An Attempt to Cultivate Parasitic Protozoa from Malignant Tumours, Vaccinia, Molluscum Contagiosum, and Certain Normal Tissues, together with Infection Experiments Carried out with the Culture Media, and a Note on the Treatment of Cancer. [Abstract]
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"An Attempt to Cultivate Parasitic Protozoa from Malignant Tumours, Vaccinia, Molluscum Contagiosum, and certain Normal Tissues, together with Infection Experiments carried out with the Culture Media, and a Note on the Treatment of Cancer." By SAMUEL G. SHATTOCK and CHARLES A. BALLANCE. Communicated by Sir JAMES PAGET, Bart., F.R.S. Received March 25,—Read May 2, 1895.

(Abstract.)

In a previous communication the authors showed that no organism belonging to the protophyta could be cultivated from malignant new growths, and in addition that carcinomatous and sarcomatous tumours from the human subject could not be transplanted so as to produce infection in the lower animals.

About this time the researches of Nils Sjöbring and Soudakewitch, and in this country, of Ruffer and others, showed that in sections of carcinoma stained by special methods there were present certain bodies which the above observers alleged to be parasitic protozoa. The authors then determined to try whether any protozoon could be cultivated from malignant new growths; and as it is well known that the *habitat* of the common amœba is damp sand or pond water, they decided to select sand and water as the medium for their investigation. Their first experiments were imperfect, for the reason that sufficient care was not used in the sterilisation of the materials and in the precautions taken during microscopic examination.

The only experiments in which they found living amœbæ were certain of the earlier, in which a possibility of external contamination was not rigidly excluded.

The following is their final method of procedure:—

Sand.—Silver sand, from which the finest part had been removed by sifting, was baked in a shallow thin iron dish over a large ring Bunsen for an hour. It was then transferred to the small deep capsules and Petri dishes about to be used, which had been previously baked for an hour at 150° C. in the hot-air steriliser. The capsules so charged were then baked for an hour at 150° C.; on removal from the steriliser the sand was heaped up on one side by shaking the capsule so that when the water was added part of the sand was submerged and part remained above the level of the fluid. The object of this proceeding was to obtain a littoral in order to ensure better aëration for any protozoa that might develop.

Water.—This was distilled and collected in a sterilised flask; it was subsequently boiled for from four to five hours with the object of rendering it quite sterile.

Transference of the Tumour.—The malignant tumour was received fresh from the operating theatre, the redundant tissue around was removed with sterilised scissors; then, with knives previously sterilised at 150° C. for an hour in an iron box, pieces of the growing edge were cut away, and transferred with sterilised forceps to the capsules; they were laid on the sand just beneath the water level. Two kinds of capsules were used: one, the ordinary Petri, the other considerably deeper, of less diameter, and furnished sometimes with a cover, like the Petri, at other times not.

Storage of the Capsules.—The capsules thus prepared and "infected" were placed between sterilised double dishes; the covers of these dishes were raised for a short distance by means of blocks of wood which had been soaked in solution of corrosive sublimate; the height was such as to allow free entrance of air, but not sufficient to expose the mouth of the lower dish. The double dishes were first sterilised by washing with sublimate solution, absolute alcohol and by heat.

The double dishes were finally placed, each pair, upon a sheet of glass beneath a capacious shade, both of which had been cleansed with sublimate solution. Most of the small, deep capsules had their covers removed as they were placed between the double dishes. The Petri capsules remained covered throughout. All the experiments were conducted in a private laboratory continuously heated.

Method of Microscopical Examination.—A glass rod and slide were sterilised in the flame, and allowed to cool. The shade was removed and the upper dish raised sufficiently to allow of the passage of the rod to the capsule. A little sand was then taken from three or four places along the littoral or from the neighbourhood of the piece of tumour and transferred to the slide; occasionally a hole was dug with the rod above the water level, and some of the deeper sand removed. The sand so removed was gently stroked with the rod on the slide until displaced from one end to the other; the slide was finally inclined so that enough fluid left the sand to make a microscopic preparation.

The examination was made with 1/12 apochromatic oil immersion, Zeiss, oculars 4 and 8. Occasionally a few drops of beef peptone broth were added to the capsule; and as the water became low from evaporation more was supplied.

In all the capsules bacteria developed, a fact which the authors regard as important inasmuch as such would furnish a pabulum for the growth of any protozoa that might develop.

The authors then give a table exhibiting the results of experiments made in twenty-three capsules; there were used nine scirrhous carcinomata of the breast, and five sarcomata from different sources; the sarcomata comprised one from the human biceps, one a mela-

notic growth of the cheek, two melanotic sarcomata from horses, and a spindle-celled mammary sarcoma from a dog. In the case of carcinomata the authors confined themselves to the typical scirrhus of the breast for the reason that in new growths involving superficial parts as the lip, tongue, &c., there is not only a chance of protophytic contamination, but also of protozoic, especially as certain protozoa are normal inhabitants of such mucous passages as the vagina and intestine.

The result of all these experiments was negative. No traces of protozoic life, whether as spores or amœbæ were encountered, although the examinations were made at regular intervals and repeated for periods of many months.

It may be added that a similar method of investigation carried out with *normal tissues* was equally negative of result.

The experiments so made were nineteen in number: seven were with human tissues (five subjects), muscle, pancreas, spleen, mamma; and twelve with the tissues of three dogs, submaxillary salivary gland, muscle, testicle, pancreas, kidney, and spleen.

The authors obtained equally negative results with vaccinia, molluscum contagiosum, the pancreas of Salamandra maculata, and muscles of the frog.

Vaccinia.—The experiments were made with freshly excised skin of the calf on which mature vesicles had been raised. They were carried out because bodies similar to those viewed as parasitic in carcinoma have been demonstrated in the epithelium of the vaccine and variolous vesicle.

Molluscum contagiosum was experimented with, because certain observers have held that the "molluscous bodies" in the lesions are protozoa.

Pancreas of Salamandra maculata was used because it has been alleged that the paranucleus seen in certain of the epithelial cells is a protozoon.

Muscles of the frog; because it has been stated that active amœbæ may readily be raised by some such method as that described from the tissue in question.

The experiments of the authors, conducted with the precautions detailed in the paper, especially the avoidance of contamination from the integument, prove that this is untrue.

The general conclusion the authors draw from the different series of experiments recounted in the paper is that by the method adopted no protozoa can be cultivated from the healthy living tissues, from malignant tumours (at least such as are not directly exposed to external contamination), from the lesions of vaccinia and molluscum contagiosum, from the salamander's pancreas, and from the muscles of the frog.

The authors record, in addition, a certain number of experiments made upon animals by means of sand infected with carcinoma and sarcoma, and containing amœbæ which later experiments showed to be adventitious. These experiments, which were all negative in result, included intravenous injection (dogs), the repeated "vaccination" of skin (white rats), intraperitoneal insertion (white rats).

Having previously found it impossible to raise a growth of carcinoma in any of the lower animals by transplantation of recent human carcinoma, they thought it possible that if the tumour was first incubated outside the body, the hypothetical protozoon might pass into some phase which would enable it to convey the infection. With this object, pieces of carcinoma incubated at the room temperature in milk, potassium oxalate plasma, and dilute broth were inserted into the peritoneal cavity of white rats, but with negative result.

In the case of two rats, the material used consisted of scirrhous carcinoma, which had been buried in a country garden for six weeks; the animals were kept alive for six months, but remained unaffected with the disease.

Under the head of Treatment are recorded the negative results following the subcutaneous injection of fresh aqueous extract of carcinoma and sarcoma in cases of both these diseases, as well as the similar injection of fresh sheep-serum.