TABLE III. Non-Cancer Serum.

Diagnosis	Total cases	Positive reactions with heated serums 56°C	64°C
	705	275	71
Rheumatoid arthritis, active	96	38	6
Endocrine disturbances + medication	143	59	16
Allergy disease + medication	113	32	12
Cirrhosis	14	10	4
Gastric ulcer	30	11	3
Duodenal "	13	3	0
Active tuberculosis	25	16	4
Bronchiectasis	7	4	0
Diabetes *on* insulin	66	33	7
" *no* "	46	10	2
Pregnancy	14	6	1
Chronic cystic mastitis	7	2	1
Menorrhagia	31	19	2
Infection with temperature	20	12	10
Keratosis	26	4	0
Bone cyst	4	0	0
Lipogranuloma	2	1	0
Benign prostatic hypertrophy	48	15	3
% positive		39%	10%

variety of other pathological conditions (non-malignant group) was also considerably diminished (38% to 10%). The persistence of positive reactions in this group might suggest the presence of some change in plasma which is similar to that occurring in malignancies.

Summary. A method is described for an improved laboratory aid in mass screening for invasive neoplasms. The modification of the described serofloccculation test consisted essentially in heating sera at 64°C for 30 minutes.

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Transfer of Lymph Node Cells to Recipient Rabbits Pre-Injected with Blood Leucocytes of Donors.*† (23107)

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Studies of antibody formation in this laboratory have recently involved the technic of transfer of lymph node cells from donor to recipient animals, as described by Chase (1,2). In earlier studies, cells to be transferred were obtained from lymph nodes draining sites of injection of antigen into donor rabbits(3,4); more recently lymph node cells obtained from uninjected donors (or from donors injected with heterologous antigens) were incubated *in vitro* with the antigenic substance and then transferred to X-irradiated recipient rabbits(5,6). In each case antibody to the antigen referred to appeared subsequently in sera of recipients. In both systems it was found that living cells were necessary, and that antibody did not appear in recipients of injured cells. In studies with other systems involving humoral antibody, or resistance to tumor homografts, various workers also found that injury to lymph node cells prior to transfer results in failure of antibody to appear in the recipient(1,2,7-9). However, the role of transferred cells in the formation of the antibody subsequently found in the recipient has not been clarified. In the present study the relation of transferred cells to such antibody was approached by attempts to alter the host-tissue environment of the transferred cells, rather than by manipulation of the cells themselves. This approach was suggested by

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the work of Medawar(10,11) who showed that after rejection of a skin homograft from a given donor by a given recipient, a second skin graft from the same donor to the same recipient was rejected in a substantially shorter period of time (the "second-set phenomenon").

In the present study experiments were carried out in the lymph node cell transfer system, in which some of the recipient rabbits were injected with blood leucocytes obtained from donor rabbits, prior to lymph node cell transfer, and the curves of antibody concentrations with time after transfer were compared in the two groups of rabbits.

Materials and methods. Pre-injection of donor blood leucocytes. Adult rabbits scheduled to become donors of lymph node cells were bled by cardiac puncture. The heparinized blood was mixed with one volume of 3% gelatin solution in tall narrow tubes, and the mixture allowed to stand one hour at room temperature. The layer above the sedimented erythrocytes was removed by suction, and the leucocytes contained therein were collected by centrifugation. The cell sediment was suspended in a convenient volume of Tyrode's solution containing 7½% normal rabbit serum and heparin. Recipient rabbits were injected intradermally in the back in 6-8 sites. *Cell transfer.* The popliteal and axillary lymph nodes of donor rabbits were excised and cells obtained from them by technics described elsewhere(6). The lymph node cells were incubated *in vitro* with filtrates of trypsin-treated suspensions of dysentery bacilli as described earlier(12). After ½ hour incubation at 37°C, the lymph node cells were washed and transferred to X-irradiated recipients by intravenous route. Thereafter recipients were bled at regular intervals and their sera tested for agglutinins to dysentery bacilli. No recipients were irradiated at time of pre-injection of donor leucocytes, but all were given 425r of deep X-ray treatment 24 hours before transfer of incubated lymph node cells.

Results. Effect of injection of donor leucocytes; variations in time interval and dosage. In some experiments recipients were injected intradermally with blood leucocytes from

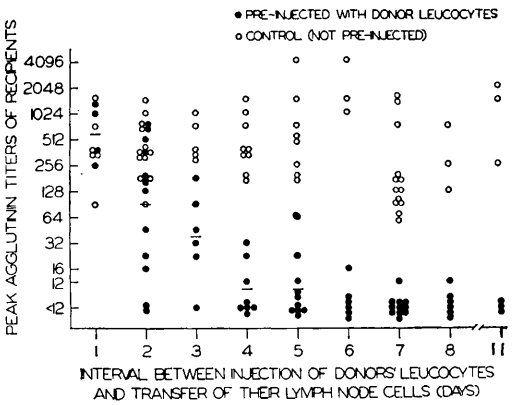

FIG. 1. Peak agglutinin titers of individual recipients of lymph node cells incubated *in vitro* with filtrates of trypsin-treated suspensions of dysentery bacilli. Recipients in the experimental group had been inj. intradermally with donor leucocytes at intervals, as indicated, prior to transfer of the incubated lymph node cells. The bar indicates geometric mean titer of group of pre-inj. recipients.

prospective donors several days before cell transfer. On day of transfer, lymph node cells of these donors were incubated *in vitro* with filtrates of trypsin-treated suspensions of dysentery bacilli. These cells were washed and transferred to irradiated recipients which had been pre-injected at various times (1-11 days earlier), and also to control recipients not pre-injected. The results of such experiments, summarized in Fig. 1, show that whereas control recipients developed agglutinin titers in the usual range, recipients pre-injected with leucocytes on fourth to eleventh day before transfer showed only very low titers of agglutinin. Recipients which had been pre-injected 3 or 2 days before transfer showed some reduction in agglutinin titer, and serum titers of those which had been pre-injected one day before transfer were indistinguishable from controls which had not been pre-injected. In most of these experiments the number of leucocytes pre-injected was 10 million. In the first experiments the number of donor blood leucocytes injected into recipients ranged between 30 and 100 x 10^6. In experiments each involving the range of 100 x 10^6 to 0.1 x 10^6 an injection of approximately 10^6 leucocytes was adequate to produce the effect described when injected 6 days prior to cell transfer.

TABLE I. Peak Agglutinin Titers of Recipient Rabbits Pre-Injected with Various Blood Cells.

	Control rabbits, not pre-inj.	Recipient rabbits pre-inj. with: Blood leucocytes of Rabbit	Man	Horse	Cow	Chicken	Rabbit erythrocytes
	256	64	64	256			384
		<12	16	64			384
		<12					16
	96	12					48
	96	<12					48
		<12					
	128	<12	384				512
		<12	32				192
		16	24				32
			24				
	192	12			384	384	768
	192	<12			192	384	384
	768				128	192	128
	192				128	128	96
					96	24	
	768	<12	256	384			1024
	384	<12	64	384			512
	128	<12	16	256			384
	96	12	<12	128			96
	64			96			
Geometric mean titer of group ($\log_2$)	7.6	3.0	5.3	7.5	7.3	7.2	7.4

Peritoneal exudates were obtained from donor rabbits 2 days after the intraperitoneal injection of either of 2 irritants: heavy mineral oil, which yielded an exudate predominantly of monocytes (75-81%), or an emulsion of lanolin and light mineral-oil in saline, which yielded a cell population largely of polymorphonuclear leucocytes (60-78%). The cells thus obtained, as well as leucocytes obtained from blood, were injected intradermally into recipient rabbits (approximately 10^7 cells/animal) 7 days before cell transfer. Recipients pre-injected with cells of either type of peritoneal exudate failed to develop agglutinins or did so in low titer, the distribution of titers being similar to that in the group of recipients pre-injected with leucocytes obtained from blood. The group of recipients not pre-injected developed a mean peak titer of 768.

Pre-injection of rabbit erythrocytes or of blood leucocytes of other animal species. Leucocytes prepared from chicken, cow, horse, and human blood, as well as erythrocytes and leucocytes from rabbits, were injected intradermally into prospective recipients 6 days before cell transfer. As can be seen in Table I, following transfer of incubated lymph node cells, the recipients which had been pre-injected with leucocytes obtained from rabbit blood developed no measurable amounts of agglutinin, or very low titers. On the other hand, of 16 recipients pre-injected with rabbit erythrocytes, 9 developed agglutinin titers as high or higher than control recipients, 6 developed somewhat lower titers and one developed a barely detectable level. The geometric mean of the maximum titers of the whole group of recipients was similar to the controls. Recipients pre-injected with leucocytes obtained from blood of chickens, cows and horses also developed agglutinin titers similar to those of the control (non-pre-injected) recipients. Of 10 recipients pre-injected with leucocytes obtained from human blood, the sera of 2 showed agglutinin titers in the range of control recipients, 6 were lower and 2 were barely detectable. The geometric mean titer of this group was distinctly lower than that of the control group.

Discussion. In sera of control rabbits of above experiments, as in recipient rabbits of earlier studies, agglutinins to dysentery bacilli were found after transfer of lymph node cells

incubated *in vitro* with antigenic material derived from those organisms. In the present study recipients which had been pre-injected intradermally with leucocytes from donor animals did not develop agglutinins after transfer of incubated lymph node cells. This failure of appearance of antibody is presumably due to an alteration, brought about by pre-injection of donor leucocytes, in the environment of the transferred cells within the recipient. The following data are consistent with an hypothesis that this alteration is due to an immunologic effect:

1. *Rabbit erythrocytes.* The geometric mean antibody titer of recipients pre-injected with donors' erythrocytes was similar to that of the control group. (It is felt that the occurrence of lower peak titers in some animals of this group may have been due to leucocytic contamination of the erythrocyte suspension, which was generally more than one/thousand rbc.)

2. *Heterologous leucocytes.* The appearance of antibody in recipient rabbits was not affected by pre-injection of leucocytes obtained from cow, chicken or horse. In the case of human leucocytes there was evidence of reduction in antibody concentration in sera of more than half of the recipients. This suggests the possibility of a similarity in structure between some antigen(s) in human and rabbit leucocytes.

3. *Time relations of pre-injection to cell transfer.* An interval of 2-3 days between pre-injection and cell transfer was required for substantial suppression of antibody formation. This period, added to the 3-day interval after the transfer of *in-vitro*-incubated lymph node cells before antibody would normally appear, yields a total of 5-6 days, an interval which would be consistent with that required for an appreciable immunologic reaction of the recipients' tissues to antigens in leucocytes of donors. While the above data suggest an immunologic mechanism for this suppression of cell-transfer effect, the data available thus far have not proven that an immunologic mechanism is involved, or indicated its nature.

The observations reported here are parallel to those of Medawar(10) and of others in tissue homografting, in that the response of the recipient is different according to whether it is involved in the first or second contact with the tissue (or cells) of the donor, and that the effect of first contact could be brought about by pre-injection of leucocytes from the donor and not by erythrocytes(11).

There is a difference, however, between the phenomena observed in the 2 kinds of experiments. In one case (skin homograft) the second graft is rejected earlier; in the other case (cell transfer) antibody fails to appear. This probably reflects the difference in the 2 experimental situations. In the case of a second skin graft the interaction between the grafted cells and the immunologic agent in the host tissue must await vascularization of the graft; death of the grafted tissue follows within a few days. In the lymph node cell transfer cells are injected directly into the blood stream, so that injury to these cells could occur within a short time. Such injury might well result in complete failure of antibody to appear, since antibody does not normally appear until the third day after cell transfer (in the present system).

The experiments described above give further evidence for some active function of the transferred lymph node cells in the formation of the antibody which appears subsequently in the sera of recipient animals, since the prior injection of donor leucocytes, which presumably leads to a specific alteration in the host tissue environment of the transferred cells, affects the appearance of such antibody.

Summary. As observed in previous studies the transfer to irradiated recipient rabbits of donor lymph node cells incubated *in vitro* with soluble antigenic material derived from *Shigella paradysenteriae* was followed by the appearance of agglutinins to these bacilli in the sera of the recipients. If, 4-6 days prior to cell transfer, the recipients were injected intradermally with leucocytes obtained from blood or from peritoneal exudates of the donors, agglutinins failed to appear in the recipients' sera, or appeared in low titer. This effect could be brought about consistently with the pre-injection of 10^6 to 100 x 10^6

leucocytes; not by the pre-injection of rabbit erythrocytes or of leucocytes of horse, cow and chicken blood; and occasionally by the pre-injection of human blood leucocytes.

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Comparative Absorption of Vit. B_{12} Analogues by Normal Humans. III. 5,6-Dichlorobenzimidazole, 5,6-Desdimethylbenzimidazole and 5-Hydroxybenzimidazole Analogues. (23108)

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Preceding communications(1,2) have reported oral absorption of several non-cyanocobalamins to be inferior to cyanocobalamin. Furthermore, all cobalamins were equally effective, upon injection, in inducing excretion of orally administered cobalt-60 labeled forms. These findings indicate the importance of the cyanide group for cobalamin absorption, but offer no suggestion as to influence of structural modifications on oral absorption. It is known that pseudo-vit. B_{12} and its 2-methyl derivative(3), Factor III (3) and the desdimethyl analogue(3,4) of cyanocobalamin exhibit inferior growth activity in chicks. However, direct absorption tests of substituted benzimidazole derivatives in man remain to be performed. Accordingly, urinary excretion tests have been performed with 3 analogues of vit. B_{12} with altered substituents in the benzimidazole moiety. These compounds are (1) the 5,6-dichlorobenzimidazole analogue (DCBA)(5), (2) the 5,6-desdimethylbenzimidazole analogue (DDMBA)(5,6) and (3) 5-hydroxybenzimidazole analogue (Bernhauer's Factor III)(7).

Materials. Cobalt-60 labeled analogues (specific activity $\simeq$ 180 μc/mg) for oral administration were prepared by fermentation in medium containing cobalt-60 and appropriate precursors. Products were isolated by methods previously described for vit. B_{12}, followed by chromatography on cellulose(8).* The DCBA was prepared in the Merck Sharp & Dohme Research Laboratories.†

Method. Urinary excretion method of Schilling(9) was employed as index of absorption. This was(1,2) valid for cobalamins by comparison with 3 other test methods. An oral dose of 2 μg (except for 4 subjects as shown in Table I) of labeled derivative was fed in aqueous solution to normal young adult males, each receiving 1 mg injection (except where otherwise stated) of unlabeled derivative 2 hours after the oral dose. Urine was collected for 24 hours, and, in Study 6, for

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