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The Passive Transfer of Delayed Hypersensitivity in Guinea Pigs by the Transfusion of Isotopically-Labelled Lymphoid Cells

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Summary. Delayed hypersensitivity to tuberculin and contact sensitivity to picryl chloride were transferred passively to normal guinea pigs by the intravenous injection of spleen, lymph node and peripheral blood mononuclear cells labelled *in vitro* with ^{51}Cr or *in vivo* with ^{32}P or ^{3}H-thymidine. No significant difference was found between the number of injected cells arriving at the passive lesions and actively induced lesions in controls, after 24 hours. The proportion of labelled cells in the exudate was the same as that in the peripheral blood and they showed a random distribution throughout the lesion.

The fate of labelled cells in the body was followed, especially their role of elimination from the lungs and peripheral blood.

It was concluded that no special affinity of the transferred cells for antigen could be demonstrated after 24 hours.

INTRODUCTION

Cellular transfer of delayed hypersensitivity in the guinea pig was demonstrated by Landsteiner and Chase in 1942. Since then there has been considerable speculation as to the mechanism of the reaction. This has become of increasing interest as the concept of delayed hypersensitivity has been widened to include not only contact sensitivity to simple chemicals (Landsteiner and Chase, 1942) and bacterial allergy (Chase, 1945), but also the homograft reaction (Brent, Brown and Medawar, 1958) and experimental auto-immune diseases (Waksman, 1959).

It has been suggested that, in the tuberculin reaction, circulating sensitized cells and locally injected antigen interact to produce the local reaction (Metaxas and Metaxas-Bühler, 1959). The aim of the present investigation was to find out whether lymphoid cells, injected intravenously to confer passive sensitivity to tuberculin and picryl chloride, showed any evidence of a specific localization at the site of application of antigen after the transfusion in animals' skin tested at the same time. It was also considered important to determine the fate of the injected cells in the body, in case this might throw some light on the mechanism of the cellular transfer.

MATERIALS AND METHODS

ANIMALS. Female albino guinea pigs of the Hartley strain weighing 450 g. initially were used throughout.

RADIOACTIVE ISOTOPES. ^{51}Cr-sodium chromate (CJS1), ^{32}P-orthophosphate (PBS2) and ^{3}H-thymidine (sp. activity 0.63 C/mM) were obtained from the Radiochemical Centre, Amersham, Bucks.

MEASUREMENT OF RADIOACTIVITY. ^{51}Cr was measured in a well-type scintillation counter and ^{32}P in either the scintillation counter or, if a higher efficiency of counting was desired, in a liquid sample β counter.

Measurement of radioactivity from ^{51}Cr in a skin lesion was performed on the whole lesion, which was cut out and placed in a standard glass counting tube. This was compared with the radioactivity in a piece of normal skin of the same weight taken from the opposite side of the same animal.

In the case of lesions containing ^{32}P the skin was wet ashed in concentrated H_2SO_4 and H_2O_2 (100 vol. A.R.), and brought to a volume of 10 ml. before the radioactivity was measured in the liquid sample β counter. Comparison was also made of the radioactivity in the lesion and the same weight of skin from the opposite side of the same animal.

Autoradiographs for detecting labelling with ^{3}H-thymidine were prepared from smears or sections by the stripping film technique (Pelc, 1956). The preparations were exposed 21 days and stained through the emulsion with Giemsa stain. Blocks of tissue were fixed in Carnoy's solution for 1 hour, followed by 10 per cent formol saline. Smears were fixed in methanol for 15 minutes.

ACTIVE SENSITIZATION OF GUINEA PIGS. Donor guinea pigs were sensitized to tuberculin with water in oil emulsion of killed tubercle bacilli and to picryl chloride as described by Chase (1954). Control recipient guinea pigs were sensitized to human γ globulin (HGG — prepared on DEAE cellulose) by the injection of antigen-antibody precipitates in incomplete Freund's adjuvant (Difco) (Turk and Humphrey, 1961). Active delayed reactions to HGG were induced by the intradermal injection of 10 μg. HGG 5 days after sensitization. Other control recipients were actively sensitized to picryl chloride by painting a 4 per cent solution of picryl chloride in ethanol on the back of the neck daily for 7 days and skin tested by painting with 1 per cent picryl chloride in olive oil within 1 week.

PREPARATION OF CELL SUSPENSIONS AND PASSIVE TRANSFER. Lymph-node, spleen-cell suspensions and suspensions of peripheral blood leucocytes were prepared according to Turk (1960, 1961a). Spleen and lymph-node suspensions contained between 5×10^8 and 1×10^9 cells and peripheral blood leucocyte suspensions contained 2.5×10^8 cells. All suspensions were injected intravenously. Immediately after transfusion the recipients were skin tested with 40 μg. tuberculin (Mammalian PPD from the Ministry of Agriculture's Veterinary Laboratory, Weybridge, which had been previously dialysed to remove the glycerol), or by painting the skin with 1 per cent picryl chloride in olive oil. Skin reactions were read 24 hours later, and reactions to picryl chloride were graded as follows— ++++, pink usually very slightly elevated; +++, pink, but either somewhat pale or macular; ++, pale pink; +, faint pink; ±, many pale spots; +++±, ++±, +±, were recognized as intermediate reactions.

TRACE LABELLING OF CELL SUSPENSIONS

(a) *^{51}Cr-sodium chromate*

Lymph-node and spleen cells were labelled with ^{51}Cr *in vitro* by a method similar to that of Shorter and Bollman (1960). Between 5×10^8 and 1×10^9 washed mononuclear cells were incubated for 1 hour in an ice and water bath with 150 μc. ^{51}Cr and then

washed three times in Hanks's solution, before transfusion. The specific activity of the suspensions was between 0.4 and $1.8 \times 10/$ counts/1000 seconds/10^4 cells, as measured in the scintillation counter.

(b) *^{32}P-orthophosphate*

Donor guinea pigs received 1 μc./g. body weight ^{32}P intravenously, in divided doses, 24 and 16 hours before being killed. Spleen and lymph-node cells prepared as described above were washed three times before transfusion. The specific activity of the suspensions was between 1.5 and 3.6×10^3 counts/1000 seconds/10^6 cells, as measured in the liquid β counter.

(c) *^{3}H-thymidine*

Donor guinea pigs received 0.75 μc./g. body weight in three divided doses intravenously 24, 20 and 16 hours before being killed. Spleen and peripheral blood leucocyte suspensions were prepared as above. Autoradiographs of the suspensions before transfusion showed that 30 per cent of the spleen cells and between 10 and 30 per cent of the peripheral blood lymphocytes were labelled.

ESTIMATE OF NUMBER OF CELLS ARRIVING IN THE LESION. In experiments using ^{51}Cr and ^{32}P it was not known what percentage of cells in a labelled cell population actually carried the label. For the purposes of comparison it was presumed that 100 per cent of the cells were labelled, though there was no evidence for this. The term 'cell equivalent' was therefore introduced and the following expression used to indicate the number of cell equivalents present in a particular tissue.

$$\text{Number of cell equivalents} = \frac{(\text{Number of counts/1000 seconds in lesion}) \times (\text{number of cells injected})}{\text{Number of counts/1000 seconds injected}}$$

Since there was always a considerable background of radioactivity in the normal skin of guinea pigs 24 hours after transfusion of ^{51}Cr- or ^{32}P-labelled cells, this had to be corrected.

$$\text{Number of cell equivalents in lesion} = \left(\text{Number of counts/1000 seconds in lesion} - \text{Number of counts/1000 seconds in same weight of normal skin}\right) \times \frac{\text{Number of cells injected}}{\text{Number of counts/1000 seconds injected}}$$

Estimates were made of the total number of circulating ^{51}Cr-labelled leucocytes from a knowledge of the specific activity of a known number of washed leucocytes and the white-cell count in the peripheral blood, assuming the total circulating blood volume to be equivalent to 8 per cent of the total body weight.

RESULTS

ESTIMATE OF THE TOTAL NUMBER OF CELLS IN ACTIVELY INDUCED TUBERCULIN AND PICRYL CHLORIDE LESIONS

Experiments were designed to give a rough estimate of the total number of lymphoid cells present in the skin 24 hours after the induction of a tuberculin or picryl chloride

lesion. Guinea pigs were given 75–100 μc. ^{32}P intravenously in two doses 24 and 16 hours before skin testing. Skin lesions were removed 24 hours after skin test and the radioactivity estimated. Cell preparations were made from the lymph nodes and spleen at the same time for the purpose of obtaining an estimate of the specific activity of these cells. The number of labelled mononuclear cell equivalents was determined according to the following expression:

$$\text{Number of mononuclear cell equivalents in lesion} = \frac{\text{(Number of counts/1000 seconds in lesion)} - \text{(Number of counts/1000 seconds in same weight of normal skin)}}{\text{Mean specific activity of spleen and lymph node cells at the same time (counts/1000 seconds/cell)}}$$

It would not be expected that circulating polymorphonuclear cells would be labelled so soon after administration of ^{32}P, but in order to check this point a peritoneal exudate was induced by the injection of 20 ml. 3 per cent peptone 24 hours before killing and the radioactivity in the exudate cells was compared with the radioactivity of lymph-node and spleen mononuclear cells. The exudate contained 84 per cent polymorphonuclear leucocytes, but the radioactivity present could be accounted for completely by the mononuclear cells present in the exudate. It was therefore presumed that polymorphonuclear leucocytes were not significantly labelled with ^{32}P in these experiments.

In Figs. 1 and 2 the number of mononuclear cell equivalents present in tuberculin and picryl chloride lesions are compared with the intensity of the lesion. In both cases there

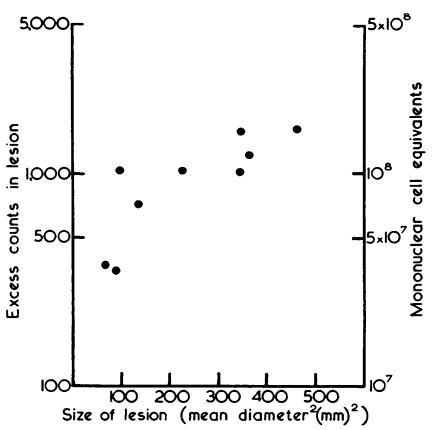

FIG. 1. Estimate of the total number of cells in actively induced tuberculin lesions. Excess counts standardized to specific activity equivalent to 10 counts/10^6 cells.

is good correlation between the number of labelled cell equivalents present and the intensity of the lesion. 10^8 cell equivalents were present in a tuberculin lesion of size $12 \times 12 - 15 \times 15$ mm. or a picryl chloride lesion with a $+++$ intensity.

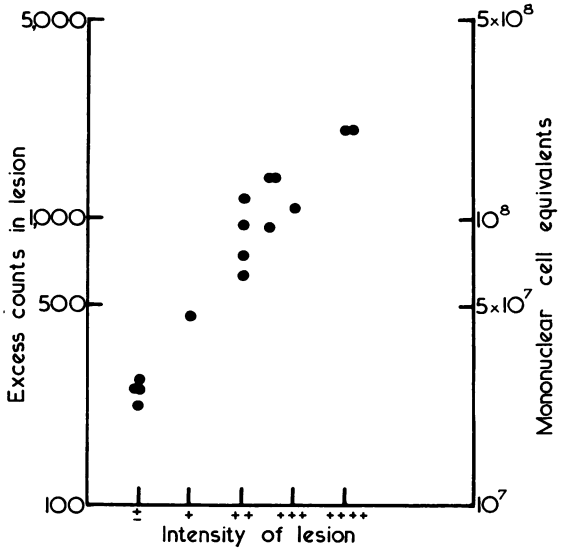

FIG. 2. Estimate of the total number of cells in actively induced contact lesions to picryl chloride. Excess counts standardized to specific activity equivalent to 10 counts/10^6 cells.

TABLE 1

COMPARISON OF THE ARRIVAL OF TRANSFUSED ^{51}Cr- AND ^{32}P-LABELLED LYMPHOID CELLS FROM TUBERCULIN-SENSITIVE GUINEA PIGS AT PASSIVELY INDUCED DELAYED SKIN LESIONS TO TUBERCULIN AND ACTIVELY INDUCED DELAYED SKIN LESIONS TO HGG

Weight of lesion (mg.)	*Diameters of lesion (mm.)*	*Cell equivalents per lesion*	*Cell equivalents per mg. skin*
(a) *Tuberculin lesion — cells labelled with ^{51}Cr*			
533	14 × 15	8.2×10^5	15.4×10^2
240	12 × 10	3.1×10^5	12.8×10^2
187	11 × 9	2.0×10^5	10.7×10^2
295	12 × 10	1.13×10^5	3.9×10^2
272	11 × 11	1.12×10^5	4.1×10^2
183	10 × 8	0.97×10^5	5.3×10^2
338	11 × 11	0.93×10^5	2.75×10^2
207	12 × 10	0.82×10^5	4.00×10^2
235	11 × 11	0.70×10^5	3.00×10^2
(b) *Tuberculin lesion — cells labelled with ^{32}P*			
260	12 × 10	3.9×10^5	15.0×10^2
350	13 × 13	3.95×10^5	11.3×10^2
250	12 × 10	1.45×10^5	5.8×10^2
260	10 × 10	0.58×10^5	2.2×10^2
(c) *HGG active lesion — cells labelled with ^{51}Cr*			
580	24 × 24	4.7×10^5	8.1×10^2
307	15 × 12	0.94×10^5	3.0×10^2
371	17 × 17	0.88×10^5	2.33×10^2
336	12 × 12	0.82×10^5	2.45×10^2
408	17 × 17	0.45×10^5	1.1×10^2
260	13 × 13	0.55×10^5	2.1×10^2
301	13 × 13	0.4×10^5	1.3×10^2
(d) *HGG active lesion — cells labelled with ^{32}P*			
322	15 × 15	1.63×10^5	5.0×10^2

Animals transfused with cells and skin tested at same time. Reactions read and radioactivity measured 24 hours later.

ARRIVAL OF ^{51}Cr- AND ^{32}P-LABELLED CELLS AT PASSIVELY INDUCED TUBERCULIN LESIONS 24 HOURS AFTER TRANSFUSION (TABLE 1)

Normal guinea pigs were transfused with lymph-node and spleen cells, labelled with ^{51}Cr and ^{32}P, from donor guinea pigs actively sensitized to tuberculin. The recipients were tested with 40 μg. PPD immediately after the transfusion. The amount of radioactivity present in the lesion after 24 hours in excess of that present in the same weight of normal skin was found to be equivalent to between 0.7 and 8×10^5 cells. This was between 0.3 and 1.4×10^3 'cell equivalents' per mg. of skin, and represented the radioactivity of between 0.005 and 0.07 per cent of the cells injected. Similar results were obtained with both labels.

Guinea pigs actively sensitized so as to produce delayed-type responses to HGG were also transfused with lymphoid cells labelled with ^{51}Cr and ^{32}P from similar tuberculin-sensitive donors and were skin tested with 10 μg. HGG immediately after transfusion. The numbers of 'cell equivalents' arriving at the actively induced lesions were found to be slightly less but of the same order as those arriving at the passively induced tuberculin lesions. The apparent small difference between lesions of the same size is probably of no significance and due to the fact that HGG lesions were more diffuse than the tuberculin lesions.

ARRIVAL OF ^{51}Cr-LABELLED CELLS AT PASSIVELY INDUCED PICRYL CHLORIDE LESIONS 24 HOURS AFTER TRANSFUSION (TABLE 2)

Normal guinea pigs were transfused with lymphoid cells, labelled with ^{51}Cr, from donor guinea pigs actively sensitized to picryl chloride, and were skin tested by painting with

TABLE 2

COMPARISON OF THE ARRIVAL OF TRANSFUSED ^{51}Cr-LABELLED LYMPHOID CELLS FROM GUINEA PIGS AT PASSIVELY INDUCED CONTACT LESIONS TO PICRYL CHLORIDE, WITH THE ARRIVAL OF SIMILARLY LABELLED CELLS FROM TUBERCULIN-SENSITIVE GUINEA PIGS AT ACTIVELY INDUCED LESIONS TO PICRYL CHLORIDE

Weight of lesion (mg.)	*Intensity of lesion*	*Cell equivalents per lesion*	*Cell equivalents per mg.*
(a) *Passively induced picryl chloride lesions — cells from picryl chloride sensitive donors*			
388	+++	2.73×10^5	7.0×10^2
830	+++	4.35×10^5	5.25×10^2
330	+++	2.1×10^5	6.4×10^2
217	++±	0.96×10^5	4.45×10^2
282	++±	1.03×10^5	3.66×10^2
233	++	0.59×10^5	2.5×10^2
249	++	0.6×10^5	2.4×10^2
(b) *Actively induced picryl chloride lesion — cells from tuberculin sensitive donors*			
692	+++	2.75×10^5	4.0×10^2
649	+++	1.98×10^5	3.2×10^2
866	++±	2.43×10^5	2.8×10^2
503	++	1.6×10^5	3.2×10^2
904	++	2.55×10^5	2.8×10^2
712	+±	0.9×10^5	1.27×10^2

Animals transfused with cells and skin tested at same time. Reactions read and radioactivity measured 24 hours later.

1 per cent picryl chloride in olive oil immediately after transfusion. The radioactivity in the lesion, 24 hours later, was between 0.6 and 4.5×10^5 'cell equivalents' representing between 0.01 and 0.05 per cent of the total number of cells in the exudate as estimated above.

Guinea pigs actively sensitized to picryl chloride by a technique which did not employ tubercle bacilli were transfused with ^{51}Cr-labelled lymphoid cells from tuberculin-sensitized donors, and their skin was tested with 1 per cent picryl chloride immediately after transfusion. The number of 'cell equivalents' arriving at the lesions was again of the same order as that arriving at the passively induced lesions.

ARRIVAL OF LYMPHOID CELLS LABELLED WITH ^{3}H-THYMIDINE AT PASSIVELY INDUCED TUBERCULIN LESIONS 24 HOURS AFTER TRANSFUSION (TABLE 3)

Spleen cells and peripheral blood leucocytes, labelled with ^{3}H-thymidine, from tuberculin-sensitized donors were transfused to normal recipients, which were skin tested with 40 μg. PPD immediately after the transfusion. Twenty-four hours later, the lesion was

TABLE 3

ARRIVAL OF ^{3}H-THYMIDINE-LABELLED DONOR CELLS AT SITE OF PASSIVELY TRANSFERRED TUBERCULIN LESION

Donor cells		*No. of cells injected*	*% of injected cells labelled*	*Diameters of lesion (mm.)*	*% labelling of mononuclear cells in peripheral blood of recipient 24 hours after transfusion*	*% labelling of lymphoid cells in lesion 24 hours after transfusion*
Spleen	Donor 1	1×10^9	33	20 × 20	2.75	2
	Donor 2	6.6×10^8	29	15 × 15	1.0	1
Peripheral-blood leucocytes	Donor 1	2.5×10^8	30	14 × 14	1.4	0.78
	Donor 2	2.2×10^8	11	12 × 12	0.5	0.4

removed for histological examination and smears were made from preparations of peripheral-blood leucocytes. Autoradiographs were then prepared from each. Labelled cells were found not only in the exudate round the blood vessels between the musculus carnosus and the dermis, but also throughout the dermis (Figs. 3 and 4). Their distribution was apparently random, in the sense that foci consisting mainly of labelled cells were not observed. A rough attempt was made to estimate what proportion of lymphoid cells in the skin lesions were labelled with ^{3}H, for comparison with the mononuclear cells in the blood. The percentages were very similar.

FATE OF TRANSFUSED CELLS

(a) *^{51}Cr-labelled cells (Table 4)*

The amount of radioactivity present in the liver, spleen, lungs and peripheral blood was determined 10 minutes, 1 hour and 24 hours after the transfusion of ^{51}Cr-labelled lymphoid cells. Between 40 and 50 per cent of the injected radioactivity was in the liver

within 10 minutes of transfusion and this proportion remained constant throughout this period. The proportion in the spleen increased from 1 per cent to 6 per cent and the proportion in the lungs fell from 35 per cent 10 minutes after transfusion to 7.5 per cent after 24 hours. The percentage of injected cells in the circulation was only 3.5 per cent 10 minutes after transfusion and fell to 1 per cent after 24 hours.

TABLE 4

PERCENTAGE OF RADIOACTIVITY PRESENT IN TISSUES 10 MINUTES, 1 HOUR AND 24 HOURS AFTER TRANSFUSION WITH ^{51}Cr-LABELLED LYMPHOID CELLS

	% of radioactivity injected			*Peripheral blood*	
	Liver	*Spleen*	*Lung*	*% cells injected*	*% circulating cells*
10 minutes	44	1.16	34.6	3.4	7.3
1 hour	38	3.73	17.7	2.65	2.5
24 hours	49	6.0	7.65	0.81	1.94

The calculated percentage of circulating cells which were labelled was 7.5 per cent after 10 minutes and 2 per cent after 24 hours. This figure corresponds well with the percentage of circulating cells which were actually observed to be labelled with ^{3}H-thymidine in autoradiographs prepared from smears of peripheral blood 24 hours after transfusion.

(b) *^{3}H-thymidine-labelled cells*

Autoradiographs of sections of liver and lungs of animals transfused with ^{3}H-thymidine-

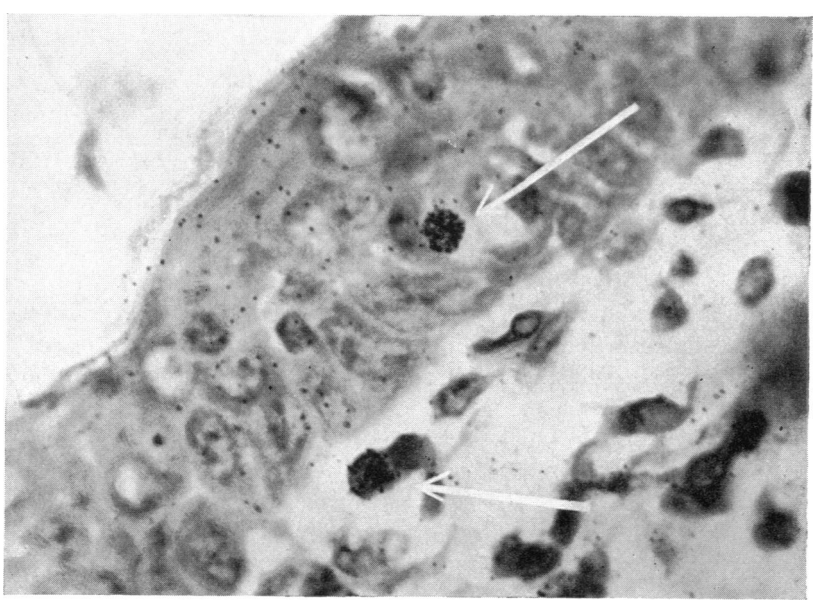

FIG. 3. ^{3}H-labelled lymphocytes in passively transferred tuberculin lesion — subepidermal dermis. (×860).

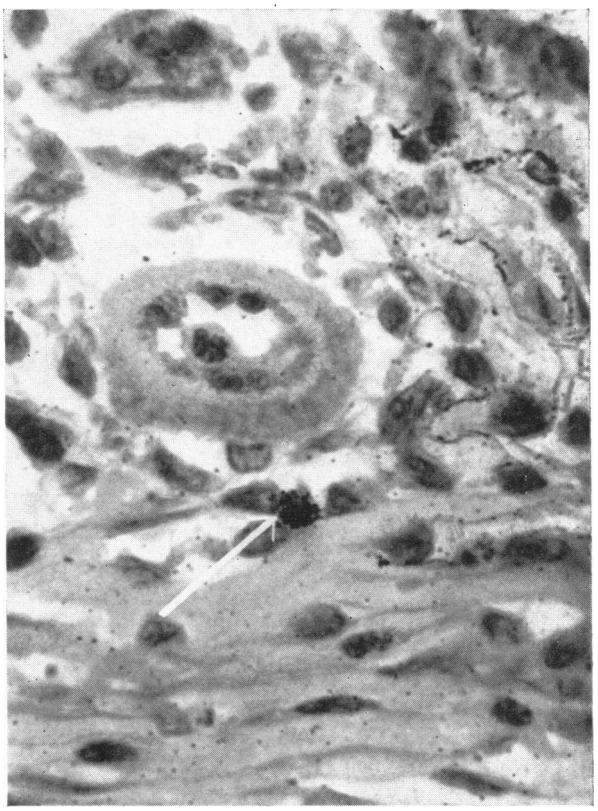

FIG. 4. ^{3}H-labelled lymphocyte in passively transferred tuberculin lesion — dermis superficial to musculus carnosus. (×860).

labelled lymphoid cells 24 hours previously were examined. In the liver the Kupffer cells were prominent; there was some periportal accumulation of lymphoid cells which were present also scattered throughout the liver. However, only a small proportion of the Kupffer cells contained traces of the ^{3}H label and only a few of the lymphoid cells were labelled.

The lungs were infiltrated with lymphoid cells, macrophages and occasional eosinophils, which were predominantly peribronchiolar in distribution. Only a few of the lymphoid cells were labelled and a small proportion of the macrophages contained traces of the ^{3}H label.

DISCUSSION

In a recent review, Metaxas and Metaxas-Bühler (1959) discussed five hypotheses to explain the cellular transfer of tuberculin hypersensitivity.

(1) Circulating cells and locally injected tuberculin interact to produce a local lesion.
(2) Transferred cells carry specific 'tuberculin antibody', presumably in their surface.

(3) Cells, mediating transfer, carry antibody but release it only when they come into contact with tuberculin.

(4) Donor cells within the recipient gradually elaborate and set free antibody which is taken up by the recipient tissues.

(5) Transferred cells 'activate' host cells, possibly by passing on to them some 'enzymic mechanism' connected with antibody formation.

When delayed-type reactions were elicited in actively sensitized guinea pigs whose cells were labelled endogenously with ^{32}P, the number of lymphoid cells estimated to arrive after 24 hours in a 13×13 mm. tuberculin reaction would be about 10^8 'cell equivalents' and in a $+++$ picryl chloride lesion about the same (Figs. 1 and 2). When reactions of similar size and intensity were elicited after passive transfer of labelled sensitized cells, the number of donor cells arriving at a corresponding reaction site was estimated to be $1-3 \times 10^5$ (Tables 2 and 3). According to such calculations less than 1 per cent of the mononuclear cells which arrive in a lesion appear to have come from the donor. Moreover, no clear difference could be found between the number of cells arriving at sites of actively induced lesions (heterologous system) and arriving at passively induced (homologous) lesions.

The passive transfer experiments with ^3H-thymidine-labelled cells, in which a direct estimate was made of the percentage of labelled mononuclear cells in tuberculin lesions, indicated that some 3 per cent of the lymphoid cells were derived from the sensitized cells transferred.

There is thus no evidence for any marked accumulation of transferred cells in response to the antigen at the site of a mature 24-hour delayed lesion to tuberculin or picryl chloride. The possibility is not excluded that some of the transferred cells might have initiated the reaction during the previous 24 hours, and after doing so have been destroyed, leaving no trace, or have returned to the circulation. It is also possible that the donor cells, although they appear to arrive in lesion in a random manner, are in some way mediating the reaction without forming any obvious focus.

There is an apparent discrepancy between the rapid accumulation of ^{51}Cr-labelled cells in the liver and the presence of only small amounts of ^3H-labelled cells in the Kupffer cells 24 hours after transfusion. The ^{51}Cr label, however, would tend to remain in the liver Kupffer cells after breakdown of labelled cells (Mollison and Veall, 1955), whereas the breakdown products of DNA would be released. From the results with either label it would appear that about 40 per cent of labelled cells pass within 10 minutes of transfusion to the liver, where they are probably broken down quite rapidly by the Kupffer cells. There is also evidence of breakdown products of donor cells in the lung macrophages. The possibility cannot be excluded that donor cells release some specific sensitizing material when they are taken up by the reticulo-endothelial system, and that this material is taken up by host cells, which then pass back into the circulation and eventually reach the site of antigen application.

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