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Studies on the filterability of mouse sarcoma.

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Rous[1] first pointed out that he was able to transmit the sarcoma of the Plymouth Rock hen to other fowls of the same species by means of a tumor extract which had been rendered cell free by passage through a Berkfeldt filter. Numerous attempts to obtain a similar filterable extract from mammalian tumors were uniformly negative until Gye[2] and Barnard[3] published their reports concerning the filterability of the mouse as well as the Rous chicken sarcoma. In their exhaustive studies of these filtrates they suggest the possibility, and offer experimental data to prove that the infective nature of the Rous tumor depended upon the adjustment of two agents: one, a filterable ultra-microscopic virus, common to all tumors, the other, an accessory or unstable chemical substance derived from the tissue itself, which they called the "specific factor." It was deemed advisable to repeat their experimental work, especially upon the mammalian tumors.

For the first point of attack, mouse sarcoma No. 37 was selected, which was the original tumor Gye and Barnard worked with at the laboratory of the National Research Institute. The tumor is a spindle cell sarcoma, grows very rapidly in practically 100 per cent, is very cellular, with very slight central necrosis, and attains full growth within 10 to 12 days.

Experiment No. 1: the tumor-bearing mouse was killed with gas, the carcass dipped for a few moments in a 4 per cent lysol solution, and the tumor was then excised by means of a cautery to insure perfect asepsis. One to two grams of the tumor tissue were placed in a tube containing 5 cc. of Hartley's broth plus 1 cc. of fresh rabbit serum. The tubes were put in a MacIntosh-Fildes jar for anaerobic incubation, the air was evacuated by a motor pump, and hydrogen gas was then allowed to enter the jar, in order to combine with any remaining oxygen under the

[1] Rous, P., *J. Exp. Med.*, 1911, xiii, 397.

[2] Gye, W. E., *Lancet*, 1925, July 18, 109.

[3] Barnard, J. E., *Lancet*, 1925, July 18, 117.

influence of electric sparking. To control complete anaerobiosis, a tube of methylene blue was also placed in the jar to indicate the absence of oxygen. The jar was placed in an incubator at 36.5 degrees C. and left for 23 to 24 hours. In almost every instance the indicator remained colorless, thus proving complete anaerobiosis. The culture tubes were then removed from the jar, and the sterility tested by stab and slant cultures on agar. The tumor cultures were now centrifuged for 10 to 12 minutes at 2700 revolutions per minute and only the supernatant fluid pipetted off, and 1 cc. injected into normal mice.

Several of these experimentally produced tumors have been inoculated into normal mice, and up to the present have gone through nine generations.

To rule out the possibility that an occasional living cell in the supernatant fluid may have given rise to tumor production, the fluid was filtered through a fine Berkfeldt filter. In this manner it was thought to meet the requirements of a cell free fluid, and to test the filterability of Mouse Sarcoma No. 37. With this end in view the following experiment was carried out:

TABLE I.
Injections of Mouse Sarcoma No. 37 Broth Serum Culture.

Cage No.	Date	Animals injected	No. tumors
12	10/28/25	4	3
13	10/29	4	3
15	10/30	4	4
17	11/3	4	2
19	11/6	4	3
21	11/11	6	0
23	11/12	1	0
26	11/14	1	0
41	12/5	10	2
45	12/9	11	8
46	12/10	11	2
48	12/14	7	0
85	1/19/26	1	0
102	2/2	3	1
104	2/2	3	0
106	2/5	2	0
108	2/6	4	1
110	2/11	1	0
112	2/13	5	0
114	2/16	4	1
116	2/18	4	2
117	2/20	3	2
120	2/23	4	0
Totals		105	34

Experiment No. 2: the tumor was excised aseptically and placed in 5 cc. tubes of Hartley's Broth plus rabbit serum, and incubated under anaerobic conditions for 23 to 24 hours. The tubes were then removed from the jars, and the fluid from these cultures filtered through Berkfeldt Filter "N", which had been tested against *B. prodigiosus*. The filtrate thus obtained was injected subcutaneously in doses varying from 1 cc. to 2 cc. into 164 mice, with 6 successful tumor productions.

TABLE II.
Injections of Filtrate of Mouse Sarcoma No. 37.

Animals injected	Tumor takes	Percentage tumor takes
164	6	3.66

To find out whether the anaerobically incubated tumor tissue was still alive after 24 hours incubation and capable of proliferation, the incubated tumor was removed and washed free of the serum broth by centrifuging it for 30 minutes in 3 changes of Ringer's or Tyrode's solution. The usual size fragments of this incubated tumor tissue were then inoculated into 43 normal mice, with but one successful "take". In the instances where the washing of the incubated tissue was omitted, there were two takes out of 38 inoculations. In other words it is safe to assume from these experiments that the tumor tissue itself is not sufficiently viable to account for the usual 30 per cent of takes when the centrifuged supernatant fluid of the tumor cultures is injected.

TABLE III.
Inoculation of Anaerobically Incubated Tumor Tissue.

Washed tumor tissue		Unwashed tumor tissue	
No. injected	Takes	No. injected	Takes
43	1	38	2

Experiment No. 3: Other experiments were conducted upon Mouse Carcinoma No. 63, supplied by the Imperial Cancer Research Fund of London. This carcinoma is somewhat hemorrhagic, does not grow as rapidly as the Mouse Sarcoma No. 37, requiring 14 to 21 days for full growth. The same technique of anaerobic cultivation was used with the Mouse Carcinoma

No. 63, as with the Mouse Sarcoma No. 37. After 23 to 24 hours incubation, the tubes were centrifuged for 10 to 12 minutes, the supernatant fluid pipetted off, and 1 to 1.5 cc. of the fluid was injected subcutaneously into 81 mice with 2 "takes".

TABLE IV.

Injection of Broth Serum Cultures of Mouse Carcinoma No. 63.

Mice injected	Tumor takes	Percentage tumor takes
81	2	2.5

Experiment No. 5: We applied the same technique as in Experiment No. 2, namely, we filtered the fluid of the incubated mouse Carcinoma No. 63. Out of 27 subcutaneous injections of the carcinoma filtrate there has not been up to the present a single successful tumor "take". This work, however, is still in progress.

Another point of interest is the time of the first appearance of the tumor nodule. The tumors produced by the injection of the supernatant fluid appeared from the 12th to the 24th day, averaging 17 days. The tumors produced by the injection of the cell-free filtrate of Mouse Sarcoma No. 37 were first observed on the 20th, 18th, 26th, 34th, 20th, and 22nd day respectively, averaging the 23rd day. All experimentally produced tumors upon excision were sectioned and examined microscopically, revealing the typical picture of the original Mouse Sarcoma No. 37.

For the present we do not wish to draw conclusions from our results, as the work had to be carried on under rather adverse conditions. For instance on account of the scarcity of supply of mice at one time during the winter, the mice had to be obtained from different sources, and irregular strains used. This seriously affected the behavior of our stock inoculations. It is only fair to assume that this had some effect upon the experimental work. At another time an incidental epidemic carried off a great many of the normal experimental animals, and therefore we are reporting only those experiments where the mice survived the period of 2 weeks after the experiment was initiated.

CONCLUSIONS.

1. We have been successful in obtaining 34 mouse sarcoma No. 37 out of 105 injections, when supernatant fluid of cultures of Mouse Sarcoma No. 37, incubated anaerobically for 23 to 24 hours, was used.

2. The Berkfeldt filtrate of Mouse Sarcoma No. 37 also incubated anaerobically yielded 6 tumors out of 164 injected animals.

3. Tumor tissue incubated anaerobically does not seem to be sufficiently viable to produce tumors when inoculated subcutaneously.

4. Mouse Carcinoma No. 63 yielded 2 tumors out of 81 injections of the supernatant fluid from incubated mouse carcinomata.

5. The filtered fluid from Mouse Carcinoma No. 63 so far has not produced a single successful tumor formation.

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Functioning autoplastic suprarenal transplants.

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No proof has as yet been presented that free transplants of the suprarenal gland function. Our experiments, with the view of obtaining evidence of function, were carried out on both the rat and the guinea pig. We realize the difficulties of interpreting results in favor of function in the rat, particularly if the clinical condition of the animal is taken as an index, because of the frequent occurrence of accessory suprarenal tissue. For crucial evidence of functioning free transplants we used the guinea pig because continued survival of this species after complete bilateral suprarenal ablation has never been reported. The best results are Rogoff's,[1] who, in a series of 17 animals with an interval of 2 to 3 weeks between the removal of the right and left glands, reports that 1 pig lived 28 days, while 14 of the 17 died during the first 8 days.

PROCEDURE.

In the rat both glands were removed in one sitting and placed in sterile physiological saline at about 39° C. Each gland was cut in half and the four parts were immediately transplanted into

[1] Rogoff, J. M., quoted by Stewart, G. N., *Physiol. Rev.*, 1924, iv, 167.