TRANSFORMATION OF ADULT ALLOGENEIC SMALL LYMPHOCYTES AFTER TRANSFUSION INTO NEWBORN RATS*

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PLATES 111 TO 113

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Immunological tolerance of tissue homografts can be induced in rats if they are injected with living cells from the prospective donors during the neonatal period (1–3).

When the cell suspension used to bring about tolerance contains a high percentage of immunologically competent cells, many of the treated animals, after an initial period of apparently normal growth, become ill, fail to grow at a normal rate, and soon die (3–5). This syndrome, first described in mice, was christened "runt disease" by Billingham and Brent (6).

Recently it has been shown that runt disease can be induced in rats by an injection of cells from thoracic duct lymph (7, 8). Since about 95 per cent of the cells in such lymph are typical small lymphocytes considered to be incapable of division, and the other 5 per cent are medium and large lymphocytes known to divide in culture (9), it has been generally assumed that the large lymphocytes are the immunologically active cells in the inoculum responsible for any ensuing illness (7).

In this paper evidence is presented which suggests that in fact the large lymphocytes are relatively inactive in this situation and that it is the small lymphocytes which initiate the reaction against the host.

Materials and Methods

Rats.—The rats used belong to laboratory sublines of two isogenic strains originating at the Agricultural Research Council, Compton, Berkshire, England: albino A and black-hooded B.

Collection of Lymph.—Under ether anaesthesia the thoracic duct was cannulated just below the diaphragm with polyethylene tubing (9, 10). The conscious animal was then placed in a restraining cage, saline introduced slowly into the femoral or jugular vein, and the

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lymph allowed to drip into buffered saline solution containing 100 units of penicillin and 1 μg of streptomycin per ml. During cannulation the animals were given small doses of heparin to prevent clotting of the lymph.

Injection of Cells.—Each recipient was given into the heart (usually the left ventricle) 0.08 ml of suspension containing, in all but the first two experiments, $30(\times 10^6)$ nucleated cells. The injection equipment (Fig. 1) consisted of a mounted tuberculin syringe connected to a length of fine polyethylene tubing ending in a hubless 30 gauge needle with a short bevel. In order to limit the depth of the injection, only 3.5 mm of the needle was left protruding from the protective sleeve of polyethylene tubing. Mortality due to the operative procedure was 5 per cent.

Labelling of Cells with Isotope.—Thoracic duct lymphocytes, after preliminary concentration by gentle centrifugation, were labelled *in vitro* by incubation for 1 hour at 37°C with either 3 μc/ml of ^{3}H-thymidine (specific activity: 1.9 curies/millimole, Schwartz Laboratories, New York), or 5 μc/ml of ^{3}H-adenosine (specific activity: 0.68 curies/millimole, Radiochemical Centre, Amersham, England), or 5 μc/ml of ^{3}H-DL-leucine (specific activity: 576 microcuries/millimole, Radiochemical Centre, Amersham, England). Before injection the cells were washed once in isogenic lymph to remove any uncombined tritiated material.

In vivo labelling was achieved by injecting the prospective donor rat intraperitoneally with 0.5 μc of ^{3}H-thymidine per gm of body weight. The injections were given every 12 hours for 3 weeks.

Autoradiographs.—Material for histology was fixed for 1 hour in acetic acid–alcohol (1:3), transferred to formol-saline for 24 hours, dehydrated, embedded in paraffin wax, sectioned at 5 μ, and autoradiographs were prepared by the stripping film technique (11), using British Kodak AR10 film. Autoradiographs were exposed for 7 to 42 days, developed, and the sections stained through the emulsion with neutral red, or with Erhlich's haematoxylin and eosin, or with methyl green pyronin. Cells were only considered labelled if they had 3 grains or more above representative background areas.

Terminology Used.—The term allogeneic is used to mean genetically different as proposed by Gorer *et al.* (12). Isogenic is used to mean genetically similar.

In smears, by "large lymphocytes" we mean cells measuring 10 μ in diameter or more; by "medium lymphocytes" we mean cells more than 7 μ and less than 10 μ in diameter; and by "small lymphocytes" we mean cells less than 7 μ in diameter.

In sections we use the term "large lymphocyte" to describe a cell with basophilic cytoplasm and a nucleus measuring 6 μ or more in diameter; the term "medium lymphocyte" to describe a similar cell with a nucleus measuring more than 4.5 μ and less than 6 μ in diameter; and the term "small lymphocyte" to describe a cell with scanty basophilic cytoplasm and a nucleus measuring 4.5 μ or less in diameter.

EXPERIMENTAL PLAN

Five experiments were undertaken.

Experiment 1 was to ensure that injection of large doses of adult allogeneic thoracic duct lymphocytes into newborn rats would, under our conditions, consistently produce runt disease, and to become familiar with the histological changes during the course of the illness. 101 A strain rats, divided into 6 groups, were given, within 24 hours of birth, an intracardiac injection of nucleated cells from thoracic duct lymph derived from adult B strain donors. The dosage varied from 2 to 30 ($\times 10^6$) cells (see Table I).

Experiment 2 was to determine the effect on the incidence of fatal runt disease of

varying the number of large and small thoracic duct lymphocytes injected. For this experiment adult donor B strain rats were used, some with recent thoracic duct fistulas and some with long-standing fistulas. The 2 sorts of lymph obtained, differing in their relative content of large lymphocytes, were injected in various dosages into newborn A strain recipients, and the number of animals subsequently dying from runt disease was recorded (see Table I). In this experiment 146 newborn rats were used.

Experiment 3 was to see where the injected thoracic duct cells from an adult rat went in the first 24 hours after being injected into a newborn rat of a different strain. For this purpose as many of the cells as possible were labelled with the isotope tritium

TABLE I

Incidence of Fatal Runt Disease Following Intracardiac Injection of Newborn A Strain Rats With Various Doses of Large and Small Lymphocytes from B Strain Rat Thoracic Duct Lymph

No. of cells injected ($\times 10^6$)			No. of animals	Incidence of fatal runt disease
Total	Small lymphocytes	Large and medium lymphocytes		
				per cent
30	28.5	1.5	40	100
30	24.0	6.0	10	100
20	19.0	1.0	20	80
16	15.2	0.8	12	67
16	12.8	3.2	10	60
8	7.6	0.4	12	50
8	6.4	1.6	10	40
4	3.8	0.2	9	11
4	3.2	0.8	10	10
2	1.9	0.1	8	0
2	1.6	0.4	5	0

by incubating them with tritiated leucine or adenosine. These labelled compounds were incorporated by small, medium, and large lymphocytes and enabled the cells to be traced in their wanderings through the recipients' tissues by autoradiographs prepared at various intervals after the injection. In this experiment 15 newborn rats were used.

Experiment 4 was to see where the large and medium lymphocyte component of the thoracic duct cell suspension went and what they did in the days following their injection into a newborn recipient. For this experiment the large and medium lymphocytes were selectively labelled by incubating the lymph suspension with ^{3}H-thymidine *in vitro*. Under these conditions small lymphocytes did not become labelled. In this experiment 25 newborn rats were used. As a control an additional 8 newborn rats were injected with labelled isogenic thoracic duct cells.

Experiment 5 was to trace the small lymphocyte component of thoracic duct lymph from an adult B strain rat in the days following their injection into a newborn A strain

rat. The cells were labelled *in vivo* by injecting the prospective donor repeatedly with ^{3}H-thymidine. When a suitable interval was left after the last injection before cannulating the thoracic duct, very few medium or large lymphocytes retained any isotope. In this experiment 20 newborn rats were used. As a control an additional 8 newborn rats were injected with labelled isogenic thoracic duct cells.

RESULTS

Effect of Injecting Adult Allogeneic Thoracic Duct Lymphocytes into Newborn Rats (Experiment 1).—

To produce fatal runt disease consistently in all our A strain recipients, it was found necessary to inject 30 ($\times 10^6$) B strain thoracic duct cells (see Table I). Lesser dosages produced a lower percentage of rapidly fatal runt disease, although several of the survivors went through a period of wasting.

The pathological changes seen in the affected rats have been fully described by Billingham *et al.* (8) and will now only be mentioned briefly.

The first sign of something untoward happening to the recipient of the foreign cells was a dermatitis around the urogenital and anal orifices which later involved the ears, tail, and abdomen; this was accompanied by ruffled fur, crusting of the eyes and nose, and sometimes diarrhoea. Failure to gain weight became obvious from about the 10th day after birth. Those animals that died usually did so within the first 3 weeks and at necropsy all the lymph nodes were swollen, the spleen was enlarged, the thymus was often atrophied, and the small intestine distended and thin walled.

The earliest histological changes occurred in the white pulp of the spleen and in the centres of the lymphoid nodules and could be detected about 24 hours after the injection of large numbers of the foreign cells. In these areas many cells appeared which characteristically had cytoplasm which stained bright red with methyl green pyronin, a large pale nucleus with a fine chromatin network, and one or two prominent nucleoli. There was considerable variation in the size of these cells and many were in mitosis. After 10 days most of the lymphocytes in the cortex of the lymph nodes and in the white pulp of the spleen had disappeared but the medulla of the nodes and the red pulp of the spleen contained a proliferating mass of the pyroninophilic cells. Later a few animals showed necrotic changes in the mass of cells and some mature plasma cells were seen.

Effect of Varying the Numbers of Large and Small Lymphocytes Injected (Experiment 2).—

If the drainage of lymph from the thoracic duct of an unanesthetized rat is continued for more than 2 to 3 days, the daily output of small lymphocytes falls sharply (9, 13). However, the output of large lymphocytes is less affected and lymph collected 5 to 6 days after cannulation of the thoracic duct of our rats often contained about 20 per cent large lymphocytes. This change in cellular composition of the lymph was used to study the effects of varying the numbers of large and small lymphocytes injected. It can be seen from Table I that there is a direct relationship between the number of small lymphocytes injected and the incidence of fatal runt disease, but no such relationship between the large lymphocyte content of an inoculum and its runt-inducing potentiality.

Effect of Injecting Thoracic Duct Lymph Cells, the Majority of Which Have Been Labelled Isotopically (Experiment 3).—

85 to 90 per cent of the small and large lymphocytes in the thoracic duct lymph draining from our rats become heavily labelled after incubation with tritium-labelled leucine or adenosine for 1 hour *in vitro* at 37°C. Thoracic duct cells from our black-hooded B strain rats labelled in this way were injected into newborn albino A strain rats and autoradiographs of their tissues examined 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours later. Because of the relative instability of these labels it was felt that to examine tissues later than 24 hours might, through transference of label, give misleading results.

At 2 hours a few labelled cells were found lying in the alveolar walls of the lung. Many heavily labelled cells were present in the spleen, and even greater numbers in the lymph nodes (Fig. 2) and lymphoid tissue of Peyer's patches (Fig. 3). In the spleen the greatest accumulation of labelled cells was in the middle and outer zones of the perivascular lymphoid sheaths; in the lymph nodes and Peyer's patches it was in the middle and peripheral zones of the lymphoid nodules. Labelled cells were also found close up against the endothelium of the postcapillary venules in the cortex of the lymph nodes and in the tissues adjacent to these vessels. The transfused cells showed no predilection for any particular group of lymph nodes. Some labelled cells were also found in the bone marrow. Most of these cells were non-pyroninophilic small lymphocytes. Very rarely a labelled cell was found in the liver; a few were present in the stroma of the villi and in the lamina propria of the small intestine, and none in the thymus. In both liver and gut the labelled cells were predominantly large and medium lymphocytes.

At 4 hours only isolated labelled cells were left in the pulmonary alveolar walls and by 8 hours they had disappeared completely. The distribution of cells in the lymphoid tissues, however, remained much as at 2 hours.

At 12 hours labelled cells were present in the central as well as the outer zones of the splenic perivascular lymphoid sheaths and the centres of the lymphoid nodules of the other lymphoid tissue. Although most of these cells were still small lymphocytes, a few larger labelled pyroninophilic cells were present in the central zones. A few cells in mitosis were labelled. The liver was now devoid of labelled cells.

At 24 hours labelled large pyroninophilic cells in the spleen and other lymphoid tissues were more frequent.

Effect of Injecting Isotopically Labelled Large and Medium Thoracic Duct Lymphocytes (Experiment 4).—

About 40 per cent of the large and medium lymphocytes in thoracic duct lymph become labelled after exposure to tritiated thymidine *in vitro* for 1 hour at 37°C (Fig. 4). In this experiment 2 types of thoracic duct lymph were used. The first, collected during the 12 hours following cannulation of a rat, contained about 5 per cent of large and medium lymphocytes; the second, collected after the thoracic duct fistula had been draining continuously for 5 to 6 days, contained about 20 per cent of large and medium lymphocytes.

(*a*) *Allogeneic large and medium lymphocytes:* Radioactively labelled cells from these

two different types of B strain rat lymph were injected into newly born A strain litters. Autoradiographs were prepared from sections of the recipient rats' tissues at 4 hours, 12 hours, and at daily intervals up to 18 days.

At 4 hours small numbers of labelled cells were to be seen in the spleen, lymph nodes, and Peyer's patches. A few were also present in the small intestine and in the bone marrow. None were present in the thymus, liver, or lungs. Within the spleen some of the cells lay in the red pulp and a few at the periphery of the perivascular lymphoid tissue. In the lymph nodes the labelled cells tended to lie in the medulla, at the periphery of the lymphoid nodules, and in the adjacent region close to the subcapsular sinus. In the Peyer's patches the labelled cells were lightly scattered throughout the mass of lymphoid tissue. Within the small intestine the labelled cells were again located in the stroma of the villi and in the lamina propria (Fig. 5).

Between 12 hours and 2 days the number of heavily labelled cells and their distribution in the tissue did not alter appreciably, despite obvious histological changes in the white pulp of the spleen, the cortex of the lymph nodes, and the lymphoid collections in the intestinal wall. At the 2nd to the 3rd day there was a drop in the number of heavily labelled cells visible in the tissues, and from then onwards labelled cells declined steadily, but appreciable numbers were still present at 10 days (Fig. 6) and a few at 18 days. Up to the 2nd day large and medium lymphocytes predominated among the labelled cells, but from then onwards some less heavily labelled small lymphocytes were present (Fig. 7).

When "late" lymph containing 20 per cent large lymphocytes was used, heavily labelled cells were easier to find but their distribution within the various tissues remained the same, and again some labelled small lymphocytes subsequently appeared.

(*b*) *Isogenic large and medium lymphocytes:* Radioactively labelled cells from the 2 different types of A strain rat lymph were injected into newly born litters of the same A strain. Autoradiographs were prepared at 1, 2, and 4 days. The distribution of labelled large lymphocytes was the same as in the newborn rats injected with labelled allogeneic cells, and labelled small lymphocytes were ultimately found in moderate numbers.

Effect of Injecting Isotopically Labelled Small Lymphocytes (*Experiment 5*).—

If a rat is given for 3 weeks twice daily intraperitoneal injections of ^{3}H-thymidine, about 40 per cent of the small lymphocytes in the thoracic duct lymph become labelled (14) (Fig. 8). If the thoracic duct cannulation is postponed for 4 days after the last injection of isotope very few large lymphocytes carry appreciable label.

(*a*) *Allogeneic small lymphocytes:* Small lymphocytes from our black-hooded B strain rats labelled in this fashion were injected into newborn albino A strain rats and autoradiographs of their tissues examined at 12 hours, 24 hours, 2 days, 3 days, 5 days, and 7 days.

At 12 hours a large number of labelled cells was found in the lymphoid nodules in the cortex of the lymph nodes, and in the lymphoid tissues in the intestinal walls. Smaller numbers were present in the white pulp of the spleen. A very occasional labelled cell was found in the stroma and lamina propria of the small intestine. None were present in the thymus, liver, or lungs. Most of these radioactively labelled cells

were small lymphocytes, but a few were large cells with pyroninophilic cytoplasm.

24 hours after their injection, although most of the labelled cells were still small lymphocytes, about 30 per cent were larger cells with a thin rim of pyroninophilic cytoplasm and a large pale nucleus with a solitary nucleolus (Figs. 9 to 12). A few were even bigger cells with plenty of densely pyroninophilic cytoplasm and 1 or 2 prominent nucleoli. Very rarely labelled cells in mitosis were seen (Fig. 13).

At 2 days very lightly labelled large cells were now abundant, but heavily labelled unchanged small lymphocytes were still to be found.

After 3 days the label had disappeared from the pyroninophilic cells and only remained in a light scattering of small lymphocytes.

A few labelled small lymphocytes were still to be found in the tissues at 7 days.

(*b*) *Isogenic small lymphocytes:* Small lymphocytes from our albino A strain rats, labelled *in vivo* as described above, were injected into newborn rats of the same A strain. Recipients were killed at 12 hours, 24 hours, 2, 3, 5, and 7 days and their tissues examined autoradiographically. Many labelled small lymphocytes were found just as in the previous experiment in the cortical zones of the lymph nodes, in the Peyer's patches, and in the white pulp of the spleen. However, no pyroninophilic cells appeared and at no time was label found in any cells other than small lymphocytes.

DISCUSSION

The ability of adult allogeneic thoracic duct lymph to produce lethal runt disease when injected into the circulation of a newborn rat seems from these experiments to depend entirely upon the small lymphocytes in the cell suspension. The large and medium lymphocytes appear innocuous, confirming the tentative findings of Billingham *et al.* (8). A similar conclusion has also recently been reached by Gowans *et al.* (15) who found that the lethal effect of parental thoracic duct cells when injected into young adult F1 hybrid rats was not due to their content of large lymphocytes. It could legitimately be argued, however, that the large lymphoid cells which we and Gowans have used come from a severely stressed animal with a long-standing fistula, and although able to incorporate tritiated thymidine into their nuclear material, differ functionally in some way from those in lymph from a recently cannulated rat. This possibility seems unlikely as the cells are not only morphologically normal, but when observed by phase contrast microscopy they also seem to be moving and behaving in a normal fashion.

This indictment of the small lymphocytes conflicts with a report (16) that F1 hybrid mice can be killed by injecting them with whole blood from parental strain animals which have 24 hours earlier been given a single massive dose of cortisone. Such blood apparently contains very few small lymphocytes, but a relatively large number of mononuclears measuring more than 9.3 μ in diameter. However, in this experiment it is difficult to exclude some carrying over of cortisone with the donated cells, which would cause damage to the host's

lymphoid tissue and favour the establishment of even a few small lymphocytes. Moreover, a recent and extensive study (17) indicates that purified preparations of small lymphocytes from the peripheral blood of adult mice, freed from all large cells, are still capable of producing a lethal graft-*versus*-host reaction in newborn mice of a different strain.

Our experiments with isotopically labelled materials show that the small thoracic duct lymphocytes migrate in large numbers into those very areas of the lymph nodes and the spleen in which reactive and necrotic changes later occur as runt disease pursues its course. Once within the lymphoid tissue of these organs some of the small lymphocytes injected appear to change without division into large pyroninophilic cells. An alternative explanation would be that the large cells are of host origin and that they become labelled through phagocytosis, and incorporation into their nuclei, of fragments of tritiated DNA liberated by the destruction of injected cells (18). It is certainly known that effete small lymphocytes are phagocytosed by reticular cells in the germinal centres of normal lymphoid tissue (19), and if after appropriate stimulation these host reticulum cells are able to differentiate into pyroninophilic cells it is possible to see how confusion might arise. Although we consider this latter interpretation improbable, at present we cannot dismiss it, especially in view of a recent suggestion that the splenic enlargement in some graft-*versus*-host reactions is almost entirely due to proliferation of host cells (20).

This concept that some small lymphocytes are primitive cells capable of transforming into other cell types is by no means a new one; it was first put forward by Bloom (21) on the basis of tissue culture studies of thoracic duct cells, and more recently it has been the object of a review by Trowell (22). Within millipore diffusion chambers some of the small lymphocytes from rabbit thoracic duct lymph have been seen to change into large pyroninophilic cells after stimulation with foreign protein antigen (23). In our experiments it is tempting to think that in a similar fashion a small proportion of the small lymphocytes interact early with antigens of the host and that this interaction results in their transformation into a new cell type capable of forming antibody. What subsequently happens to the large pyroninophilic cells so formed is hard to say from our observations. The rapid loss of label and the abundant mitoses suggest that they can divide and many of them may later die reacting with host cells (24). Because of the loss of label their link, if any, with the scattered mature plasma cells which appear in the terminal phases of runt disease, is unknown. Those labelled small lymphocytes which do not change their form appear to go on recirculating, as in the normal animal, for a considerable time.

The large lymphocytes appear to play a very minor or no part in this early reaction by the injected cells against the host. Although many of them divide to form small lymphocytes, some of the heavily labelled large lymphoid cells either recirculate, or just linger for considerable periods in the red pulp of the

spleen and medulla of the lymph nodes. In our newborn rats only a few labelled large lymphocytes were found in the stroma of the intestinal villi. This is interesting because Gowans *et al.* (15) working with 5-week-old F1 hybrid rats, found that most of their injected large lymphocytes accumulated at this site—which raises the question: do these cells, which presumably have been formed in the lymphoid tissues draining the lymphatic bed of the gut, only return to the gut in large numbers after the baby animal has ingested food for a few days?

In view of the role we suspect the small lymphocyte to play in runt disease, it is also relevant that Hildemann and Haas (25) have found that in bullfrog larvae large and medium lymphocytes are present during the period when tolerance to homografts can still be induced, but that the appearance of small lymphocytes coincides with the onset of the ability to mount a homograft reaction. These authors infer from their observations that the induction of tolerance may well depend upon the absence or relative scarcity of small lymphocytes in foetal or neonatal animals at the time when allogeneic cells or other antigens are first introduced. Nothing in our experiments contradicts this hypothesis and we would agree that some small lymphocytes seem to be immunologically competent cells and may well be mainly responsible for homograft recognition and destruction.

SUMMARY AND CONCLUSIONS

Newborn rats of one inbred strain were given an intracardiac injection of adult thoracic duct lymphocytes from another inbred strain. It was found that although there was a direct relationship between the number of small lymphocytes injected and the incidence of fatal runt disease, there was no particular relationship between the large lymphocyte content of an inoculum and its runt-inducing potentiality.

Using tritiated thymidine and an autoradiographic technique, the small lymphocytes in the inoculum were labelled and found to migrate in large numbers into the cortex of the lymph nodes and into the Peyer's patches of the host animal, and in smaller numbers into the white pulp of the spleen. Within 24 hours isotope, previously present in the DNA of small lymphocytes, appeared in a number of the large pyroninophilic cells which were a characteristic feature of the spleen and lymph nodes in this early phase of runt disease.

When the large lymphocytes in the inoculum were labelled they were found to migrate to the red pulp of the spleen, medulla of the lymph nodes, and the Peyer's patches and the lamina propria of the small intestine. Later some labelled small lymphocytes appeared at these sites.

These findings suggest that:

(*1*) Some small lymphocytes are immunologically competent cells, and

(*2*) After introduction into the circulation of a newborn rat, these same small lymphocytes are the first cells to react with the antigens of the host, and in the

process they become transformed into large pyroninophilic cells capable of division. The large lymphocytes seem to play little part in this initiating of an immunological response, but do give rise to some small lymphocytes.

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EXPLANATION OF PLATES

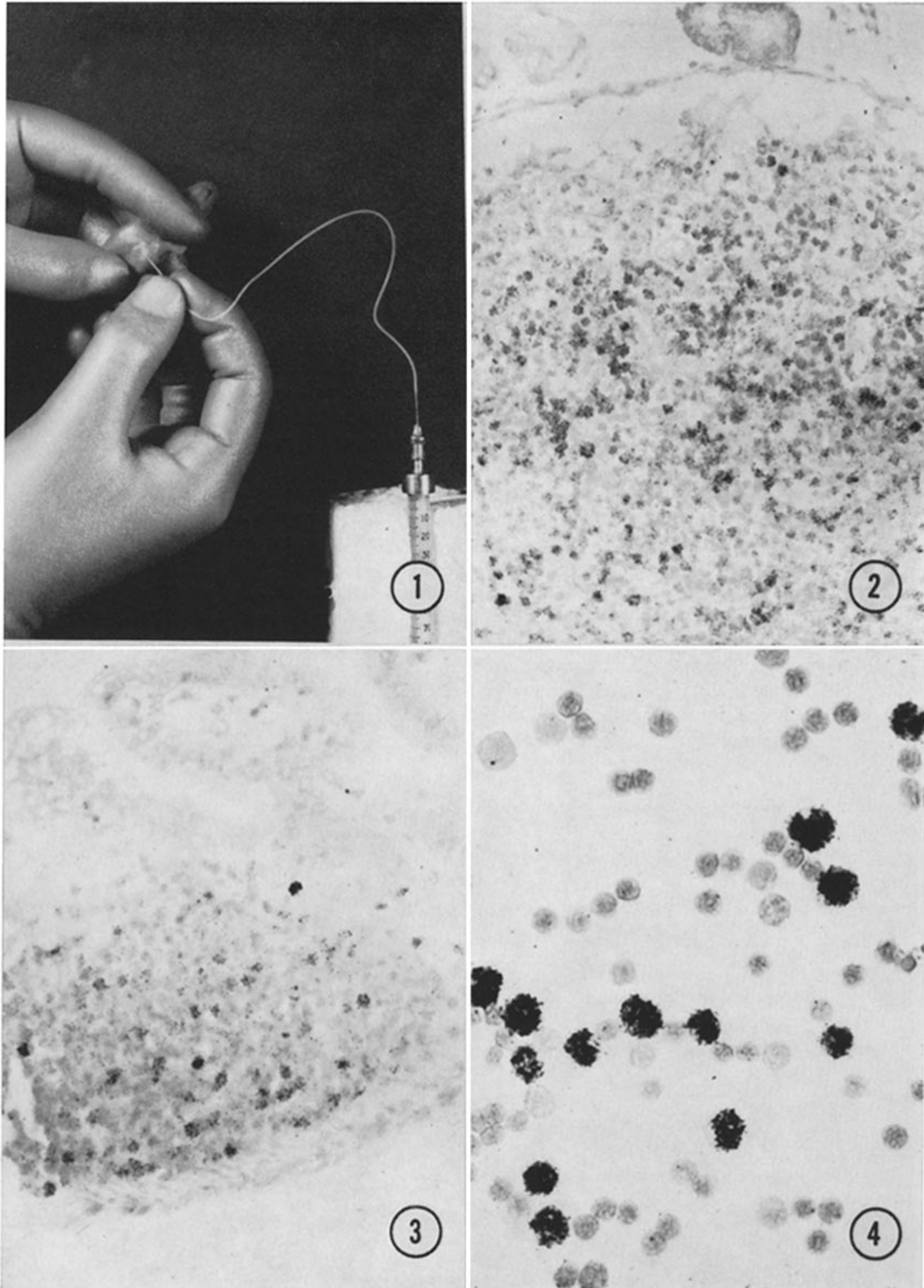
Plate 111

Fig. 1. Intracardiac injection of a newborn A strain rat with 0.08 ml of a suspension of thoracic duct cells from an adult B strain rat.

Fig. 2. Autoradiograph prepared from a mesenteric lymph node of a newborn rat which had received 2 hours previously 30 ($\times 10^6$) allogeneic thoracic duct lymphocytes, all labelled with tritiated adenosine. Many radioactive cells are present: most lie in the cortex, but some are present in the medulla. Haematoxylin and eosin. $\times$ 100.

Fig. 3. Autoradiograph of a Peyer's patch from the small intestine of a newborn rat which had received 2 hours previously 30 ($\times 10^6$) allogeneic thoracic duct lymphocytes labelled with tritiated adenosine. Labelled cells are scattered throughout the lymphoid tissue. Haematoxylin and eosin. $\times$ 100.

Fig. 4. Autoradiograph of a smear of thoracic duct lymphocytes. The cells had previously been incubated for 1 hour with $3\mu c/ml$ of tritiated thymidine. About 40 per cent of the large and medium lymphocytes are heavily labelled: none of the small lymphocytes are labelled. Neutral red. $\times$ 800.



(Porter and Cooper: Transformation of small lymphocytes)

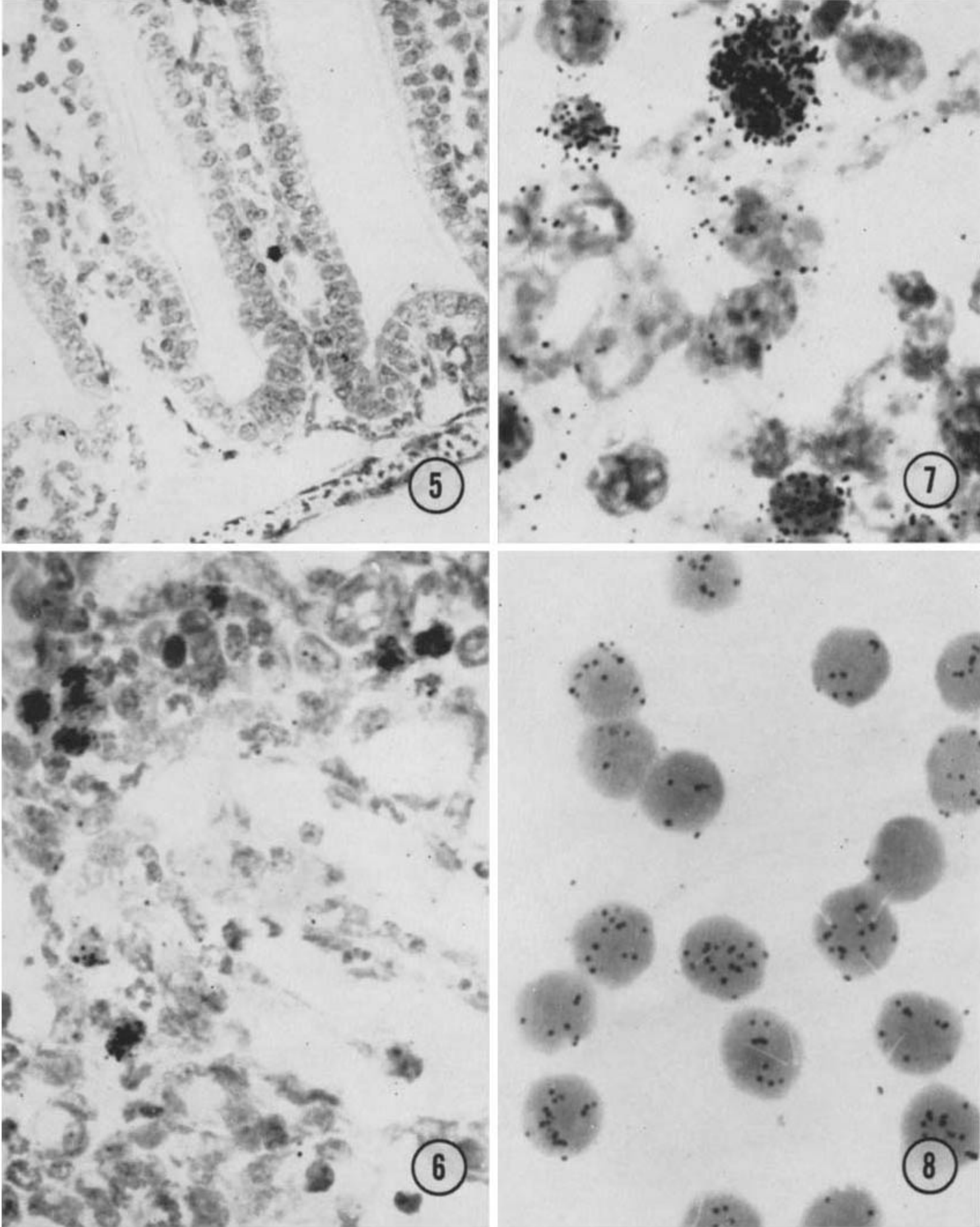
Plate 112

Fig. 5. Autoradiograph prepared from a section of small intestine of a newborn rat which had received 4 hours previously allogeneic thoracic duct lymphocytes. The large and medium lymphocytes amongst the injected cells had been labelled *in vitro* with tritiated thymidine. A solitary heavily labelled cell is present in the stroma of the central villus. Haematoxylin and eosin. × 600.

Fig. 6. Autoradiograph of a cervical lymph node of a newborn rat which had received 10 days previously tritiated thymidine-labelled large and medium thoracic duct cells. Heavily labelled large lymphocytes are still present in the medulla. Haematoxylin and eosin. × 800.

Fig. 7. Autoradiograph of the spleen of a newborn rat which had received 4 days previously tritiated thymidine-labelled large and medium thoracic duct cells. A very heavily labelled large lymphocyte and two less heavily labelled small lymphocytes are present. Haematoxylin and eosin. × 1500.

Fig. 8. Autoradiograph of a smear of thoracic duct lymphocytes from a rat which had been given daily injections of tritiated thymidine for the previous 3 weeks. Many of the small lymphocytes are moderately labelled. Giemsa. × 1000.



(Porter and Cooper: Transformation of small lymphocytes)

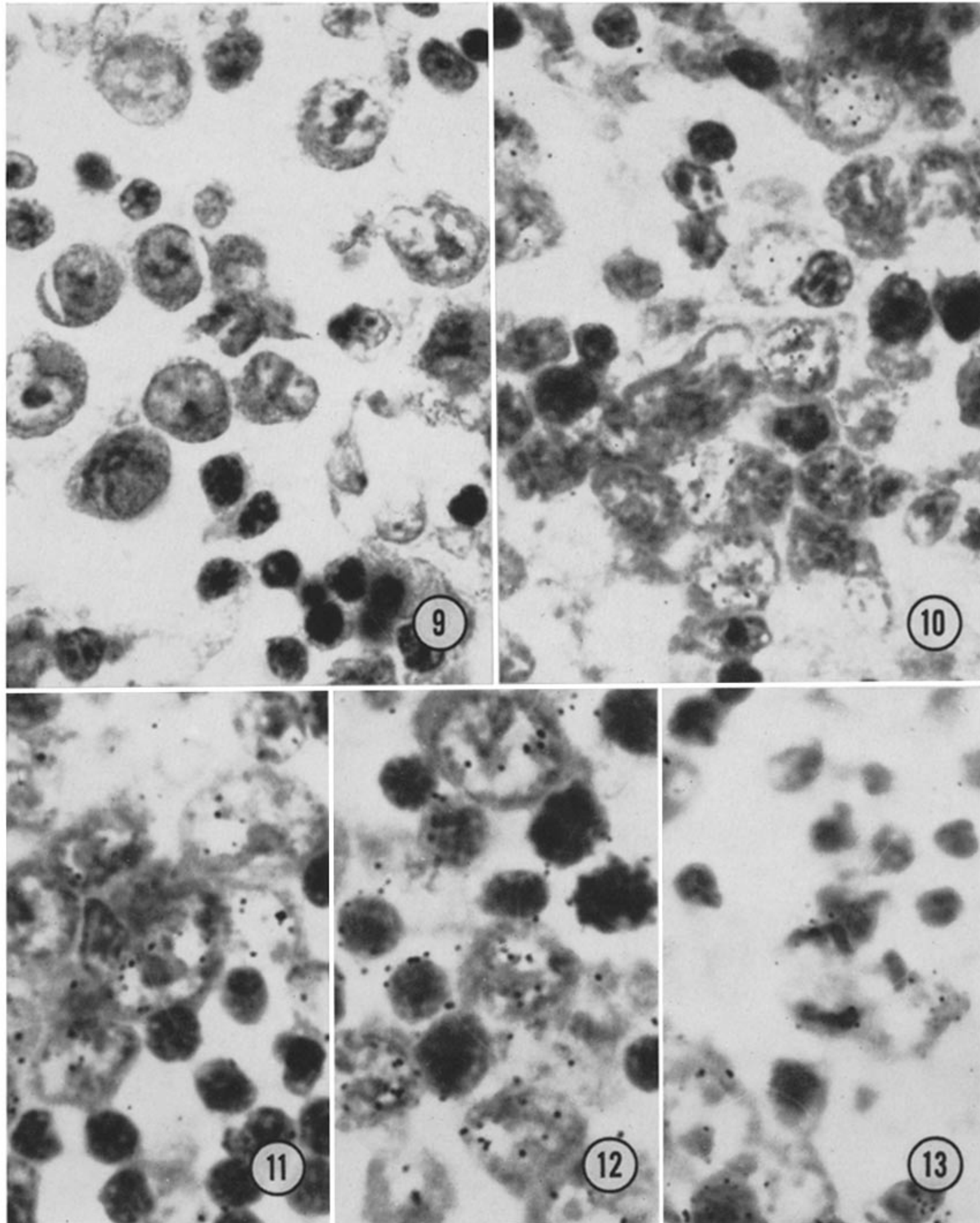
PLATE 113

FIG. 9. Spleen of a newborn rat 24 hours after the animal had received 30 ($\times 10^6$) allogeneic thoracic duct lymphocytes. In addition to small lymphocytes there are large cells with a thin rim of pyroninophilic cytoplasm, a large pale nucleus, and 1 or 2 prominent nucleoli. Methyl green pyronin. $\times$ 1500.

FIG. 10. Autoradiograph from the spleen of a newborn rat 2 days after the animal had received allogeneic thoracic duct lymphocytes. The small lymphocytes amongst the injected cells had been labelled *in vivo* with tritiated thymidine. Many lightly labelled large cells with pyroninophilic cytoplasm are present. Methyl green pyronin. $\times$ 1500.

FIGS. 11 and 12. Autoradiographs from the same spleen showing more of the lightly labelled large pyroninophilic cells. Methyl green pyronin. $\times$ 1500.

FIG. 13. Autoradiograph from the same spleen. A labelled cell in mitosis is present. Haematoxylin and eosin. $\times$ 1500.



(Porter and Cooper: Transformation of small lymphocytes)