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D. A. Darcy

Phil. Trans. R. Soc. Lond. B 1952 **236**, 463-503
doi: 10.1098/rstb.1952.0008

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A STUDY OF THE PLASMA CELL AND LYMPHOCYTE REACTION IN RABBIT TISSUE HOMOGRAFTS

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(*Communicated by A. C. Hardy, F.R.S.—Received* 14 *December* 1951—
Revised 9 *February* 1952)

CONTENTS

	PAGE
Introduction	465
Materials and methods	467
(*a*) General	467
(*b*) Routine procedure	468
(*c*) Implantations	468
(*d*) Intradermal pockets	468
(*e*) Biopsies	468
(*f*) Histological technique	468
(*g*) Cell counts	469
(*h*) The serial biopsy assumption	469
(*i*) Statistical methods	470
Results	470
(*a*) Summary of behaviour of submaxillary gland grafts	470
(i) Autografts	470
(ii) Homografts (first-set)	470
(*b*) The plasma cell and lymphocyte reaction in the grafts	471
(i) In autografts	471
(ii) In homografts	471
(1) Description of the cells	471
(2) Occurrence and behaviour of cells	471
(*c*) Cell counts in first-set grafts	472
(i) Lymphocytes and pyronin cells	472
(1) Their ratios	472
(2) Their concentrations	475
(ii) Immature and mature pyronin cells	476
(1) Their ratios	476
(2) Their concentrations	479
(3) Comment	480
(*d*) Second-set homografts	481
(i) Introductory	481
(ii) Lymphocyte and pyronin-cell counts	482
(1) Their ratios	482
(2) Their concentrations	482
(iii) Immature and mature pyronin-cell counts	484
(1) Their ratios	484
(2) Their concentrations	485
(iv) Lymphocytes and pyronin cells in relation to speed of graft destruction	485
(v) Correlation tests	486
(1) Significant correlations within second-set grafts	486
(2) Significant correlations between first-set and second-set grafts	487
(*e*) Differential counts of immature pyronin cells	489
Summary and general discussion	490
(*a*) Whether the lymphocytes and plasma cells are a cause of graft destruction	490
(i) Conditions to be fulfilled	490
(ii) Evidence from the first-set grafts	491
(iii) Evidence from the second-set grafts	492
(iv) Conclusion	494
(*b*) The role of the lymphocytes and plasma cells in the grafts	495
(*c*) The maturation of plasma cells in these grafts	497
(*d*) Whether the invading lymphocytes become plasma cells	497
(*e*) The percentage of immature plasma cells as an index of the host reaction	498
Appendix	499
References	502

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The failure of tissue grafts from one animal to another of the same species (homografts) in mammals is known to result from an active immunization of the recipient animal. But attempts to show that circulating antibody is the agent of destruction have so far been unsuccessful. The present study was undertaken chiefly to test the widely entertained alternative hypothesis that the breakdown of these homografts is, in part at least, caused by the 'lymphocytes' whose infiltration into the graft is so characteristic a feature of the process.

The method was to implant a set of grafts intradermally in each rabbit from a single donor and to excise one graft for histological study every fourth day thereafter. Sections stained with methyl-green pyronin were counted for lymphocytes and plasma cells (both mature and presumptive immature). The cell counts were then subjected to various statistical tests.

The invading cells were found to include, besides small lymphocytes, a large number of cells staining strongly with pyronin. Some of the latter were characteristic mature plasma cells; the rest were, according to the evidence, probably immature ones. The immature forms were largely lymphocytic in appearance, but some had reticular cell characteristics; maturation occurred at the graft site. It appeared likely that a fate of the non-pyronin-staining lymphocytes was to become plasma cells; but others underwent destruction at the time of failure of the graft's blood vessels.

On the fourth day after implantation very few invading cells were present in the grafts. On the eighth day large numbers were present, and in most grafts the blood vessels were greatly dilated and engorged and tissue destruction was just beginning. At this time there was evidence of a positive relationship between the lymphocyte content of the grafts and the speed with which the grafts were going to be destroyed. No such relationship was found for the plasma cells.

On the twelfth day the destruction was well advanced in most grafts and vascular breakdown widespread. No relationships were found between the concentrations of the invading cells and the stage of graft destruction achieved. But there was evidence that the increase in the number of mature plasma cells in the grafts from the eighth to the twelfth day was positively related to the amount of graft tissue destroyed during that time; the lymphocyte increment, on the other hand, tended to be negatively related to the amount of tissue destroyed.

It was seen that the percentage of immature forms among the plasma cells was high in grafts at the beginning of breakdown and that it thereafter declined, but it tended to remain relatively high as long as graft tissue persisted. There was a positive relationship between this percentage in the grafts on the eighth day and the speed of graft breakdown. No graft was destroyed unless this percentage reached over fifty. It would appear to be an index of the progress of the host reaction against the graft.

The grafts in one animal contained a massive infiltration of lymphocytes and plasma cells on the eighth and twelfth day without undergoing any tissue destruction.

Lymphocytes were frequently observed in such close contact with the graft cells as to give the appearance of having penetrated them. Immature plasma cells were also found in such positions, but not frequently.

Grafts were transplanted to animals previously sensitized by a set of grafts from the same donor. The second set of grafts in general underwent an accelerated destruction and had a much greater infiltration of plasma cells, but not of lymphocytes. Those second-set grafts which were slowest to breakdown contained most plasma cells.

There was a striking correlation between the mature plasma-cell concentrations in an animal's first and second set of grafts, at any rate after graft breakdown was moderately advanced. It appeared that this cell concentration was determined by the response of the host animal rather than by the graft. Since the ranking of animals according to the speed of breakdown of their grafts was different for the first- and second-set grafts, this suggested that these cells were unlikely to be an important cause of graft destruction.

In a few animals the second set of grafts was destroyed no quicker than the first set, yet their infiltration by plasma cells was much greater. This too was taken to be evidence against an important causative role for these cells.

From these and other considerations it was concluded that the results do not support the hypothesis that the plasma cells which infiltrate these homografts are a significant cause of their destruction. The same would also appear to hold, though with less evidence, for the lymphocytes.

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At the same time it appeared likely that the plasma-cell response is specifically connected with the immune reaction. Of the main theories of plasma-cell function that of resorption would best explain the findings in these grafts. If the infiltrating plasma cells secrete specific antibody against the graft tissue this contribution to graft destruction would not appear to be significant.

INTRODUCTION

It is well established that the cause of breakdown of mammalian homografts is the immunization of the host by these grafts (Medawar 1944, 1945). The actual mechanism of the destruction has, however, not yet been discovered. There has so far been a failure to demonstrate that circulating antibodies are responsible for grafts destruction (cf. Medawar 1948*a*; Harris 1943), and evidence has appeared that they are not responsible for regression of homografts of a rabbit tumour (Kidd 1946).

It seemed important therefore to test the most widely entertained alternative hypothesis (cf. Woodruff & Woodruff 1950), namely, that destruction of homograft is brought about by the characteristic 'lymphocyte' infiltration which these grafts undergo and for which no satisfactory explanation has been offered. Such an infiltration has been thought to play an important part in immunity to tumour homografts (e.g. by Wade 1908; Da Fano 1912; Mottram & Russ 1917; Murphy 1926), and this has not been altogether refuted (Woglom 1929), although it was based on nothing more than the observation of associations between the cells and tumour destruction. The suggestion of Loeb (1930) that the invading cells assist in homograft breakdown in a physical manner (e.g. by mechanical pressure) has not been supported; this was part of Loeb's purely 'local' theory of homograft breakdown which has been disproved by Medawar's demonstration that the effect is a systemic one. A more likely hypothesis for a causal relationship between the cellular invasion and graft destruction is that the cells liberate specific antibody at the site of the graft. This hypothesis has already been considered briefly (Darcy 1949); it is possible to reconcile such a mechanism with the systemic effect of postulating that lymphocytes and plasma cells, specifically modified through the action of the graft antigen on the haemopoietic centres, are liberated into the circulation and carried to the graft. A subsidiary hypothesis would be required to explain the actual infiltration into the graft. Alternatively, a slight reaction of circulating antibody with the graft might attract the cells.

A few years ago it seemed possible that the lymphocyte might be a vehicle for antibody and might also produce it (Ehrich & Harris 1945; White & Dougherty 1946). Since that time this hypothesis has been criticized from many sides (e.g. Fishel, Le May & Kabat 1949; Habel, Endicott, Bell & Spear 1949; Thatcher, Houghton & Ziegler 1948; Craddock, Valentine & Lawrence 1949), and there seems to be no sure evidence that the lymphocyte either produces antibody or contains any significant amount of it (see, for example, Habel *et al.* 1949; Erslev 1951). Recently Ehrich, Drabkin & Forman (1949) attributed the earlier results to an admixture of plasma cells in the lymphocyte preparations.

In the meantime an increasingly strong case has been built up for the plasma cell as an antibody producer. The main lines of evidence are:

(1) The repeatedly observed association of plasma-cell proliferation with a rise in antibody titre (Kolouch 1938; Bjørneboe & Gormsen 1941; Bing & Christensen 1944; Gormsen 1942; Rich, Lewis & Wintrobe 1939).

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(2) When extracts were made of the various tissues of hyperimmunized animals it was found that those tissues with the highest concentration of plasma cells, notably the fat of the renal pelvis, also had the highest antibody titre, whereas the organ with the highest concentration of lymphocytes (and very few plasma cells), the thymus, had no antibody (Bjørneboe, Gormsen & Lundquist 1947).

(3) Bing, Fagraeus & Thorell (1945) showed that plasma cells have the cytochemical organization which, according to Caspersson (1947) and his co-workers, promotes synthesis of cytoplasmic protein for cell growth or for secretion. They might therefore produce antibodies. One of the main features of this organization is a high content of pentose-nucleic acid in the cytoplasm, and it is this which causes plasma cells to stain so heavily with basiphilic dyes. The common small lymphocyte was poorly equipped in this respect (Thorell 1945).

(4) Fagraeus (1948*a*, *b*) found that in the spleen of rabbits undergoing 'secondary response' to an intravenously injected antigen there was a great increase in plasma cells simultaneously with the increase in circulating antibody. These cells were confined almost exclusively to the red pulp and appeared to originate from reticulum cells, passing through a chain of development which she lists as: transitional cell, immature plasma cell, and mature plasma cell. Fragments of spleen excised at different times during the period of antibody formation contained very little antibody, but if the pieces were grown *in vitro* significant amounts were obtained. Fragments of red pulp produced much more antibody than white pulp. When the red-pulp fragments contained only the 'transitional' type of cell antibody production was comparatively poor; it was greatest when numerous 'immature plasma cells' were present and receded somewhat when mature plasma cells predominated.

Plasma-cell development in the various tissues of rabbits under antigen treatment, somewhat similar to that reported by Fagraeus, have been described by Kolouch (1938), Kolouch, Good & Campbell (1947), Marshall & White (1950), and Ringertz & Adamson (1950). Keuning & van der Slikke (1950) have, in addition, confirmed the results of the *in vitro* experiments of Fagraeus, and extended them with the important observation that immature plasma cells (but not small lymphocytes) isolated from the spleens of immunized rabbits liberated antibody into the culture medium in amounts significantly greater than was obtained when the cells were assayed without culturing. Their technique makes adhesion of serum antibody an unlikely explanation, especially in view of their negative results with the lymphocytes. The only other alternative explanation to that of antibody production is one of resorption of antibody (either as such or combined with antigen) followed by liberation *in vitro*.

(5) Reiss, Mertens & Ehrich (1950) showed that cells from lymph nodes of immunized animals will specifically agglutinate on their surface, *in vitro*, the bacteria against which the animals have been immunized. The cells which did this were of the plasma-cell series, the immature cells being particularly active. Typical small lymphocytes did not exhibit the effect.

(6) Antibody transmission from mother to young in cattle is by way of the colostrum rather than the placenta (Smith & Little 1922); the concentration of the immune globulin fraction of the colostrum is many times that of the maternal serum globulin (Smith 1948);

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a strong plasmacytosis occurred in the milk-secreting tissue at the time of colostrum formation, these cells disappearing shortly after calving; if the cows were not milked for a period the antibody content of their milk rose to the level of that in colostrum; at the same time there was a reoccurrence of the plasmacytosis. The evidence for this suggestive argument is given in a preliminary note by Campbell, Porter & Petersen (1950); only three cows have so far been used for the plasma-cell determinations.

It can be objected against the evidence listed above that all of it might be explained by the co-occurrence of phenomena which may not be causally related. Its variety, however, is impressive. The alternative possibility, that antibodies evoke plasma-cell formation, was tested by Fagraeus (1948*b*). She found that passive immunization of rabbits did not noticeably alter the plasma-cell content of their tissues.

There remains the possibility that it is the reaction of antibody with antigen that calls forth plasma cells. This hypothesis would dovetail with the views of Dubois-Ferrière (1951), who holds that the plasma cell's main function is resorption and neutralization of foreign proteins. The evidence for this at present is slight; protein inclusions of several kinds are known to occur in plasma cells, but it is not known whether they are products of secretion or resorption (cf. Kabelitz 1951).

In addition to the above hypotheses of plasma-cell function there may be other possibilities, for there seems to be no overwhelming reason why they should not have somewhat different functions in different situations, especially since they probably represent functional states of the lymphocyte or reticulum cell rather than a genetically distinct cell lineage (Burnet & Fenner 1949; Dubois-Ferrière 1951). It seems generally agreed that the stimulus which evokes them in normal animals is foreign protein, and that they are in some way concerned in its destruction or neutralization.

For the present purpose the important question is whether the cellular infiltration is causally connected with homograft destruction. The results obtained make it unlikely that the cells are a significant cause, and fit better the hypothesis that they are an effect of the immune reaction, especially in the case of the mature plasma cells. This argues against the hypothesis that these cells produce antibody in the homografts.

MATERIALS AND METHODS

(*a*) General

The animals used throughout were the ordinary dealer's stock rabbits (and hence genetically heterogeneous). It is possible that the cellular reactions against homografts may be stronger in rabbits than in some species, but no species of mammal has been reported to lack them. It must also be stressed that although most of the observations and all the cell counts presented in this paper were made on homografts of submaxillary gland tissue, the same general picture of graft destruction, including a similar lymphocyte-plasma cell invasion, was found for thyroid gland and kidney homografts. Autografts of all these tissues were studied as controls.

A feature of the present experimental work (as in Medawar's with skin) was the use of serial biopsies. This withdrawing of graft samples at regular intervals made it possible to

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follow the course of graft evolution in each animal. It also brought to light some cases of atypical graft behaviour which were most valuable in illuminating normal behaviour.

The general method of the investigation was to study the grafts histologically, make counts of the invading lymphocytes and plasma cells, and treat these counts statistically.

(*b*) Routine procedure

This was to remove a submaxillary gland from the donor, cut six graft pieces from it and transplant these at the same operative session to separate intradermal pockets in one recipient. The grafts were equal in volume to cubes of between 3 and 4 mm edge, and six of them gave a graft dosage of the order of 250 mg/kg of rabbit. Sterile technique was used throughout and no infection was encountered. Ether anaesthesia was used for operations and biopsies.

(*c*) Implantations

Implantations were made on the rabbit's side in or near the thoracic area. The left side was used for the first set of grafts and the right for a second set. To prepare for implantation, a large area of the thorax and adjoining abdomen was shaved clean and, a few minutes before implantation, was swabbed with Dettol.

(*d*) Intradermal pockets

Intradermal pockets were prepared by making incisions in the prepared skin about a centimetre in length and down to the 'splitting layer' of the dermis (cf. Medawar 1944). Here the connective tissue splits easily, and pockets about 3 cm long were made by forcing the closed points of a pair of curved haemostatic forceps parallel to the skin surface and then opening them to widen the resulting tunnel. The graft fragments were inserted as far into the pockets as possible by means of fine forceps, and the incision was closed with one or two stitches. The site of the graft was marked by painting a circle around it on the skin surface with gentian violet.

(*e*) Biopsies

Biopsies were made on the 4th, 8th, 12th, etc., days after the implantation until the last graft was removed at an autospy on the 24th day. A small block of skin enclosing the graft and including the underlying panniculus carnosus muscle was excised. The wound was closed with stitches or Michel's clips.

(*f*) Histological technique

All specimens were fixed for at least 2 days in mercuric chloride formaldehyde. They were embedded in paraffin wax. Short ribbons of sections 8 μ thick were cut vertically to the skin surface at evenly spaced intervals throughout the specimen. The ribbons (usually five in number) were laid out in order of cutting and slides were prepared each with a series of five sections (one from each ribbon).

One slide was stained with methyl-green and pyronin (Unna-Pappenheim), the procedure of Carleton & Leach (1947) being followed except that the stains were made up as separate solutions (Baker 1941) because of the instability of the mixture. On sections thus stained are based most of the observations and all of the cell counts given in this

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paper. In half of the second-set grafting experiments, viz. 302, 304, 306, 308, the experimental and control slides were stained back to back.

A second slide was stained with Ehrlich's haematoxylin, counter-stained with aqueous-orange G and alcoholic eosin; two others were stained with Heidenhain's haematoxylin, one being counter-stained with picrofuchsin (van Gieson) and the other with xylidene red and light green (Masson).

(*g*) Cell counts

Three kinds of count were made. In one the ratio of 'pyronin cells' (plasma cells and their suspected immature forms) to non-pyronin-staining lymphocytes was determined. For this a $\frac{1}{6}$ in. objective was used and a $6\times$ ocular containing a glass plate on which was cut a square subdivided into 100 small squares by lines numbered 1 to 9 vertically and *a* to *i* horizontally. The cells enclosed in the sixteen squares, *bd* 24, *bd* 68, *fh* 24 and *fh* 68 were counted, and four of the sections through each graft were treated in this way; the total area thus counted was 0·098 mm^2. The count was made where the invading cells were densest, usually at the peripheral parts of the graft epithelium. The two cell types were for the most part well mixed together and the counts were made consistently in such areas, avoiding any special accumulations of either type. In this way a total of about 300 cells was counted on the average. Only in a few specimens did the count fall below 200 cells.

A second type of count was made to determine what proportion of the pyronin cells were of the immature kind, the rest being characteristic mature plasma cells. For this the oil-immersion ($\frac{1}{12}$ in.) objective was used, and the same ocular and grid system as for the first count. Fifty cells were counted per graft; this involved counting at least four fields (sixteen squares of the grid per field), each section on the slide being taken in turn until fifty cells were made up. The counts were made whenever possible on cells surrounding the graft epithelium and, failing this, wherever the pyronin cells were densest. The specimens were taken in random order for counting, the source of the particular specimen being unknown to the observer.

The third cell count, a differential one on the immature forms of pyronin cell, will be described later in the text.

Two points concerning the cell counts must be underlined. First, all counts were made on similarly fixed and treated specimens. It will be seen below that a change of fixative can make a considerable difference. The second point is that some lymphocytic cells were always found which it was difficult to classify because of the faint pyronin staining of their cytoplasm. These were so few, however, as to introduce a negligible error into the counts.

(*h*) The serial biopsy assumption

In interpreting the serial biopsy specimens the assumption has been generally made in this work that changes take place more or less simultaneously in all grafts of a certain tissue transplanted from a given donor to a given recipient at the same operative session, i.e. that at any time these grafts will be at approximately the same stage of evolution. This has been shown to be true for skin homografts by Medawar (1944), for submaxillary gland homografts by the present author (unpublished) and by many other workers (e.g. Loeb 1945).

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(i) Statistical methods

As the data were in the form of rather heterogeneous cell counts logarithmic transformations were made before applying the tests other than χ^2. The purpose of this is to produce comparable variances in all groups (cf. Quenouille 1950). Percentages were subjected to angular transformation for the same reason. Percentages of pyronin cells which were derived from variable total cell counts were weighted with these counts on the assumption that a high count would give a more reliable estimate of the percentage than a small one. Where increments in cell counts and percentages occurring between the eighth and twelfth day were being treated, the increment column was constructed by subtracting the transformed values for 8 days from those for 12. A result was described as 'significant' when the probability of its being due to chance was 5% or less.

RESULTS

(a) Summary of behaviour of submaxillary gland grafts

(i) *Autografts* (controls)

Fourth day. Only the surface layer of the graft has survived transplantation. This has been revascularized, many of the new vessels being dilated and engorged.

Eighth day. The surviving surface layer of the graft has undergone a powerful burst of proliferation, a scaffolding being provided for the new tissue by a strong fibroblastic reaction on the part of the surrounding dermis. Dilation and engorgement of the graft vessels had subsided. The dead core of the graft has been partly absorbed.

Twelfth day. The newly proliferated tissue has begun to differentiate from its condition of multilayered nests or solid buds of cells towards a duct-like or vesicle-like condition. Further absorption of the core.

Sixteenth day (*and onwards*). Further differentiation of the regenerated graft tissue and complete absorption of the dead core.

A fuller account of these autografts will be given in a later report. But two other facts are relevant here:

(1) graft survival time is, for practical purposes, indefinite,

(2) a small number of lymphocytes and even fewer pyronin-staining cells were found in most of the graft series; the number of lymphocytes appeared to reach a peak at 16 days and thereafter to fall off.

The number of grafts on which the above description is based was not less than six (representing six different rabbits) at any stage.

(ii) *Homografts transplanted to non-immunized animals* (*'first set' grafts*)

The features which differentiated these grafts from the autografts were as follows:

Fourth day. There was some indication of a stronger vascular reaction in the average homograft than in the autografts.

Eighth day. Graft invaded by numerous lymphocytes and plasma cells; its blood vessels greatly dilated and engorged; destruction of the regenerated tissue about to begin; lymphocytes vesicular, plasma cells (strictly, 'pyronin cells') predominantly immature.

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Twelfth day. Graft blood vessels largely broken down; graft epithelium partly destroyed. Lymphocytes and plasma cells still numerous; many of the lymphocytes now pyknotic; plasma cells predominantly mature.

Sixteenth day. Graft together with host cells invading it completely destroyed.

This time-table applied to the majority of the homografts; the others went through the same series of events but took either a longer time ('slow reactors') or a shorter time ('rapid reactors'). Not less than eight grafts (representing eight different rabbits) at each stage were examined. The actual numbers were 4th day, 23; 8th day, 23; 12th day, 23; 16th day, 8.

The behaviour of second sets of homografts will be described below.

(*b*) The plasma cell and lymphocyte reaction in the grafts

(i) *In autografts*

As noted above, there were very few of these cells in the autografts, and it is possible that their appearance there is non-specific. Thus Loeb (1945) notes that lymphocytes were no more numerous in autografts than around implants of inert materials, and Blumenthal (1939) found that the rise in blood lymphocytes following autografting was no higher than when inert materials were implanted.

(ii) *In homografts*

(1) *Description of the cells*

The invading non-pyronin-staining lymphocytes were mainly of the small variety. A full description has been given of the invading cells which stained strongly with pyronin (Darcy 1949). These will be referred to as 'pyronin cells'. They include forms corresponding to the 'transitional cells' and 'immature plasma cells' of Fagraeus, but these were not numerous. Much more common were cells answering to the description of small, medium and, less commonly, large lymphocytes (cf. Maximow & Bloom 1942), whose cytoplasm stained with pyronin, often with the same intensity as that of mature plasma cells. Finally, there were the typical mature (Marschalko) plasma cells and forms intermediate in appearance between these and all the foregoing types. Cells with two or more nuclei were numerous in a few specimens.

(2) *Occurrence and behaviour of cells*

The presence of pyronin cells and lymphocytes in the graft blood vessels (including arterioles) and the occurrence of mitoses in the pyronin cells were described in the earlier report. The great majority of the invading lymphocytes and pyronin cells were found in the stroma immediately supporting the graft epithelium and in the connective tissue closely surrounding the graft as a whole.

A striking and common phenomenon was the curiously intimate penetration of the homograft epithelium by lymphocytes (small) and to some degree by pyronin cells. The lymphocytes in particular frequently gave the appearance of having actually penetrated an epithelial cell. Thyroid homografts showed this particularly well. The pyronin cells found amongst the graft epithelial cells were nearly always the immature kinds, rarely mature plasma cells.

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No shedding of cytoplasm by the pyronin cells in the grafts was observed. But, as was noted above, many of the lymphocytes become pyknotic, and sometimes fragmented, during graft destruction. Vacuoles were seen in the pyronin cells, but no cell inclusions.

Four grafts (the 8- and 12-day members of the two first-set homograft series) were cut in two; one-half was fixed in mercuric chloride formaldehyde according to the routine procedure, and the other half was fixed in Helly's fluid. Cell counts revealed a ratio of pyronin cells to lymphocytes about three times as great in the Helly-fixed specimens as in their control halves. The χ^2 test showed that the difference was highly significant in each case. In two of the four grafts it was possible to compare the percentage of immature forms among the pyronin cells in the differently treated halves. In both cases the percentage of immature forms was higher in the Helly-fixed half; further, this difference in percentage of immature pyronin cells was sufficient to account for the difference in total pyronin cells. It is inferred from this that many cells which appeared as lymphocytes after mercuric chloride formaldehyde fixation were classifiable as immature pyronin cells of the lymphocytic type after Helly fixation. Other elements in the tissue also stained more strongly with pyronin after Helly, so that the sections were red to the naked eye; nevertheless, non-pyronin-staining lymphocytes still predominated over the pyronin cells in these Helly-fixed grafts.

(c) Cell counts in first-set grafts

(i) *Lymphocytes and pyronin cells*

(1) *Their ratios*

Table 1 gives the number of 'pyronin cells' and non-pyronin-staining lymphocytes found in the counted area in first-set submaxillary gland homografts; the percentage which is given for each graft as well as the difference in this percentage between the 8- and 12-day grafts of each series. The last three columns give estimates of the degree of destruction of the grafts at either stage.

The graft series (each from one rabbit) are arranged in a spectrum according to their speeds of breakdown, three grades being defined as follows:

Slow: little or no destruction of graft tissue by the twelfth day.

Average: destruction well in progress by the twelfth day.

Rapid: destruction complete by the twelfth day.

Within these groups there is likewise a ranking according to speed of breakdown, the criterion being the amount and condition of the graft epithelium existing at 12 days. When grafts were identical by this standard then the condition of the graft blood vessels was invoked, and in one or two cases where this failed the 8-day specimens of the series were taken into account. It will be noted that this ranking is more subjective than the above division into groups. Both systems are merely approximations, but they are independent of the numbers of invading lymphocytes and pyronin cells.

No counts were made on the 4- and 16-day specimens, as on the fourth day the cells had not begun their invasion, and on the sixteenth day destruction was complete in most series.

There are several points of interest in table 1. First it will be noted that on the average there is roughly one pyronin cell for every three or four lymphocytes. Further, as the

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TABLE 1. CELL COUNTS OF LYMPHOCYTES (*ly*) AND 'PYRONIN CELLS' (*py.c.*) IN SUBMAXILLARY GLAND HOMOGRAFTS

Graft series ranked in order of speed of breakdown. Amount of graft tissue destruction indicated by 0 = none, 1 = slight, 2 = moderate, 3 = much, 4 = complete

	graft series	8 days				12 days				12 − 8 days			graft tissue destruction (days)		
		py.c.	*ly*	total	% *py.c.*	*py.c.*	*ly*	total	% *py.c.*	% *py.c.*	χ^2	*P*	8	12	12 − 8
slow	130*h*	232	563	795	29·2	183	915	1098	16·7	−12·5	45·1	< 0·001	0	0	0
	105*h*	49	54	103	43·7	101	205	306	33·0	−10·7	6·3	< 0·02	0	0–1	½
	99*h*	31	81	112	27·7	113	160	273	41·4	13·7	6·0	< 0·02	0	1	1
average	198*h*	63	453	516	12·2	51	125	176	29·0	16·8	26·8	< 0·001	1	1–2	½
	166*h*	138	201	339	40·7	91	145	236	38·5	− 2·2	not significant		1	2	1
	132*h*	35	130	165	21·2	98	488	586	16·7	− 4·5	not significant		0	2	2
	61*h*	112	352	464	24·2	64	243	307	20·8	− 3·4	not significant		1	2	1
	245*h*	23	186	209	11·0	88	586	674	13·1	2·1	not significant		1	2	1
	64*h*	112	363	475	23·6	139	424	563	24·7	1·1	not significant		1	2	1
	95*h*	81	321	402	20·1	55	297	352	15·6	− 4·5	not significant		1	2	1
	144*h*	83	218	301	27·6	168	160	328	51·2	23·6	34·9	< 0·001	1	3	2
	56*h*	24	214	238	10·1	85	191	276	30·8	20·7	32·3	< 0·001	1–2	3	1½
	63*h*	90	365	455	19·7	114	173	287	39·7	20·0	34·4	< 0·001	1	3–4	2½
	197*h*	106	531	637	16·6	30	141	171	17·5	0·9	not significant		1	3–4	2½
	97*h*	12	188	200	6·0	91	320	411	22·0	16·0	25·1	< 0·001	1	3–4	2½
	243*h*	60	316	376	15·9	52	187	239	21·7	5·8	not significant		1	3–4	2½
	306*h*	58	357	415	14·0	220	156	376	58·5	44·5	176·5	< 0·001	1	3–4	2½
	304*h*	192	608	800	24·0	154	475	629	24·5	0·5	not significant		3	3–4	½
rapid	302*h*	79	369	448	17·6	51	269	320	15·9	− 1·7	not significant		0–1	4	3½
	101*h*	104	468	572	18·2	157	372	529	29·7	11·5	20·1	< 0·001	1	4	3
	183*h*	210	292	502	41·8	30	254	284	10·5	−31·3	84·0	< 0·001	3	4	1
	308*h*	56	380	436	12·8	20	344	364	5·5	− 7·3	11·8	< 0·001	4	4	0
	average	86·6	318·6	407·2	21·3	98·0	301·4	399·4	24·5	3·2	—	—	—	—	—

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grafts evolve from 8 to 12 days their proportion of pyronin cells may either increase or decrease.

The 8- to 12-day changes in the proportion of pyronin cells were investigated statistically by subjecting the cell counts of each graft series to a χ^2 test for a two-by-two table, Yates's correction for continuity being applied. Significant decreases in the percentage of pyronin cells were shown by the two most rapidly destroyed series and also by the two slowest, while significant increases occurred mainly in the 'rapid' half of the average group. It is worth pointing out that, in terms of the pyronin cell to lymphocyte ratio, these increases were considerable. Thus in series 63*h*, to take an average example, the increase from 20 % pyronin cells to 40 % means an increase in the ratio from 1:4 to 1:1·5.

Within the rapid group, the two series which showed the significant decreases, 308*h* and 183*h*, had graft destruction completed and well advanced respectively at 8 days. But series 101*h*, which showed a significant increase, and series 302*h*, which showed no significant change, had only slight destruction at that time. This suggests that the change in the proportion of pyronin cells may be associated with the amount of tissue destruction.

The average group lends support to this suggestion. Five of the six significant increases observed here occurred in the half of the group showing almost complete destruction on the twelfth day; this half underwent the greatest amount of destruction during the period, the amount of destruction being estimated by taking the difference of the 8- and 12-day 'destruction' figures as shown in the table.

Of the slow group, series 130*h* was remarkable for its massive concentration of lymphocytes and pyronin cells at both 8 and 12 days (this is reflected in its cell counts). At either stage it showed no definite epithelial destruction and only mild vascular dilatation. It underwent a significant decrease in the percentage of its pyronin cells from 8 to 12 days. Series 105*h* and 99*h* had low concentrations of the cells at 8 days; at 12 days 99*h* showed evidence of slight epithelial destruction and vascular disturbance, while 105*h* showed even less. In 99*h* there was a significant increase in the percentage of pyronin cells and in 105*h* there was a significant decrease.

Summarizing these results, significant increases in the proportion of pyronin cells between 8 and 12 days were found principally among those graft series which underwent the greatest amount of destruction during this period. Significant decreases in the proportion of pyronin cells occurred among those series in which destruction was least, viz. series 308*h* and 183*h* of the rapid group in which the breakdown reaction was over or on the wane, and series 130*h* and 105*h* of the slow group in which the reaction was practically stationary. Although the nature of the grading system precludes the obtaining of reliable statistical information, yet there is a strong suggestion in these results that the increase in the percentage of pyronin cells (decrease in the percentage of lymphocytes) is positively related to the amount of destruction that took place during the period. A somewhat different and more rigorous treatment will appear below.

The question arises whether there is any relationship between the speed of breakdown of the graft series and the percentage of pyronin cells which they exhibited either at 8 or 12 days or the increment in the percentage during the interval, or the average percentage of the two stages. This was examined by testing whether the regression coefficient of the percentages on their rank (as given in table 1) differed significantly

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from zero. No significant values were obtained. This is not unexpected, since the ranking according to speed of breakdown would not be the same as the ranking according to amount of graft-tissue destruction during the 8- to 12-day interval. The difference is caused chiefly by the three series 304*h*, 183*h* and 308*h*, which showed advanced destruction at 8 days (see table 1). If these three series are omitted from the treatment, thus giving a ranking roughly in the order of amount of destruction, the regression coefficient for the 8- to 12-day increments in percentage pyronin cells now differs significantly from zero ($t_{17}=2\cdot84$, P approaching 0·01), the greatest increases in the percentage being associated with the greatest amount of destruction.

If these three series are also omitted from the 8-day array a significant ($P=0\cdot02$) negative relationship between percentage of pyronin cells and speed of breakdown is obtained (the faster series having the lowest percentage). Here the omission would have for its justification the fact that these three series are sharply different from all the others at 8 days in their advanced stage of destruction; also, it seems very probable that they have already at 8 days undergone the increase in percentage of pyronin cells noted above.

(2) *Their concentrations*

Introduction. Although the cell counts were primarily designed to give the ratio of pyronin cells to lymphocytes they also provide a measure of the concentrations of these cells in the grafts, for the counts were made on equal areas in all grafts and the sections were cut at 8 μ in every series except 243*h* and 245*h* (which were cut at 5 μ). The measure is subject to several errors, such as the variation in the thickness of the supposedly 8 μ sections, errors due to variations in degree of pyronin staining, and especially the possibility that the particular areas counted may not have been accurately representative of the graft (although the use of four levels in the graft to some extent obviates this). However, there is no reason to suppose that these errors are other than randomly distributed, so that while there may be some inaccurate representations of individual specimens, it is unlikely that the overall trend will be misrepresented.

In order to include the grafts of series 243*h* and 245*h* in the tables and in the statistical treatment their cell counts were multiplied by factors derived by the method of Abercrombie (1946), which takes into account the mean length of the nuclei upon which the counts were based. In practice it made a negligible difference to the statistical results whether the crude factor or the derived ones were used. Abercrombie's criticism that observed nuclear counts do not represent the true nuclear population of a section owing to the inclusion of fragments of all sizes does not affect any of the results presented in this work.

Tests for regression. Tests were made to see whether there was any relationship between the speed of breakdown of the graft series and their cell concentrations as represented by the counts. To do this the lymphocyte counts, the pyronin-cell counts, and the total lymphocytes plus pyronin cells of the graft series as ranked in table 1 were each subjected to the regression test. The 8- and 12-day counts were treated separately, as were their differences (the 8- and 12-day increments), and their sums. Of these tests only one gave a significant result, viz. that on the lymphocyte counts for 8 days ($t_{20}=2\cdot34$, $P<0\cdot05$), the 'slower' series having the lowest counts (i.e. positive slope). The total cells at 8 days

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(lymphocytes plus pyronin cells) gave a value just under the 5 % significance level ($t_{20}=2{\cdot}034$, where the 5 % P level is $t=2{\cdot}086$), thus showing the slight masking effect of including the pyronin cells.

The 8- to 12-day lymphocyte increments of the ranked graft series did not exhibit a significant regression. However, if the three series 304*h*, 183*h* and 308*h* were omitted, thus giving a ranking roughly in the order of amount of destruction undergone between 8 and 12 days, then the probability value obtained was about 1 in 19, the slope being negative.

Again omitting these three series, the pyronin-cell counts for 8 and 12 days still showed no sign of regression, and, what is particularly interesting, the 8- to 12-day increments in pyronin cells showed none either. This last fact, taken in conjunction with the possible negative regression of the lymphocyte increments, throws light on the previously noted association between the increment in percentage of pyronin cells and the amount of graft destruction. This association now appears likely to have been more the result of a relatively smaller lymphocyte increment in the grafts undergoing most destruction rather than a greater increase in pyronin cells. The cell counts showed that lymphocytes actually decreased in the grafts undergoing most destruction but increased in those undergoing least.

t tests. No significant difference between the 8-day and the 12-day grafts in their average count of lymphocytes, pyronin cells, or total cells, was found when *t* tests were applied.

(ii) *Immature and mature pyronin cells*

(1) *Their ratios*

Table 2 gives the percentage of the invading pyronin cells which are 'immature' (presumed immature plasma cells), the rest being mature (Marschalko) plasma cells. The graft series are arranged according to their speed of breakdown as before. All counts are recorded; where there are gaps in the table it is either because there was no methyl-green and pyronin preparation available (54*h*, 56*h*, 61*h*, 64*h*, 65*h*), or because the series stopped at 12 days (all others).

The main point of interest is that in the majority of graft series there is a high proportion of immature pyronin cells at 8 days (they easily outnumber the mature forms), while at 12 days these are equalled or outnumbered by the mature cells. *In almost every series there has been a shift in the pyronin-cell population towards mature plasma cells.* In later stage grafts (16 days and onwards) a preponderance of the mature cells is maintained or increased in most.

The interest of this phenomenon lies in its relation with the general homograft reaction; at 8 days destruction of the graft is just starting (average series); at 12 days it is well in progress; at 16 days it is complete on most specimens. An hypothesis may therefore be put forward for trial, viz. that the immature forms compose a high percentage of the invading pyronin cells at the beginning of breakdown of the graft epithelium, and as this breakdown proceeds they decline in percentage. This can be tested by reference to the slow and rapid series, and those of the average series which do not follow strictly the pattern of their class. That the immature to mature shift is not something which automatically occurs between 8 and 12 days is shown by series 130*h*, 99*h*, 132*h*; that it occurs

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automatically and without reference to graft breakdown, once a high immature percentage is attained, is disproved by the behaviour of series 65*h*, 56*h* and 64*h* described below. Reference will be made to table 1 for the amount of destruction in each case.

Slow series. 130*h*. This series had an exceptionally large concentration of both lymphocytes and pyronin cells both at 8 and 12 days. It showed no signs of destruction at either stage (cf. table 1). The fact that its percentage of immature pyronin cells was 42 % at both times is in agreement with the hypothesis. It is particularly interesting (in view of the failure of graft breakdown) that the immature forms do not gain a predominance.

Table 2. Percentage of immature forms among the 'pyronin cells' in submaxillary gland homografts

Graft series ranked in order of speed of breakdown. * indicates stage at which graft epithelium was found completely destroyed.

	graft series	age of graft (days) 8	12	16	20	24	12–8
slow	130*h*	42	42	—	—	—	0
	105*h*	56	16	—	—	—	−40
	99*h*	40	76	—	—	—	36
average	198*h*	82	28	34*	26*	24*	−54
	166*h*	58	42	—	—	—	−16
	132*h*	48	70	—	—	—	22
	61*h*	86	24	34*	—	40*	−62
	245*h*	82	76	—	—	—	− 6
	64*h*	78	54	56	—	—	−24
	95*h*	64	16	—	—	—	−48
	144*h*	70	28	—	—	—	−42
	56*h*	70	46	—	56	—	−24
	63*h*	74	64	30*	26*	30*	−10
	54*h*	—	—	—	24*	10*	—
	197*h*	88	58	20*	22*	6*	−30
	97*h*	66	44	—	—	—	−22
	243*h*	66	34	—	—	—	−32
	306*h*	96	20	—	—	—	−76
	65*h*	—	64	26*	60	20*	—
	304*h*	60	22	—	—	—	−38
rapid	302*h*	90	42*	—	—	—	−48
	101*h*	76	26*	—	—	—	−50
	183*h*	38	18*	—	—	—	−20
	308*h*	86*	56*	—	—	—	−30
	average	69	41	34	36	22	−28

105*h*. There was here a decline in percentage of immature forms without accompanying graft-tissue destruction (there was only very doubtful signs of destruction at 12 days). The hypothesis is not necessarily contradicted by the low percentage of immature cells at 12 days, since it was by no means certain that breakdown had begun. The decline in the percentage of the immature pyronin cells from 8 to 12 days may mean nothing more than that the invasion by these cells is so slow that their maturation in the graft to mature plasma cells has outpaced it.

99*h*. This is in perfect agreement with the hypothesis, showing a high percentage of immature forms at the beginning of graft breakdown (12 days, cf. table 1).

Average series. 56*h* at 20 days has a comparatively high percentage (56 %) of immature pyronin cells. Graft-tissue destruction in this series, unlike the majority, was not quite

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complete at 16 days, and at 20 days there were still persisting some fragments of what appeared to be graft epithelium. This persistence would account for (on the basis of the hypothesis) the high proportion of immature cells.

64*h* at 16 days had a comparatively high percentage (56 %) of immature pyronin cells. It will be noted that it also had some epithelium still surviving at this time and therefore supports the hypothesis.

65*h*. The behaviour of this series was unusual in that, although there was no surviving epithelium in its 16-day graft, the 20-day graft had a few scraps of surviving epithelium. The pyronin-cell population of these grafts shows a striking correlation with this behaviour, being predominantly mature at 16 days and predominantly immature at 20 days—a remarkable reversal of the customary behaviour and entirely in agreement with the hypothesis. This strongly suggests that the 'maturation' of the pyronin-cell population is not an event which occurs automatically and without reference to the progress of graft destruction.

132*h*. This series shows excellent agreement with the hypothesis. It was the only member of the average graft series which reached its maximum percentage of immature pyronin cells at 12 days instead of 8 days. It was also the only one in which graft destruction had not begun at 8 days (cf. table 1).

Rapid series. 302*h* and 101*h* at 8 days showed as little destruction as the average series, but their 12-day grafts showed complete destruction. Their percentages of immature forms (high at 8 days, low at 12) agrees with the hypothesis. 183*h* showed rapid destruction; this was well advanced at 8 days and complete at 12. The low percentage of immature pyronin cells (high percentage of mature plasma cells) on both these days is in perfect agreement with the hypothesis.

308*h*. While this series does not disagree with the hypothesis the high percentage of immature forms persisting after graft destruction is complete (8 days), is unexpected. Maturation of the pyronin-cell population does not necessarily keep exactly in step with graft destruction (series 105*h* showed this), and here the extremely rapid destruction would appear to have outpaced the maturation.

It may be concluded therefore that the behaviour of the rapid series, the slow, and the 'irregular' series of the average group, strongly support the hypothesis that the immature pyronin cells form a high percentage of the total pyronin cells at the beginning of destruction of the graft epithelium, and that as this breakdown proceeds they decline in percentage. Further, the figures in table 2 show that this high percentage was a majority percentage in every case (in series 183*h* destruction was well advanced at 8 days); later the mature forms become predominant. This suggests that there may be a critical percentage (over 50 %) of immature pyronin cell associated with graft breakdown and that destruction does not occur without this percentage first being reached. The lowest figure in the table is 58 % (in series 166*h*). It is interesting that series 130*h*, which underwent a massive invasion of lymphocytes and pyronin cells without showing epithelial destruction, did not attain this percentage. Nor does series 105*h*, which was the only other series without definite signs of destruction, quite reach it.

Tests for regression. To test whether there was any correlation between speed of breakdown of the grafts and the percentage of immature pyronin cells regression tests were

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performed, as described above, on the ranked data. The 8- and 12-day percentages and their differences were tested. None of these yielded a significant result.

If the three series 304*h*, 183*h* and 308*h* which, unlike all the others, showed advanced destruction at 8 days are again omitted from the treatment then significant values are obtained. It will be remembered that the effect of this omission is to give a ranking roughly in the order of amount of destruction during the 8- to 12-day interval. The new value given by the 8- to 12-day increments in the percentage of immature pyronin cells is $t_{17}=2\cdot16$ ($P=0\cdot05$), the slope being negative; i.e. the greatest decrease in the percentage occurred in those grafts showing most destruction during the interval. The new value given by the 8-day percentages was quite significant, $t_{17}=3\cdot18$ ($P<0\cdot01$), and here there would seem to be additional justification for omitting the three series with advanced destruction, since it was shown that the percentage falls after the beginning of graft destruction. The remaining eighteen series are comparable in their early stage of destruction (see table 1). Thus the statement can be made that in the grafts at 8 days (the beginning of breakdown) there is a relationship between the percentage of immature forms among their pyronin cells and the speed of graft breakdown, the higher this percentage the faster the breakdown.

This would be interpreted to mean either that there is a certain maximum percentage which is the same for all grafts but that the 'rapid' grafts reach it sooner; or that the maximum is lower in the slow grafts; or, alternatively, that the rapid grafts have a higher maximum and reach it sooner. Table 2 shows that the three highest percentages recorded were in the 'rapid' half of the ranked graft series; further, the only two increases in the percentage (from 8 to 12 days) occurred in the slow half. This suggests, therefore, that the third of the interpretations given is the most likely.

(2) *Their concentrations*

An estimate of the concentration of immature and mature pyronin cells in the grafts was obtained by applying the percentage figures in table 2 to the pyronin-cell counts in table 1. The estimated numbers are given in table 3.

Immature pyronin cells. There appears to have been no increase or decrease in their average concentration from 8 to 12 days. Although sixteen of the twenty-two series showed decreases a *t* test failed to show a significant difference between the cell numbers for the two days ($t_{21}=-1\cdot652$).

Tests for regression failed to reveal a relationship between speed of breakdown and the estimated concentrations of immature pyronin cells at 8 days, 12 days and the difference (8- to 12-day increment). The average of the two counts was found, on inspection, to be not worth testing. Omission of the three series 308*h*, 183*h* and 304*h* had no effect.

Mature plasma cells. A *t* test gave a highly significant value ($t_{21}=3\cdot684$, P approaching $0\cdot001$) for the difference between the 8- and 12-day numbers. This is strong evidence that there is a true increase in mature plasma cells on the average from 8 to 12 days. The actual averages were thirty-one cells at 8 days and fifty-nine at 12 days.

There was no indication of a relationship between the speed of breakdown of the grafts and these estimated numbers of mature plasma cells for 8, 12, $8+12$ or $12-8$ days. However, if the three series with advanced destruction at 8 days were omitted, a significant

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regression was found ($t_{17}=2\cdot135$, $P=0\cdot05$, positive slope) for the 8- to 12-day increment, thus suggesting a relationship between the amount of graft-tissue destruction from 8 to 12 days and the increase in mature plasma-cell concentration during that time—the greater the one the greater the other.

TABLE 3. CALCULATED NUMBERS OF IMMATURE AND MATURE PYRONIN CELLS IN COUNTED AREA

Graft series ranked in order of speed of breakdown.

	graft series	immature pyronin cells (days)			mature plasma cells (days)		
		8	12	12−8	8	12	12−8
slow	130*h*	97·5	77·0	−20·5	134·5	106·0	−28·5
	105*h*	27·4	16·2	−11·2	21·6	84·8	63·2
	99*h*	12·4	86·0	73·6	18·6	27·0	8·4
average	198*h*	51·6	14·3	−37·3	11·4	36·7	25·3
	166*h*	80·0	38·2	−41·8	58·0	52·8	− 5·2
	132*h*	16·8	68·6	51·8	18·2	29·4	11·2
	61*h*	96·4	15·4	−81·0	15·6	48·6	33·0
	245*h*	18·9	66·9	48·0	4·1	21·1	17·0
	64*h*	87·4	75·1	−12·3	24·6	63·9	39·3
	95*h*	51·8	8·8	−43·0	29·2	46·2	17·0
	144*h*	58·1	47·1	−11·0	24·9	120·9	96·0
	56*h*	16·8	39·1	22·3	7·2	45·9	38·7
	63*h*	66·5	73·0	6·5	23·5	41·0	17·5
	197*h*	93·4	17·4	−76·0	12·6	12·6	0
	97*h*	7·9	40·0	32·1	4·1	51·0	46·9
	243*h*	39·6	17·7	−21·9	20·4	34·3	13·9
	306*h*	55·7	44·0	−11·7	2·3	176·0	173·7
	304*h*	115·2	33·9	−81·3	76·8	120·1	43·3
rapid	302*h*	71·1	21·4	−49·7	7·9	29·6	21·7
	101*h*	79·0	40·8	−38·2	25·0	116·2	91·2
	183*h*	79·8	5·4	−74·4	130·2	24·6	−105·6
	308*h*	48·2	11·2	−37·0	7·8	8·8	1·0
	average	53·0	38·9	−14·1	30·8	58·7	27·9

Correlation tests. An attempt was made to find interrelationships between the different types of cell described above. A positive correlation was found between the lymphocyte and the immature pyronin-cell concentrations in the grafts at 8 days ($r_{20}=0\cdot7225$, $P<0\cdot001$). Certain other relationships were found which could be reasonably explained as results of this one; for example, there was a positive correlation between lymphocytes and total pyronin cells at 8 days (there being no correlation between lymphocytes and mature plasma cells). Apart from these no significant correlations were found, even though every legitimate combination of the various quantities was tested (see appendix).

(3) *Comment*

Series 130*h* which showed such a massive cellular infiltration deserves special comment. Its concentrations of lymphocytes and of immature and mature pyronin cells were among the highest for any graft series at 8 or 12 days. If the serial biopsy assumption (that grafts of a uniform population are at the same stage of evolution at a given time, see Methods) is accepted, the high concentration of these cells presumably existed for at least 4 days, during which no visible graft destruction and very little vascular dilatation occurred. This is strong evidence against the hypothesis that a high concentration of these cell types is sufficient to cause breakdown of the homografts.

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At 8 days after transplantation series 130*h* had a ratio of pyronin cells to lymphocytes which was above the average, at 12 days it had dropped to the average level apparently as a result in part of a decrease in pyronin cells but more especially of an increase in lymphocytes. The decrease in percentage of pyronin cells was highly significant. The percentage of immature forms among the pyronin cells was 42 % at both stages, the mature and immature cells having decreased proportionally.

It is possible to offer an explanation of this pyronin-cell behaviour in series 130*h* solely on the basis of the resorptive theory of plasma-cell function. An antigen-antibody reaction, let it be assumed, began in the graft, and toxic products were formed which caused to be attracted a large invasion of immature pyronin cells. These started to 'mature' by filling up with the toxic substance. Then for some reason the immune reaction stopped and the cells were left with little to resorb, so that they no longer matured and the percentage of immature forms remained constant.

On the basis of the antibody production theory the pyronin-cell changes in 130*h* could be accounted for by assuming that continued invasion of immature forms (or their development from lymphocytes) replaced the ones which became mature, although it is strange that mature cells did not increase in numbers as in most other graft series. But to account for the failure of graft destruction on this basis it would have to be assumed that 130*h* is a special case; for example, that the cells produced a defective antibody, or that some accessory factor (e.g. complement) was lacking, or else that the cells were here mobilized against a microbial infection of the graft tissue or a secretory product of the graft and were, therefore, not the customary invasion. If the more economical assumption is made that this is not a special case it follows that the pyronin cells (and lymphocytes) are not likely to be an important cause of graft destruction; there is even a suggestion here that they might protect the grafts in some way.

(*d*) Second-set homografts

(i) *Introductory*

Twenty-four days after an animal had received a set of six homografts in the ordinary way it was given a second set of three grafts of the same tissue from the same donor. These (second-set grafts) were removed as biopsy specimens after 4, 8 and 12 days' residence. They were found to have undergone an accelerated process of destruction as expected, thus giving further confirmation of the immunity hypotheses of graft destruction (cf. Medawar 1944). The state of epithelial destruction in the eight second-set grafts and their controls is indicated in table 4. The second-set grafts at 8 days showed at least a moderate amount of destruction and the accompanying vascular disruption and lymphocyte pyknosis at this time.

The 4-day-old grafts of the second set were compared with their controls of the first set. All the slides were mixed thoroughly, with their numbers concealed, so that their identity was unknown to the observer. Points were assigned for the degree of vascular reaction (number of vessels, dilatation, engorgement and hemorrhage) and the lymphocyte-pyronin-cell reaction. There was no clear difference between the two sets with regard

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to the vascular reaction, but the cellular reaction was greater in the second set in every case but one (and in this the scores were equal). This cellular invasion in the second sets at 4 days was composed principally of lymphocytes and constituted only a mild reaction; the lymphocytes outnumbered the pyronin cells by at least ten to one. There was no very clear-cut tissue destruction in either set at 4 days.

(ii) *Lymphocytes and pyronin-cell counts*

Table 4 gives the lymphocyte and pyronin-cell counts made on second-set grafts and their controls (the corresponding first-set grafts from the same animal). The percentage of pyronin cells is given and the results of an analysis of the difference in this percentage between the first and second-set grafts.

(1) *Their ratios*

The second-set grafts had a higher percentage of pyronin cells than the corresponding first-set grafts. The statistical significance of this difference is amply demonstrated by the χ^2 tests. The size of the difference is noteworthy. The average percentage difference at 8 days is 25 (from an average of 20·5 % in the first sets to 45·5 % in the second sets) and at 12 days is 31 (first-set average 25·4 %, second-set average 56·4 %). Both at 8 and 12 days the second-set average was double that of the first set. In terms of cells this means that at 8 days there are about four lymphocytes to every pyronin cell in the first-set grafts, while in the second-set grafts there are only about 1·2 lymphocytes to each pyronin cell; at 12 days there would be about 3·0 lymphocytes in the first-sets, and only about 0·8 lymphocyte in the second-sets, to every pyronin cell. The last figure, showing the lymphocytes to be outnumbered by the pyronin cells, is particularly interesting.

Since the second-set grafts were at a more advanced stage of destruction at 8 and 12 days than their controls of the same age, a better comparison would be between the first set at 12 days and the second set at 8 days, these being at roughly the same stage of breakdown (cf. table 4). Here too the second-set grafts were superior, on the average, in percentage of pyronin cells ($t_7 = 3{\cdot}53$, $P < 0{\cdot}01$).

The percentage of pyronin cells in the second-set grafts increased significantly from 8 to 12 days in five of the eight series and decreased in one (see appendix).

(2) *Their concentrations*

Again, with the provisos stated earlier it will be assumed that the cell counts are representative of the actual population densities of their grafts. It follows from the cell counts therefore, that besides their superiority in percentage of pyronin cells, the second-set grafts have actually greater concentrations of these cells. Furthermore, this occurred in the face of a higher total cell count in the first-set grafts in over half of the cases. The one example of a higher pyronin-cell count in a first-set graft (166*h* at 8 days) occurred in a case where the total cell count of the control was higher. The average of the cell counts at 8 days was 80 pyronin cells for the first-sets and 160 for the second-sets; at 12 days it was 99 for the first sets and 254 for the second sets. t tests on the paired data showed that the difference was significant at $P < 0{\cdot}02$ ($t_7 = 3{\cdot}22$) at 8 days, and at $P < 0{\cdot}001$ ($t_7 = 6{\cdot}24$) at 12 days.

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TABLE 4. CELL COUNTS OF LYMPHOCYTES (*ly*) AND PYRONIN CELLS (*py.c.*) IN SECOND-SET GRAFTS AND THEIR CONTROLS (FIRST-SET GRAFTS)

Amount of graft tissue destruction: 0 = none, 1 = slight, 2 = moderate, 3 = much, 4 = complete, 4? means doubtful fragments remaining.

	graft series	first-set grafts				second-set grafts				second-set – first set			graft-tissue destruction	
		py.c.	*ly*	total	% *py.c.*	*py.c.*	*ly*	total	% *py.c.*	% *py.c.*	χ^2	*P*	1st set	2nd set
8 days	99*h*	31	81	112	27·7	94	142	236	39·8	12·1	4·3	< 0·05	0	2
	166*h*	138	201	339	40·7	130	179	309	42·1	1·4	not significant		1	2
	245*h*	23	186	209	11·0	120	246	366	32·8	21·8	25·0	< 0·001	1	3
	243*h*	60	316	376	15·9	112	199	311	36·0	20·1	28·0	< 0·001	1	4?
	306*h*	58	357	415	14·0	247	84	331	74·6	60·6	286·0	< 0·001	1	2
	304*h*	192	608	800	24·0	422	184	606	69·7	45·7	293·0	< 0·001	3	3–4
	302*h*	79	369	448	17·6	95	150	245	38·8	21·2	36·0	< 0·001	0–1	3–4
	308*h*	56	380	436	12·8	60	357	417	14·4	1·6	not significant		4	3
	average	79·6	312·2	392·0	20·5	160·0	192·6	352·6	49·1	28·6	—	—	—	—
12 days	99*h*	113	160	273	41·4	149	220	369	40·4	−1·0	not significant		1	4
	166*h*	91	145	236	38·5	276	98	374	73·8	35·3	75·0	< 0·001	2	3–4
	245*h*	88	586	674	13·1	168	255	423	39·7	26·6	79·0	< 0·001	2	4
	243*h*	52	187	239	21·7	260	256	516	50·4	28·7	42·0	< 0·001	3–4	4
	306*h*	220	156	376	58·5	555	69	624	89·0	30·5	123·0	< 0·001	3–4	3–4
	304*h*	154	475	629	24·5	362	263	625	57·9	33·4	145·0	< 0·001	3–4	4?
	302*h*	51	269	320	15·9	162	147	309	52·4	36·5	92·0	< 0·001	4	4
	308*h*	20	344	364	5·5	100	259	359	27·9	22·4	64·0	< 0·001	4	4
	average	98·6	290·2	388·8	25·4	254·0	195·9	449·9	56·4	31·0	—	—	—	—

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What is especially interesting is that the lymphocytes are not in higher concentration in the second-set grafts but even show some tendency to be lower. *t* tests showed, however, that the difference was not significant at 8, 12 or for 8+12 days.

With regard to total cells (lymphocytes plus pyronin cells) there was no significant difference between first-set and second-set grafts.

On testing the 8-day grafts of the second set with the 12-day members of the first, i.e. the two most comparable stages with regard to graft destruction, the second-set grafts were again superior in concentration of pyronin-cells ($t_7 = 3{\cdot}26$, $P < 0{\cdot}02$). The lymphocyte concentrations did not differ significantly.

Within the second-set series there was a significant increase in pyronin cells from 8 to 12 days ($t_7 = 4{\cdot}42$, $P < 0{\cdot}01$) and in total lymphocytes plus pyronin cells ($t_7 = 2{\cdot}99$, $P = 0{\cdot}02$) but not in lymphocytes alone.

TABLE 5. PERCENTAGE OF IMMATURE FORMS AMONG THE PYRONIN CELLS IN SECOND-SET GRAFTS AND THEIR FIRST-SET CONTROLS; CALCULATED NUMBERS OF IMMATURE AND MATURE PYRONIN CELLS IN COUNTED AREA

	% immature *py.c.*			calculated numbers immature *py.c.*			calculated numbers mature *py.c.*		
graft series	1st set	2nd set	2nd − 1st set	1st set	2nd set	2nd − 1st set	1st set	2nd set	2nd − 1st set
8 days 99*h*	40	46	6	13	43	30	19	51	32
166*h*	58	64	6	80	83	3	58	47	−11
245*h*	82	80	− 2	19	96	77	4	24	20
243*h*	66	58	− 8	40	65	25	21	47	26
306*h*	96	40	−56	56	99	43	2	148	146
304*h*	60	36	−24	115	152	37	77	270	193
302*h*	90	32	−58	71	30	−41	8	65	57
308*h*	86	56	−30	48	34	−14	8	26	14
average	72·3	51·5	−20·8	55·3	75·3	20·0	24·6	84·7	60·1
12 days 99*h*	76	14	−62	86	21	−65	27	128	101
166*h*	42	52	10	38	144	106	53	133	80
245*h*	76	64	−12	67	108	41	21	61	40
243*h*	34	48	14	18	125	107	34	135	101
306*h*	20	18	− 2	44	100	56	176	455	279
304*h*	22	30	8	34	109	75	120	253	133
302*h*	42	30	−12	21	49	28	30	113	83
308*h*	56	46	−10	11	46	35	9	54	45
average	46·0	37·8	− 8·2	39·9	87·7	47·8	58·8	166·5	107·7

(iii) *Immature and mature pyronin-cell counts*

Table 5 gives the percentage of immature forms among the pyronin cells of second-set grafts and their controls of the first set. The numbers of immature and mature forms have been calculated by applying the percentages to the pyronin-cell counts of table 4.

(1) *Their ratios*

At 8 days the percentage of immature forms tends to be higher in the first-set grafts, but a *t* test on the differences of the percentage showed that the mean was not significantly different from zero. At 12 days there was no consistent difference in the percentage between the two sets. However, the combined 8+12 day percentages were significantly higher on the average in the first set (see appendix).

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Within the second-set grafts, there was a drop from 8 to 12 days in the percentage of immature forms (increase in percentage of mature plasma cells) in every series. This would be expected from the first-set results. The average decrease was shown by a t test to be significant ($P<0\cdot01$).

(2) *Their concentrations*

Immature pyronin cells. There is a tendency towards a higher count in the second-set grafts both at 8 and 12 days. This, however, was not significant at 8 days and just failed to reach the 5 % level of significance at 12 days (with $t_7=2\cdot319$, P about 1 in 19). The combined 8+12 day counts were significantly higher on the average in the second sets ($t_7=2\cdot495$, $P<0\cdot05$); analysis of variance showed that this effect was genuine ($F_{1,21}=7\cdot95$, $P<0\cdot05$). There was an average of forty-seven cells in the counted area in the first sets and eighty-one in the second sets.

When again the two graft sets were compared at the stages when their degree of destruction most nearly coincided, viz. 12th day of the first set and 8th day of the second set, it was found that the second sets had a higher concentration of immature pyronin cells on the average ($t_7=2\cdot828$, $P<0\cdot05$), even though their proportion of immature forms (to mature) was not higher.

Finally, the greater concentration of mature plasma cells in the second-set grafts (see below) implies a previously greater concentration of immature forms.

Mature plasma cells. The concentration of these cells as represented by the calculated numbers was significantly higher in the second-sets both at 8 days ($t_7=3\cdot30$, $P<0\cdot02$) and at 12 days ($t_7=9\cdot51$, $P<0\cdot001$). Within the second-set grafts there was a significant increase from 8 to 12 days ($t_7=5\cdot65$, $P<0\cdot001$). The average numbers at 8 days were: first set, 25; second set, 85. And at 12 days: first set, 59; second set, 166. The increase in mature plasma cells from 8 to 12 days was not significantly different in first and second-set grafts.

The 8-day members of the second set had on the average a higher concentration of mature plasma cells than the 12-day members of the first set ($t_7=2\cdot653$, $P<0\cdot05$).

(iv) *Lymphocytes and pyronin cells in relation to speed of graft destruction*

There was no satisfactory method of ranking the second-set graft series in order of speed of breakdown so that they could be treated statistically. However, it is of interest to observe the cell counts of the three series in which, unlike the rest, graft destruction was not quite complete at 12 days. They are series 166*h*, 306*h* and 304*h* (table 4). They had a higher percentage of pyronin cells than the others at both 8 and 12 days. t tests confirmed the significance of this, yielding $t_6=2\cdot976$ ($P<0\cdot05$) at 8 days and $t_6=3\cdot207$ ($P<0\cdot02$) at 12 days. Their cell counts of pyronin cells were higher than in the others: $t_6=3\cdot135$ ($P=0\cdot02$) at 8 days and $t_6=3\cdot478$ ($P<0\cdot02$) at 12 days. Their cell counts of lymphocytes were not significantly different at 8 or at 12 days, but their combined 8+12 days lymphocyte counts were significantly lower than the other five series ($t_{14}=2\cdot44$, $P<0\cdot05$).

Further, it can be seen from table 5 that while their percentage of immature pyronin cells is no different from the others, their estimated counts of mature plasma cells tend to be higher. The values obtained for the latter were $t_6=2\cdot486$ ($P=0\cdot05$) at 8 days, and

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$t_6 = 2{\cdot}695$ ($P < 0{\cdot}05$) at 12 days. Immature pyronin-cell counts were higher in these 'slow' series than in the others at 8 days, $t_6 = 2{\cdot}556$ ($P < 0{\cdot}05$), but not at 12 days nor for 8+12 days. With regard to the increments between 8 and 12 days in cell counts and in percentages there were no differences.

The superiority of the 'slow' second-set graft series in pyronin cells appears to tell against the pyronin cell as a destructive agent. It cannot be argued that the faster series being further advanced in destruction may have undergone a decline in pyronin-cell concentration since there is no evidence of a decline, but rather of an increase between 8 and 12 days. However, the possibility was investigated that pyronin-cell infiltration may have begun later in the slow grafts (and then become more intense than in the others). All the second-set grafts were at a fairly advanced stage of destruction at 8 days. The 4-day grafts, examined and assigned scores according to the 'blindfold' test mentioned above, showed that the slow second-set grafts had more pyronin cells than the others and possibly more lymphocytes; both immature and mature pyronin cells were well represented in the slow series, and neither was in a concentration inferior to that in the faster series.

An interesting consideration is whether the animals whose first-set grafts are slowest to break down are also those whose second-set grafts are slowest, i.e. whether there is a positive correlation between the speed of breakdown in the two sets. There was no indication of this in the present experiments. The three slowest second-set graft series were not the three slowest according to the first-set ranking (which is used in table 4). Indeed, it would be very surprising if they were, since the graft dosages were not controlled. The ranking system is not a very efficient test of speed of breakdown, but there can be little doubt that series 306*h*, for example, has changed from being a comparatively rapid series with respect to the speed of breakdown of its first set to a slow series with regard to its second set.

(v) *Correlation tests*

The data of the second-set experiments were examined in the same manner as the first-set data by means of correlation tests to see if interrelationships could be found between the different cell types. All combinations of the various arrays (again excluding those correlated by an obvious relationship) were tested. First the data for the second-set grafts were tested separately and then in relation to the first-set data. The statistically significant results obtained are indicated below.

(1) *Significant correlations within second-set grafts*

(*a*) There was a positive correlation between the lymphocyte counts in the 8- and 12-day second-set grafts ($r_6 = 0{\cdot}7311$, $P < 0{\cdot}05$). As the average count of lymphocytes in the second-set grafts did not change significantly between 8 and 12 days, this correlation means that the concentration of lymphocytes in the grafts of any one animal is approximately the same at 8 and 12 days.

(*b*) The concentration of mature plasma cells in an animal's grafts at 8 days showed a high degree of correlation (positive) with that in the 12-day grafts of the same animal ($r_6 = 0{\cdot}8807$, $P < 0{\cdot}01$). It was already seen that the numbers at 12 days were significantly higher on the average than those at 8 days. The actual average numbers were 85 at 8 days and 166 at 12 days. It appears, therefore, that the concentration of mature plasma cells

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has approximately doubled in each animal's grafts between 8 and 12 days. In the first-set grafts, while there was no correlation between the 8- and 12-day numbers, the 12-day average was significantly higher—also by a factor of approximately two.

There are two interpretations of the meaning of the correlation in the second-set grafts. The first is that for all grafts the maximum count of mature plasma cells is approximately the same, and it is reached at a definite stage in graft destruction. This is disproved by the fact that the two largest maxima recorded occur in the 'slow' grafts, while at the same time the rapid series show increases from 8 to 12 days, i.e. they have apparently yet to reach their maxima (a violent upsurge and decline in five separate cases would be most unlikely to escape detection). This leaves the other interpretation, viz. that the maximum concentration of mature plasma cells reached varies from one animal to another. It is apparently attained at or near the end of graft destruction (although this may vary too since the one 8- to 12-day decrease in cell count that occurred was in a slow series, 304*h*), and appears to be greatest in those animals in which destruction is more retarded.

(*c*) A positive correlation was found in second-set grafts between the concentration of immature pyronin cells at 8 days and that at 12 days ($r_6 = 0{\cdot}7209$, $P < 0{\cdot}05$). Since the concentration in the 12-day grafts was not significantly different, on the average, from that in the 8-day grafts, this indicates that the concentration is approximately the same in the 8- and 12-day grafts of any animal. This could be interpreted to mean either that these cells remain physically undisturbed in the grafts during the period, i.e. a static interpretation, or that a dynamic equilibrium is maintained over the period, the organism keeping the level of these cells up by replacing ones which are removed or transformed. The first interpretation is made unlikely by the fact that a great increase in mature plasma cells took place.

(*d*) There was a negative correlation between the concentration of lymphocytes at 8 days and the increase of total cells between 8 and 12 days ($r_6 = -0{\cdot}8051$, $P < 0{\cdot}02$). This could not be shown to be a mere mathematical effect. It is interesting that there were no significant correlations between the lymphocytes at 8 days and any of the constituent parts of the total cell increment, nor between total cells at 8 days and their increment.

The only other significant correlations in an exhaustive survey were ones that could be reasonably explained as resulting from those given above. For example, there was a positive correlation between the total pyronin cells at 8 days and at 12 days ($r_6 = 0{\cdot}8508$, $P < 0{\cdot}01$), and between the percentage of immature pyronin cells at 8 and 12 days ($r_6 = 0{\cdot}8157$, $P < 0{\cdot}02$).

(2) *Significant correlations between first-set and second-set grafts*

(*a*) Two interesting relationships will be considered together: there was a positive correlation between the cell counts of mature plasma cells in the first-set grafts at 12 days and those in the second-set grafts at both 8 days ($r_6 = 0{\cdot}8794$, $P < 0{\cdot}01$) and at 12 days ($r_6 = 0{\cdot}9541$, $P < 0{\cdot}001$). The mature plasma-cell counts in the second-set grafts were, on the average, 3·4 times as high as their controls at 8 days and 2·8 times as high at 12 days. The 12-day relationship is shown graphically in figure 1.

From this it would appear that the mature plasma-cell infiltration is characteristic of the animal, at any rate in grafts with moderate or more destruction; it was seen to be

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exceedingly unlikely that all graft series have the same maximal concentration of these cells. There is the possibility, however, that the characteristic infiltration is determined not by the host animal but by the graft tissue itself or partly by it. To test this hypothesis two rabbits (130 and 132) were given their second set of grafts not from their original donors, but from others—in fact, their donors for the second set were exchanged. The resulting infiltration in these 'false' second-set grafts was clearly characteristic of the host animal rather than of the tissue grafted (see figure 1).

(*b*) The concentration of mature plasma cells in the first-set grafts at 12 days are positively correlated with the concentration of immature pyronin cells in the second-set at 8 days ($r_6 = 0{\cdot}7151$, $P = 0{\cdot}05$).

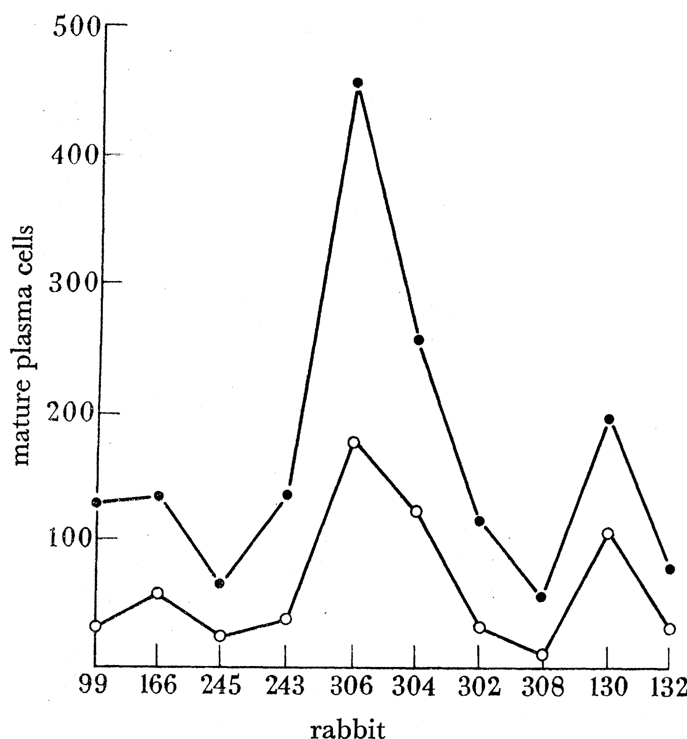

FIGURE 1. The concentration of mature plasma cells in the first and second set of grafts of ten rabbits 12 days after transplantation. ○, first set. ●, second set. In each animal, except 130 and 132, the first and second set came from one donor.

Comment. All other correlations found were related directly or indirectly with the three described above (see appendix). Each of the three involves the mature plasma cells in the first set at 12 days; all the constituent cellular reactions of the 8-day-old second-set grafts are correlated with them, but only the mature plasma-cell reaction of the 12-day-old second sets. It is remarkable that there were no correlations between the 8-day grafts of the first set and either stage of the second set. This may well be a reflexion of the fact that only at 12 days was the first set at a stage of destruction comparable with the second set; the two stages which are most alike in degree of destruction (twelfth day of the first set and eighth day of the second set) show most correlations. It is also noteworthy that the first- and second-set grafts were not correlated in respect of their lymphocytes or immature

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pyronin cells but only the mature plasma cells; however, these other cells may have been correlated in the pre-breakdown period which the observations did not cover—for a correlation of mature cells tends to imply a previous correlation of immature ones.

For the correlation between mature plasma-cell concentrations in the first- and second-set grafts there are two main lines of interpretation. One is that the actual production of these cells is characteristic for each grafted animal, the other that it is merely some property of retaining (or admitting or attracting) the cells at the graft site which determines the characteristic cell concentration. The retaining force might be, for example, the animal's ability to dispose of the cells via the lymphatic vessels or some characteristic which caused the animal's plasma cells to adhere (or be attracted) to the graft site. To account for the higher concentration of mature plasma cells in the second-set grafts on this basis alone it would have to be assumed that the force in question was not only greatly increased in second-set grafts but increased proportionally in each animal. In the case of an attracting force this would probably be not too much to assume. Otherwise it would be far simpler to suppose that increased cell production is involved and that the characteristic cell level in the grafts is obtained either by a characteristic retaining force acting on this or, more simply, by a characteristic cell production in each animal. The characteristic cell production could refer either to the intensity or to the time of beginning of production.

(*e*) Differential counts of immature pyronin cells

Differential counts were made of these cells on fifteen first-set graft series (two of them being the 'slow' series 99*h* and 130*h*) and six second-set graft series. These counts were designed simply to give the approximate proportion of the various cell types in the 8- and 12-day grafts. A few counts were also made on later stage grafts.

Three cell types were recognized: (1) the lymphocyte type of pyronin cell, (2) the reticular cell type (i.e. forms corresponding to the 'transitional cell' and the 'immature plasma cell' of Fagraeus), and (3) forms intermediate between the two foregoing classes and mature plasma cells.

An oil immersion ($\frac{1}{12}$ in.) lens was used and four fields (one per section) counted in each graft, so that a constant area rather than a constant total of cells was counted in each case. The total was small, averaging twenty-two cells per graft in the first sets and thirty per graft in the second sets. However, certain statements could be made with confidence from the results:

(1) Intermediate forms were nearly always the most numerous (in forty-two grafts out of the forty-seven examined), usually outnumbering the other two types added together (in thirty-seven out of forty-seven). The great majority of them were intermediate between the *lymphocytic* forms and mature plasma cells.

(2) Reticular forms were less numerous than lymphocytic forms in all but two or three out of forty-seven grafts.

(3) In the first-set grafts there was an increase in the proportion of intermediate forms as the grafts evolved from 8 to 12 days. This is exactly what might be expected in view of the shift in the pyronin-cell population from immature to mature forms during graft breakdown. The two 'slow' series (99*h* and 130*h*) did not show this change.

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It is interesting that the series 65*h* showed at 20 days a resurgence of lymphocytic forms and a decrease in the proportion of intermediates, coinciding with the suddenly increased proportion of total immature pyronin cells at this stage (table 2). Similar behaviour was shown by series 64*h* at the 16-day stage.

SUMMARY AND GENERAL DISCUSSION

(*a*) Whether the lymphocytes and plasma cells are a cause of graft destruction

(i) *Conditions to be fulfilled*

The main question under investigation was whether the lymphocytes and plasma cells ('pyronin cells') which invade these rabbit-tissue homografts were a cause or merely an effect of graft destruction. If the cells are a cause (agent) of destruction they would be expected to fulfil certain *a priori* conditions: (1) They must be present in the graft before any tissue destruction or degeneration takes place (if they are the sole cause). (2) If the cells are the principal or an important contributory cause, then in their absence a delay in graft destruction might be expected. (3) There should be in general a positive relationship between the speed of breakdown of the grafts and the speed or strength of their invasion by these cells, assuming that if variation in the potency of the cells should exist it is not such as to mask this relationship (principal or important contributory cause). (4) If the cells in exerting their effect must undergo disruption then there should be in general a positive relationship between the amount of destruction of the cells and of the graft tissue (conditions as in (3)).

On the other hand, if the cells are merely an effect of graft destruction, they might be expected to fulfil these requirements: (1) They should invade the graft only after destruction has begun. (2) Complete graft destruction could occur without them. (3) Their concentration in the grafts at a given time might be expected to be, in general, proportional to the intensity of graft-tissue destruction. (4) Their increase in concentration within a definite interval should be in general proportional to the amount of destruction that takes place during that interval. For the last two conditions it must be assumed that the time lag between the two events is reasonably short, e.g. about a day, and also that variations in response are not such as to mask the relationship; they cannot therefore be regarded as rigorous requirements.

With regard to condition (1) in both lists, no definite information was obtained: the cells were present before the graft tissue showed any obvious signs of degeneration, but this is no guarantee that degenerative changes had not begun; vascular dilatation and engorgement were nearly always present when the cells were first observed. One graft (97*h*, see table 1), which showed incipient destruction, had a negligible number of plasma cells.

No graft destruction occurred in the present experiments without the accompanying lymphocyte and plasma-cell reaction. But cancer investigators find that homografts of tumours sometimes regress without the usual cellular infiltration (e.g. Potter & Findley 1935). Skin homografts appear to undergo a very mild and variable round-cell infiltration (Gibson & Medawar 1943; Dempster, Lennox & Boag 1950) and especially second-set

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grafts (Medawar 1944). But since in these grafts the main inflammatory reaction (apparently with much round cell infiltration) occurred in the graft 'bed' (i.e. the adjoining host dermis), the fact that the graft proper was not significantly invaded is weakened as an argument.

(ii) *Evidence from the first-set grafts*

The only relationship found between the speed of breakdown of the present homografts and their cellular infiltration was for the lymphocytes in the grafts at 8 days after transplantation, i.e. just at the beginning of breakdown for the average set of grafts; the faster the speed of breakdown the greater was the lymphocyte concentration at this time ($P<0{\cdot}05$). This result can be interpreted in terms either of cause or effect (a strong vascular inflammation indicated that the immunity reaction had begun). It is not surprising that such a relationship was not found at 12 days, since the destructive reaction would then be on the wane in the more rapidly destroyed grafts. The fact that no relationship to speed of graft destruction was found for the immature or mature plasma cells, or their sum, means that in these experiments they did not fulfil a requirement which would be expected of an important cause of the destruction. This of course depends on the assumption about variation in cell potency from animal to animal, cell potency being defined as the ratio of the destructive power of the cell to the resistance of the graft tissues. It can be remarked on this point that it is more economical to assume that the variation (if any) is randomly distributed rather than to make the two assumptions: (*a*) that the cell potency is greater in those animals whose grafts are destroyed faster and (*b*) that this effect is strong enough to mask a relationship between cell numbers and speed of graft breakdown.

Mature plasma cells alone fulfilled the fourth condition listed above for an effect, in that there was a positive relation ($P=0{\cdot}05$) between the amount of graft tissue destruction occurring between the eighth and twelfth day after transplantation and the increase in concentration of these cells during that time. The fact that during this interval the lymphocytes usually increased in concentration in the slower-destroyed graft sets and decreased in the faster—and that there was a suggestive negative relationship (P about 1 in 19) between lymphocyte increment and amount of graft destruction—does not necessarily mean that they have fulfilled the fourth requirement listed for a cause. The decrease in lymphocytes may have occurred in other ways than by disintegration in the grafts, although some disintegration clearly took place. In this connexion, it should be observed that the immune reaction had begun—to judge from the strong inflammation—before the lymphocyte disintegration occurred.

The percentage of pyronin cells (of the total lymphocytes plus pyronin cells) in the graft was, by the nature of the cell counts, more accurately estimated than the cell concentrations. The percentage was found to be lower in the grafts at 8 days the faster the grafts were going to break down ($P=0{\cdot}02$). This implies that the pyronin cells are much less likely than the lymphocytes to be a cause of graft destruction, since at 8 days destruction was just beginning and the lymphocytes had not yet disintegrated. The positive relationship ($P=0{\cdot}01$) between the increase of this percentage and the amount of graft destruction during the 8- to 12-day interval appeared likely to be the result more of the changes in lymphocyte concentration than in pyronin-cell concentration.

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The percentage of immature forms amongst the pyronin cells at 8 days was in general highest in those grafts which were going to be destroyed most quickly and vice versa ($P<0\cdot01$); and it was found to remain relatively high so long as any graft tissue persisted. This means that the mature plasma cells are much less likely to be a cause of graft destruction than the immature. As far as the immature forms are concerned this behaviour can be interpreted in terms of effect as well as of cause in spite of its correspondence with Fagraeus's evidence that immature plasma cells may be stronger antibody producers than mature ones. (It is to be noted, incidentally, that the present immature forms are predominantly lymphocytic while those of Fagraeus were reticular cell type.) The suggestive evidence ($P=0\cdot05$) that the decrease in the percentage (increase in percentage of mature plasma cells) is positively related to the amount of graft-tissue destruction also favours the hypothesis that the mature plasma cells appear in the graft as an effect of graft-tissue destruction.

The grafts in animal 130 (see table 1) were the slowest to undergo destruction and yet had a more massive infiltration of pyronin cells and lymphocytes than almost any of the others at both 8 and 12 days after transplantation. Unless it is assumed that some abnormal factor was operating here it follows that the invading cells (both lymphocytes and plasma cells) are unlikely to be an important cause of graft destruction. It is in any case strong evidence that their presence in large numbers is not a sufficient cause.

(iii) *Evidence from the second-set grafts*

When a second set of grafts was transplanted from the same donor to the same recipient 24 days after the first set, there was an accelerated destruction of this second set. If the cells are the principal instrument of graft destruction a greater infiltration of them in second-set grafts would be demanded, the assumption being made (and justified below) that even though the cells were to have an increased potency in the second-set grafts it would not be great enough to prevent such a difference. It was found that pyronin cells, and their mature and immature forms taken separately, were in significantly higher concentration in the second-set homografts than in the first-set grafts in the same animals, and not merely when grafts of the same age were compared but also grafts at approximately the same stage of breakdown. Lymphocytes, on the other hand, showed no evidence of being in higher concentration in the second-set grafts during the breakdown period. This behaviour qualifies the pyronin cells to be either cause or effect. It would appear to rule out a significant causal role for the lymphocytes as such, unless it could be shown that they underwent destruction in considerably greater numbers in second-set grafts (of which there was no indication); or that they were actually in higher concentration in second-set grafts just at the beginning of breakdown and then declined more sharply (say by transformation to pyronin cells), which is improbable; or, finally, that they have a much greater potency in second-set grafts, and so much greater that the accelerated second-set breakdown is caused by no more lymphocytes than that of the first set—which seems unlikely. There is, however, the possibility that without achieving a higher concentration in the second-set grafts during the breakdown period the lymphocytes brought about the accelerated destruction simply by a more rapid invasion. As a more rapid invasion probably occurred in second-set grafts this objection holds. It is

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also possible that the mechanism of graft destruction differs for the first- and second-set grafts and that the lymphocytes while playing an important part in the first set might have a smaller part in the second set.

Mature plasma cells were found to be in highest concentration in the second-set grafts, not at the time of active graft destruction (8 days) but rather at the state when destruction was complete or almost so (12 days). This fits better the character of an effect rather than a cause.

There are three other points in the second-set grafting experiments which argue against a causal role for the pyronin cells and especially the mature ones:

(1) Three second-set graft series underwent a slower destruction than the rest in that they were not completely broken down at 12 days. This slow group had a higher concentration of pyronin cells at 4, 8 and 12 days (i.e. from beginning to end of the cellular invasion). Their superiority was especially one of mature plasma cells, but not entirely, since the concentration of immature forms was significantly higher in the slow group at 8 days ($P<0.05$); further, a superiority of mature forms at 8 days implies a superiority of immature forms previously. Lymphocytes tended to be in lower concentration in the slow group. This suggests that if the pyronin cells are causally connected with graft destruction it can only be in a very minor contributory role.

(2) The mature pyronin-cell concentrations of the first-set and second-set grafts in the same animal showed a significant positive correlation. The total pyronin cells did also, mainly through the effect of the mature cells. This means (it was seen) that the mature and total plasma-cell infiltration of these grafts is characteristic of the host animal—at any rate over the period of the observations which was from the time when graft destruction was moderate in extent. That this cellular infiltration is not related to the speed of graft breakdown is suggested by the fact that the ranking of the animals in the order of the speed of breakdown of their grafts was different for first-set and second-set grafts.

(3) In two animals (304 and 308) the speed of breakdown of the first and second set of grafts was practically identical (see table 4). Yet the concentration of pyronin cells in three out of the four comparisons (8 and 12 days for each) is considerably greater in the second sets. This superiority holds for the immature and mature pyronin cells alike, and especially for the proportion of pyronin cells to lymphocytes (since lymphocytes are correspondingly lower in the second sets, the total cells being remarkably even). The case is strengthened by a consideration of animals 306 and 302. The second-set specimen was stained back to back with its corresponding first-set member in every instance.

If the pyronin cells were a cause of graft destruction it would be reasonable to expect a difference in the speed of breakdown corresponding to this difference in concentration of cells. This, incidentally, makes it exceedingly doubtful that the second-set pyronin cells could have a greater 'potency' than those in the first-set grafts. The assumption made above would therefore appear to be justified.

Another feature of this example that is worth observing is the enormous variation in pyronin-cell concentration between two animals whose grafts broke down at approximately the same speed. Such a difference could not possibly be attributed to errors in the counting method. There are many other examples of this in both the first- and second-set grafts.

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Finally, this example and a consideration of the second-set grafts as a whole (e.g. a comparison of the 12-day first-set grafts with the 8-day ones of the second set, these being at approximately the same stage of breakdown) strongly suggests that the increased plasma cell response in second-set grafts is over and above what would be demanded merely by a more intense breakdown of graft tissue and that it has some specific connexion with the immune response.

In the first two of these three examples the lymphocytes are not open to the objections brought against the plasma cells as a cause of graft destruction, but in the third example the tendency for the second-set grafts to have lower lymphocyte concentrations raises the same objection that was brought against the plasma cells. The simplest interpretation of the phenomenon of a high pyronin-cell concentration being accompanied by a low lymphocyte concentration in the same graft is that many lymphocytes have developed into pyronin cells.

There is the possibility that the present classification of the cells is wrong from the functional point of view, and that the active group is composed of the lymphocytes and immature pyronin cells. Such a group would make a better stand than the two separately against some of the above objections to the causal hypothesis, but by no means against all of them, especially if it is held in mind that a high concentration of mature plasma cells implies a previously high concentration of immature forms. To take the total lymphocytes and pyronin cells as the functional group would be to weaken the case through the effect of the mature plasma cells.

(iv) *Conclusion*

It is to be concluded therefore that the present results do not support the hypothesis that the immature or mature plasma cells which invade these rabbit-tissue homografts are a cause, or at any rate a significant cause, of their destruction. In the case of the mature plasma cells the evidence makes the hypothesis particularly unlikely. Indeed, it is possible to make the interpretation that plasma cells protect the grafts.

As far as the invading lymphocytes are concerned most of the evidence can be interpreted in terms either of cause or effect, but there is a residue against the hypothesis that the lymphocyte invasion is a significant cause of breakdown. Furthermore, if, as appears probable (see below), the invading lymphocytes give rise to plasma cells, then it is not likely that the action of the two in the grafts would be greatly different. Finally, the recent work referred to in the introduction makes it improbable that the lymphocyte as such is concerned in antibody production or transport.

As noted in the introduction certain cancer investigators, struck by the close association between immunity to transplanted tumours and the presence of lymphocytes and plasma cells, believed that the cells played an important part in the immunity. Woglom (1929) after a careful weighing of the evidence inclined against the hypothesis. The associations observed by these authors can all be interpreted in terms of effect as well as of cause.

Da Fano (1912) observed (in contrast to the present findings) that while lymphocytes and plasma cells appeared in large numbers around a first inoculation of carcinoma into resistant animals they did not reappear or only in small numbers around the second inoculation. However, in a few cases where immunity was weak the cell reaction did occur at the second inoculation, and this may be the key to the discrepancy, viz. a more powerful

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immunization in Da Fano's experiments. The animals he used were mice and rats, but Mottram & Russ (1917) obtained a strong cellular reaction about 'second-set' tumour grafts in rats. Da Fano obtained the same results again—this time using mice only—when the first (immunizing) graft dose was of mouse embryo skin and the second was either embryo skin or tumour. Whatever the explanation of the discrepancy, Da Fano's findings are evidence against a causal role for the infiltrating cells in homograft destruction, and especially for second-set homografts.

Medawar (1948*b*) placed together in the anterior chamber of the eye skin homografts and mesenchymal tissue from an animal which had reacted specifically against samples of that skin. The mesenchymal tissue was the dermis from the graft beds of the destroyed grafts, and hence presumably contained numerous 'specific' lymphocytes and plasma cells (although the plasma cells at this stage would have been largely mature). The mesenchymal tissue was entirely without effect on the skin in these circumstances, even though the two tissues became firmly knitted together. Medawar (1948*a*) also found that skin grown *in vitro* in contact with a variety of mesenchymal tissues from a specifically immunized animal, including spleen, lymph node and leucocytes, was unaffected; here again the 'immune' tissues became firmly united to the skin, and cells from the lymph node showed a strong tendency to invade the dermis. These findings argue against a causal role for the cells.

In the course of the above work Medawar (1948*b*) demonstrated that the failure of the blood vessels of homografts is not causally connected with graft destruction. Unless, therefore, it is eventually shown that circulating antibodies are involved, the immediate cause of homograft destruction would appear to be none of the candidates so far tested.

(*b*) The role of the lymphocytes and plasma cells in the grafts

If these cells are not a significant cause of homograft destruction, what then is their role? There remain the possibilities that they are a very minor contributory cause or else purely an effect. If they are a minor contributory cause—and the findings with tumours suggest that homograft destruction could take place without them—they would be of little interest for the main problem here. The results obtained in animal 130 suggest that even a massive infiltration of them is without effect on graft breakdown.

This is not necessarily to say that the plasma cells in the graft do not produce antibody, but it means that such a contribution is unlikely to be of any importance to graft destruction. This in itself is a criticism of the antibody production theory of plasma-cell function because the numbers of these cells at the graft site was large. It suggests the possibility that in spite of all the striking association between plasma cells and antibody production listed in the introduction the plasma cells may not necessarily produce the antibody, and, indeed, none of the evidence for that theory is proof against this objection. Keuning & van der Slikke's work seems to establish clearly enough that immature plasma cells can secrete antibody *in vitro*, but the possibility that these cells previously resorbed it in some form remains to be eliminated. There remains, finally, the possibility that plasma cells may have different functions in different situations.

The hypothesis that the cellular infiltration is an effect of homograft breakdown emerges from the present investigation strengthened. The evidence suggests, however, that the

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plasma-cell infiltration is more than a quantitative response to a certain intensity of breakdown of foreign tissue and that, independently of this, it has a specific connexion with the immune response. This is supported by the fact that, although there is a large amount of cell death in the original graft immediately after transplantation, the infiltrating cells do not appear until about 6 days later (first-set grafts). It is further supported by the observation of Sanders (1950) that while frozen-dried killed homografts of nerve fail to immunize a rabbit and to elicit a cellular response, if placed in an animal already immunized by a live nerve homograft from the same donor, they then elicit a round cell infiltration. A hapten would appear to be involved here.

Nevertheless, in the present grafts the plasma-cell reaction was closely related to the actual tissue destruction, and the key to its meaning undoubtedly lies in the question of why a predominance of immature plasma cells is always associated with graft tissue which is on the point of breaking down. On the whole this phenomenon fits better the resorption hypothesis, which would predict it, rather than the antibody production hypothesis by which the maturation might be expected frequently to run ahead of the start of graft-tissue destruction. Since by the latter hypothesis plasma-cell maturation should proceed independently of what is happening to the graft tissue, the above phenomenon would have to be explained by a continuous emergence of immature plasma cells and displacement of mature, whereas on the resorption hypothesis nothing of the sort is required. A similar criticism of the antibody production theory made by Dougherty (1948) is discussed by Marshall & White (1950).

A resorption function for the graft plasma cells is more in keeping with the phagocytic properties of other mesenchymal cells in such tissue reactions; for example, the polymorphonuclear leucocytes whose phagocytic activities depend so greatly on the presence of antibody (opsonin) and whose numbers are known to be greatly increased in immunized animals. This last phenomenon could, by analogy, account for the increased response of plasma cells in second-set homografts. Another advantage of this hypothesis is that the attraction of the cells to the homografts could be explained by a mechanism similar to that for the leucocytes, whereas on a purely antibody-production theory this mechanism could not so justifiably be invoked.

A difficulty for the resorption theory, on the other hand, is to account for the great proliferation of what appear to be plasma cells in the cancerous disease of multiple myeloma. What do the cells resorb here? It would be possible to resolve the difficulties and reconcile the two theories if both functions, resorption and antibody production, were performed by the cells commonly called plasma cells, but each being exercised under different circumstances; the dual origin of the plasma cells might explain this.

A difficulty for the antibody production theory is that the mature plasma cells have, to judge from their staining reaction, a large amount of pentose nucleic acid in their cytoplasm, possibly even more than the immature forms. Yet these are the cells which, according to Fagraeus's results, show a relatively diminished antibody production and which in the present grafts appear most likely to be an effect rather than a cause of graft tissue destruction. For a resorption theory of plasma-cell function the large amount of pentose nucleic acid in mature plasma cells, so far from being a difficulty, would be expected.

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As for the lymphocytes which infiltrate these homografts there is evidence (summarized below) suggesting that they may give rise to plasma cells. The resorption theory would obviously offer a simple explanation of this transformation. In addition, it is certain that the fate of some of the lymphocytes is destruction in the graft. It is also possible that the lymphocytes might aid in the destruction of graft proteins and other products (cf. Moor & Newport 1939) by means of the enzymes they are known to possess (cf. Barnes 1940). The curious 'penetration' of graft cells by lymphocytes (and to a lesser extent by immature plasma cells) awaits a satisfactory interpretation (Andrew & Sosa 1947).

The simplest explanation of the lymphocyte destruction is the anoxia resulting from failure of the graft blood vessels. Lymphocytes are known to be particularly susceptible to oxygen deficiency (Trowell 1952), and their destruction in the grafts was observed for the most part only where vascular failure was evident. This resulted in areas with live lymphocytes and other areas with pyknotic ones in the same graft—since vascular failure was regional—and argues against a hormonal mechanism of lymphocyte destruction.

To summarize, a resorption theory of plasma-cell function could explain the present results, including the behaviour of the lymphocytes, better than an antibody production theory. If these invading cells produce antibody this would appear to have no significant effect on graft destruction.

(*c*) The maturation of plasma cells in these grafts

There can be little doubt from the results that mature (Marschalko) plasma cells develop from immature forms in these grafts. There was a striking increase in the proportion of mature plasma cells among the 'pyronin cells' during the period of graft destruction. This occurred in both first- and second-set grafts and was accompanied by an increase in the actual concentration of the mature plasma cells but not of those tentatively called immature. No mature plasma cells were observed in the blood vessels, and it is generally recognized that they are rare in the blood except in certain pathological conditions. There was no evidence that they came by migration or were produced by division of other mature plasma cells. Added to this is the occurrence of large numbers of forms intermediate between the mature and the very immature forms, and the evidence that the intermediate forms increased in proportion to the very immature during the breakdown period in the first-set grafts.

It is to be concluded therefore that the cells termed for convenience 'immature pyronin cells' are immature plasma cells, or at any rate that the majority are. Now the majority of them were lymphocytic in appearance, the minority being of reticular cell aspect and not present in many grafts. This supports both the lymphocytic and reticulum cell origin of plasma cells, but suggests that the lymphocytic route predominates in these grafts. A dual origin of plasma cells has probably most evidence in its favour (see Dubois-Ferrière 1951).

(*d*) Whether the invading lymphocytes become plasma cells

There remains the question as to whether the immature plasma cells in the grafts arrive by way of the vascular system or whether some or all of them originate from lymphocytes in the graft. The presence of immature plasma cells in the graft blood vessels, including the arterioles, indicates that some of them at least come directly from the blood. However,

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several pieces of evidence suggest that immature plasma cells may arise from lymphocytes in the grafts:

(1) In four experiments one-half of a graft was fixed in Helly's fluid and the other half in formol-sublimate. A considerable number (but by no means all) of the cells that were non-pyronin-staining lymphocytes in the formol-sublimate-fixed half were classifiable as immature pyronin cells in the Helly-fixed half.

(2) Most grafts were found to contain a few lymphocytes which were difficult to classify because of the faint pyronin-staining of their cytoplasm.

(3) There was a highly significant positive correlation between the cell counts of lymphocytes and immature plasma cells in the first-set grafts at 8 days (although not at 12 days, or in the second-set grafts); no such correlation was found between lymphocytes and mature plasma cells.

(4) The fact that the second-set grafts had a much higher plasma-cell concentration than the first set, and at the same time tended to have a lower concentration of lymphocytes—so that the total cell concentration of the two was the same—is most simply explained on the basis of transformation of lymphocytes to plasma cells.

(5) The intimacy of association of the lymphocytes and plasma cells observed in these grafts and elsewhere by others (cf. Dubois-Ferrière 1951) in itself suggests that they together form a series and that the transformation may occur in the grafts.

(6) There was in many cases a large increase in the proportion of plasma cells to lymphocytes between the two stages at which cell counts were made (the eighth and twelfth day after transplantation). This increase occurred in five out of the eight second-set graft series and in certain of the first sets which underwent most destruction during the interval. In either case the actual concentration of plasma cells increased significantly without a significant change in the lymphocytes. This is no evidence in itself for the transformation *in situ*, but it is a condition that would be expected if such occurred.

(7) The fact that the proportion of plasma cells to lymphocytes was very low in the second-set grafts at the start of infiltration (4 days) and thereafter increased greatly is again a condition to be expected if the transformation occurred.

It is to be concluded therefore that while some of the immature plasma cells arrive as such at the graft site via the vascular system, it is likely that others may originate at the site from lymphocytes. Lymphocytes can apparently become transformed into histiocytes after a short sojourn in the field of acute inflammation (Rebuck 1950). It would not be too much to suppose that with a different stimulus they could become transformed into plasma cells.

(*e*) The percentage of immature plasma cells as an index of the host reaction

The percentage of immature forms among the graft plasma cells appears to indicate the state of the breakdown reaction. Thus it is high at the beginning of graft-tissue destruction and thereafter declines sharply. But in grafts where portions of the epithelium persists for a long time the percentage of these cells remains relatively high. None of the present grafts broke down without their percentage of immature plasma cells reaching a predominance. The minimum observed was 58 %, and the only two series which

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showed no definite destruction during the experiments failed to attain this percentage, one of them being the massively infiltrated series 130*h*. The percentage of immature plasma cells might therefore be of use as an index of the progress of the host reaction against the graft. The fact that the plasma cell response may be a characteristic of the individual would not necessarily affect it, but a low concentration of the cells would probably make it less reliable.

I would particularly like to express my thanks to Professor P. B. Medawar, F.R.S., and Dr F. K. Sanders, for help and encouragement throughout this work; to Dr M. R. Sampford for advice on statistical procedure; to Professor A. C. Hardy, F.R.S., for facilities provided in the Department of Zoology and Comparative Anatomy of Oxford University; and to the Beit Medical Fellowship for financial support.

APPENDIX: ADDITIONAL STATISTICS AND COMMENTS

(1) *First-set grafts*

The graft ranking. Evidence that the ranking of the graft series according to speed of their breakdown is unlikely to have been biased, or at any rate biased positively, to any significant degree by the cell populations of the grafts is provided by the fact that the 12-day grafts (on which the ranking was based almost entirely) yielded no significant regression, while the 8-day grafts did. Sections stained with haematoxylin and eosin were used to decide the ranking, so that the pyronin cells were indistinguishable from the lymphocytes.

Analysis of variance. As an alternative to the regression method of testing for relationships between the speed of graft breakdown, or the amount of graft destruction, and the cell content of the grafts, analysis of variance was performed on the percentages and cell counts as they occurred in the three groups of grafts (slow, average and rapid) defined in the text.

With regard to the percentage of pyronin cells and the cell counts of pyronin cells and lymphocytes, no significant results were obtained. It is perhaps worth mentioning, however, that if series 130*h*, which showed such massive concentrations of lymphocytes and pyronin cells and no definite tissue destruction, was omitted from the statistical treatment then significant values were given by lymphocytes at 8 days ($P<0{\cdot}001$) and 8+12 days (P almost 0·001), total cells at 8 days ($P<0{\cdot}001$) and 8+12 days ($P<0{\cdot}01$). The mean of the three groups in every instance followed the trend indicated by the regression time.

Omitting series 130*h* also improved the results given by the regression method: lymphocytes at 8 days and total cells at 8 days yielded a value of $P<0{\cdot}01$, and two other cell arrays gave regression coefficients differing significantly from zero, viz. the sum of the lymphocytes (8+12 days) with $P<0{\cdot}02$, and the sum of the total cells ($P<0{\cdot}02$), both having positive slopes. However, as there appears to be no sound reason for omitting 130*h* these results cannot be considered valid.

The correlation between speed of breakdown of the graft sets and their percentage of immature pyronin cells at 8 days found by the regression method was confirmed by analysis of variance. The value obtained was $F_{2,19}=3{\cdot}64$, $P=0{\cdot}05$, the means and their standard errors for the slow, average and rapid groups being respectively $42{\cdot}67\pm5{\cdot}70$,

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$59{\cdot}13 \pm 2{\cdot}55$ and $59{\cdot}60 \pm 4{\cdot}94$. If the three series omitted from the regression test were omitted here no change in the P value would result. The 8-day and $12-8$ day figures did not yield significant results.

The analysis of variance method applied to the cell counts of immature and mature plasma cells yielded no significant results.

Correlation tests. There were twenty-eight variates to be compared, not all of which were independent: lymphocytes, pyronin cells, total cells, percentage of pyronin cells, percentage of immature pyronin cells and calculated numbers of immature and mature pyronin cells, each for 8, 12, $8+12$ and $12-8$ days.

The significant results are indicated in the text. Some of the negative results are also interesting. The failure to find a correlation between lymphocytes and immature pyronin cells in the grafts at 12 days, though present at 8 days, argues against the interpretation that the immature pyronin cells are merely staining artifacts, i.e. that a certain proportion of the lymphocytes happen for some accidental reason, such as nearness to the surface of the section, to stain with pyronin. Results from the second-set experiments likewise showed no correlation between lymphocytes and immature pyronin cells in the same graft.

The fact that no correlation was found between the concentration of mature plasma cells at 12 days and that of immature pyronin cells at 8 days does not argue against the transformation of the latter into the former; the period of transformation may be considerably less (or more) than 4 days, and other factors could prevent such a correlation. In the same way the absence of a correlation between lymphocytes at 8 days and immature pyronin cells at 12 days would not be evidence against the transformation of the former into the latter within the grafts.

t test. A t test showed that the average percentage of pyronin cells did not change significantly between 8 and 12 days.

(2) *Second-set grafts*

The percentage of pyronin cells. This percentage increased from 8 to 12 days in seven of the eight second-set graft series. Five of these increases were found by χ^2 tests to be significant and highly so ($P=0{\cdot}001$ or less). The one decrease that occurred (in series 304h) was also significant at $P<0{\cdot}001$. The two series without significant changes were 99h and 245h. When a t test was applied the average increase was seen not to differ significantly from zero.

The second-set grafts had a higher percentage of pyronin cells on the average than the corresponding first-set grafts at 8 days ($t=3{\cdot}616$, $P<0{\cdot}01$) and at 12 days ($t_7=7{\cdot}358$, $P<0{\cdot}001$).

The percentage of immature pyronin cells. This percentage tends to be lower in the second-set grafts than in their controls of the first set. Thus when χ^2 tests were applied to the pairs the only significant results showed a higher percentage in the first sets: 302h ($\chi^2=35$, $P<0{\cdot}001$), 304h ($\chi^2=5{\cdot}27$, $P<0{\cdot}05$), 306h ($\chi^2=35{\cdot}5$, $P<0{\cdot}001$), 308h ($\chi^2=10{\cdot}4$, P almost $0{\cdot}001$) all at 8 days; at 12 days only 99h ($\chi^2=38{\cdot}3$, $P<0{\cdot}001$). While t tests showed no significant difference between the two sets on the eighth or on the twelfth day, the combined $8+12$ day percentages were significantly higher on the average in the first set

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($t_7 = 2{\cdot}848$, P almost 0·02), and analysis of variance showed that the effect was genuine, yielding $F_{1,21} = 6{\cdot}08$, $P < 0{\cdot}05$. A lower percentage would be expected in the second sets because their destruction was more advanced. When the two stages which were most comparable in degree of destruction, viz. the 12-day stage of the first set and the 8-day stage of the second, were compared there was not a significant difference.

The concentration of immature pyronin cells. Within the second-set grafts there was no significant difference between the average numbers of immature pyronin cells at 8 and at 12 days; this was also the case for the first-set grafts. There was no significant difference between first- and second-set grafts in their increments of immature pyronin cells between 8 and 12 days.

Correlations within second-set grafts. A point of interest is that there were no correlations within the 8-day grafts of the second-set series, nor within the 12-day members. This is in contrast to the first-set grafts where at 8 days there were several positive correlations, notably one between lymphocytes and immature pyronin cells. The difference between the two sets may depend on the fact that the second-set grafts at 8 days were in a much more advanced state of destruction than the first set, and that at an earlier stage (say 6 days) the second set might correspond with the first.

With regard to the positive correlation between the lymphocyte counts in the 8- and 12-day second-set grafts, in the first-set grafts there was no such correlation between 8 and 12 days. It is interesting, however, that the seven most rapid of the first-sets did appear, by inspection, to show a positive correlation between their 8- and 12-day lymphocyte counts. The rest tended towards a negative correlation. The second-set grafts would therefore appear to correspond with the more rapid of the first sets.

The reason why no correlations were found between the 8- and 12-day numbers of the first-set grafts such as were found in the second sets lies at least partly in the comparative lack of homogeneity of the first-set grafts with regard to the state of destruction at a given time, some of them being in the earliest stages while others were in the last stages.

A negative correlation was found in the second-set grafts between lymphocytes at 8 days and mature plasma cells at 12 days ($r_6 = -0{\cdot}8566$, $P < 0{\cdot}01$), that is to say, the more lymphocytes found in the second-set grafts of an animal at 8 days the fewer will be the mature plasma cells at 12 days. This suggests some sort of 'antagonism' between lymphocytes and mature plasma cells—which would not be surprising, since lymphocytes predominate in the early infiltration (4 days) and mature plasma cells in the late. However, no significant correlation between these two cell types was found within the 8- or the 12-day stages of the second-set grafts, or within either stage (or between them) of the first set. And while the 'slow' second-set grafts had most plasma cells and possibly least lymphocytes, no significant relationship was found between the ratios of the two cell types and the speed of graft breakdown when the full first-set data were tested. The most likely explanation of this correlation is that both variates are correlated with a third; when the effect of mature plasma cells at 12 days in the first set—to which both are correlated—was eliminated by partial correlation analysis, the resulting value was shown to be not significant.

Correlations between first-set and second-set grafts. A negative correlation was found between the number of mature plasma cells in the animal's first set of grafts at 12 days and the

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number of lymphocytes in its second set at 8 days ($r_6 = -0{\cdot}7877$, $P = 0{\cdot}02$). This is another example of the lymphocyte-mature plasma-cell 'antagonism' noted above. When, by partial correlation analysis, the effect of the mature plasma cells in the second set at 8 days (with which the two arrays showed some correlation) was eliminated—the resulting value for r could be shown to be not significant.

As examples of correlations related to the three given in the text, there were positive correlations between mature plasma cells at 12 days in the first-set grafts and the percentage of pyronin cells in the second set at 8 days ($r_6 = 0{\cdot}9866$, $P < 0{\cdot}001$), and between percentage of pyronin cells in the first set at 12 days and the lymphocyte concentration in the second set at 8 days ($r_6 = 0{\cdot}9120$, $P < 0{\cdot}001$). There was also a positive correlation between the total pyronin cells at 12 days in the first- and second-set grafts ($r_6 = 0{\cdot}7974$, $P < 0{\cdot}02$), and at 8+12 days ($r_6 = 0{\cdot}9096$, $P < 0{\cdot}01$).

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