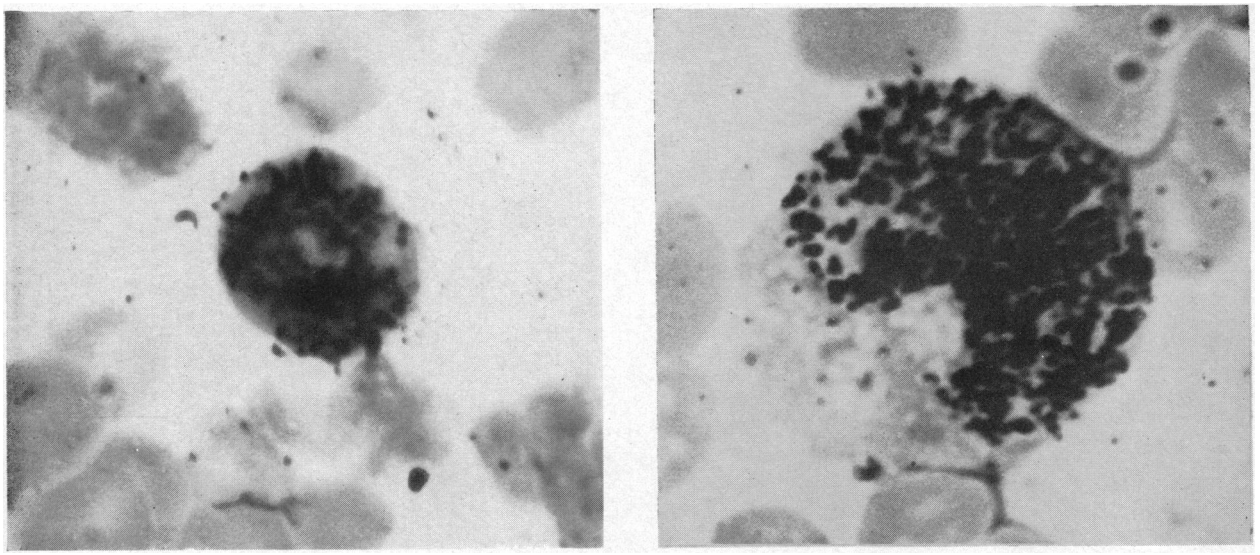

FIG. 1.—Examples of peripheral blood cells labelled *in vitro* by incubating with tritiated thymidine. (×3,550.)

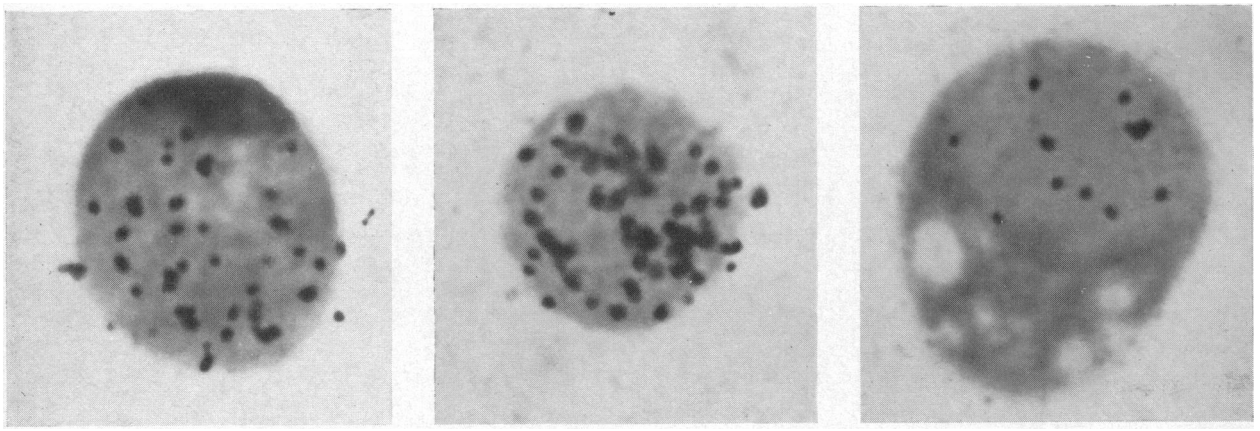

FIG. 2.—Examples of peripheral cells labelled by two intravenous injections of tritiated thymidine. (×3,350.)

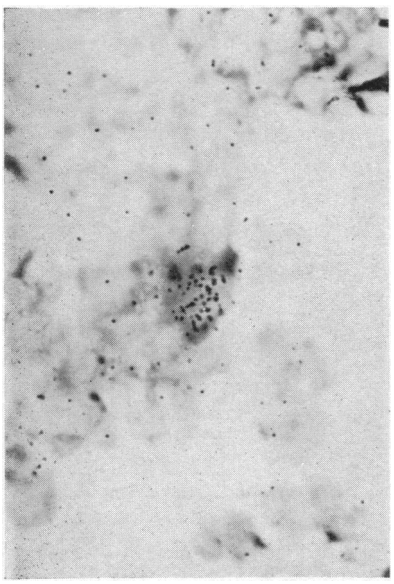

FIG. 3.—Typical example of isolated heavily labelled pyroninophilic cells in homotransplanted kidney. (Methyl-green–pyronine. ×1,050.)

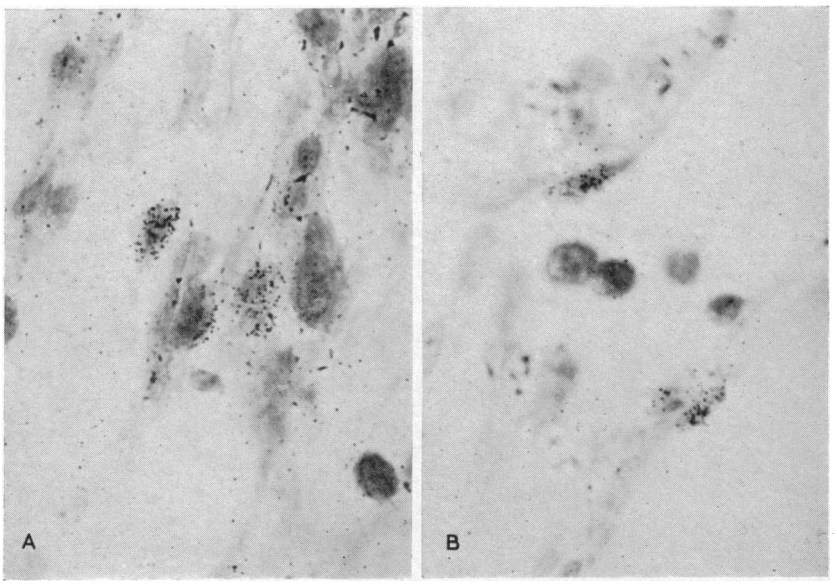

FIG. 4.—Examples of cells in homotransplanted kidney labelled by injecting tritiated thymidine into renal artery before removal. A. Large fibroblastic type of labelled cells and possibly endothelial cells. B. Definite labelling of endothelial cells. (Methyl-green–pyronine. ×900.)

42-year-old man had a cerebellar metastasis excised in 1954. Several months later a bronchial carcinoma was discovered and treated with a course of irradiation. At necropsy 14 months after the operation there were no cerebral metastases and no recurrence at the operation site.

Finally we return to the possibility of the spontaneous regression of tumours, a subject fully discussed by Everson and Cole (1956), and recently commented on by Smithers (1962). A 53-year-old woman was seen in 1938 complaining of headaches for six weeks with gradual onset of dysphasia and right hemiparesis. A well-demarcated tumour was found at a depth of 3 cm. in the left parietal cortex and was enucleated. Sections of the tumour were seen by several histologists, who were agreed that this was a metastasis from a hypernephroma: in 1942 the sections were reviewed and the diagnosis was upheld. After the operation the dysphasia and hemiparesis cleared almost completely. Later in 1938 a laparotomy was performed at St. Mary's Hospital, but both kidneys appeared normal. Intravenous and retrograde pyelograms in 1941 were normal. In 1955, at the age of 70, she was alive and well, and a regular attender at the follow-up clinic with her husband, from whom an acoustic neuroma had been excised.

Conclusions

The surgical excision of an apparently single intracranial metastasis is worth while if the patient is in good general physical condition, and particularly if a primary tumour of breast or kidney has previously been excised.

Biopsy of intracranial metastases carries a very high mortality and morbidity; if it were possible to make a confident diagnosis that an intracranial tumour was a metastasis by other means, suitable cases could be selected for total excision of the secondary, while the majority could be spared useless and time-consuming surgical procedures.

Patients from whom a metastasis of uncertain origin has been excised should be followed up regularly, with particular reference to the diagnosis of a bronchial carcinoma as soon as it becomes detectable, and before it is inoperable.

Very occasionally years of reasonable health may follow excision of an intracranial metastasis, even if the primary tumour is not found and excised.

Summary

In an analysis of 389 patients with intracranial metastases the commonest primary sites were found to be lung (65%), breast (5.9%), gastro-intestinal tract (4.9%), and kidney (2.8%), while 17% were of uncertain origin. Up to 13 months after operation 213 necropsies were performed, and in 33 no other intracranial or visceral metastases were found.

It was usually not possible to be certain clinically or radiologically that a tumour was a metastasis. Biopsy was associated with a high mortality and morbidity: 68.5% of those with carcinoma of breast, bronchus, kidney, or uncertain origin who were submitted to it lived only one month or less. The mortality for total excision and incomplete excision of the metastasis in these groups was 32.2% and 41% respectively.

Twenty-two patients survived for one year or longer, 10 with carcinoma of the bronchus, six with carcinoma of the breast, four of uncertain origin, and one each with carcinoma of colon and kidney. Total excision of the metastasis was performed in 18 cases, incomplete excision in two, biopsy in one, and in one case total excision of cerebral metastasis was followed a year later by total excision of a cerebellar metastasis. One man was still alive 6 years after the total excision of a cerebral metastasis.

Operation was not rewarding on patients in poor general physical condition on admission: 9.3% of 43 with minimal neurological signs and in good physical condition survived more than one year, as did 9.2% of 163 with marked neurological signs and in moderately good general condition. Only 1.6% of 183 admitted when drowsy, disorientated, and in poor general condition survived more than one year. There was no significant difference in the survival of patients in these groups whether or not they had papilloedema.

We are grateful to Professor T. Crawford for Fig. 3.

REFERENCES

Bryson, C. C., and Spencer, H. (1951). *Quart. J. Med.*, **20**, 173.
Everson, T. C., and Cole, W. H. (1956). *Ann. Surg.*, **144**, 366.
Flavell, G. (1949). *Brit. med. J.*, **2**, 736.
Galluzzi, S., and Payne, P. M. (1956). *Brit. J. Cancer*, **10**, 408.
Globus, J. H., and Meltzer, T. (1942). *Arch. Neurol. Psychiat. (Chic.)*, **48**, 163.
Gutmann, C. (1904). *Fortschr. Med.*, **22**, 141.
Livingston, K. E., Horrax, G., and Sachs, E. (1948). *Surg. Clin. N. Amer.*, **28**, 805.
Meyer, P. C., and Reah, T. G. (1953). *Brit. J. Cancer*, **7**, 438.
Russell, D. S., and Rubinstein, L. J. (1959). *Pathology of Tumours of the Nervous System*, p. 219.
Smithers, D. W. (1962). *Lancet*, **1**, 493.
Störtebecker, T. P. (1954). *J. Neurosurg.*, **11**, 84.
Willis, R. A. (1952). *Spread of Tumours in the Human Body*, p. 255. Butterworth, London.

CELLULAR INFILTRATION IN HOMOTRANSPLANTED KIDNEYS

BY

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[WITH SPECIAL PLATE]

It has been known for several decades that homotransplanted kidneys in dogs become infiltrated with cells. Some authors have considered that this cellular infiltration reflected an infection which terminated the natural life of homotransplanted kidneys (Williamson, 1926; Wu and Mann, 1934). Autotransplanted kidneys, on the other hand, do not become infiltrated with cells. In more recent papers it has been demonstrated that in the early stages of the natural life of a homotransplanted kidney the infiltrating cells are pyroninophilic (Simonsen *et al.*, 1953; Dempster, 1953a). It has also been demonstrated that the cell population radically alters during the period of oliguria and final anuria (Dempster, 1955). In the oliguric-anuric stage the infiltrating cells are easily recognized as polymorphs, histiocytes, and lymphocytes. This cell population, indisputably of host origin, is associated with parenchymal liquefaction in the rejecting kidney.

The morphology of the pyroninophilic cells suggested that they originated from renal reticulum cells. Darmady *et al.* (1955) actually demonstrated what could be interpreted as stimulated reticulum cells. In addition, these authors demonstrated that the renal vascular endothelial cells were also pyroninophilic.

Simonsen *et al.* (1953) interpreted the pyroninophilic infiltration as a reaction of the renal reticulum cells against host antibody—that is, as an anti-antibody reaction. Dempster (1953a) regarded the cellular infiltration as a reaction to an undefined factor in the host blood. Both authors concluded that the cellular infiltration was a graft against host reaction. Dempster (1955) later dismissed the presence of the pyroninophilic cells as essential to rejection and put forward the suggestion that the renal tubule cells themselves were reacting against unknown host factors. This attitude was based on Ehrlich's concept that all cells can attempt to defend themselves if need be. Dempster (1955) postulated that in trying to defend themselves an intracellular imbalance occurred which suddenly led to renal failure. It is this kind of reaction which Dempster (1955) regards as the graft against host reaction. The more obvious sign of this reaction is an interstitial oedema, and the less obvious and the more significant changes are perhaps within or on the renal tubule cells.

From the very beginning the interpretation of the pyroninophilic cellular infiltration in homotransplanted kidneys was controversial. Several authors have quite rightly voiced doubts about the origin of the cells, and the most important evidence against a renal origin has recently come from Porter and Calne (1960). These authors labelled the prospective host dog with tritiated thymidine three hours prior to transplanting a kidney to it. The result of their studies was apparently a clear demonstration of heavily labelled host cells in the three dogs studied. These authors implied that the cells infiltrating homotransplanted kidneys were derived from those circulating lymphocytes which can take up thymidine during *in vitro* incubation (Bond *et al.*, 1958 ; Cronkite *et al.*, 1959), and hence, theoretically, are capable of division. These cells, Porter and Calne (1960) considered, must enter the homotransplanted kidney before the third day.

It was decided to repeat and extend their work because it raised a number of interesting points, perhaps the most important of which is their assumption that circulating lymphocytes can transform into immature plasma cells within the homotransplanted kidney itself. Porter and Calne (1960) did not, in fact, establish that cells similar to those described by Bond *et al.* (1958) and Cronkite *et al.* (1959) circulated in the blood-stream of dogs.

Materials and Methods

Greyhounds (wt. = 22–25 kg.) and mongrel dogs (wt. = 10–12 kg.) were used.

The technique of transplanting kidneys has been described elsewhere (Dempster, 1953a). The technique of skin grafting in dogs has been described elsewhere (Dempster, 1953b). Skin grafts of about 6 g. weight were used ; they were of full thickness and were fitted grafts. The general plan was as follows.

1. *To Transplant Kidneys and Skin to Dogs under Various Conditions.*—The types of experiments were as follows: (*a*) Kidney and skin transplanted three hours after two pulses of tritiated thymidine. (*b*) Kidney and skin transplanted 24 hours after two pulses of tritiated thymidine. (*c*) Kidney and skin transplanted 48 hours after two pulses of tritiated thymidine. (*d*) Having transplanted kidneys to dogs under one or other of the above conditions to then return the kidney after 48 hours to the original donor for three days. This is called a homo-autotransplant (Simonsen *et al.*, 1953 ; Dempster, 1955). (*e*) Having first sensitized the hosts by repeated skin grafting to inject tritiated thymidine and then transplant a kidney from the same donor as supplied the skin. This elicits a second kidney reaction in the transplanted kidney (Dempster, 1953a). (*f*) To transplant kidneys to hosts bearing surviving skin grafts from the kidney donor. (*g*) Prior to removing a homotransplanted kidney 200 μc. tritiated thymidine was injected into the renal artery. The renal circulation was clamped so as to trap the thymidine and contain it for 10 minutes within the kidney. Before releasing the clamps 400 mg. of "cold" thymidine was injected intravenously. (*h*) A thoracic-duct fistula was established in one dog and 160 ml. of chyle was collected in aliquots of about 30 ml. at a time over a period of about one and a half hours. Each aliquot was spun and the residue was incubated with Eagle's medium + 20 μc. of ^{14}C thymidine. The residue appeared sometimes as a clot and for this reason adequate contact with the ^{14}C thymidine was not always perhaps attained. After incubation for one hour the cells were resuspended in a mixture of normal saline and dog plasma, and injected intravenously half an hour after a kidney had been homotransplanted to the dog. Forty-eight hours later the kidney was removed and histological sections prepared for autoradiography. Sample smears were taken of each aliquot of thoracic-duct cells injected.

2. Having transplanted the kidneys, to remove them later and to examine histological preparations for evidence of labelled cells.

TABLE I.—*Assessment by Autoradiography of Labelled Pyronine-positive Cells in Kidneys Homotransplanted to Labelled Recipients*

No. of Dog	Type of Experiment (see text)	Dosage of Labelled Thymidine μc./kg. Body Wt.	Interval of Time between Labelling Recipient and Kidney Transplantation (hrs)	Time of Kidney Sampling. (b = Biopsy)	Assessment of Labelling in 500 Pyronine + Cells Counted. Percentage Labelled with > 3 Grains/Nucleus (i.e., 10 × Background)
TT8	1a	190	3	3 days	6·8
TT1a	1b	90	24	3 ,, (b)	4·6
TT1b	1b	90	24	9 ,, (b)	2·2
TT2	1b	90	24	24 hours	6·0
TT4	1b	90	24	3 days	7·3
TT7	1b	220	24	3 ,,	3·7
TT3	1c	100	48	3 ,,	< 1·0
TT9	1d	180	24	5 ,,	0
TT10	1d	160	24	5 ,,	0
TT13	1e	70	3	30 hours	7·1
TT14	1e	95	3	30 ,,	4·0
TT11	1f	90	24	3 days	2·4
TT12	1f	95	24	3 ,,	1·8

TABLE II.—*Relation of Size of Nucleus to Degree of Labelling in Circulating Mononuclear Cells. (Monocytes and Lymphocytes of All Sizes)*

No. of Grains per Nucleus	Size of Nucleus < 7μ	Size of Nucleus 7μ–10μ	Size of Nucleus > 10μ
3–6	17	21	1
7–9	2	2	0
10–19	0	6	3
> 19	1	5	3

Total number of cells examined in each dog = 500. Percentage labelled = 12·2%.
The more heavily labelled cells are those in the range > 7μ. This agrees with the results of Schooley *et al.* (1959).

3 *Tritiated Thymidine.*—(*a*) *In-vivo labelling:* The labelling thymidine was obtained from the Radiochemical Centre, Amersham, England. The specific activity of the H3-thymidine was 2.8–4.4 curies/mM. Both batches of thymidine were injected in two equal doses at intervals of four hours. (*b*) *In-vitro labelling:* 9 ml. of blood from a vein was made up to 11 ml. with 20 μc. of tritiated thymidine in heparinized saline and incubated for one hour (Bond *et al.*, 1958). Autoradiographs were prepared by the stripping film technique of Doniach and Pelc (1950), using British Kodak AR 10 stripping film. The autoradiographs were exposed for 7 to 14 days.

4. When the transplanted kidneys were removed they were immediately fixed in 10% formol-saline. Sections were prepared at 5 μ and autoradiographs set up by the stripping-film technique. The sections were placed on slides which were reputedly low in background. The autoradiographs were exposed for 30 to 90 days. In one instance a kidney was biopsied at three days and later at nine days. We preferred to remove the kidneys because biopsies provoked local polymorph infiltration into homotransplanted kidneys.

After appropriate exposure the autoradiographs were developed and the sections were stained through the emulsion with methyl-green–pyronine.

Ordinary histological preparations were also made; they were stained by several preparations, including haematoxylin and eosin and methyl-green–pyronine. Serial sections were used for both the autoradiographs and ordinary sections. At least 12 sections from each kidney were exposed as autoradiographs.

Assessment of Autoradiographs

The grain count in these experiments is generally low. If the kidney was transplanted three hours after the second pulse of tritiated thymidine a few isolated heavily labelled cells were observed (TT8, 13, 14).

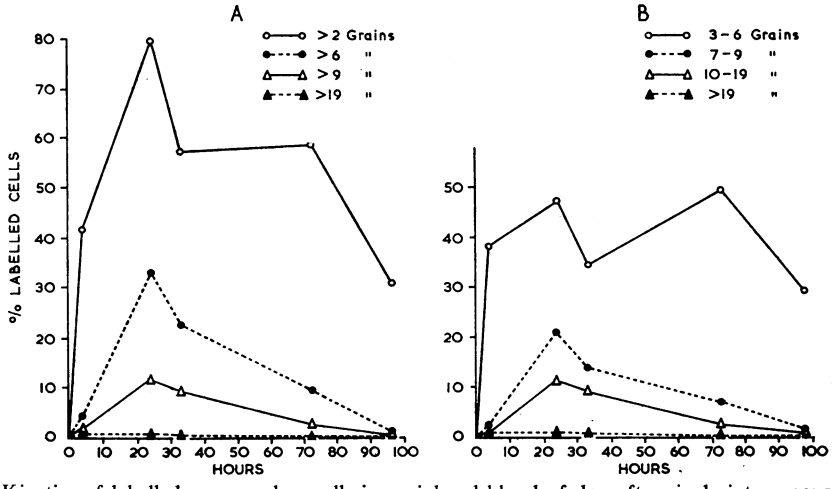
The following criteria could be accepted as evidence of labelling: (1) A grain count/cell which is more than 10× background of the slide. (2) A grain count/cell greater than a given number of grains. In the present experiments a number greater than three was accepted on the basis of the following argument: The magnitude of the slide background and its distribution were assessed. The magnitude in general was 0.1–0.2 grain per immature plasma-cell nucleus. The degree of "clumping" was assessed in two ways: (1) grains were counted per square using an eyepiece graticule, and (2) grains were counted per proximal convoluted tubule cell nucleus.

The results of these assessments were tested against the expected Poisson distribution for the mean grain count of any particular slide using the index of dispersion. In most instances the distribution was significantly non-random. The actual distribution obtained was found to approximate closely to a negative binomial expansion (David and Moore, 1954). This indicates a degree of "clumping" of grains. The expected frequencies of cells with four or more grains are smaller than 10^{-4} irrespective of whether a Poisson distribution or a negative binomial expansion is applied. Thus counts of more than three grains per cell may be taken as representing significant labelling in that cell.

Results

Examination of Blood of Recipient Animals

Labelling of the white blood cells by incubation with tritiated thymidine *in vitro* resulted in labelling a very variable percentage of mononuclear cells. These were almost entirely >8 μ in nuclear diameter. The mean percentage of labelled mononuclear cells for 10 normal dogs was 7.13 (S.D. 8.6). Some of these labelled cells



Kinetics of labelled mononuclear cells in peripheral blood of dog after single intravenous injection of 2 mc. of tritiated thymidine. A. Total grain counts/nucleus. B. Differential grain counts/nucleus.

are shown in Special Plate, Fig. 1. Individual animals seemed to possess characteristically high or low values for a period of some days.

Intravenous injection of tritiated thymidine results in the dog in the appearance of labelled mononuclear cells in the peripheral blood. The labelled cells are confined almost entirely to the group with nuclei >7 μ. Special Plate, Fig. 2, shows labelled cells at 48 hours. Considerably more labelled mononuclear cells were present in the peripheral blood at 24 hours than at three to four hours. The Charts above show the appearance of labelled cells in the peripheral blood of a dog after a single intravenous injection of 2 mc. of tritiated thymidine. Similar results were obtained with two other dogs.

Assessment of Number of Labelled Cells in Interstitium of Kidneys (Homotransplanted) and Skin (Homografted) to Labelled Recipients

When the kidneys were transplanted three hours after the second pulse of tritiated thymidine small numbers of heavily labelled non-pyroninophilic cells were seen. Similar cells were not seen when the kidneys were transplanted 24 hours after the second pulse. In both types of experiments lightly labelled—that is, 4–9 grains/nucleus—pyroninophilic cells were seen. It would appear that both pyroninophilic and non-pyroninophilic cells were more heavily labelled in kidneys transplanted three hours after the second pulse than in kidneys transplanted 24 hours after the second pulse (Special Plate, Fig. 3). It was not practical to substantiate this

observation quantitatively because of the very small percentage of cells actually labelled.

The kidney transplanted 48 hours after the injection of tritiated thymidine showed less than 1% of the pyroninophilic cells labelled. This number was not considered significant.

The homo-autotransplanted kidney showed a massive infiltration as described previously (Dempster, 1955). No labelled cells were recorded.

Kidneys homotransplanted to recipients already in possession of a surviving skin homograft from the same donor showed no second-set reaction and were removed while functioning well. No difference in the number of labelled pyroninophilic cells was observed when compared with first-set kidneys (1b).

Kidneys homotransplanted to recipients already sensitized with two successive skin grafts (from the same donor), both of which had been allowed to reject, showed extensive glomerular and tubular damage, cellular infiltration, and oedema. The cellular infiltration at 30 hours was light. Grain counts of pyroninophilic cells showed a percentage of labelled cells very similar to that in the appropriate first-set control (1a). Some non-pyroninophilic cell types showed occasional well-labelled cells as in 1a.

The skin homografts examined did not show heavy cellular infiltration. Occasional lightly labelled pyroninophilic cells were seen. It was not deemed worth while to assess the percentage cells bearing label.

Results of Incubating Thoracic-duct Cells with ^{14}C Thymidine.—In vitro sampling indicated that only lymphocytes with nuclei >7 μ were labelled. No labelled small lymphocytes were seen. Autoradiographs of the kidney removed after 48 hours revealed an occasional labelled cell of the larger lymphocyte type. No quantitative data are available because of the paucity of labelled cells actually seen.

Assessment of Extent of Division of Interstitial Cells in Homotransplanted Kidneys

In ordinary histological preparations we observed mitosis of pyroninophilic cells on a few occasions. In an effort to demonstrate whether the interstitial cells were capable of division, tritiated thymidine was injected into two homotransplanted kidneys while still functioning prior to their removal. The circulation was clamped for 10 minutes so as to allow the tritiated thymidine to diffuse into the interstitium and so to come in contact with cells about to divide.

Two kidneys removed on the fourth day were used for this study. In each histological autoradiograph three series of 1,000 interstitial cells were studied. The counts were made over different areas of the histological section. The counts of labelled cells ranged from 1.5 to 8.5%. The maximum grain count recorded was 30 grains/nucleus. There appeared to be areas in which heavily labelled cells were frequently found. This may be a problem of physical diffusion.

TABLE III

	Kidney No. 1	Kidney No. 2
Lymphocytic type cells	4	3
Plasma cells (immature)	3	4
Cells of capillary endothelium (Special Plate, Fig. 4 B)	0	2
Cells with oval nuclei and cytoplasmic processes	23	21
	30	30

In each of the two kidneys labelled with tritiated thymidine, 30 labelled cells were critically examined histologically, and identified so far as was possible. The majority of labelled cells defied ready classification—a problem encountered with electron-microscopic identification (Galle and Montera, 1962). These cells could vaguely be described as fibroblastic type cells (Special Plate, Fig. 4 A). Many cells possessed several cytoplasmic processes and commonly an oval nucleus. The results are shown in Table III.

Discussion

The premises involved in the use of tritiated thymidine as a D.N.A. precursor for autoradiography and the interpretation of the results obtained with it have been discussed (Hughes *et al.*, 1958; Cronkite *et al.*, 1959). The following points are generally accepted. (1) Thymidine is a specific precursor for D.N.A. and is available for only a short period after injection. (2) Thymidine is incorporated into D.N.A. only during actual synthesis prior to cell division and the label is distributed to the daughter cells without loss. (3) The label present in a non-dividing cell formed from a labelled cell is stable until the death of that cell. (4) Significant reutilization of labelled D.N.A. in other cells has not been convincingly demonstrated. It thus seems highly likely that a labelled cell observed in an autoradiograph is either a cell labelled at the time of injection of the radioactive thymidine or one of its direct descendants.

In the light of these statements it is possible to examine the results obtained in the present experiments. In experiments 1a and 1b a small percentage of labelled cells was observed in the kidneys homotransplanted to previously labelled recipients. It is clear that these cells contain D.N.A. that was labelled during its formation in the host. The most likely explanation is that these cells are direct descendants of cells labelled in the host animal and which travel via the blood-stream to the transplanted kidney. The effect of transplanting a kidney at different times after the injection of the thymidine does suggest that the labelled cells entering the kidney are available for only a relatively short period—compare Nossal and Mäkelä (1961). The most positive result was obtained when transplantation was effected three hours after the last injection of isotope. However, many of the labelled cells observed in the transplanted kidney three hours after the injection of isotope were not pyroninophilic cells. The total percentage and degree of labelling of pyroninophilic cells were relatively consistent in "three-hour" and "24-hour" experiments (2.2–7.3%). This percentage of labelled pyroninophilic cells agrees reasonably with the data reported by Fowler and West (1961) for first kidney transplants. In no instance did the percentage of pyroninophilic labelled cells contain the grain counts illustrated by Porter and Calne (1960), but some non-pyroninophilic cells in the "three-hour" experiments had similar grain counts. Labelled cells similar to those observed in the peripheral blood were not seen in homotransplanted kidneys. It was never possible to make a confident statement about the cell of origin.

Kidneys transplanted to hosts previously sensitized by skin grafts showed a percentage of labelled cells comparable to that of the "three-hour" first kidneys. These kidneys showed gross histological damage typical of second kidneys (Dempster, 1953a) and were rendered

anuric by 30 hours. Our results on second kidneys, therefore, did not confirm those reported by Fowler and West (1961) using rabbits, and because of the limitations of the technique of injecting tritiated thymidine we fail to see how 100% labelling could ever be obtained.

It is clear that first kidney homotransplants show a small but fairly consistent percentage of pyroninophilic cells that bear host D.N.A. This small percentage must obviously be regarded as a minimal figure, since only a proportion of the precursor cells could be manufacturing D.N.A. at the time when the thymidine was injected, and a proportion of the radioactive cells may bear too little label to be detected in autoradiographs. The fact that neither the presence of pre-existing grafts from the same donor nor kidneys transplanted to a sensitized donor increased the percentage of labelled pyroninophilic cells throws doubt on the notion of a continuous release of antigen from the graft eliciting the proliferation of a specific body of cells—that is, clonal selection. There is some evidence that antigen is not released from first kidneys and skin homografts (Dempster *et al.*, 1963).

Examination of the blood of recipients by *in vitro* incubation with thymidine confirms the presence of cells manufacturing D.N.A. and presumably, therefore, able to divide. These cells fall almost entirely into the categories of medium lymphocytes (nucleus >7 μ) and monocytes. Labelling of the recipient by intravenous injection of tritiated thymidine results in labelling a similar group of large mononuclear cells. In neither case was the small lymphocyte significantly labelled. Evidence from one experiment involving the *in vitro* labelling of thoracic duct cells indicates that small lymphocytes were not labelled but lymphocytes with nuclei >7 μ were labelled; cells corresponding to the latter type were very occasionally seen in a homotransplanted kidney at 48 hours. Thus, any labelled cells observed within homotransplants in these experiments cannot have originated from small lymphocytes which are considered to be the mediators of homograft rejection. Or alternatively, since only a low percentage of labelled small lymphocytes were found in the blood, an explanation is provided for the consistently low percentage of labelled cells in homotransplanted kidneys. However, this does not explain the inability to obtain an increased percentage of labelled cells when the animal is bearing a surviving skin homograft from the same donor, or in an animal that has been sensitized.

It is obvious that tritiated thymidine is not a suitable isotope to use for this particular problem when only two pulses are given. We would be cautious about drawing too enthusiastic conclusions about the labelled cells in homotransplanted kidneys, particularly since we, in a much larger series of experiments, have not confirmed the degree of labelling reported by Porter and Calne (1960).

A further criticism of the method is the fact that the tritiated thymidine was injected prior to the antigen—assuming that a homotransplanted kidney acts as an antigen immediately. This being so, any cells that are labelled prior to the transplant are merely those able to divide and need have no direct connexion with the transplant.

Finally, we have failed to label the endothelial cells of homotransplanted kidneys which also swell up and become pyroninophilic. Some of these cells were labelled when tritiated thymidine was injected just prior to removing a kidney some days after transplantation. This indicates that at least some of these cells are capable of division. We have not been able to determine the origin of the star-shaped cells which are usually the first cells to appear (Dempster, 1957). These experiments have not provided sufficient evidence to eliminate our previous attitude that these cells are stimulated renal reticulum cells. It follows that there are perhaps two populations of cells differentiating in the kidney interstitium—one of host origin and one of renal origin. It has never been denied that the occasional non-pyroninophilic cell is of host origin. The percentage labelling in first and second kidneys is the same and yet the histology is quite different and hence the significance of these cells remains in doubt. They may be present in long-surviving human transplants (Hamburger *et al.*, 1962) or they may be virtually absent in rejected transplants in dogs (Dempster, 1953c). Similar pyroninophilic cells are commonly seen in various chronic renal diseases and no one has suggested that the plasma cells have originated from infiltrating white blood cells. It is generally accepted that these cells are of renal origin, and since they are associated with such a variety of lesions in the kidney it is possible that they are non-specific.

Summary

Kidneys were homotransplanted to hosts previously labelled with two pulses of tritiated thymidine. Small percentages of labelled pyroninophilic cells were recorded in autoradiographs of kidney homograft sections. These figures are minimal, and represent 15–58% of the level of labelled mononuclear cells (12%) recorded in the peripheral blood of the recipient animals. It is clear that the results cannot exclude the view that a proportion of the pyroninophilic cells are of renal origin. Such labelled cells as are recorded in the transplant are probably originating from those mononuclear cells in the blood with a nuclear diameter greater than 7 μ. Many of such cells are apparently able to divide.

Transplants to recipients which bore, or had rejected, skin homografts from the same donor showed no increase in the percentage of labelled cells. Thus no evidence was obtained for proliferation of a specific clone of cells directed against the transplant.

Difficulty has been experienced in differential counts of the cells infiltrating homotransplants. Even the electron microscope fails to identify a large proportion of the cells.

While the stability of the label makes the use of tritiated thymidine desirable in many problems, when given in one or two pulses it is not an ideal cell label for this problem. A more reliable estimate of the number of infiltrating host cells would be recorded after many weeks of tritiated thymidine administration or after injecting an isotope which would label 100% of the lymphocyte population instantaneously.

It is possible that two populations of cells are differentiating within the kidney homograft, one of host origin and one of renal origin. The relative magnitude of the two populations is not known. It is perhaps significant that no labelled cells were recorded in homo-autotransplanted kidneys.

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REFERENCES

Bond, V. P., Cronkite, E. P., Fliedner, T. M., and Schork, P. (1958). *Science*, **128**, 202.
—— Fliedner, T. M., Bond, V. P., Rubini, J. R., Brecher, G., and Quastler, H. (1959). *Ann. N.Y. Acad. Sci.*, **77**, 803.
Darmady, E. M., Dempster, W. J., and Stranack, F. (1955). *J. Path. Bact.*, **70**, 225.
David, F. N., and Moore, P. G. (1954). *Ann. Bot. (Lond.)*, **18**, 47.
Dempster, W. J. (1953a). *Brit. J. Surg.*, **40**, 447.
—— (1953b). *Brit. J. plast. Surg.*, **5**, 228.
—— (1953c). *Arch. int. Pharmacodyn.*, **95**, 253.
—— (1955). *Brit. J. Urol.*, **27**, 66.
—— (1957). *Introduction to Experimental Surgical Studies*. Blackwell, Oxford.
—— Calnan, J. S., and Kulatilake, A. E. (1963). *Brit. med. J.*, **1**, 23.
Doniach, I., and Pelc, S. R. (1950). *Brit. J. Radiol.*, **23**, 184.
Fowler, R., and West, C. D. (1961). *Aust. J. exp. Biol. med. Sci.*, **39**, 537.
Galle, P., and Montera, H. de (1962). *Rev. franç. Étude clin. biol.*, **7**, 40.
Hamburger, J., Vaysse, J., Crosnier, J., Auvert, J., Lalanne, C. M., and Dormont, J. (1962). Ibid., **7**, 20.
Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G. (1958). *Proc. nat. Acad. Sci. (Wash.)*, **44**, 476.
Nossal, G. J. V., and Mäkelä, O. (1961). *J. exp. Med.*, **115**, 209.
Porter, K. A., and Calne, R. Y. (1960). *Transplant. Bull.*, **26**, 458.
Schooley, J. C., Bryant, B. S., and Kelly, Lola S. (1959). *Kinetics of Cellular Proliferation*, edited by F. Stohlman jun., p. 208. Grune and Stratton, New York.
Simonsen, M., Buemann, J., Gammeltoft, A., Jensen, F., and Jorgensen, K. (1953). *Acta path. microbiol. scand.*, **32**, 1.
Williamson, C. S. (1926). *J. Urol.*, **16**, 231.
Wu, P. P. T., and Mann, F. C. (1934). *Arch. Surg.*, **28**, 889.

TIME OF ANTIGEN RELEASE FROM HOMOGRAFTS

BY

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It is generally believed that, within a short period after grafting, antigen is in some way constantly transported from homografts to the reticulo-endothelial system of the host. We had no particular reason for challenging this concept until evidence apparently to the contrary gradually emerged from our work. The first doubts arose after the publication of work on kidney homotransplantation by Gordon and Richards (1957). These authors showed that when a second kidney was transplanted on the day that a first kidney had become anuric no accelerated rejection occurred. Indeed, the second behaved like a first kidney. It was not clear from their paper whether the second kidney had come from the same donor as the first; Gordon (personal communication, 1957) clarified the point that both first and second kidneys in each case had originated from the same donor. Evidence from previous work of our own indicated, in retrospect, that a first kidney removed on the fourth day while still fuctioning had no obvious sensitizing effect on a second kidney transplanted 4 to 10 days later (Dempster, 1955).

For several reasons we considered it opportune to gather some further information about the time at which antigen was released from skin and kidney homografts in dogs. In addition, it was considered worth while to gather some comparative data on skin grafts in rats and mice from which quantitative statistical analysis could be made.

Materials and Methods

Dogs.—Greyhounds of weight varying from 22 to 25 kg. were used. Kidneys were transplanted by a technique previously described (Dempster, 1953a). Large (3 by 2 in.; 7.5 by 5 cm.) fitted full-thickness skin grafts were applied to the same recipient. Experiments done under such exacting conditions were difficult to achieve, and kidneys becoming anuric during week-ends were all lost to these experiments. The plan was: (1) To transplant second kidneys on the day that the first kidneys became anuric or oliguric. This was a repeat of the experiments reported by Gordon and Richards (1957). Four experiments were performed which exactly corresponded to conditions laid down. (2) To apply skin homografts and to remove them while in an apparently healthy state on the eighth day. To wait 14 days and then apply a second skin homograft. Four experiments were performed.

Rats.—Forty-two Wistar rats of average weight 119.7 g. (S.D. 31.9) were used. The rats were obtained from two sources, and at neither was inbreeding practised. Designated Tuck's and St. Thomas's, they may be considered as not isogenic. Fitted full-thickness skin grafts were sutured to the facial layer of the back by the technique previously described (Calnan and Fry, 1962).

Mice.—Skin homografts were exchanged between nine mice of T.T. strain (white) and nine of C57 strain (black).

Experimental Design

Rats.—A random block design was executed in such a way that the assessment of viability of any particular skin graft was made without knowledge of its origin in order to remove any bias ("double - blind trial"). (1) Skin homografts of 1.5 cm. diameter were exchanged between a pair of rats (Fig. 1) and at five, six, seven, or eight days later, while the grafts were still viable, returned

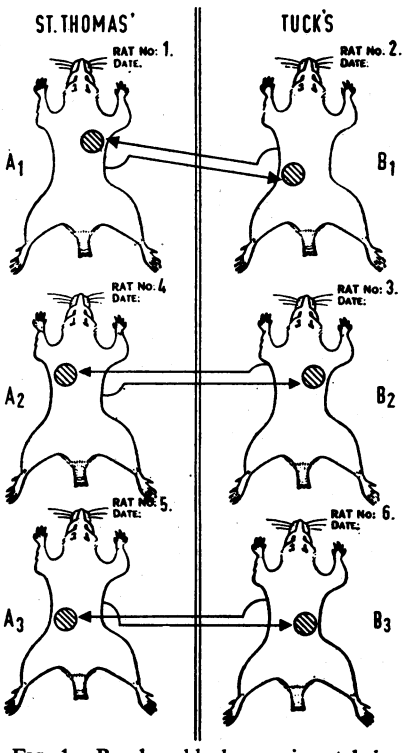

FIG. 1.—Random block experimental design. First homografts (belly skin to defect on back).