nervous connections(3,15). Further experimentation will be necessary to resolve these paradoxical findings.

Summary. Repeated doses of ergotamine and hexamethonium had no effect on increase in plasma non-esterified fatty acid (NEFA) concentration which occurs during fasting. Dibenzyline was similarly without effect on this response. These findings suggest that the stimulus to increased NEFA mobilization during fasting may not be mediated by the adrenergic nervous system.

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Effect of Injection of Rabbit Leucocytes into Neonatal Rabbits on Subsequent Lymph Node Cell Transfer.* (25294)

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If rabbit lymph node cells are incubated with filtrate of trypsin-treated *Shigella paradysenteriae* and transferred to fresh rabbits, agglutinins to *Shigella* appear in sera of recipients in a few days. It has also been shown (1) that if blood leucocytes obtained from prospective donor rabbits are injected into recipients a week before cell transfer, such agglutinins do not appear. Failure of agglutinins to appear indicates that injection of leucocytes before cell transfer has altered the host environment with respect to transferred cells, with the result that these cells become ineffective in production of agglutinins. This suppressive effect on transferred lymph node cells was considered the result of a homograft reaction, as originally described by Medawar (2) in the case of skin grafts. The present study arose from attempts to produce in the lymph node cell transfer system another, essentially opposite, effect of tissue transplantation, that of acquired tolerance. This state was first experimentally induced by Billingham, Brent and Medawar(3) by injection of fetal mice of one inbred strain with tissue cells obtained from another strain of mice; after birth, injected mice showed tolerance to tissues of donor strain by accepting skin homografts from this strain. Similar results were reported by these authors with rabbits injected during fetal life with rabbit spleen cells(4). Later Billingham and Brent(5) established acquired tolerance even in mice injected soon after birth with spleen cells of donor strain. In rabbits these authors reported that intravenous injection of the newborn with homologous thymocytes or spleen cells resulted in induction of weak tolerance, in only a small

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TABLE I. Maximum Agglutinin Titers of Recipients of *In Vitro* Incubated Lymph Node Cells; Comparison between Recipients Injected with Donor Leucocytes Near Birth, and Those Not Injected with Donor Leucocytes.

Age of rabbits at time of:			Max agglutinin titers of recipients inj. with donor leucocytes:		
Inj. of donor leucocytes	Cell transfer	No. of cells transferred, $\times 10^6$	Soon after birth and again 1 wk before cell transfer	Soon after birth only	Max agglutinin titers of control, 1-kg, recipients not inj. with donor leucocytes
1 day	80	152	<12, <12, 24		128, 128
1	70	179	<12, <12, <12, 12		128
2	67	"	<12		256
2 hr	64	"	<12		384
10 "	74	206		16	96
2 days	81	204		24	384, 384
8 hr	75	206		<12, 48, 64	128, 192, 384, 512
1 day	60	"		12, 32, 64, 64	192, 384
1	52	172		<12, <12, 32, 48	128, 128, 512, 512
1	36	"		<12, <12, <12, 32, 96	256
1	43	222		<12	

proportion of the animals(6). The present study was begun to determine whether injection of leucocytes of adult rabbits into neonatal rabbits would lead to a state, referable to immunologic tolerance, in which transferred lymph node cells would be better accepted by tissues of recipient, with the result of higher agglutinin titers in sera of these recipients. This was not achieved; on the contrary, evidence was obtained that injection of leucocytes very early in life induced the lymph-node-cell-suppressive reaction.

Materials and methods. Injection of newborn rabbits with donor blood leucocytes. Prospective donors of lymph node cells were bled by cardiac puncture, and the heparinized blood was mixed with one volume of 3% gelatin solution in tall narrow tubes. After one hour at room temperature the layer above the sedimented erythrocytes was removed by suction and leucocytes contained therein were collected by centrifugation. The cell sediment was suspended in a convenient volume of Tyrode's solution containing 0.5% bovine plasma albumin and heparin. Newborn rabbits were injected intravenously with 10 x 10^6 donor leucocytes at an interval after birth of a few hours to 2 days. *Cell transfer.* The popliteal and axillary lymph nodes of donor rabbits were excised, and cells obtained from them were incubated *in vitro* with filtrates of trypsin-treated suspensions of *Shigella paradysenteriae,* as described elsewhere(7). After ½ hour incubation at 37°C, lymph node cells were washed and transferred to X-irradiated recipients (425 r of deep X-ray treatment 24 hours earlier) by intravenous route. Thereafter recipients were bled at regular intervals and their sera tested for agglutinins to *Shigella.*

Results. 1. *Attempts to induce tolerance to donors' lymph node cells by injection of neonatal prospective recipients with blood leucocytes from prospective donors.* Newborn rabbits (2 hours to 2 days after birth) were injected intravenously with leucocytes of prospective donors. About 2 months later the same young rabbits were again injected, intradermally, with leucocytes from these donor rabbits, and one week later the young rabbits were given *Shigella*-antigen-incubated lymph node cells from same donors. Injection of leucocytes prior to cell transfer could be expected to result in suppression of transferred cells, as indicated above, unless injection of leucocytes near birth had made the rabbits tolerant of these donor cells. As shown in first 4 rows of Table I, these recipients either developed no measurable agglutinins to *Shigella,* or a very low level of agglutinins, in contrast to control recipients, which had not been injected with donor leucocytes at any time. Suppression of transferred cells in leucocyte-injected animals indicated that tolerance to donors' tissue antigens had not been induced by neonatal injection of recipients with such cells.

The possibility was considered that injection of leucocytes prior to cell transfer might have constituted too severe a test of tolerance to tissue antigens of donors in young rabbits. Accordingly, in the next series of experiments neonatal rabbits were injected with leucocytes of adult rabbits only near the time of birth, and some time later these young rabbits were given antigen-incubated lymph node cells from adult rabbits. It was thought that if a state of tolerance existed which was too weak to overcome the effects of pre-injection of donor leucocytes prior to cell-transfer, this might be indicated by agglutinin titers higher in these recipients than in control recipients, which were not injected during neonatal life with the donors' leucocytes. However, results of these experiments indicated quite the reverse: maximal agglutinin titers of recipients injected with donors' leucocytes early in life were considerably lower than those of control recipients which had not received such an injection (lower part of Table I). These data not only failed to give evidence of a greater degree of acceptance of transferred cells by tissues of the recipient, as a result of neonatal injection of leucocytes, but indicated a suppressive effect on transferred lymph node cells, presumably due to that injection.

With older recipient animals(1) it was found that maximal suppression of lymph node cells occurred when the interval between injection of leucocytes and transfer of lymph node cells was between 7 and 20 days, and that with increasing length of this interval there was gradual decrease of suppressive effect. In the present experiments the interval between injection of leucocytes and transfer of lymph node cells was 36-81 days. To see whether the lymph-node-cell-suppressive effect could be more clearly demonstrated, the following experiments were performed:

2. *Induction of lymph-node-cell-suppressive effect by neonatal injection of leucocytes.* Litters of new-born rabbits were divided into 2 halves. Rabbits in one half of each litter were injected (at age between 8 hours and 1 day) with blood leucocytes from prospective donors of lymph node cells. At various intervals thereafter lymph node cells obtained from these donor rabbits were incubated *in vitro* with *Shigella*-trypsin filtrate and transferred to all rabbits of the litter. Data are shown in Table II. In all cases the anti-*Shigella* agglutinin titers of recipients which had been injected with leucocytes were markedly lower than those of their control littermates. Even with injection of leucocytes at 8 hours of age, and cell transfer on fourth day of life, suppression of transferred lymph node cells was found. Clearly, injection of donor leucocytes near birth had actively induced a reaction of the homograft type against the transferred lymph node cells.

From another type of experiment involving cell-transfer Sterzl deduced that newborn rabbits can react to antigens of leucocytes of other rabbits. Spleen cells were transferred to 5-day-old recipients, *Brucella suis* antigen alone was injected at various intervals thereafter, and agglutinins were subsequently sought in sera of recipients. From the finding that 4 days was the maximum interval consistent with appearance of agglutinins, it was concluded that spleen cells survived no more than 4 days in these neonatal rabbits(8).

In view of this evidence of immunologic activity of the neonatal rabbit, it is of interest that newborn rabbits have been found not to respond (prior to 14th day of life) with active formation of antibody to antigens of *Brucella suis*(9) or to agglutinogen of *Shigella*.

3. *Transfer of* in vitro-*incubated lymph node cells to neonatal rabbits.* Data of control rabbits of Table II, including litters of 8 and 4 days of age, also showed that young rabbits could develop agglutinins after transfer of *in vitro*-incubated lymph node cells. This was explored further, in age range of 1-11-day-old litters. All neonatal rabbits were X-irradiated 24 hours before cell-transfer, then were given lymph node cells which had been incubated *in vitro* with *Shigella*-trypsin filtrate. In these recipients agglutinins developed by fifth day, generally reached a peak by 7th day, and began to decline between 9th and 12th day after transfer. Peak agglutinin titers are shown in Table III, in comparison with those of 1-kg recipients. Agglutinins appeared in neonatal recipients in a wide

TABLE II. Comparison of Maximum Agglutinin Titers of Littermates after Transfer of *In Vitro* Incubated Lymph Node Cells; Part of Litter Injected with Donor Leucocytes Near Birth, Remainder Not Injected.

Age of rabbits at time of:				
Inj. of donor leucocytes	Cell transfer	No. of cells transferred, $\times 10^6$	Max agglutinin titers of recipients inj. with donor leucocytes soon after birth	Max agglutinin titers of littermates not inj. with donor leucocytes
1 day	31	222	<12, <12, 32	384, 512, 512, 768, 3072
10 hr	30	"	<12, 64	512, 512, 768, 2084
1 day	8	182	32, 48, 64	1024, 1536, 1536
1 *	8	144	<12, 12, 12	128, 256
8 hr	4	140	16, 16	128, 256
		70	16	

* Donor leucocytes inj. intradermally. All other groups were inj. intrav.

range of titer, often at a relatively high level and occasionally near the threshold of measurement.

In these experiments neonatal recipients were X-irradiated prior to cell-transfer, as were the 1-kg recipients. In internal comparisons within litters, agglutinin titers of irradiated recipients were higher than those of their non-irradiated littermates. In other experiments comparisons were made of transfer to neonatal rabbits of lymph node cells obtained from uninjected donor rabbits and from rabbits which had previously been injected in the foot pad with a heterologous antigen (human gamma globulin) to increase the yield of lymph node cells. No differences were found between geometric mean peak titers of groups of littermates receiving cells from these 2 sources.

Finding of agglutinin titers, especially in a relatively elevated range, in neonatal recipients indicates that these recipients can, to some extent at least, provide a suitable environment for the functions of transferred cells. A full evaluation of the relative effectiveness of lymph node cells obtained from adult donors in 1-kg recipients and in neonatal recipients would require data which are not yet at hand. However, preliminary evaluation can be made by calculations involving the numbers of cells transferred and relative weights of recipients of the 2 groups. Relative effectiveness of cells in the 2 situations was estimated by multiplying for each type of recipient, the geometric mean maximal agglutinin titer, per million cells, by mean body

TABLE III. Transfer of *In Vitro* Incubated Lymph Node Cells to Irradiated Neonatal Recipients (1-11 Days) and to 1-kg Rabbits.

Age of neonatal rabbits at time of cell transfer (days)	Mean wt of rabbits (g)	Lymph node cell transfer to neonatal recipients			Lymph node cell transfer to 1-kg recipients		
		No. of cells transferred ($\times 10^6$)	Max agglutinin titers of recipients: Individual peak titers	Geometric mean ($\log_2$)	No. of cells transferred ($\times 10^6$)	Max agglutinin titers of recipients: Individual peak titers	Geometric mean ($\log_2$)
11	150	138	96, 192, 192, 256, 384, 384, 384, 512, 512, 1024	7.5	138	96, 96	6.5
9	130	184	128, 384	7.8	184	384, 1024, 1024	9.5
4	80	87	256, 256, 384, 512, 768, 1024	8.8	194	96, 384	7.5
4	80	115	24, 384, 384, 512, 1024	8.3	156	128, 128, 384	7.3
2	60	43	192, 384, 384, 768	8.5	127	96, 1024	8.3
1	50	187	384, 384, 384, 512, 512, 768	8.8	187	128, 384	7.8
1	50	152	768, 768	9.5	152	64, 256	7.0

weight of recipients (Table III). In 5 of 7 experiments the ratio of effectiveness of cells when transferred to 1-kg recipients, to that found on transfer to neonatal recipients, ranged from 2.2 to 4.9. In the other 2 experiments this ratio was 10 and 24, respectively.

In a study by Dixon and Weigle(10) lymph node cells obtained from donor rabbits injected 2 hours earlier with *Shigella*-trypsin filtrates were transferred subcutaneously or intramuscularly into 5-day-old recipient rabbits. Agglutinins failed to appear in sera of 5-day-old rabbits, although agglutinins were found subsequently in sera of adult control recipients. A possible basis for this difference in findings may be that in our experiments lymph node cells were incubated *in vitro* with the antigen, and were transferred by intracardiac injection, and that neonatal recipients had been irradiated. However, in a more recent study Dixon and Weigle did not observe substantial differences in peak titers of neonatal recipients whether they were irradiated or not, or whether lymph node cells were given by subcutaneous or intracardiac injection (11).

In other studies involving transfer to neonatal rabbits of cells of rabbit lymph or spleen, Sterzl(12) transferred to 5-day old rabbits, by intraperitoneal injection, spleen cells which had been incubated *in vitro* with *S. paratyphi* and found agglutinins subsequently in sera of recipients, in range of titers of 8 to 128. Also, Holub(13) transferred to 2-5-day-old rabbits, by the same route, cells collected from the cisterna chyli or spleens of adult rabbits and incubated *in vitro* with *Brucella suis* or *Salmonella paratyphi B*. Agglutinins appeared in these recipients in range of 2-64.

Summary. 1. Newborn rabbits were injected with leucocytes obtained from adult rabbits which were prospective donors of lymph node cells. When antigen-incubated lymph node cells from these donors were transferred to young rabbits 1-2½ months later, agglutinins failed to appear, or did so in low titer. This suppression of transferred lymph node cells indicated that tissues of newborn rabbits had reacted to antigens of leucocytes of donor animals. 2. Transfer of lymph node cells incubated *in vitro* with *Shigella*-trypsin filtrate to uninjected newborn rabbits (1-11 days of age) resulted in the appearance of anti-*Shigella* agglutinins.

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Mechanism of Aminonucleoside-Induced Nephrosis in the Rat. I. Metabolism of Tritiated Aminonucleoside.* (25295)

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Structural similarity of adenosine and the aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β - D - ribofuranosyl)-purine, suggests that the mechanism by which the lat-

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