result of the operation, is of more importance in this connection than the cutting of the nerves. But, whatever may ultimately prove to be the real cause of the condition, it has been observed in eight cats that the operation is followed by marked change in the coagulability of the blood. Two of the eight cats died after two and three days of continuous bleeding from a needle prick in an ear vein; a third cat had the bleeding from a similar wound stopped only by the local application of cephalin after the cat had been bleeding for two days. This phenomenon occurred in these cats 4 to 6 weeks after the operation, when they had regained their original weight and were apparently in good condition. In the remaining 5 cats the condition was not as severe, but with them it was often difficult to determine the clotting time of the blood because only a thin clot would be formed. We are of the opinion, however, that the clotting time is practically normal, although the amount of fibrin formed is decidedly less than normal. Unfortunately, there has been no time for further analysis and this preliminary report is made because of the interest of this condition in connection with the problem of hemophilia.

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A Study of the Rous Chicken Sarcoma No. 1.

CARL F. CORI.

From the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.

Gye[1] concluded that malignant growth is a specific disease caused by a living organism. His crucial experiment was carried out as follows. A piece of chicken sarcoma was placed into a culture medium composed of Hartley's broth, 0.2 per cent KCl and rabbit serum, and was incubated at 37° C. After several days the supernatant fluid of this "culture" was no longer able to produce tumors in chickens. Next a piece of fresh tumor was ground up with sand and extracted with salt solution. The clear filtrate was then treated with chloroform. After removal of the chloroform *in vacuo* the filtrate proved to be inactive. How-

ever, if a mixture of chloroformed filtrate and culture fluid was injected into chickens, a typical sarcoma was produced. Gye assumed that the living organism which was supposed to have multiplied in the culture was in itself ineffective. It became active only when aided by a specific chemical factor, which was present in the chloroform treated filtrate. According to Gye, the chloroform treatment of the fresh tumor extract destroys the living organism contained in it, but leaves the chemical factor unimpaired. The present experiments deal with the conditions under which reactivation occurs and with the question whether "cultures" of other tissues than maligant tissue contain reactivating substances.

EXPERIMENTAL.

Gye's prescriptions, unless stated otherwise, were exactly followed and need, therefore, not be repeated here. It soon developed that the chief difficulty was the proper chloroformation of the sand filtrate. After three and one half hours incubation of 10 cc. filtrate at 37° C. with 0.2 cc. chloroform, the filtrate was generally inactive in doses of 1 cc. that were used by Gye, but it produced tumors when 2 to 5 cc. were injected. Since Gye's method of chloroformation seemed unsatisfactory, when applied to our tumor material, various changes were tried. It was found that the amount of chloroform used, and the number of times that the filtrate was mixed with the chloroform during the course of the incubation had a decided influence on the inactivation. The technique finally adopted was as follows: 10 cc. lots of clear sand filtrate were placed in test tubes kept in a water bath at 37° C. One cc. of chloroform was added and the filtrate mixed four times during 2 hours of incubation.

The next step was the preparation of the tumor cultures. All media used and all cultures made were tested repeatedly for sterility. It was found, in confirmation of Gye's results, that when 0.5 to 1 gm. of chicken sarcoma were placed in test tubes containing 6 cc. of rabbit serum KCl broth and incubated aerobically at 37° C., the clear supernatant fluid still produced tumors after 3 to 5 days incubation. Here again the dose of the supernatant fluid that was injected was found to be of importance. Cultures that proved to be inactive in the doses used by Gye gave rise to tumors, when larger doses were administered. Therefore, only 6 to 8 days old cultures were used. Similar cul-

tures were also prepared using the same medium and about 1 gm. of 14 days old chicken embryo.

Since so many uncertain factors seemed to play a rôle in the reactivation experiment of Gye, rather extensive control tests were necessary. It should be noted that there exists a difference in the susceptibility of the chickens for tumor material of a low virulence. It seemed, therefore, preferable to test the chloroformed filtrate, the supernatant fluid of the tumor culture, and the mixture of both on the same chicken, instead of using different chickens, as was done by Gye. Furthermore, different untreated sand filtrates, though prepared in exactly the same way, showed a variation in their activity.

In 11 reactivation experiments only 1 positive result was obtained. The course of these experiments was as follows: Untreated sand filtrate was tested on 1 chicken in doses of 0.05, 0.2, 0.5 and 1 cc. In the musculature of both wings and both breasts of 3 to 5 Plymouth Rock hens were then injected 5 cc. of chloroformed sand filtrate, 5 cc. of the supernatant fluid of the tumor culture, 4 cc. of a mixture of equal parts of treated filtrate and of culture fluid, and finally, 4 cc. of a mixture of equal parts of treated filtrate and of the supernatant fluid of an embryonic culture. The injections were made in the same order, starting at a different place in each chicken. The positive experiment (No. 4 of this series) gave the following results: The untreated sand filtrate produced tumors in doses of 0.2 to 1 cc. 5 cc. of the chloroformed filtrate, and 5 cc. of the fluid of the tumor cultures were inactive in all chickens. The mixture of treated filtrate and culture fluid from the chicken tumor produced a typical sarcoma in the right breast of chicken 1, and in the right wing of chicken 3. The mixture of treated filtrate and of fluid from the embryonic culture was negative in chickens 1 and 2, but produced a rapidly growing tumor in the left wing of chicken 3. The two tumors of chicken 3 were noticeable after 2 weeks, and killed the animal after 3 weeks. Chicken 1 developed a large tumor and died after 5 weeks.

DISCUSSION.

Obviously Gye's reactivation experiment is very hard to duplicate and works only under very strict experimental conditions. The cause for this is undoubtedly the difficulty with which the

proper degree of chloroformation is obtained. The margin between incomplete inactivation and irreversible inactivation due to an excess of chloroform action seems to be very narrow. It was thought safer to adjust conditions in such a way that they were close to an irreversible inactivation of the tumor producing agent rather than to have incomplete inactivation. This may account for the many negative experiments that we obtained. On the other hand, it is evident from our positive experiment that the reactivation is an unspecific process, since an embryonic culture proved just as effective in this respect as a tumor culture, yet it is a postulate of Gye's theory of the origin of cancer that the power to reactivate be confined exclusively to malignant tissue. The reactivation of the chloroformed filtrate can, therefore, not be regarded as proof that a culture of tumor tissue contains a living organism.

SUMMARY.

The supernatant fluid from a culture of embryonic tissue was just as effective in reactivating a chloroformed extract from a fresh tumor as was the supernatant fluid from a tumor culture. Due to the unspecific nature of the reactivation process, Gye's interpretation that a living organism is involved in this type of experiment, is not warranted.

[1] Gye, W. E., *The Lancet*, 1925, ccix, 109.