STUDIES ON THE TRANSFER OF LYMPH NODE CELLS

VIII. The Transfer of Cells from Blood and Peritoneal Exudates Incubated in Vitro with Antigenic Material Derived from Shigella paradysenteriae*

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In earlier studies of this series data were presented on the appearance of antibodies in rabbits which had received cells obtained from lymph nodes draining sites of injection of antigens in donor rabbits (1). More recently, the appearance of agglutinins to *Shigella paradysenteriae* has been described in rabbits which received lymph node cells incubated *in vitro* with suspensions of *Shigella*, or with antigenic material derived from these organisms (2, 3). In the earlier, or donor injection system, it had been necessary to use cells from lymphatic organs at an appropriate site and time relative to those of the injection of antigen. Thus a few days after the injection of antigens into the pads of hind feet and fore feet, the draining lymph nodes (popliteal and axillary, respectively) could be used as sources of cells for transfer, and similarly, after intravenous injections of bacterial suspensions, the spleen (4). However, the observation that the cell transfer effect could be obtained with cells incubated with antigenic material *in vitro* made it possible to examine suspensions of cells other than those obtained from fixed organs of the lymphatic system. The experiments to be described below were carried out with cells from two sources, blood and peritoneal inflammatory exudates, which yield cell populations of different composition than do suspensions of cells obtained from lymph nodes.

Materials and Methods

Animals.—The rabbits used as donors of cells for transfer were adult animals, of approximately 2 kg., which had not previously been injected with dysentery organisms or with materials prepared therefrom. Recipient rabbits were approximately 1 kg.

Antigenic Material.—All antigenic materials used in these experiments were prepared from

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suspensions of *Shigella paradysenteriae*. The earlier experiments with blood cells were done with *Shigella*-treated serum; that is, serum which had been incubated with one-tenth volume of a 10 per cent suspension of *Shigella* for 30 minutes at 37°C. and then overnight at 4°C., and from which the organisms were then removed by centrifugation and Seitz filtration. The majority of the experiments were performed with filtrates of trypsin-treated suspensions of *Shigella*, as described in the previous paper (5).

White Blood Cells.—Blood was obtained from presumably normal rabbits by cardiac puncture, approximately 40 ml. being obtained from each animal. Glassware used in collecting and handling the blood was pretreated with silicone (SC87, General Electric Company). In a few instances fibrin was removed from the blood as it was formed by shaking the blood with glass beads. In most instances, however, coagulation was prevented, either by the addition of heparin (3 mg. of heparin per 50 ml. of blood, with rapid cooling of the mixture) or by passing the blood directly from the puncture needle through polyvinyl plastic tubing into a column of cation exchange resin (6) in order to remove calcium (amberlite 150, in Na phase, the column 5 cm. high and 1.5 cm. in diameter).

In order to accelerate the sedimentation of erythrocytes, 30 ml. portions of the whole blood were added to large test tubes containing 15 ml. of a 3 per cent solution of human fibrinogen, or, more usually, 30 ml. of a 3 per cent solution of gelatin[1] in 0.75 per cent NaCl. After the tubes had been kept for 1 hour in the vertical position in an ice bath, the extent of sedimentation of erythrocytes was such that it was possible to remove by suction an overlying column of fluid, which contained most of the leucocytes, with relatively few erythrocytes. The cells were collected from this suspension by centrifugation for 10 minutes at 2000 R.P.M., and were then washed once with Tyrode's solution. To the suspension of washed cells was added the solution of antigenic material in a volume 1 per cent of the cell suspension and at a concentration 100 times that desired for incubation of the cells. The flask containing this suspension was maintained in a 37°C. water bath, with frequent agitation, for 30 minutes, after which the cells were collected by centrifugation, washed twice in Tyrode's solution and suspended for injection in serum-Tyrode's. Cell counts and smears for cytologic examination were made of this suspension, and a part of it was treated with sodium iodoacetate at a concentration of 0.01 M for 30 minutes. This treatment had been found to inactivate the lymph node cells in cell-transfer experiments.

Peritoneal Exudates.—Two preparations were used as irritants for the production of peritoneal exudates. The first was an emulsion of a concentrate of lanolin (falba, Pfaltz and Bauer, Inc., New York) in water, thinned with light mineral oil (atreol 9, Atlantic Refining Co.). This mixture, which will be designated below as FMO, was prepared by melting falba and allowing a given volume of it to solidify (at room temperature) in a layer adherent to the inner surface of the lower half of a wide test-tube. An equal volume of isotonic salt solution was then added to the tube and the contents of the tube were stirred by a motor driven stirring rod until uniform opaque whitish paste was produced. Four additional volumes of the light mineral oil were then added and the stirring was continued until a uniform viscous liquid was obtained. This procedure of emulsification is that described by Freund and McDermott (7). Of this mixture 10 ml. or, in some later experiments, 20 ml. was injected into the peritoneal cavity of donor rabbits. The other irritant used in the production of peritoneal exudates was a heavy mineral oil (drakeol, Pennsylvania Refining Co., Butler, Pennsylvania) which was injected intraperitoneally in doses of 100 ml.

At various intervals after the injection of these irritants the donor animals were sacrificed

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[1] The gelatin solution was kindly supplied by the Kind and Knox Gelatine Co., Camden, New Jersey. For use with ion exchange resin–treated blood this solution was previously passed through a similar column in order to remove traces of divalent cations.

and placed on the operating board in a supine position. A midline skin incision from the xiphoid process to the pubis was made and the peritoneum was exposed. The peritoneum was then incised under sterile conditions and the peritoneal exudate was withdrawn by hypodermic syringe with the use of large volumes of Tyrode's solution containing 6 units per ml. of heparin to wash out the cells of the exudate (up to 200 ml. per rabbit in the case of those injected with heavy oil). When necessary the oil phase was separated from the cell suspension by means of a separatory funnel. The cells were collected by centrifugation, washed, treated with antigen, washed again, and resuspended for injection as in the case of the cells derived from blood, and portions for control injections were similarly treated with sodium iodoacetate. Recipient animals received an intravenous injection of 1000 units of heparin shortly before the transfer of the cells.

Irradiation of Recipient Rabbits.—The animals were exposed on the day before cell transfer to 425 r of deep Roentgen rays, with the following factors: 200 kv., 20 ma., 67.5 cm. distance to the bottom of the container yielding 18 r per minute in air. Filtration was by 1 mm. aluminum + 0.5 mm. copper.

Cytologic Examination.—Smears of the suspensions of cells transferred were made before injection and stained by May-Gruenwald-Giemsa or with Wright's stain. Differential blood counts were made of either 200 or 400 cells, and the results were expressed in percentages, as shown in the tables.

EXPERIMENTAL

1. Incubation of Leucocytes of the Blood with Shigella-Treated Serum.—

In the first group of experiments leucocytes obtained from the blood of normal rabbits were incubated with a preparation of *Shigella*-treated serum as described above. After being washed twice the cells were transferred to irradiated recipients. In a total of 4 such experiments, 12 recipients were injected with an average of 220×10^6 of fresh (untreated) cells per rabbit, and 6 rabbits with iodoacetate-treated cells.

Of the 12 recipients of untreated cells, 9 developed no agglutinins to dysentery bacilli within the 1st week after transfer. Of the remaining 3 recipients, 2 developed a peak titer of 24 and the remaining animal, a peak titer of 48. None of the 6 control rabbits showed agglutinins to dysentery bacilli. In view of the occurrence of barely measurable agglutinin titers in the sera of some of the recipient animals it was considered possible that the infrequent occurrence and low values of these titers might be due to an inadequate concentration of antigenic material in *Shigella*-treated serum. Accordingly, experiments were undertaken with soluble antigen derived from *Shigella paradysenteriae* by incubation with trypsin.

2. Incubation of Leucocytes of the Blood with Filtrates of Trypsin-Treated Bacilli.—

In 13 experiments leucocytes were prepared from the blood of donor rabbits by the methods described above. These cells were incubated *in vitro* with filtrates of trypsin-treated suspensions of dysentery bacilli, washed, and transferred to irradiated recipients. The numbers of cells transferred ranged between 45×10^6 to 217×10^6, with an average of 113×10^6. Table I summarizes the cytologic and serologic findings in these experiments. Of a total

of 47 recipients, 8 animals, or 17 per cent, developed no measurable level of agglutinins; 16, or 34 per cent, developed titers in the range of 16 to 48; 8, or 17 per cent, developed a level of titer of 64; and 15, or 32 per cent developed titers of 96 or higher. Among the 16 recipients of iodoacetate-treated cells 12 showed no agglutinins at our threshold of measurement, 3 developed barely detectable titers of 12, and in one such animal a titer of 24 was found.

TABLE I

Cytologic and Serologic Data of Experiments Involving Transfer of Leucocytes of Blood

Exper-iment No.	Differential cell counts of suspensions of leucocytes transferred				No. of cells transferred × 10⁶	Serologic data										
	Lymphocytes	Monocytes	Plasmacytes	Neutrophils		Maximum agglutinin titers in sera of recipients of:										
						Untreated cells						Injured cells				
						No. of re-cipients	<12–12	16–48	64	96–192	256 or over	No. of re-cipients	<12	12	16	24
	per cent	per cent	per cent	per cent												
267	77	2	1	20	100	2			1	1		1			1	
274	92	2	—	6	90	2	1			1		2	2			
279	65	3	1	31	109	1		1				1	1			
281	77	4	—	19	200	3	1	2				2	2			
285	69	5	—	26	110	4		2		1	1	1	1			
344	64	3	—	33	135	4		1	1	1	1	2	2			
348	87	3	—	10	125	4		1	2	1		2	1	1		
349	93.5	2	—	4.5	96	3			1	2						
351	69	2	—	29	105	5		1		3	1					
354	78	1	—	21	70	6	1	2	1	1	1					
356	71	2	—	27	45	9	2	6	1							
458	63	2	—	35	75	1	1					4	2	1		1
464	56.5	4.5	—	39	217	3	2			1		1	1			

The differential cell counts of smears made from the various cell suspensions transferred are also shown in Table I. As can be seen, there is considerable variation among experiments in the percentage distribution of the respective cell types, the range of percentage occurrence of polymorphonuclear neutrophils being from 4.5 to 39, that of monocytes from 1 to 4.5, and that of lymphocytes from 63 to 93.5.

Within a number of these experiments comparisons were made of blood treated by various methods. In some such experiments half the blood collected from each rabbit was defibrinated and the remaining blood heparinized, and in others similar comparisons were made between collection with heparin and that by ion exchange resin. In these experiments leucocytes were obtained from each pool of blood and treated similarly thereafter until the transfer to recipient animal. There appeared to be no consistent differences in the results obtained by the different methods of blood collection.

3. Experiments with Leucocytes of Peritoneal Exudates Obtained Shortly after the Injection of Falba Mineral Oil.—Peritoneal exudates were used as another source of leucocytes, partly with the objective of obtaining cell populations with a greater predominance of a single cell type. Thus, with the plan of obtaining primarily polymorphonuclear leucocytes, peritoneal exudates were obtained from donors 1 or 2 days after the intraperitoneal injection of falba mineral oil. The resultant suspensions were, in fact, predominantly of poly-morphonuclear neutrophilic leucocytes, as can be seen in Table II. These

TABLE II

Experiments with Cells of Peritoneal Exudates Obtained at Various Intervals after the Injection of Falba Mineral Oil

Time after injection of FMO	Differential Cell Counts of suspensions transferred					Cell transfer												
						No. of experiments	Average No. of cells transferred, × 10⁶	Maximum agglutinin titers in sera of recipients of:										
								Untreated cells						Injured cells				
	No. of slides	Lymphocytes	Monocytes	Plasmacytes	Neutrophils			No. of recipients	Titers					No. of recipients	Titers			
									<12 -12	$16-$ 48	64	$96-$ 192	256 or over		<12	12	16
days		*per cent*	*per cent*	*per cent*	*per cent*												
1	3	7	10	1	82	2	170	5	4	1				2	2		
2	4	9.7	15.8	1.5	73	3	154	14	11	3				6	5	1	
3	3	22	18.4	1.6	58	2	255	5	4	1				4	4		
4	2	46.5	19.5	2	32	2	135	4	3	1							
5	3	51	16	1.5	31	2	153	5	1	4							
6	2	62.5	17.5	2	18	2	122	6	4	2				3	2		1
9	7	72.8	15.2	1.8	10.2	12	144	54	13	22	3	12	4	24	21	3	

cells were incubated with filtrates of trypsin-treated bacilli, washed, and transferred to irradiated recipients. Agglutinins to dysentery bacilli did not appear in the sera of a substantial majority of the recipients; in a few recipients agglutinins appeared in low titer.

4. Experiments with Peritoneal Exudates Obtained at Longer Intervals after the Injection of FMO.—In an attempt to obtain peritoneal exudates with a cell population largely composed of monocytes, donor rabbits were sacrificed 9 days after the intraperitoneal injection of FMO. These cells were incubated with antigenic material, washed, and transferred. The sera of the recipients of the first such experiment developed high titers of agglutinin to dysentery bacilli. Differential counts of the smears prepared from the cell suspension transferred indicated that the monocyte was not the predominating cell type. This finding led to experiments in several directions: first, repetitions of the experiment at a 9 day interval between the injection of FMO and the collec-

tion of the exudate; second, experiments in which this interval was varied between 1 and 9 days.

(a) *Experiments with Cells Obtained 9 Days after the Injection of FMO.*— Thirteen experiments were carried out with cells from peritoneal exudates obtained 9 days after the injection of this irritant. These experiments involved a total of 54 recipients of untreated cells and 24 recipients of iodoacetate-treated cells. Of the recipients of untreated cells, 24 per cent developed no agglutinins to dysentery bacilli; 46 per cent, titers of 16 to 64; and 30 per cent, titers of 96 or higher. Almost all of the recipients of iodoacetate cells, or of cells otherwise injured, failed to develop measurable levels of agglutinins. Cytologic examination of the smears of these suspensions yielded the following average percentages: lymphocytes, 72.5; monocytes, 15; polymorphonuclear leucocytes, 10.4; and plasmacytes, 1.7.

In four of these experiments, in addition to some portions of cells treated with iodoacetate, other portions were subjected to milder degrees of injury, such as were known to inactivate cell transfer by lymph node cells. In three such experiments portions of the cell suspensions were injected directly into irradiated recipient animals, while other portions were maintained overnight at 37°C., under conditions of bacterial sterility in tissue culture roller tubes, and transferred to irradiated recipient rabbits on the following day. In a fourth experiment an additional part was subjected to 300 r of x-irradiation before transfer. In all of these experiments the distribution of agglutinin titers in the recipients of overnight-incubated or irradiated cells did not appear to differ from those of recipients of iodoacetate-treated cells. In order to examine the overnight-incubated and irradiated cell suspensions for evidence of injury to the monocytes present, the latter were tested for migration and phagocytosis *in vitro* in the following experiments. Portions of the fresh or treated leucocyte suspension were mixed with an equal volume of chilled heparinized rabbit plasma and of rabbit spleen extract (10 per cent). Of this chilled mixture 0.01 ml. was placed in the center of a shallow depression in a glass slide. After allowing a few minutes for coagulation of this mixture, 0.05 ml. was added of a chilled mixture of equal parts of the heparinized rabbit plasma, rabbit spleen extract, and a 1:10,000 solution of neutral red dye. The drop added of the second mixture flowed over and entirely around the small clot in the center of the depression, and then underwent coagulation. The depression was now sealed with a coverslip by the use of a ring of vaseline-paraffin mixture, and the slides were incubated at 37°C.

On microscopic examination of such preparations of the fresh cell suspensions it was found that within a few hours of incubation, cells could be found at the periphery of the inner clot which contained red granules and vacuoles. On the next day a substantial number of mononuclear cells could be found in the outer clot, but not far from the periphery of the inner clot, and these contained red granules in greater number and of deeper color than the granules seen on the day before. 3 days after setting up of the cultures many mononuclear cells with very deeply stained red granules and vacuoles were found uniformly distributed throughout the entire area of the outer clot. The background, between cells, did not contain detectable red or pink color, and only rarely did cells show diffuse pale pink staining throughout their cytoplasm. On examination of such preparations made from irradiated suspensions or suspensions incubated 24 hours at 37°C. no differences could be observed in the time or extent of migration or uptake of the dye by such cells in comparison with those of fresh suspensions.

(b) *Experiments Involving Periods of Peritoneal Irritation between 2 and 9 Days.*—In pursuit of the original objective of obtaining exudates with mono-

cytes as the predominant cell type, experiments were carried out with inter-mediate intervals between the injection of FMO and the collection of cells. At no part of this range, however, was evidence obtained of a preponderance of monocytes in the exudate. The mean percentage of monocytes, which had increased to 15.8 during the period of preponderance of neutrophilic cells (the first 2 days) was found to rise only slightly, to 19.5, during the next 2 days, and then to subside gradually to a value of 15. Rather, it was the per-centage of lymphocytes which increased in a manner complementary to the decrease in frequency of the polymorphonuclear cells, the lymphocyte per-centage increasing from 6 to 72.5 as the polymorphonuclear percentage de-creased from 82 to 10.4.

TABLE III

Experiments Involving the Transfer of Peritoneal Cell Exudates Obtained after the Injection of Heavy Mineral Oil

Time after injection of mineral oil	Differential counts of suspensions transferred						Cell transfer					
	No. of Slides	Lymphocytes	Monocytes	Plasmacytes	Neutrophils	No. of experiments	Average No. of cells transferred, ×10⁶	Maximum agglutinin titers in sera of recipients of:				
								Untreated cells			Injured cells	
								No. of recipients	Titers		No. of recipients	Titers
									<12	12		<12
days		*per cent*	*per cent*	*per cent*	*per cent*							
2	2	6.0	78.5	0.5	15.0	1	122	2	2		1	1
3	2	9.3	81.2	0	9.5	2	114	4	3	1	4	4
4	3	12.7	74.6	0.7	12.0	3	197	11	9	2	6	6

The serologic data obtained in this group of experiments, which are also presented in Table II, showed no greater agglutinin titer among recipients of cells obtained after 3 or 4 days of peritoneal irritation than after 1 or two, and only a suggestion of higher agglutinin titers on the 5th and 6th day.

5. Experiments with Leucocytes Obtained from Peritoneal Exudates Following Injection of Heavy Mineral Oil.—Since peritoneal exudates predominating in macrophages had not been produced by the injections of the mixtures of falba and light mineral oil, attempts were made to obtain such a population by the intraperitoneal injection of a heavy mineral oil (8). Peritoneal exudates were collected 2, 3, and 4 days after the injection of 100 ml. of heavy mineral oil, and the differential counts of the cell suspensions transferred in these experi-ments showed a preponderance of monocytes, with a percentage frequency varying between 75 and 81. Cells prepared from these exudates were incu-bated with antigen, washed, and transferred as described above. The majority of recipients of such cells (82.5 per cent) did not develop agglutinins to dysen-

tery bacilli, and when agglutinin appeared it was only at the mimimum detectable titer of 12, in the present system of measurement. The recipients of iodoacetate-treated cells also did not develop measurable levels of agglutinin. These data are summarized in Table III.

DISCUSSION

The earlier studies in this series, both those in which the antigenic material had been injected into the donor of the transferred cells and those in which such cells had been incubated *in vitro* with the antigenic material, had been carried out with cells obtained from lymph nodes (1, 3). The suspensions of cells thus obtained had included a variety of cell types, but with a substantial preponderance of members of the lymphocytic series (5, 9). The present study was undertaken in the interest of extending such experiments to involve cells of other sources than lymphatic tissue, particularly suspensions with some similarities and some differences from the populations of cells obtained from lymph nodes.

The cell transfer experiments with leucocytes from blood indicated that the transfer of such cells could be followed by the appearance of antibody in the serum of recipients. Approximately 17 per cent of these recipients showed no antibody above the present threshold of measurement, and of the remainder 51 per cent developed low titers of agglutinin and 32 per cent showed titers of agglutinin of the order which have been found in the lymph node cell transfer experiments. The lower distribution of titers in comparison with those found in lymph node cell transfer experiments will be discussed below. However, regardless of the quantitative relation of the peak agglutinin titers observed in recipients of blood leucocytes to the total number of cells or to the number of cells of any given type transferred, it appeared that one or more of the types of leucocytes of blood could be incubated with soluble antigen and transferred to recipient animals with the subsequent appearance of homologous antibody in the latter.

Peritoneal exudates were then used as a second source of leucocytes, partly with the intention of being able to obtain suspensions in which a single cell type would predominate, and thus to obtain data suggesting which of the leucocytes of the blood could be associated with the cell-transfer effect.

The transfer of cells from exudates obtained 1 or 2 days after the injection of falba and light mineral oil failed to give rise to agglutinins in the sera of recipient rabbits. In these cell suspensions polymorphonuclear leucocytes were predominant (73 to 82 per cent). These experiments would suggest, then, that these cells had not been the effective cell type in the experiments with the leucocytes of blood.

The experiments involving a 9 day period of irritation by FMO were undertaken in an effort to obtain suspensions predominantly of monocytes. Experi-

ments involving peritoneal irritation for this period had in an earlier study been found to give such suspensions (10). (In the earlier studies bacterial cells had been included in the irritant.) In the present study, the irritant of oxycholesterins and light oil was not found to produce a predominantly mono-cytic exudate at any time. Rather, the percentage of monocytes showed only minor changes between the 2nd to the 9th day, and it was the percentage of lymphocytes which rose from mean values below 10 per cent in the 1st and 2nd days to 72.5 per cent on the 9th day.

Most of the experiments involving peritoneal irritation by FMO were performed with cells obtained 9 days after the injection of this irritant. Approximately 24 per cent of the recipients of fresh cells showed no measurable agglutinin titers, 46 per cent developed low titers of agglutinin, and 30 per cent showed titers within the range encountered in cell transfer studies with lymph node cells. The frequency distribution of peak titers of recipients in the experiments with peritoneal exudates following 9 days of irritation was thus fairly similar to that found with leucocytes of blood. The distribution of cell types in the suspensions from 9 day FMO peritoneal exudates was similar to that found in the suspensions of blood leucocytes in the average percentage of lymphocytes present, 74 and 72.5 per cent respectively, but different in that the remainder were largely polymorphonuclear cells in the case of blood cells, but included more monocytes in the peritoneal exudates.

The experiments with leucocytes both of blood and of 9 day FMO peritoneal exudates yielded distribution of agglutinin titers which, while similar to each other, were substantially lower than those found after the transfer of lymph node cells similarly incubated. In part these lower titers might have been due to a greater degree of injury of these cells than in the case of lymph node cells, since the preparation of leucocytes from blood or peritoneal exudates required substantially more manipulation and greater centrifugal fields than did the preparation of lymph node cells. It is conceivable also that the different sources of the cells—in one case an organized tissue and in the others suspensions of cells removed from the parent tissue—might be involved in the difference in relative effectiveness of the cells in the two types of experiments.

In connection with the blood leucocyte experiments it is of interest to recall the study of Erslev (11), who gave series of intravenous injections of pneumococci to rabbits and then collected leucocytes from the blood of these animals and examined extracts of these leucocytes for agglutinins to pneumococci. No significant amount of antibody was found in such extracts, although antibody was detected in the blood plasma at the same time. The implications of these data as to the ability of any of the leucocytes of blood to form antibody are not in agreement with those of the present study. However, it should be borne in mind that in experimental situations in which antigen is injected into the animal body the tissue to be examined for evidence of antibody formation must be collected from a site which could be expected to be involved in active production of antibody, and at an appropriate time relative

to the injection of antigen. It is questionable whether leucocytes of blood, which constitute a pool of cells originating in various tissues throughout the body could be expected to provide an appropriate tissue for such examination following the injection of antigen into the animal body. On the other hand, in the experimental situation involving *in vitro* incubation of cells and antigen, one is independent of the source of the cells in terms of sites of antibody formation in the animal body. The *in vitro* incubation system should thus afford a better test of the antibody-forming capacity of the cells under investigation.

The peritoneal exudates obtained from 2 to 4 days after the injection of heavy mineral oil provided a cell population which was predominantly monocytic. After incubation of these cells with antigenic material and transfer to irradiated recipients, agglutinins did not appear in the sera of these animals above our threshold of measurement. This would imply that monocytes obtained from inflammatory exudates are not effective in cell transfer under the present experimental conditions. This finding is consistent with that of Roberts, who allowed macrophages from normal or immunized rabbits to engulf typhoid bacilli and then explanted such cells *in vitro* or transferred them into recipient animals, and was not able to obtain evidence of antibody formation by these cells (12).

On the other hand, the data in this study on antibody formation by the monocyte are not in agreement with those of Dixon *et al.*, who have reported recently that the transfer either of lymph node cells or of cells of peritoneal exudates similar in cell composition to those described here were followed by the appearance of antibody to bovine serum albumin in irradiated recipients (13). Although it is not possible at present to explain the reasons for this difference, it may be noted that the experimental conditions of Dixon *et al.* differ in some respects from those of the present study. In the experimental system used by Dixon a secondary antibody response is involved, the transferred cells are injected subcutaneously, and antigen is injected into the donor animal for primary stimulation, and into the recipient for the secondary contact, whereas in the present study the cells are transferred by the intravenous route and the cells are exposed to antigenic material only *in vitro* and then washed, no antigen being injected into either the donor or recipient animal. To what extent these differences in experimental conditions can account for the differences in the findings reported cannot be stated at present.

SUMMARY

Leucocytes were obtained from the blood of rabbits, incubated *in vitro* with soluble antigenic material derived from *Shigella paradysenteriae*, washed, and transferred to irradiated recipient animals. On the 4th day after cell transfer, agglutinins to *Shigella* appeared in the sera of the majority of such recipients. The distribution of maximum agglutinin titers was substantially

lower in these animals than in recipients of lymph node cells similarly incubated, as reported in earlier studies. Recipients of iodoacetate-treated cells showed no agglutinins. Similar results were obtained with cells of peritoneal exudates obtained 9 days after the intraperitoneal injection of lanolin and light mineral oil.

When cells of peritoneal exudates obtained 1 or 2 days after the injection of this irritant were incubated with the antigenic material and transferred to irradiated recipients, agglutinins did not appear in the sera of the latter. Similar results were obtained with cells from peritoneal exudates obtained a few days after the intraperitoneal injection of heavy mineral oil. The preponderant cell types in the suspensions from these two sources were the polymorphonuclear neutrophils and monocytes, respectively.

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