Transfer to X-Irradiated Rabbits of Lymph Node Cells Incubated *in vitro* with *Shigella paradysenteriae*.* (21161)

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In an earlier paper it was reported that if 4 days after the injection of dysentery bacilli into the foot pad of the rabbit, cells were teased from the lymph node draining the site of injection and transferred into fresh recipient rabbits, the latter would show detectable serum titers of agglutinins to the dysentery organisms(1). A later study explored the time interval between the injection of antigen into the donor and the collection of its lymph node cells(2). It was found that if the lymph node cells were obtained as early as 10 minutes after the injection of the antigen and transferred to X-irradiated recipients, agglutinins would appear in the recipients' sera a few days later. Because of the shortness of this interval the possibility was explored that contact between the antigen and the cells of the donor could be reduced even further, *i.e.*, to contact *in vitro*. Preliminary results of this study are reported below.

Materials and methods. Lymph node cells were obtained from the popliteal and axillary lymph nodes of rabbits weighing 2-2½ kg. These rabbits had received either no injection of antigen or a subcutaneous injection of a heterologous antigen into the fore and hind foot pads. This heterologous material, 0.2 ml of packed sheep red blood cells was injected 4 days prior to sacrifice in order to stimulate hyperplasia of the local lymph node and thereby increase the yield of cells. The lymph nodes were excised and bathed in Tyrode's solution (made up without bicarbonate) containing 0.12% gelatin. After trimming the nodes the cells were teased free by means of 2 dissecting needles. At this time the number of cells was determined by the standard method for white blood cell counts. Each group of 225 x 10^6 cells present in the suspension was selected as the amount to be injected into one recipient rabbit. Subsequently, a given amount of antigen was added according to the number of recipients involved. The state of viability of the cells was determined by a cell count for which the cells were diluted in normal rabbit serum containing 0.7% trypan blue dye. Those cells failing to take up the blue dye were considered viable, while those stained blue were considered non-viable.

For serologic testing serial 2-fold dilutions of rabbit serum were made in volumes of 0.4 ml. To these were added 0.2 ml of a 0.05% suspension of alcohol-treated *Shigella paradysenteriae*. After shaking and after one hour of incubation at 37°C, the tubes were stored at 4°C for 48 hours, and then examined for evidence of agglutination.

The recipient rabbits were irradiated 24 hours prior to cell-transfer at a dosage of 425 r whole body X-irradiation (200 Kv.; 20 ma; 67.5 cm distance from the target to the bottom of the container, yielding 18 r (air) per minute; 0.5 mm copper plus 1 mm aluminum filtration; half-value layer 1 mm copper).

Results. The popliteal lymph nodes of rabbits which had not previously been injected with dysentery bacilli were excised and trimmed, and the cells were teased free. To the suspension of cells was added a given amount of a suspension of killed dysentery bacilli (0.07 ml of a 1% suspension of organisms per 225 x 10^6 cells). The mixture was incubated at 37°C for one hour, filtered through a number 80 stainless steel mesh and washed in a large volume of Tyrode-gelatin solution. After centrifugation at 1400 RPM for 5 minutes, the supernate was removed and the sediment resuspended in 20 volumes of

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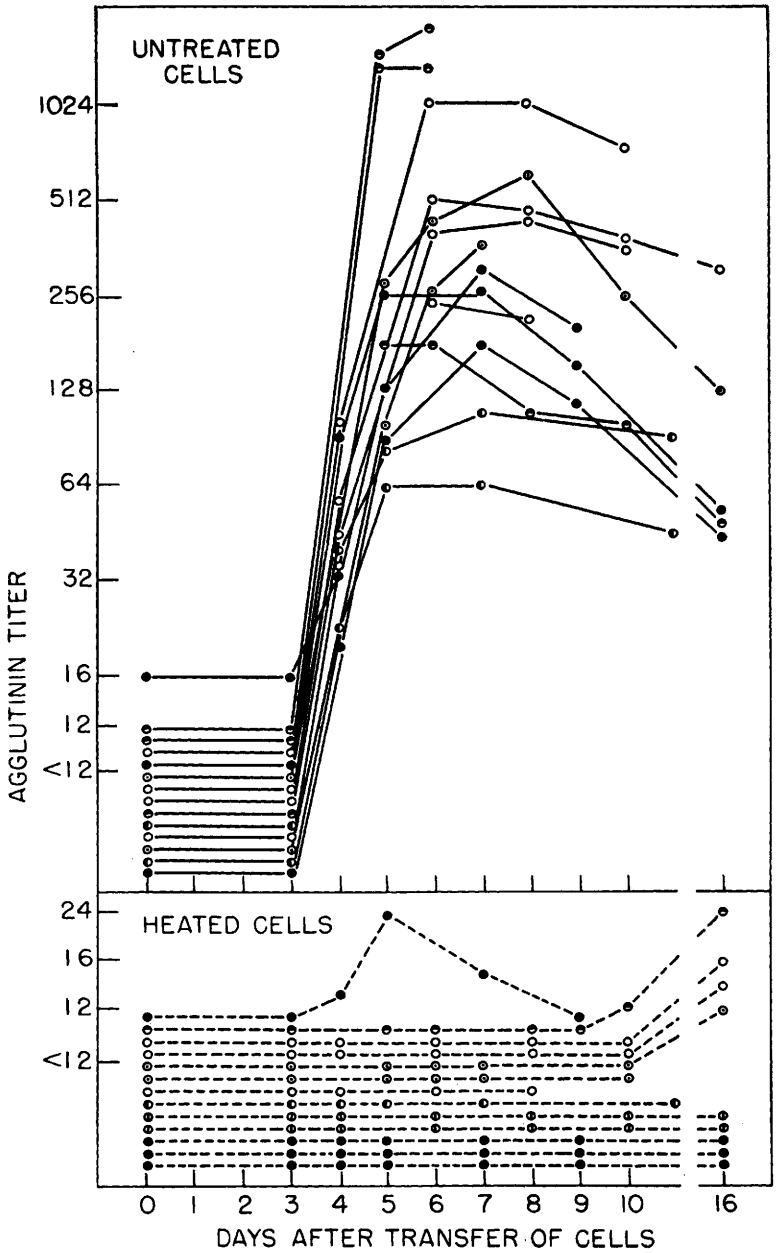

FIG. 1. Serum agglutinin titers of X-irradiated recipients of untreated and heated lymph node cells. Prior to transfer cells had been incubated *in vitro* with dysentery bacilli. Various symbols indicate different experiments. Termination of a curve before the end of the graph is due to death of the animal.

Tyrode-rabbit serum. This suspension was centrifuged at 600 RPM for 5 minutes, and the sediment resuspended in 5 additional volumes of Tyrode-rabbit serum mix. Of this cell suspension 0.9 ml was injected intravenously into each of the recipient rabbits which had received 425 r of X-irradiation 24 hours earlier. A portion of the cell suspension was heated for 60 minutes at 60°C, and of this portion 0.9 ml was injected into each of a group of recipient rabbits previously X-irradiated. Blood samples were obtained from all

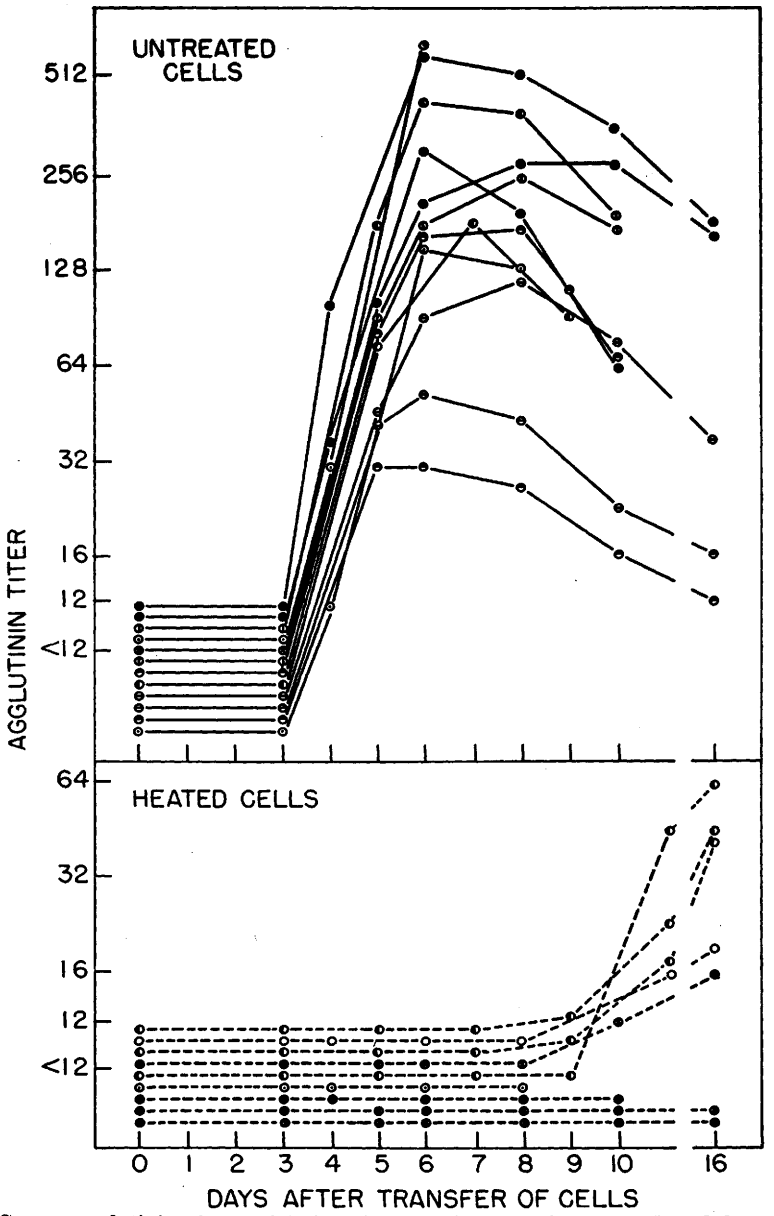

FIG. 2. Serum agglutinin titers of X-irradiated recipients of untreated and heated lymph node cells. Intravenous injection of washed cells into recipients was followed immediately by intravenous injection of dysentery bacilli into those animals.

recipients at regular intervals following the transfer of cells.

The sera of the recipients were tested for agglutinins to dysentery bacilli. No such agglutinins were detectable in the sera of these recipients up to and including the third day after transfer. On the fourth day agglutinins appeared in sera of the recipients of untreated cells. The level of this antibody increased on the fifth day and usually reached its peak by the sixth to eighth day. In the sera of the recipients of heated cells no detectable antibody appeared during the first week. Results of a group of such experiments are shown in Fig. 1.

In another series of experiments, cells de-

rived from the popliteal lymph nodes of rabbits not previously injected with dysentery bacilli were teased and suspended as for a typical cell transfer experiment (approximately 250 x 10^6 cells per ml). Of this suspension 0.9 ml was injected into the marginal ear vein of previously irradiated recipient rabbits, and this was followed immediately by injection of 1 ml of a 0.005% suspension of dysentery bacilli in the opposite marginal ear vein. The same procedure was also followed with cells which had been previously heated for 60 minutes at 60°C.

Again in this series of experiments, agglutinins appeared in the sera of the irradiated animals receiving untreated cells but not in the recipients of heated cells. Antibody generally appeared on the fourth day and thereafter followed a pattern similar to that observed in the experiments described above in which lymph node cells were incubated with dysentery bacilli. Fig. 2 shows the data obtained in typical experiments of this series.

Discussion. In the experiments described above there was no contact between the antigen and the cells obtained from the donor animal except for contact *in vitro,* and subsequently within the body of the recipient animal, or only the latter. The recipient animals had been irradiated in a dosage adequate to prevent active immunization by the amount of antigen present in the cell-suspensions injected. This was indicated by the fact that the injection of comparable suspensions which had been heated was not followed by the appearance of antibody titers of the same range in the sera of recipient animals.

It would appear, then, that antibody was produced in the recipient animals following the injection of uninjured lymph node cells which had had contact with the antigen only after removal of the cells from the tissues of the donor animal. There are at least 2 mechanisms which might account for these observations. One of these would involve a physiologic function of the transferred cells. In terms of such a hypothesis the cells would take up some form of the antigen during their incubation together *in vitro,* or subsequently during their coexistence in the blood or tissue fluids of the recipient animal, and then synthesize the antibody which subsequently appears in the serum. That sufficient contact between transferred cells and the antigenic material can occur in the body of the recipient animal is indicated by the fact that separate injection of cell and antigen into that animal can result in the production of antibody.

Another possible explanation of these data is that the transferred cells might release into the tissues of the irradiated animal some constituent or product of uninjured lymph node cells which enables the irradiated tissues of the recipient to carry out their normal function of antibody synthesis. A hypothesis of this nature has been offered by Jacobson and Robson in explanation of their data in a recent study. These authors have shown that if during 800 r or 500 r total-body X-irradiation the spleens of rabbits are shielded, left intact for 24 hours, and then removed surgically, the animals can form antibodies to antigens injected subsequently(3). The authors attribute this to a restoration of the functional capacity of the animal's antibody-forming cells by a humoral substance entering the general circulation from the originally shielded spleen during the 24-hour period prior to splenectomy. It is not possible, however, on the basis of the data presented in that study to exclude any role of cells entering the tissues of the irradiated animal from its shielded spleen.

Summary. Cells were teased from the popliteal lymph nodes of rabbits which had not been injected with dysentery bacilli. These cells were incubated with dysentery bacilli *in vitro* and transferred to X-irradiated recipients. Agglutinins were detected in the sera of such recipients on the fourth day after transfer. When the cells were heated prior to transfer agglutinins did not appear during the first week after transfer. Similar results were obtained when suspensions of lymph node cells and suspensions of dysentery organisms were injected separately into irradiated rabbits.

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Irradiation was administered by Dr. J. J. Smith of that department.

1. Harris, S., Harris, T. N., and Farber, M. B., *J. Immunol.*, 1954, v72, 148.

2. Harris, S., and Harris, T. N., *J. Exp. Med.*, 1954, in press.

3. Jacobson, L. O., and Robson, M. J., *J. Lab. and Clin. Med.*, 1952, v39, 169.

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Effect of Rate of Injection of Alloxan on Development of Diabetes in Rabbits.* (21162)

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Alloxan has been a useful laboratory tool in the production of hyperglycemia and the study of various aspects of diabetes, hypoinsulinism and carbohydrate metabolism. Knowledge of its use has been summarized and reviewed(1). Early in the course of our observations of animals made diabetic by alloxan, we noted that the toxic dose was very close to the adequate dose for the production of diabetes and that there was considerable variation in the mortality rate and in the number of animals which developed hyperglycemia. On carefully evaluating our technic and after reviewing the available literature, it appeared that differences in the rate of injection might explain some of the variations which we obtained. Although some authors have made note of the rapidity of injection this factor had not been studied extensively. As a result the study to be reported was undertaken.

The relatively rapid destruction of alloxan in blood and the fact that clamping the pancreatic vessels for a period of 5 minutes after injection prevented the appearance of diabetes (2) suggested that the persistence of adequate blood levels is essential in producing the desired Beta cell destruction. The observation that abnormalities in carbohydrate metabolism may occur despite an absence of demonstrable histological change(3) suggested that variable numbers of cells in any islet might be affected at any one time, or that functional changes might occur in normal appearing cells.

Materials and methods. The rabbits employed in this study were housed in our animal quarters for 3 to 8 weeks before they were used. They were fed purina rabbit chow and allowed water *ad lib.* No preliminary starvation was employed. Alloxan monohydrate was injected intravenously in a 3.3 or 5% solution in distilled water made slightly acid so that the pH was approximately 4.5. Rate of injection was controlled by means of a constant-rate infusion pump. Alloxan was injected in doses of 75, 100 and 150 mg/kg into the marginal ear vein. The rates of injection fell into 3 groups. (1) less than one minute, generally 15-30 seconds, (2) 5-20 minutes, generally about 10 minutes, and (3) between 30 and 50 minutes. After 3-4 hours, the animals were allowed food and water and 10 cc of a 10 or 20% solution of glucose was injected subcutaneously at 2 hour intervals for approximately 12 hours, to prevent the early hypoglycemia. After 24-48 hours, observations including blood sugar, urine volume and glucose were made. Those animals dying before the above studies were made are classified as dead. Few animals died between 48 hours and one week after injections, most of these being diabetic. Those animals showing definite hyperglycemia and glycosuria were classified as diabetic. A group of animals which showed transient hyperglycemia or glycosuria, or had abnormal glucose tolerance tests were classified as questionably diabetic.

Results. The results are presented in Fig.

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