

## STUDIES ON THE TRANSFER OF LYMPH NODE CELLS

### IV. EFFECTS OF X-IRRADIATION OF RECIPIENT RABBITS ON THE APPEARANCE OF ANTIBODY AFTER CELL TRANSFER\*

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In the previous report (1) on the effects of the transfer of lymph node cells from rabbits injected with antigenic material, the time interval between the injection of antigen into the donor animal and the collection of its regional lymph node cells was studied in relation to the effectiveness of those cells in transfer. The data obtained in that study suggested that with the progressive reduction of this interval there was a concomitant increase in the amount of antigen contained in the cell suspension and so transferred with the cells. At a given point in this exploration the amount of antibody actively formed by the tissues of the recipient in response to the antigen transferred was sufficient, and appeared at such time, as to render ambiguous the interpretation of the antibody levels observed in the sera of the recipient rabbits. It was therefore necessary not only to differentiate between the cell transfer effect and active immunization by transferred antigen but actually to suppress active immunization.

A number of reports in the literature (reviewed by the Taliaferros (2) and by Dixon (3)) have indicated that sufficient dosage of whole body x-irradiation can be effective in suppressing the active formation of antibodies if it is administered before the time of injection of the antigen. Accordingly, it was decided to use this means of obviating active formation of antibodies by the tissues of the recipient rabbit to antigenic material transferred with the cells. However, since the mechanism of the cell transfer effect is not understood, it became necessary to determine what effect such irradiation of the

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recipient animal might have on the cell transfer phenomenon itself. The present report will present the data obtained in the course of experiments on this subject.

#### *Methods and Materials*

The procedures used for the injection of antigen, collection of lymph node cells, serologic testing, and x-irradiation of recipient rabbits have been described in detail in the previous report (1).

#### EXPERIMENTAL

*The Effect of X-Irradiation on the Cell Transfer Effect.*—For the reasons given above, it had been decided to attempt the use of x-irradiation of recipient rabbits as a means of suppressing active immunization in these animals. It was necessary, however, to determine first whether x-irradiation would in any way affect the transfer effect itself. Experiments were set up in which cells of the popliteal lymph node were obtained 3 days after the injection of dysentery bacilli into the hind foot pads of rabbits. These cells were washed, suspended, and then injected intravenously into recipient rabbits. Portions of the suspension of cells were injected into normal recipients, and other portions into recipients which had been exposed to 425 r whole body x-irradiation 24 hours prior to the transfer. Both groups of recipients were then bled at the usual intervals.

When the sera of the recipients were tested for the presence of agglutinins to dysentery bacilli it was observed that antibodies appeared on the 1st day after transfer in both groups, irradiated and normal. The agglutinin titer rose, reaching a peak on the 3rd or 4th day and then began to decline, the pattern being similar in both groups of recipients. In the first such experiment performed there was a suggestion of higher titers occurring in the group of x-irradiated recipients. This experiment was repeated and again the pattern of antibody development was similar in both groups except that the titers were higher in the group of irradiated animals. These two experiments are shown in Fig. 1.

In further repetitions of this type of experiment it was found that the serum titers of the irradiated recipients were consistently higher than those of the controls. The degree of this difference showed some variation from experiment to experiment. The agglutinin titers of recipient rabbits, irradiated and normal, as they occurred in 9 experiments, are shown in a composite chart, Fig. 2. The agglutinin titer of the serum of each rabbit on the respective days after transfer of cells is indicated by a point, and the X marks indicate the geometric mean titer of the entire group of animals on the respective days after transfer.

It was noticed that the peak titer in both irradiated and normal recipients

was generally reached on the 3rd day, with a possible small rise in some cases on the 4th day. Therefore the curve of the normal recipients was similar to that seen previously (4) and began to decline by the 5th day. It appeared that the titers of irradiated recipients tended to remain higher for a longer period

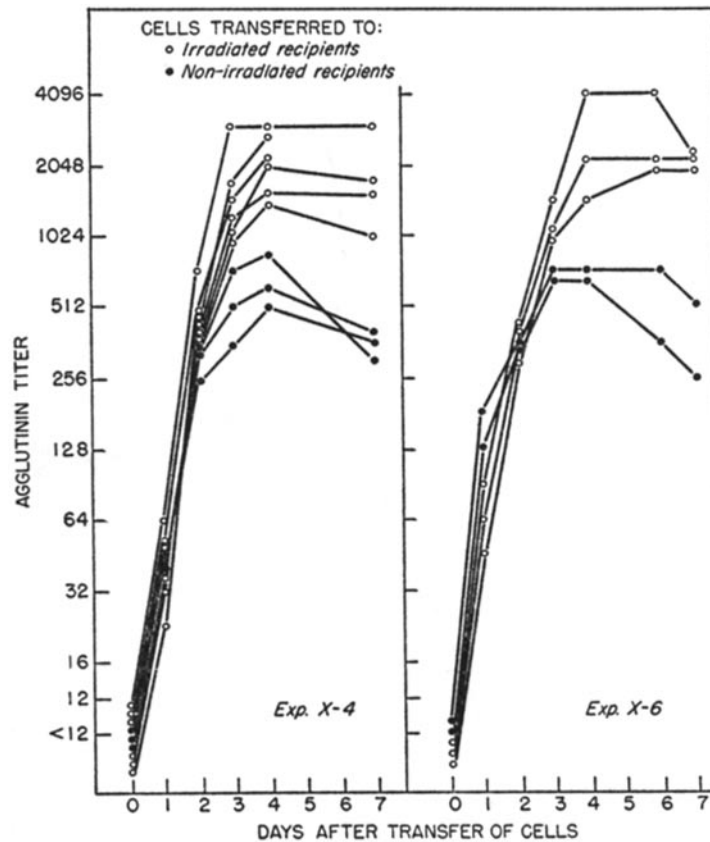


FIG. 1. Serum agglutinin titers of normal and x-irradiated recipients. Cells obtained 3 days after the injection of dysentery bacilli into the hind foot pads of donor rabbits. Two experiments are represented.

of time. Because of the variability from rabbit to rabbit data could be better examined by following the course of antibody titer in given animals and comparing the peak titer in each animal with that found 3 and 4 days thereafter. The titers of those rabbits which had all been bled on the 3rd, and 6th or 7th day after transfer were tabulated with regard to the change in titer from the 3rd to 6th and 3rd to 7th day for each such animal. These were grouped according to whether the recipient was irradiated or not and also whether the

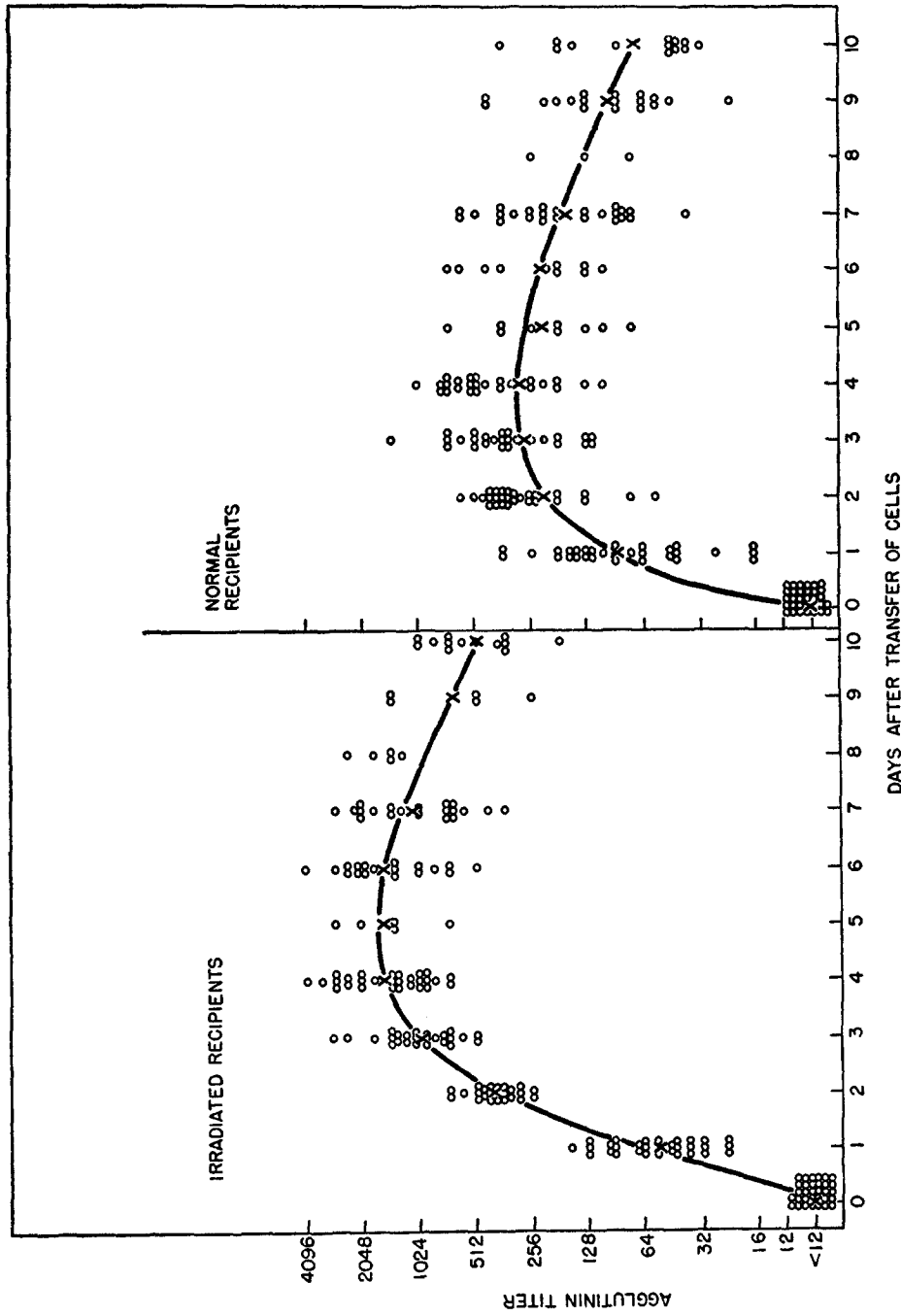


FIG. 2. Serum agglutinin titers of normal and x-irradiated recipients of 3 day cells. Each circle represents the titer of an individual recipient on the day indicated by the abscissa. The solid line connects the geometric mean titers obtained on each day within each of the two groups.

titer had dropped in these intervals or remained the same or shown a rise. Such comparisons yielded the data shown in Table I. It can be seen in this table that in each case the majority of irradiated recipients showed as high a titer on the 6th or 7th day as on the 3rd day, whereas in the case of the normal recipients the antibody titers were lower on the later days than on the 3rd day after transfer. It was thought of interest in this connection to see what happened to antibody titers in the sera of normal and irradiated recipients following the transfusion of a hyperimmune rabbit serum prepared against *Shigella paradysenteriae*. Accordingly, 14 ml. of a rabbit serum pool, having an agglutinin titer of 12,000 per ml. were injected intravenously into each of 2 normal recipients and into 2 recipients x-irradiated 24 hours earlier. These recipients were bled 2 hours after the injection and periodically thereafter. When the recipients' sera were tested for antidysentery agglutinins it was

TABLE I  
*Changes in Antibody Titers of Individual Rabbits from the 3rd to the 6th and from the 3rd to the 7th Day after Transfer of 3 Day Cells*

Recipients	3 to 6 days			3 to 7 days			Number of recipients represented in both 3 to 6, and 3 to 7 days
	Total No. of recipients	Fall in titer	Rise or same titer	Total No. of recipients	Fall in titer	Rise or same titer	
X-irradiated	17	4	13	18	4	14	12
Normal	12	10	2	21	19	2	9

observed that the rate of decline in titer was similar in both normal and x-irradiated rabbits.

*X-Irradiation after the Transfer of Lymph Node Cells.*—Since whole body x-irradiation of recipients 24 hours prior to the injection of lymph node cells did not in any way reduce or suppress the subsequent appearance of antibody in the recipients, it was thought of interest to study the effect of whole body x-irradiation given very shortly after, and 1 or more days after, the transfusion of lymph node cells.

Experiments were set up in which cells were again teased from popliteal lymph nodes 3 days after the injection of dysentery bacilli into the hind foot pad of rabbits. Aliquots of these suspensions were injected into some recipient rabbits which had not been irradiated, into others which had been x-irradiated 24 hours earlier, and into still others which were irradiated within 1 hour of receiving the cells. The sera of the recipient rabbits were tested for agglutinins to dysentery bacilli, and all showed agglutinins on the 1st day. As had been noted above, the sera of the recipients irradiated 24 hours prior to the injection of lymph node cells showed higher titers than those of non-

irradiated recipients. On the other hand those recipients irradiated within 1 hour after the transfusion of cells showed markedly lower titers, although on the 1st day after transfer the titers were similar in both groups. Two such experiments are illustrated in Fig. 3.

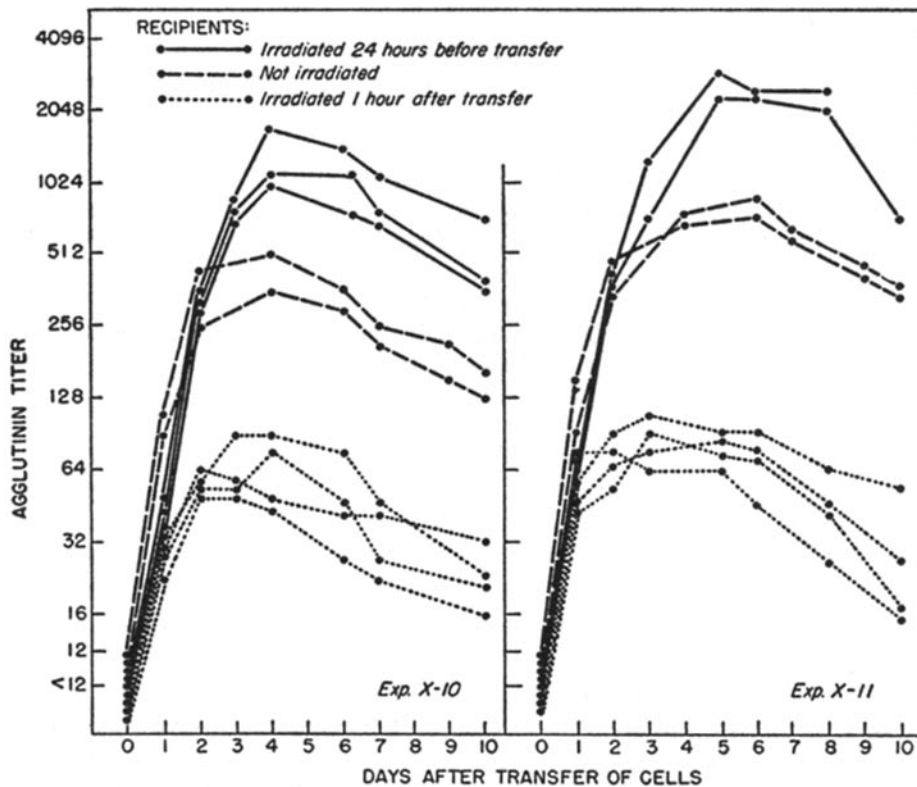


FIG. 3. Serum agglutinin titers of non-irradiated recipients and of recipients irradiated 24 hours before or 1 hour after the transfer of 3 day cells.

This type of experiment was repeated and enlarged to include recipients which received lymph node cells and were irradiated 1, 2, or 3 days later. In three such experiments it was observed that the serum titers of recipients irradiated 1 day after cell transfer did not show substantial differences from those of non-irradiated recipients. There appeared to be a small but consistent difference in the direction of lower titers in the groups irradiated 1 day after transfer. Geometric mean titers were calculated for each group of recipients, irradiated and normal, in each of the experiments, for every bleeding after the transfer of cells. As can be seen in Fig. 4, the geometric mean titers of the irradiated recipients of each experiment were lower than the non-irradiated

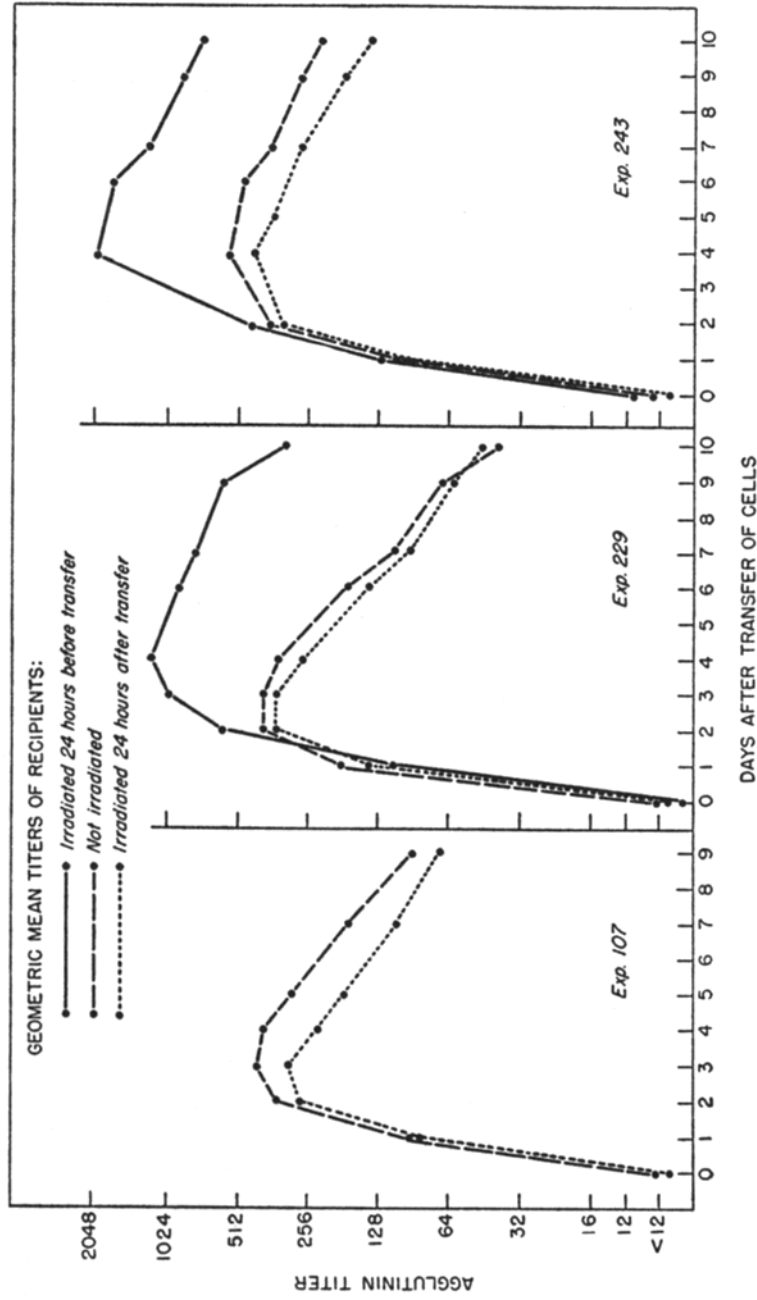


Fig. 4. Geometric mean titers of non-irradiated recipients, and of recipients irradiated 24 hours before or 24 hours after the transfer of 3 day cells. Each mean titer represents the data obtained from 3 rabbits.

rabbits. This difference was small in every case, but always in the same direction. When recipients were irradiated 2 days after the transfer of cells the serum titers observed were similar to those of non-irradiated recipients. Similar results were found in the case of recipients irradiated 3 days after the transfer of cells.

#### DISCUSSION

*The Effects of X-Irradiation Administered before Cell Transfer.*—The data presented above, on the effect of x-irradiation on the cell-transfer effect, which were obtained for the sake of application to the experiments reported in the previous paper, also have some bearing on the question of the source of the antibody found in the recipient's serum. First, the enhancement of titers due to cell transfer by a dosage of irradiation which is highly effective in suppressing antibody formation by the tissues of the animal offers further evidence against active immunization as the source of the antibody found in cell transfer studies. Second, the clear contrast between the injurious effects of the x-rays on the recipient (50 per cent mortality in a 2 week period) and the increased effectiveness of the transferred cells suggests the independence of these cells from the physiologic processes of the recipient and the passive role of the latter in the immunologic aspects of this phenomenon.

The fact that the antibody titers of the x-irradiated animals are higher than those of the normal recipients of such cells is also of interest. One physiologic effect of x-irradiation which might be considered in terms of its contribution to this effect is that of hemoconcentration due to changes in blood volume. The effects of severe irradiation on changes in blood volume which have been reported vary among species. In the case of the rabbit a recent detailed study of such effects has indicated a decrease in plasma volume, which would tend to give rise to higher observed concentration of antibodies. However, the fall in plasma volume observed by these authors was only to 80 or 85 per cent of the normal value at about 1 week after x-irradiation (5), and such decrease in plasma volume would not account for increases in antibody titer of the order observed in the irradiated rabbits in the present study.

Another physiologic mechanism which must be considered in this connection is that of phagocytosis. Although the transfused cells are homologous, they are derived from another animal than the recipient, are injected rather rapidly in considerable numbers, and have been subjected to relatively unphysiologic conditions en route from donor to recipient. It might be expected, therefore, that such cells would to some extent be treated as elements foreign to the economy of the recipient. It has, in fact, been shown by several groups of workers that homologous leucocytes introduced intravenously in large numbers are rapidly filtered out of the blood into the lung capillaries in the case of several mammalian species, and then disappear from that area (reference 6,



and see review by Fichtelius (7)). Since transfused leucocytes are thus handled by the tissues of the recipient animal as extraneous elements, they may well be subject to phagocytosis or other injury by the ordinary defense mechanisms of that animal.

As to the effects of irradiation on phagocytosis, the literature, which has been reviewed by the Taliaferros (2), contains apparently conflicting reports of work done under a variety of conditions. In a recent study by Esplin (8) suspensions of micrococci were injected intraperitoneally into mice which had been irradiated 2 to 6 days earlier (350 r). It was found that the percentage of phagocytes ingesting bacteria was increased, but more of the bacterial cells could be found free in the peritoneum of the irradiated animals than in the controls, suggesting that the actual extent of phagocytosis was less in the x-irradiated mice.

A significant decrease in the number of phagocytes in the irradiated recipients of transferred lymph node cells might account for all or part of the higher antibody titers in those animals, in comparison with the titers in non-irradiated recipients, by a sparing effect on the transferred cells. An ancillary factor to such an effect might be a "choking" of the available phagocytes by debris of the many cells of the recipient's own tissues which were severely injured by the irradiation a day before the injection. It would be consistent with this suggestion of a sparing of transferred cells by phagocytes of the irradiated recipient that the titers in these animals are no higher at the end of 1 day after transfer than are the titers in non-irradiated recipients. Thus this difference between irradiated and non-irradiated animals does not appear at the outset, but only after there has been time for the phagocytic or other defense mechanisms of normal recipient animals to injure or reduce the effectiveness of the transferred cells. This hypothesis would also be consistent with the findings that the titers of the irradiated recipients tend to remain near the level of the peak titer for that animal for a longer period than in the case of the non-irradiated control, since the greater freedom of the transferred cells from the effects of the phagocytes of the recipient would give the transferred cells a longer average period of activity per cell.

It is of course not possible to say whether phagocytosis is the mechanism which is involved in the higher antibody titers found in the irradiated recipients as compared with non-irradiated animals. However, an interpretation similar to that offered above would probably apply to any defense mechanism of the recipients' tissues which might in the normal animal reduce the longevity or effectiveness of the transferred lymph node cells, since the dosage of irradiation used in these studies would severely affect many cell systems other than the lymphatic, although probably to a lesser degree.

The possibility was also considered that the catabolism of the antibody produced in the recipient might be different in normal and irradiated animals.

However, on comparing the curves of antibody titers after transfusion of hyper-immune dysentery serum to normal and irradiated recipients there did not seem to be sufficient difference in the rate of disappearance of agglutinins from the sera of the two groups of recipients to account for the higher titers in irradiated animals after the transfer of lymph node cells.

*The Effect of X-Irradiation Administered after Cell-Transfer.*—The effect of x-irradiation of the recipient animals after the transfer of lymph node cells also presents points of interest. When recipient rabbits were irradiated within an hour after cell transfer their subsequent antibody titers were found to be lower than those of non-irradiated recipients of the same cell suspension, as can be seen in Fig. 3. On one hand it might be considered that these results were to be expected, since we are dealing with cells of high radiosensitivity, the decrease in the recipients' antibody titers being an expression of injury to the transferred cells by the x-irradiation. On the other hand, these results would perhaps not be expected in terms of what is known of the effects of x-irradiation on ordinary active immunization. The literature on this subject, which has been reviewed, with the presentation of additional data, by Dixon *et al.* (3), has indicated that irradiation can cause a failure or marked diminution of antibody response if administered before the antigen is injected, but not if administered after the injection of antigen. Thus, if the donor rabbits had been involved in ordinary active immunization, rather than in cell transfer, and if those donor rabbits had been irradiated 3 days after their injection with antigen, it would be expected that the irradiation would have no measurable effect on their subsequent antibody titers. There would thus be no indication of any suppression of antibody formation by the cells which were already engaged in that function. However, the data obtained in the present study indicated that when lymph node cells were removed from donor rabbits 3 days after their injection with antigen, and were transferred to recipient rabbits which were then irradiated an hour later, the maximal agglutinin titers observed in the recipients' sera were markedly lower than those found in the case of irradiation prior to cell transfer, or even in the absence of irradiation. Since these cells, among others, would presumably not have been affected in their ability to form antibodies had they been exposed to irradiation while in their original site, the question arises as to what factors might account for this difference. One obvious difference in the circumstances of the actual and hypothetical lymph node cells under discussion is that the latter would have experienced the irradiation and the subsequent period in their original environment of organ and host, whereas the transferred cells are present, during this period, free in suspension in the blood stream of the recipient. It may be that lymph node cells, which are at best quite radiosensitive, are even more susceptible to injury by irradiation under these circumstances than they

would be in their own organ, perhaps because the chemical environment in the irradiated animal body is altered to a greater degree in blood than in organized tissues, or perhaps because the blood collects deleterious products of tissue destruction from various tissues of the body.

This suppressive effect of the irradiation on antibody formation following transfer is of rather shorter duration than might be expected, since irradiation of the recipient only 1 day after the transfer results in a barely detectable degree of suppression in comparison with non-irradiated controls. We may allow approximately another half day for the development of the full degree of irradiation injury by the majority of the transferred cells, as would be suggested by the work of Shrek (9), but even a 2 day period of irradiation injury, of diminishing degree, to the transferred cells would seem to be at variance with the 4 day period over which these cells are presumably functioning in the recipient which is not irradiated after transfer (as judged by the time curve of rising titer in the latter).

This limited period of increased susceptibility to irradiation injury of the transferred cells would be in keeping with the hypothesis suggested above, that the blood of the irradiated animal might offer a worse environment than its tissues for the irradiated cells, if we assume that within 24 hours of cell transfer a large part of the cells have left the blood and entered the tissues. Of interest in this connection is the recent observation by Fichtelius (7) that if radiolabelled lymphocytes are transfused to an animal of the same species they can be traced predominantly to the spleen within 24 hours.

#### SUMMARY

Cells of the popliteal lymph node were teased 3 days after the injection of *Shigella paradysenteriae* into the hind foot pads of rabbits. These cells were transferred to normal and x-irradiated recipients. It was noted that the serum titers of dysentery agglutinins in irradiated recipients were higher than in normal recipients. This was represented both in a higher peak titer and a tendency to remain higher for a longer period than in normal animals.

Recipients were x-irradiated within 1 hour after receiving cells of the lymph node prepared as indicated above. The serum titers of these recipients were markedly reduced in comparison with those of non-irradiated control animals. If the irradiation of the recipients followed the transfer of cells by a day, however, this difference was much smaller and in the case of a 2 day interval after the transfer of the lymph node cells the irradiation appeared to have no effect on the resulting serum titer.

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