, necessary first of all to study the histology of the digestive gland, or gastric gland, as it is now named, and the microscopic characters of the pigment found in it. This has been done by Max Weber and Frenzel for the gland of crustacea, and by Barfurth and by Frenzel for the gland of mollusca.†

As can be gleaned from these observers, great difficulties attend the preparation of this gland for microscopic observation. I found after numerous failures that formol is the best fixative, used in stronger solution than it usually is employed in vertebrate histology. Thus it is necessary to employ solutions containing from 20 to 30 per cent. of

* 'Roy. Soc. Proc.,' vol. 35 (1883), p. 370.

† References are given in the complete paper.