A NOTE ON THE SPECIFICITY OF CYTOTOXINS.*

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Since Metchnikoff and Landsteiner, working independently, demonstrated in the serum of rabbits treated with guinea pig spermatozoa the development of substances which immobilized and dissolved spermatozoa more or less completely, many attempts have been made to obtain cytotoxic sera specific for the different types of body cells.

It was early shown by von Dungern (1) that if rabbits were injected with the tracheal epithelium of cattle, the serum of the treated animals developed the power of causing the rapid disintegration of epithelial cells of that kind. He showed further that the serum was lytic for bovine erythrocytes, but that the antibodies possessed a greater affinity for tracheal epithelium. Metchnikoff (2) found that his spermatoxic serum was likewise hemolytic, but he maintained that the hemolytic power developed as the result of the injection of hemolytic receptors. Later investigators have obtained cytotoxic sera by injecting into animals of a foreign species the cells of practically every tissue of the body; leucocytes (Metchnikoff (3), Besredka (4)), liver (Delezenne (5), Deutsch (6)), kidney (Lindemann(7), Néfédieff (8), Bierry (9), Ascoli and Figari (10)), pancreas (Surmont (11)), adrenal (Bigart and Bernard (12)), thyroid (Gontscharukov (13), Mankovski (14)), heart muscle (Centanni (15)), ovary (Ceconi and Robecchi (16)), nervous tissue (Delezenne (17)), (Centanni (18)), syncytium (Ascoli (19), Liepmann (20)), etc.

Several methods have been employed for testing the immune sera as regards their specificity for the particular cells used as antigens. The methods of exposing cell emulsions to the action of the serum, as used by von Dungern in his trichotoxin studies, and by Flexner and Noguchi (21) in studies upon the action of snake venom on various organ cells, has not been found satisfactory for testing the specificity of cytotoxins. This may be due to the fact that one is dealing with the action of antibodies on dead and dying cells instead of on living functioning ones.

The technique which has been generally used consists in injecting the immune serum into the animal body, either subcutaneously, intraperitoneally, intravenously, or into arteries leading to particular organs. Functional disturbances and histological changes in various organs have served as criteria for determining the degree of specificity. More recently several other methods have been used, especially complement deviation and the epiphanin reaction. The results with the use of these methods are briefly summarized below.

Cytotoxic sera obtained by injecting the cells of an organ into an animal of a

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foreign species are relatively specific for the species from which the antigen was derived; that is, serious or fatal symptoms develop when animals of that species are treated with suitable quantities of the serum. Animals of other species are not, as a rule, so affected. These antibodies are spoken of as showing species specificity. Reports are conflicting as to whether or not they are to any degree specific for the particular organs or tissues used as antigens. That an absolute organ specificity does not exist is generally admitted, but many authors maintain, especially from a study of the lesions found in injected animals, that a relative specificity can be demonstrated. Lüdke and Schüller (22), for example, in a recent paper describe the production in dogs of a true nephritis with insignificant lesions in organs other than the kidney by the injection of the serum of rabbits immunized with dog kidney. Pearce (23), however, called attention to the fact, several years ago, that often the most striking lesions that one finds after the injection of a cytotoxic serum may be referable not to a direct toxic action on certain parenchymatous cells, but to the hemagglutinative and hemolytic properties of the serum, causing thrombosis, embolism, and hemorrhages, with secondary necroses in organs. Pearce argues further that since the cells of different organs evidently have certain receptor characteristics in common, it is hardly conceivable that specific somatogenic cytotoxins can be produced.

Beebe (24) has suggested that by the use of nucleoproteids instead of whole cells as antigens a closer approach to specificity can be reached. Wells (25), however, has recently pointed out that Beebe's suggestion is based on the false assumption that the nucleoproteids constitute the most important and most specific part of the cell.

Apart from its biological interest the problem is important on account of its bearing on the possibility of treating certain diseases, particularly cancer and exophthalmic goitre, with specific cytotoxic sera.

EXPERIMENTAL PART.

We have used a technique which appears to possess some advantages over previous methods; namely, the cultivation of tissues *in vitro*. By this method, as was suggested in a former communication (26), active living cells may be exposed to the action of immune sera, under conditions where the effects of such disturbing factors as agglutination, thrombosis, and hemorrhages can be eliminated.

Unfortunately the tissues that have been generally used by workers with other methods—liver, kidney, and other parenchymatous organs,—can not be cultivated satisfactorily in tissue cultures and therefore can not be utilized in experiments with the new technique. After a consideration of the tissues that might be used, rat sarcoma and rat embryo skin were chosen.

The next consideration was the choice of a foreign species which could be used for immunization against these tissues. It was necessary that two conditions be fulfilled: first, the plasma from normal animals of the species should be a suitable culture medium for rat tissues; and secondly, the animals should react well to the injections, that is, they should develop a strong cytotoxic serum. Guinea pigs fulfilled these two conditions. Two groups of guinea pigs were therefore injected, one with sarcoma, the other with rat embryo skin. Plasma from the two sets of animals was later used for tissue cultures for both sarcoma and skin.

Experiment 1.—The tissue for injection was prepared as follows: A sarcoma rat was bled to death and the tumor removed aseptically. The tumor tissue, which was extremely friable but not necrotic was cut up with scissors and washed with physiological salt solution to remove the excess of blood. The embryo skin was obtained from embryos near term. The skin was carefully dissected off, washed in salt solution, and finely divided. Very little blood was present.

Guinea pig 1 was inoculated subcutaneously through a Bashford needle with 0.15 gm. of rat sarcoma. Guinea pig 2 received a similar amount of embryo skin. Ten days later a second injection in each animal was made. Twelve days after the second injection each animal was bled according to the technique previously described (27) and plasma was obtained for tissue cultures. A normal guinea pig was also bled to obtain plasma for control preparations. Six series of preparations were made composed of rat sarcoma and rat embryo skin in plasma from each of the three guinea pigs. Each series was composed of fifteen preparations. They were incubated at 37° C. and examined daily. The controls of both sarcoma and skin in normal guinea pig plasma showed after twenty-four hours an active outwandering of cells among which mitotic figures were frequent. At the end of forty-eight hours growth was luxuriant in practically every preparation. The preparations containing immune plasma were, on the contrary, much less active, though the majority showed, especially on the third and fourth days, a fair outwandering of cells. A slight but definite difference was noted in the behavior of both kinds of tissue in the plasma from the two treated animals. For each tissue there was a more marked inhibition of growth in the plasma from guinea pig 1, injected with sarcoma, than in the plasma from the animal treated with embryo skin.

Experiment 2.—In order to secure a higher degree of immunization than was obtained in the first experiment, it was decided to use larger doses of tissue for injection. Guinea pig 3 received subcutaneously 0.65 gm. of rat sarcoma. Guinea pig 4 received a similar quantity of embryo skin, prepared as in experiment 1. Twelve days later plasma was obtained in the usual way from each animal, and from a third control normal guinea pig. Small pieces of sarcoma and skin were put up in the three kinds of plasma, making six sets, each composed of fifteen preparations. Examinations were made at the end of twelve hours and daily up to the sixth day of incubation.

The controls of both tissues grew well as usual, active outwandering of cells being noticed at the end of twelve hours. There was marked inhibition in the preparations of all four sets containing immune plasma. Many of the pieces of sarcoma and skin in plasma from both immune animals not only showed no growth but presented an amorphous granular appearance. Some of the preparations, however, showed after two days an outwandering of a small number of cells. As in experiment 1, a definite difference in the toxic power of the two immune plasmas was observed. In this instance, however, the more toxic plasma was that obtained from guinea pig 4, immunized with embryo skin. This plasma acted more strongly on both sarcoma and skin than the plasma from guinea pig 3, as shown by the more complete disintegration of the tissue fragments and the smaller number of cells which wandered out in the preparations that were positive.

Experiment 3.—The two guinea pigs used in experiment 2 received after bleeding second injections of 0.65 gm. of sarcoma and skin respectively. Ten days later they were again bled and their plasma was used for cultures of sarcoma and skin as before, with controls in normal guinea pig plasma. All the fragments of both sarcoma and skin in the immune plasma of both series showed rapid disintegration with no outwandering of cells. The controls showed excellent growths.

Experiment 4.—A guinea pig was injected intraperitoneally with 15 c.c. of defibrinated rat blood. Twenty-one days later plasma was obtained and used for culture preparations of sarcoma and skin. All preparations in normal guinea pig plasma showed active growth. Both tissues showed a feeble growth in a majority of the preparations in immune plasma. In the remaining negative preparations the disintegration of the tissue fragments was not so marked as in experiments 2 and 3.

DISCUSSION.

From the first experiment it is seen that relatively small doses of rat sarcoma or embryo skin are sufficient to induce in guinea pigs an antibody reaction such that the plasma of the treated animals becomes a poor medium for the growth of either sarcoma or skin cells. There was no evidence of even a relatively specific action on the part of the cytotoxins formed, the plasma from each animal exerting an equal inhibitory action on each kind of tissue. The plasma from the animal immunized with sarcoma proved to be slightly more active against each tissue than that obtained from the guinea pig immunized with embryo.

In the second experiment in which larger doses of tissue were used for immunization a more marked toxic action was demonstrable. Again no evidence of specificity was seen. As in experiment 1 plasma from one of the animals was found to be more active against both tissues. In this case, however, it proved to be that

obtained from the guinea pig immunized with embryo. It seems clear then that these differences in toxicity are to be referred to individual differences in guinea pigs in their reacting power and not to differences in the antigenic properties of the two tissues.

The third experiment shows that a still stronger cytotoxic serum may be obtained by a repetition of large doses of either tissue. Here again no evidence of specificity was found.

The fourth experiment shows that blood may be used to immunize against both sarcoma and skin, although the cytotoxic power of such a serum seems to be not so great as when either of the other tissues is used.

A second parallel set of experiments was carried out with two chick tissues, heart and intestine, for the production of cytotoxic sera in guinea pigs. The tissues were obtained from sixteen to twenty day chick embryos. These two tissues were selected because they exhibit characteristic types of growth in tissue cultures. From the heart a radial growth of connective tissue cells is regularly seen, while from pieces of intestine wide sheets of epithelial cells stretch out. Chick tissues grow fairly well in normal guinea pig plasma. In plasma from immunized guinea pigs the results were practically the same as those obtained with rat tissues; that is, no evidence of specificity was found. The experiments are therefore not given in detail.

SUMMARY.

1. The plasma of guinea pigs treated by injections of rat sarcoma exhibits a toxic action in tissue culture preparations on the cells of both rat sarcoma and rat embryo skin. Similarly, the plasma of guinea pigs immunized by injections of rat embryo skin is toxic for cells of both types.

2. Injection of rat blood immunizes against both sarcoma and embryo skin, although not so strongly as injections of the two tissues.

3. Guinea pigs receiving injections of either chick embryo heart or intestine develop cytotoxic substances for both of these tissues.

4. The preceding findings tend to show that cytotoxins formed after the injection of different body tissues into a foreign species are to no extent specific for the tissues injected.

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