THE PRODUCTION OF MACROPHAGES IN THE RAT

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THE identity of the leucocyte in the blood which gives rise to the macrophage of inflammatory exudates is still a matter of debate. Ebert and Florey (1939) demonstrated in a rabbit ear chamber that monocytes could emigrate from the blood into a site of inflammation and evolve into macrophages. However, it is not universally accepted that emigrating monocytes are the major source of the macrophages in inflammatory exudates. One influential view is that they are derived from small lymphocytes (Rebuck and Crowley, 1955 ; Rebuck, Monto Monaghan and Riddle, 1958 ; Rebuck, Petz, Riddle, Priest and Lo Grippo, 1961). The conclusions of Rebuck and his colleagues are based on a technique in which glass coverslips (" skin windows ") are applied to areas of abraded human skin. Exudate cells adhere to the windows and can be examined microscopically by removing the coverslips at intervals. It was concluded that small lymphocytes evolved through a series of transitional forms into macrophages. A separate origin from monocytes was not excluded but Rebuck and Crowley (1955) claim to be able to distinguish morphologically between " lymphocytogenous " and " monocytogenous " macrophages. On the other hand, Riis (1959), in similar studies, did not observe a regular progression of enlarging monuclear cells on human skin windows.

Braunsteiner, Hofer and Sailer (1961) employed the skin window technique in rats and showed that the macrophages on the coverslips became labelled after multiple injections of tritiated thymidine. Braunsteiner, Paertan and Thumb (1958) also described the passive transfer of delayed hypersensitivity with human exudate cells which had been removed from skin windows. It was concluded that the lymphocyte is unquestionably the precursor of the macrophage in both rats and humans.

The present paper records an autoradiographic study on an adaptation of Rebuck's technique to the rat. The pattern of labelling among the exudate macrophages after a single injection of tritiated thymidine does not fully support the idea of a lymphocyte-to-macrophage transformation.

MATERIALS AND METHODS

Animals.—Pairs of rats for parabiosis were female littermates of the highly inbred hooded (HO) strain. Female rats belonging to a randomly-bred albino colony and weighing 100–200 g. were used in other experiments.

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Coverslip preparations

Samples of inflammatory exudates were obtained on coverslips in one of two ways :

Surface coverslips (skin windows).—Hair was removed with electric clippers from an area on each side of the dorsal thorax and the skin was cleaned and shaved.   The surface of each area was then scraped with a scalpel blade in a circle approximately ¼ in. in diameter until tiny bleeding points appeared.   A drop of human sterile old tuberculin diluted with equal parts of sterile saline was applied to each site as a stimulus to inflammation.   This application never produced vesication or necrosis.   Circular glass coverslips, ⅜–½ in. diameter, were applied to the abraded areas, covered with a dressing, and held in place with an elastic adhesive bandage which encircled the animal (Fig. 1).   Excessive pressure and strong antiseptics were avoided since they were found to interfere with emigration of cells onto the coverslip.

Subcutaneous coverslips.—Two small linear incisions were made on either side of the thorax and the tips of haemostats were inserted and spread in order to create a subcutaneous pocket of sufficient size to accommodate a coverslip.   No irritants were applied since it was found that the glass provoked an adequate inflammatory reaction when placed subcutaneously.   The wounds were closed with Michel clips.

The coverslips were removed at various intervals after application, rapidly dried in the cool air stream of a hair dryer, fixed in methyl alcohol, and stained with either May-Grünwald-Giemsa or Leishman's stain.   Preparations which were to be used for autoradiography were not stained until after exposure and development.

Autoradiography

Autoradiographs were prepared with Kodak AR.10 stripping film and sets were exposed for 2 weeks and one month.   No significant difference was noted in the proportion of labelled cells when duplicate preparations were exposed for these two periods.

Tritiated thymidine

Rats were injected by way of the lateral tail veins with a single dose of 1 μc of tritiated thymidine per g. body weight (specific activity, 4·32–9·5 c/mM ; Radiochemical Centre, Amersham).

Leucocyte concentrates

Rats were anaesthetized and bled from the heart or the aorta.   Leucocyte concentrates were prepared by the dextran sedimentation method of Skoog and Beck (1956), smeared, dried rapidly in a current of air, and fixed in methyl alcohol.

Parabiosis

Two pairs of rats were joined by a modification of the technique of Sauerbruch and Heyde (1908).   The peritoneal cavities were not in communication.   At the time of the experiment one pair had been united for 2 months and the other for 3 months.

RESULTS

Surface Coverslips

Sequence of cell-types

Polymorphonuclear leucocytes were the first cells to appear.   Mononuclear cells of varying sizes were found at about 4 hr. and persisted in increasing numbers up to 24 hr.   No strict sequence of development among mononuclear cells said to occur in human material by Rebuck and Crowley (1955) was observed ; small lymphocytes were rare at all times.   Large cells identifiable as macrophages were present from about 6 hr. and were abundant by 12 hr. (Fig. 2) ; by 24 hr. they were usually the predominating cell-type and were frequently arranged in sheets of contiguous cells (Fig. 3).   Many variations of size and shape were seen among the macrophages and it was found impossible to separate " lymphocytogenous "

and " monocytogenous " types on morphological grounds.   No mitotic figures were seen in any of the preparations.

Labelling with tritiated thymidine

The proportion of labelled macrophages on the coverslips was studied in a series of rats in which the time of injection of tritiated thymidine was varied with respect to the time of application of the coverslip.   Three groups of experiments were performed.   In a first, tritiated thymidine was given at various times after coverslip application ;   in a second, it was injected at the same time the coverslip was applied ;   and in the third it was given at various intervals before the coverslip application.   In all the experiments the coverslips were removed 24 hr. after application.

Tritiated thymidine given after coverslip application.—Each of 5 animals was given a single intravenous injection of tritiated thymidine 3, 6, 9, 12 and 18 hr. after coverslip application.   The 3, 6 and 12 hr. intervals were repeated in separate animals.   Table I shows that few macrophages became labelled, the proportion never exceeding 8 per cent.   When tritiated thymidine was given 18 hr. after the application of the coverslips no labelled cells were obtained.

Tritiated thymidine given at the same time as coverslip application.—Three rats were injected with tritiated thymidine immediately after the application of coverslips.   Table II shows that a slight but questionably significant rise in the percentages of labelled macrophages was obtained.

Tritiated thymidine given before coverslip application.—Each of 6 rats was given a single intravenous injection of tritiated thymidine at 6, 12, 15, 18, 21 and 24 hr. before coverslip application.   The 6, 12 and 21 hr. intervals were repeated in another group of rats.   A 120 hr. interval was studied in 3 rats and an 8 day interval in one.   Table III shows that when tritiated thymidine was given 18–21 hr. before coverslip application the proportion of labelled macrophages approached 60 per cent.   The proportion fell to less than 2 per cent when the interval was increased to 120 hr., and to zero with an interval of 8 days (Fig. 4).   The degree of labelling which can be achieved by giving tritiated thymidine 24 hr. before the application of a coverslip is shown in Fig. 6.


EXPLANATION OF PLATES

FIG. 1.—A circular coverslip (" skin window ") has been placed on an area of abraded skin and is being held in place with a gauze dressing attached to a strip of adhesive bandage.

FIG. 2.—Skin window removed after 12 hr.   Macrophages with vacuolated cytoplasm are abundant.   (May-Grünwald Giemsa.   × 1720.)

FIG. 3.—Skin window removed after 24 hr.   Macrophages are the predominating cell-type and many are arranged in sheets.   (May-Grünwald Giemsa.   × 110.)

FIG. 6.—Autoradiograph of macrophages on a skin window which had been in place for 24 hr.   A single injection of tritiated thymidine had been given 24 hr. before the coverslip was applied.   Most of the cells in this field are labelled, some heavily.   Over the whole coverslip about half the macrophages were labelled (Exposure 14 days.   May-Grünwald Giemsa. × 1200).

FIG. 7 and 8.—Autoradiographs of imprints of intestinal epithelium from the " donor " and " recipient " respectively of a pair of rats in parabiosis.   The pedicle between the rats was clamped immediately before the injection of tritiated thymidine into the " donor " and removed 1 hr. later.   Forty-eight hr. after the injection the donor's epithelium shows heavy labelling, the recipient's none (see text).   (Exposure, 14 days.   May-Grünwald Giemsa. × 1800).
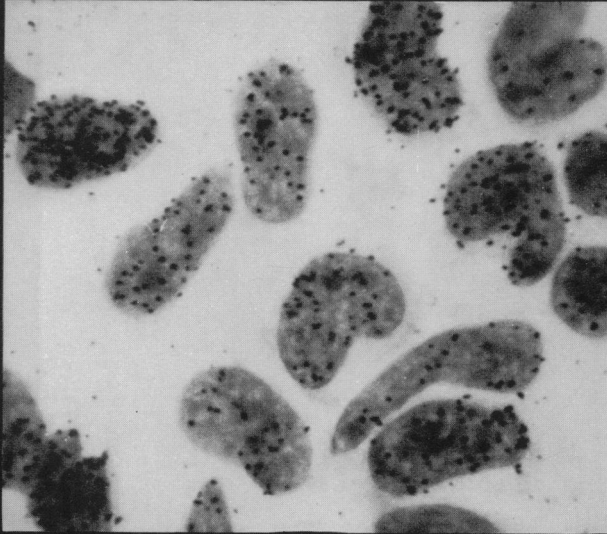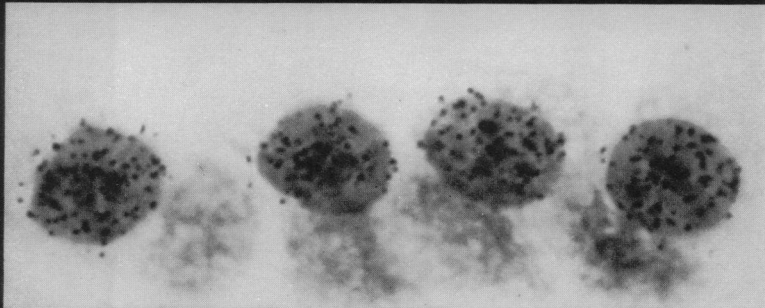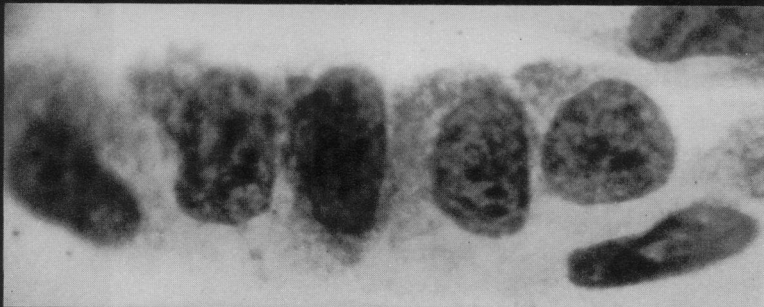
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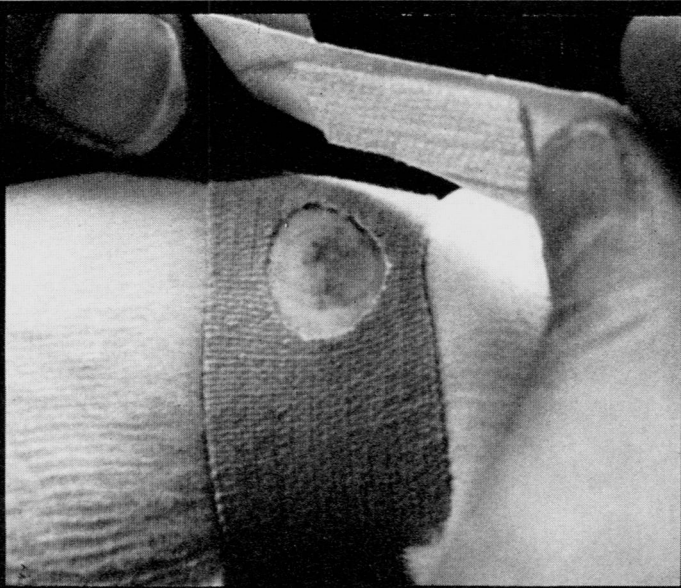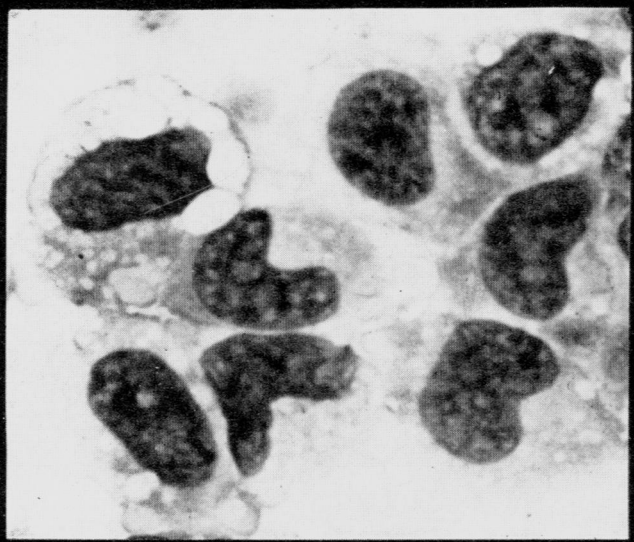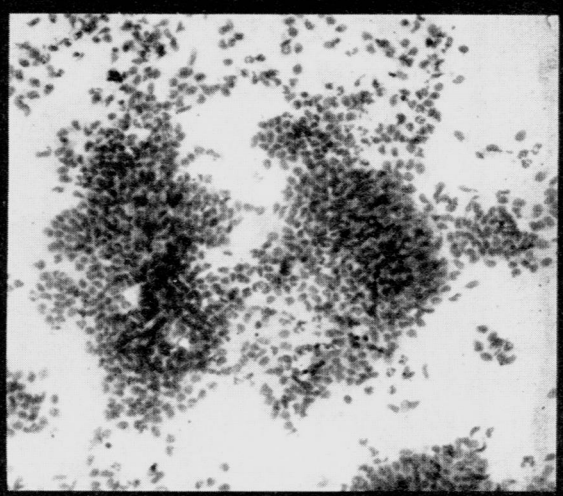
TABLE I.—*Percentage of Labelled Macrophages on Skin Windows when a Single Injection of Tritiated Thymidine was given at Various Times AFTER Application of a Coverslip (CS). Each Coverslip Removed 24 hr. After Application.*

^{3}H-thymidine: hr. AFTER CS applied	Rat no.	Macrophages*	
		No. counted	Per cent labelled
3	. 1 .	500, 1000	1·0, 5·7
	. 2 .	500, 500	4·0, 0·2
6	. 3 .	500, 500	2·4, 6·2
	. 4 .	500, 500	1·2, 1·0
9	. 5 .	100, 300	0, 0·3
12	. 6 .	500, 500	0·2, 0·6
	. 7 .	500, 500	0·2, 0·2
18	. 8 .	200	0

* Pairs of values for a single rat are counts on each of two coverslips from the same animal.

TABLE II.—*Percentage of Labelled Macrophages on Skin Windows when a Single Injection of Tritiated Thymidine was given at the Same Time as the Application of a Coverslip. Each Coverslip Removed 24 hr. After Application.*

Rat no.	Macrophages*	
	No. counted	Per cent labelled
1 .	260	7·7
2 .	500, 500	2·4, 2·2
3 .	1000, 500	2·5, 10·0

* Pairs of values for a single rat are counts on each of two coverslips from the same animal.

TABLE III.—*Percentage of Labelled Macrophages on Skin Windows when a Single Injection of Tritiated Thymidine was given BEFORE the Application of a Coverslip (CS). Each Coverslip Removed 24 hr. After Application.*

^{3}H-thymidine: hr. BEFORE CS applied	Rat no.	Macrophages*	
		No. counted	Per cent labelled
6	. 1 .	400, 500	12·3, 14·0
	. 2 .	500, 500	17·0, 21·4
12	. 3 .	200, 500	39·0, 41·4
	. 4 .	500, 500	49·4, 49·0
15	. 5 .	200, 500	53·0, 49·6
18	. 6 .	200, 100	57·5, 44·0
21	. 7 .	200, 1012	45·5, 55·4
	. 8 .	500, 500	43·8, 42·4
24	. 9 .	500, 500	40·0, 52·0
120	. 10 .	500	0
	11 .	500	1·4
	12 .	500	1·2
	13 .	500	0

* Pairs of values for a single rat are counts on each of two coverslips from the same animal.

Subcutaneous Coverslips

The exudates which were studied on subcutaneous coverslips showed a more complex mixture of mononuclear cells than were seen on the skin windows. The number of macrophages increased with time up to 24 hr. but cells which resembled small lymphocytes were found at all intervals between 4 and 24 hr. Cells which
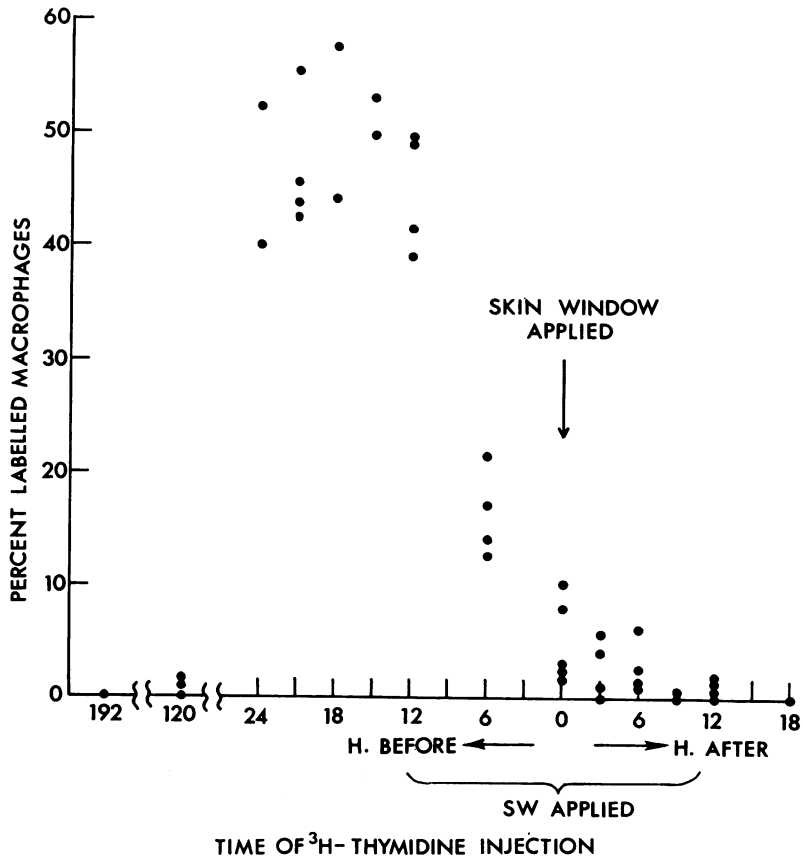

Fɪɢ. 4.—Percentage of labelled macrophages on skin windows when a single intravenous injection of tritiated thymidine was given at varying times with respect to the application of a coverslip. Each coverslip remained in place for 24 hr. (See Tables I, II and III.)

resembled blood monocytes were also seen but many of the large mononuclear cells could not be classified with certainty. Again, no simple progression of intermediates between small lymphocytes and macrophages was observed. Mitotic figures were never seen.

Experiments in which the time of tritiated thymidine injection was varied with respect to the time of insertion of the subcutaneous coverslips gave a labelling pattern for macrophages which corresponded closely to that observed on skin windows. Thus, among those cells which could be identified with certainty as macrophages, a maximum of 35 per cent were labelled when tritiated thymidine

was given 21 hr. before the insertion of the coverslips. The proportion of labelled macrophages fell to less than 2 per cent when tritiated thymidine was given either 90 hr. before or 12 hr. after coverslip insertion.
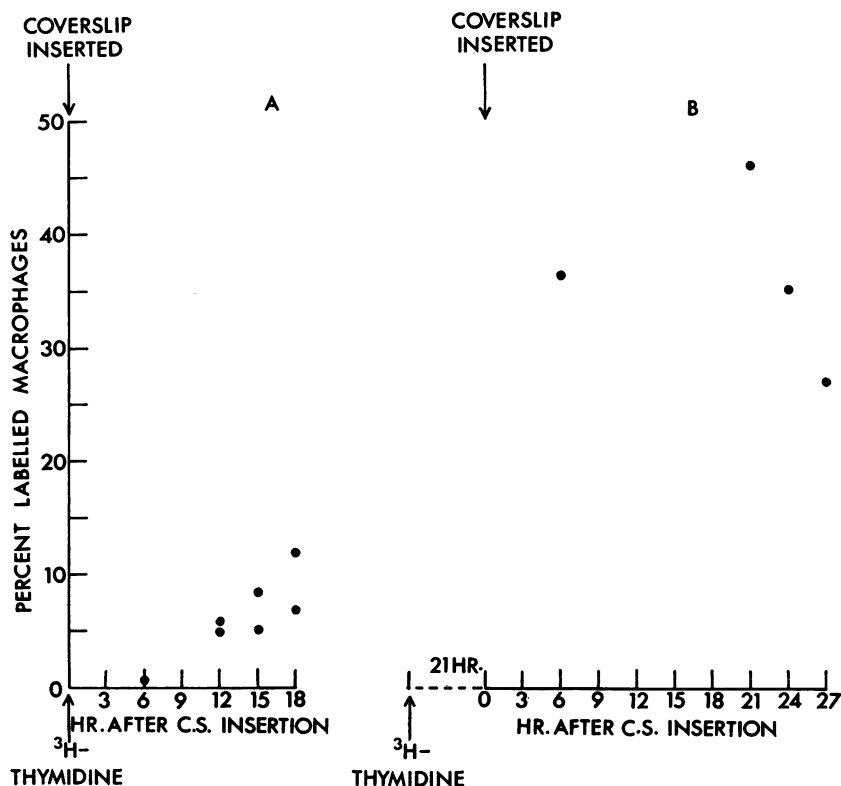

FIG. 5.—Percentage of labelled macrophages on subcutaneous coverslips when a single intravenous injection of tritiated thymidine was given, A : at the same time as coverslip insertion, and B : 21 hr. before coverslip insertion. Coverslips were removed at varying times after insertion.

The time of emergence of labelled macrophages

In the experiments with subcutaneous coverslips and with skin windows it has been shown that in order to obtain maximum labelling of the exudate macrophages it is necessary to give tritiated thymidine about one day before the application of the coverslip. This suggests that time is required for the formation of new cells and for their release, presumably into the blood. Alternatively, it could be argued that DNA-synthesizing antecedents of exudate macrophages are always available in the blood but that the process of emigration onto the coverslip takes an appreciable time. Two further experiments with subcutaneous coverslips were performed to decide between these possibilities.

In the first experiment, tritiated thymidine was injected into a rat immediately after the insertion of 3 pairs of coverslips ; these were removed 12, 15 and 18 hr.

later.   A 6 hr. sample was obtained in a similar manner from another rat.   In the second experiment, a rat was injected with tritiated thymidine 21 hr. before the insertion of 6 coverslips ; these were removed singly at 6, 15, 18, 21, 24 and 27 hr. after insertion.   The results recorded in Fig. 5 show that very few labelled macrophages were obtained at any time up to 18 hr. when the tritiated thymidine had been given at the same time as coverslip insertion.   On the other hand, coverslips removed at identical times after insertion carried a high proportion of labelled macrophages when the tritiated thymidine had been given 21 hr. before insertion : the 6 hr. coverslip already showed labelling of 36 per cent of the macrophages.

These results show that labelled macrophage antecedents emigrate rapidly if they are available, but that they do not become available until an appreciable time has elapsed after the administration of a dose of tritiated thymidine.   The simplest interpretation is that the delay is a measure of the time taken for macrophages (or their immediate antecedents) to be formed and released into the blood.

Incubation of exudate macrophages with tritiated thymidine

The pattern of labelling after single injections of tritiated thymidine and the absence of mitotic figures among the cells on the coverslips suggest that the exudate macrophages were the progeny of cells which divided at a site remote from the area of inflammation.   To determine directly whether cells already on the coverslips were able to incorporate tritiated thymidine, four coverslips were inserted subcutaneously into a rat.   Two were removed after 2 days and incubated for 1 hr. *in vitro* at 37° in 2 ml. of medium " 199 " (Glaxo Ltd.) together with tritiated thymidine at a concentration of 1 μc/ml.   The remaining two coverslips were left in place while the animal was injected with one intravenous dose of 1 μc tritiated thymidine per g. body weight.   An additional 20 μc in 0·2 ml. of saline was injected directly into each coverslip-containing pocket to insure a high local availablity of tritiated thymidine.   The coverslips were removed 2 hr. later. Both pairs of coverslips were washed in several changes of medium " 199 ", fixed, and extracted with ice-cold 5 per cent trichloracetic acid to remove acid-soluble radioactive compounds.

Autoradiographs of the coverslip preparations which had been exposed to tritiated thymidine *in vivo* and *in vitro* showed similar percentages of labelled macrophages : 0·4 and 0·5 per cent respectively were labelled in counts of 1000 cells.   It was concluded that in experiments where high proportions of labelled macrophages were obtained on coverslips they must have been almost exclusively the descendants of cells which divided at another site.

Parabiosis

A direct demonstration that the exudate macrophages were derived from the blood was obtained with parabiotic rats in the following way.

Tritiated thymidine remains available for incorporation into DNA for only a brief period after its injection into animals ; consequently, one member of a parabiotic pair, the " donor ", can be labelled while a clamp on the common skin pedicle temporarily occludes the circulation and prevents the passage of tritiated thymidine to the " recipient " (Bond, Fliedner, Cronkite, Brecher and Schork, 1958).

Two parabiotic pairs were anaesthetized with ether and the circulation through the common skin pedicle was occluded with rubber-shod intestinal clamps The donor of each pair was given a single intravenous dose of tritiated thymidine followed 1 hr. later by an intravenous injection of 3 mg. of non-radioactive thymidine. The clamps were then removed. Subcutaneous coverslips were inserted into donors and recipients 24 hr. after the injection of tritiated thymidine and were removed 24 hr. later still. As a check that no tritiated thymidine had passed into the recipient, imprints of the intestinal epithelium of both donor and recipient were made at the end of each experiment. The cells of the intestinal epithelium divide rapidly ; it could therefore be assumed that if imprints from the recipient showed no labelling, then any heavily labelled cells found elsewhere in the recipient must have originated in the donor and been delivered by the blood after the release of the clamp.

TABLE IV.—*Percentage of Labelled Macrophages on Subcutaneous Coverslips Implanted in Parabiotic Rats 24 hr. After a Single Dose of Tritiated Thymidine. Coverslips Removed 24 hr. after Implantation.*

Parabiotic pair*		Macrophages	
		No. counted	Per cent labelled
1. Donor	.	500	38·6
Recipient	.	500	7·0
2. Donor	.	500	44·2
Recipient	.	500	23·8

* Tritiated thymidine injected into "donor" of each pair and pedicle between each pair clamped during and for 1 hr. after injection.

Table IV shows that the coverslips from both donor rats had a normal proportion of labelled macrophages and it is clear that the labelled macrophages which were also present on the recipients' coverslips (Table IV) must have been derived from the donor rats because autoradiographs of intestinal epithelium (Figs. 7 and 8) show that no tritiated thymidine could have leaked from donor to recipient. The high proportion of labelled macrophages on the coverslip of one of the recipients (23·8 per cent) suggests that the blood is the major source of these cells. No particular significance is attached to the lower proportion of labelled macrophages in the other recipient : poor mixing of blood across the pedicle or damage to blood vessels during temporary occlusion of the common circulation may have prevented a free exchange of cells between the partners.

Labelling in Blood Leucocytes

Autoradiographs were prepared from smears of leucocyte concentrates at various times after a single intravenous injection of tritiated thymidine to determine the proportion of labelled mononuclear cells in the blood. By comparing these values with the proportion of macrophages which became labelled on the coverslips at corresponding times after tritiated thymidine it was hoped to obtain a clue to the identity of the circulating antecedent of the exudate macrophage.

An attempt was made to classify the mononuclear cells of the blood into three groups : small lymphocytes, large lymphocytes (including both " medium " and " large " lymphocytes) and monocytes. A sharp line of division between small

and medium lymphocytes was difficult to draw and cells in the 8–9 μ range were classified by cytological characteristics rather than by size alone. The criteria used for classifying a cell as a medium lymphocyte were : finer dispersion of nuclear chromatin, more abundant cytoplasm, and more basophilic cytoplasm than is seen in typical small lymphocytes. The identification of monocytes also presented difficulties. Only those cells with a diameter greater than 9 μ which had reniform, horseshoe-shaped, or obviously folded nuclei as well as pale cytoplasm were classified as monocytes. Such cells may have represented only a fraction of the monocyte population and their numbers have probably been underestimated.

TABLE V.—*Percentage of Labelled Mononuclear Cells in the Blood of Rats at Varying Times After a Single Intravenous Injection of Tritiated Thymidine*

	No. counted/per cent labelled		
Hr. after ³H-thymidine*	Small lymphocytes	Large lymphocytes	Monocytes
2	1000 /0	241 /6·2	293 /0·7
6	1000 /0·4	100 /26	200 /6·0
	300 /1·7	80 /18·8	80 /6·3
18	1000 /1·5	200 /29·5	200 /18·0
21	1000 /1·1	143 /27·2	200 /15·5
24	500 /1·4	100 /26	100 /13
36	300 /0·7	75 /28·0	150 /22·7
72	500 /0·8	100 /13	123 /19·2
120	500 /0·8	130 /5·4	136 /1·5

* One rat killed at each time interval for preparation of leucocyte concentrate, except at 6 hr. where 2 rats killed.

Table V shows that the proportion of labelled small lymphocytes in the blood never rose above 1–2 per cent between 2 and 120 hr. after a single injection of tritiated thymidine. This is in agreement with other reported values (Schooley, Bryant and Kelly, 1959 ; Little, Brecher, Bradley and Rose, 1962). Labelled large lymphocytes appeared rapidly and a maximum of about 30 per cent were labelled at 18 hr. Few labelled monocytes were present during the first 6 hr. at a time when labelled large lymphocytes were already abundant ; the proportion increased to a maximum of about 23 per cent at 36 hr. but few were found after 72 hr.

DISCUSSION

In the rat large numbers of macrophages accumulated on coverslips which had been applied for 24 hr. to areas of abraded skin or placed in subcutaneous pockets. Macrophages appeared on the coverslips at somewhat earlier times than in experiments on human subjects (Rebuck and Crowley, 1955) and no simple progression of cells intermediate between small lymphocytes and macrophages was observed in the rat. Nothing could be deduced about the origin of the rat macrophage from a morphological examination of coverslips removed at intervals after their application.

A striking finding in the present study was that heavy autoradiographic labelling of a substantial proportion of macrophages on skin windows and on

subcutaneous coverslips could be produced by a single intravenous injection of tritiated thymidine, providing the injection was given some hours before the application of the coverslip. Thus, up to 57 per cent of the macrophages on coverslips which had been left in place for 24 hr. were labelled by giving an injection of tritiated thymidine 18 hr. before the application of the coverslip. The percentage labelling decreased as the time of the injection approached and passed the time of application of the coverslip : when tritiated thymidine was given 12–18 hr. after the application of the coverslips, less than 1 per cent of macrophages were labelled. These observations make it clear that the exudate macrophages were derived from a precursor which was dividing rapidly. Since tritiated thymidine remains available for incorporation into DNA for only a short period after injection the results also showed that labelling of the macrophage precursors must have taken place many hours before the inflammmation was induced and their prolifertion must therefore be continuous.

A delay of at least 18 hr. separated the time at which thymidine was incorporated into the precursors from the time at which labelled macrophages appeared in high concentration on the coverslips. Several experiments showed that this delay was a measure of the time taken for the antecedents of the exudate macrophages to be formed and released into the blood. First, the experiments with parabiotic rats provided unequivocal evidence that the macrophages were derived from the blood and not from the mobilization of cells normally resident in the tissues underlying the inflamed area. Second, the delay could not have occurred at the stage of emigration from blood to coverslip because coverslips which were removed from the skin after only 6 hr. showed a high proportion of labelled macrophages, providing tritiated thymidine had been given many hours beforehand (Fig. 5). Third, the delay could not have been due to the time taken for the antecedents of the macrophages to complete their division in the blood. In the experiments where tritiated thymidine was given at intervals after the application of coverslips (Fig. 4 and Table I) ample opportunity was given for the incorporation of radioactivity into the circulating antecedents had they been dividing ; in fact, when the coverslips were removed after 24 hr. very few labelled macrophages were seen.

Very little cell division could have occurred once the macrophages had emigrated on to the coverslips. Mitotic figures were never seen and when macrophage-laden coverslips were incubated for 1 hr. *in vitro* with tritiated thymidine only 4–5 cells per 1000 became labelled. This agrees well with the value obtained by Forbes and Mackaness (1963) for the macrophages which are normally present in the peritoneal cavity of mice. On the other hand, the work of Forbes and Mackaness showed that when sensitized mice were challenged with antigen the exudate macrophages divided rapidly. The present experiments have been concerned with the response of normal (non-sensitized) rats to an inflammatory stimulus and in this situation the macrophages in the exudate were virtually all non-dividing cells.

In interpreting the results of the present study it has been useful to distinguish macrophage-" precursors " which are dividing rapidly in tissues remote from the site of inflammation from their progeny, macrophage-" antecedents ", which circulate in the blood and emigrate to become identifiable as exudate macrophages. The identity and location of the precursors is discussed in the accompanying paper (Volkman and Gowans, 1965) ; it remains to discuss the possible identity of the antecedents.

Very few labelled macrophages were found on coverslips 5 days after an injection of tritiated thymidine and none after 8 days. Thus the macrophage-antecedents, which are being continually produced, circulate in the blood for about a week. The time at which labelled monocytes appeared in the blood after a single injection of tritiated thymidine and the proportion of them which became labelled (Table V) make them possible candidates for macrophage-antecedents. On the other hand, large lymphocytes in the blood and in the thoracic duct lymph label too rapidly both *in vivo* and *in vitro* (Gowans, 1959; Cronkite *et al.*, 1959; Schooley *et al.*, 1959) to explain the delay in the emergence of labelled macrophages on the coverslips. Further evidence against the large lymphocyte as an antecedent is given by Volkman and Gowans (1965).

The most important conclusion is that small lymphocytes, in the general sense implied by Rebuck and Crowley (1959), cannot be the antecedents of macrophages. Thus, the proportion of labelled small lymphocytes in the blood never rose above 2 per cent after a single injection of tritiated thymidine, although over 50 per cent of the exudate macrophages became labelled. However, it could be argued that the inflammatory stimulus selected and concentrated the small number of newly formed small lymphocytes which circulate in the blood. A functional distinction would then have to be made between the great majority of small lymphocytes which are long-lived, recirculating cells (Gowans, 1959; Little *et al.*, 1962) and a small group of morphologically indistinguishable, newly formed cells. The existence of a special group of small lymphocytes with a shorter life than the majority has been proposed by Caffrey, Rieke and Everett (1962) and Everett, Caffrey and Rieke (1962) although their precise origin and function are not known. It is possible that a circulating leucocyte with the morphological characteristics of a " small lymphocyte " may be able to evolve into a macrophage but it is quite clear that this is not a property of the great majority of lymphocytes.

SUMMARY

In rats, large numbers of macrophages emigrated and adhered to glass coverslips which were applied to areas of abraded skin (" skin windows ") or inserted subcutaneously and then removed after 24 hr. Up to 57 per cent of the macrophages on skin windows could be labelled by giving a single intravenous dose of tritiated thymidine, providing the injection was given about 1 day before the application of the coverslip. Macrophages therefore originate from rapidly dividing precursors which are proliferating continuously.

Experiments with rats in parabiosis showed unequivocally that the exudate macrophages were derived from the blood. Further studies with tritiated thymidine showed that very few macrophages on the coverslips or macrophage antecedents in the blood were synthesizing DNA. The precursors of macrophages must proliferate at sites other than the area of inflammation and then release their progeny into the circulation.

The great majority of the small lymphocytes in the blood can be excluded as possible antecedents of the exudate macrophages.

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