SKIN TRANSPLANTATION IN THE FOETAL LAMB

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Summary

Skin autografts and homografts have been performed in foetal lambs between the ages of 80 and 117 days. By means of histological examination following grafting, visual and histological examinations after birth, and by the use of second-set homografts, it was established that homografts are actively rejected by the foetus. This rejection gave every indication of belonging to the general class of activity acquired immune responses and in all respects confirmed at the foetal stage the observations of Medewar (1944, 1945) on young and adult animals.

Homografts in which the ewe was used as donor were also rejected by the foetus, indicating that the reaction was of foetal and not maternal origin.

The experiments established that the foetal lamb is capable of making an immune response to the presence of foreign tissue. This finding is at variance with current concepts of the immunological behaviour of foetuses.

I. INTRODUCTION

It seems probable that skin transplantation techniques will be useful in obtaining a clearer understanding of some of the basic biological phenomena concerned in wool-fibre production. For numerous purposes we are interested in the possibility of establishing skin homografts, i.e. grafts from one animal to another, between individuals of normal genetic diversity. However, it has been clearly shown that such transplantations are not possible in normally diverse populations of human beings (Gibson and Medewar 1943), rabbits (Medewar 1944, 1945), cattle (Anderson *et al.* 1951), and guinea pigs and mice (Medewar, personal communication 1952).

The non-acceptance of such skin homografts has been shown unequivocally (Medewar 1944, 1945) to be due to an immunological mechanism of the general type of actively acquired immune reactions. However, evidence has been presented from immunological studies with chick embryos (Grasset 1929; Burnet 1941), which indicates that the developing foetus is incapable of producing antibodies and might therefore be expected to accept foreign tissues.

Further, it has been shown (Ferguson, Stormont, and Irwin 1942; Stormont and Cumley 1943) that, despite the large number of red-cell antigens known to exist in cattle, there is an abnormally high proportion of identical red-cell antigens in dizygotic twins, whereas identity is rare in full sibs. This is almost certainly a result of the synchorial nature of the foetal membranes

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in a high proportion of such twins, one effect of which is the sterile "freemartin" female born twin to a bull. As a result of the synchorial arrangement, vascular anastomoses are established leading to the interchange of red cells and red-cell precursors between the twins during prenatal life (Owen 1945; Owen, Davis, and Morgan 1946). Such red-cell precursors act as a homograft, giving rise to erythrocytes antigenically "foreign" to the host for the remainder of prenatal life and at least for the youthful part of postnatal life. Such dizygotic twins are therefore genetic chimaeras and contain cellular material genetically different from that acquired at fertilization. It has also been shown (Anderson *et al.* 1951) that skin grafts may be exchanged between the members of dizygotic cattle twins with a high degree of success. This is not so for full sibs born at different parturitions. It must be concluded from these observations that the dizygotic cattle twins are no longer able to recognize the "non-self" (Burnet and Fenner 1949) components of the cellular antigens of their twin sib.

It might be expected therefore that skin homografts transplanted during foetal life would have a reasonable chance of being accepted and of continuing normal development. Additionally, if the grafts were accepted at the foetal stage, then the indications are that further grafts would be accepted subsequently from the same donor during postnatal life.

The object of these observations was to determine whether or not skin homografts would succeed at the foetal stage in sheep.

II. Experimental Plan

(*a*) *General*

Sixteen pregnant merino ewes, with known conception dates, were used. With the exception of one ewe, 20G210, which was mated to a Border Leicester ram, all ewes were mated to a single Merino ram. The genetic composition of the population was therefore considerably more uniform than that of the rabbits used by Medewar (1944).

In the interests of economy of sheep it was planned to operate on two cases at a time in order to make reciprocal homografts (type *C*, Billingham and Medewar 1951) between the foetuses. Concurrently, autografts were performed on each foetus (with one exception) to act as controls for the homografts. On two occasions, one member of the pair was found to be non-pregnant when the abdominal cavity was opened. In each of these cases, the homografts were made from the abdominal wall of the ewe to the foetus. If a homograft reaction was to be encountered, these two cases provided the opportunity to determine whether it was primarily of foetal or maternal origin. Current concepts of placental transmission in the ewe make it extremely unlikely that the dam could be responsible for any such reaction, but on general grounds it seemed wise to explore this possibility.

Table 1 shows the general plan of the observations. These were planned primarily to determine whether skin homografts would succeed at the foetal stage. At the same time, it seemed desirable to obtain information on (1) the effect of age generally with donor and recipient of approximately the same age,

and (2) the effect of differences in age between donor and recipient on the success or otherwise of the transplants (Table 1). Previous observations (unpublished data) by us indicated that it was possible to make a surgical penetra-

TABLE 1
GENERAL PLAN OF OBSERVATIONS

Pair No.	Ewe No.	Operation Date	Days Pregnant	Age of Grafts at Biopsy*		Remarks
				A	*H*	
1	7F421	29.iv.52	80	n.r.	63	Homograft from ewe. Lamb born 9.vii.52. Lamb No. 38S8
	646		—	—	—	Not pregnant
2	27H516	30.iv.52	106	—	—	Aborted 11.v.52
	12G14		110	21	21	P.M. 21.v.52 for histological examination
3	13F163	1.v.52	106	81	54	Lambed 11.vi.52: lamb no. 38S1; repeat grafted postnatal
	16F323		107	47	47	Lambed 12.vi.52; lamb no. 38S2: repeat grafted postnatal
4	12H75	2.v.52	97	80	n.r.	Lambed 22.vi.52: lamb no. 38S3; repeat grafted postnatal
	16F371		98	n.r.	53	Lambed 21.vi.52: lamb nos. 38S4 (grafted), 38S5: repeat grafted postnatal
5	20G210	3.v.52	90	207	207	Homograft from ewe: lambed 26.vi.52: lamb no. 38S7
	13G342		—			Not pregnant
6	10F34	5.v.52	98	176	52	Lambed 25.vi.52: lamb no. 38S6
	20J10		91			Ewe died 11.v.52 from herniation of section of small intestine between sutures in abdominal wall
7	32J245	6.v.52	116	9	9	P.M. 15.v.52 for histological examination
	32J242		116	15	15	P.M. 21.v.52 for histological examination
8	16G136	8.v.52	117	22	22	P.M. 30.v.52 for histological examination
	12H69		115	15	15	P.M. 23.v.52 for histological examination
9	16G101	8.v.52	85	n.r.	n.r.	Lambed 10.vii.52: lamb no. 38S9
	16F365		117	33	33	Lambed 10.vi.52: lamb died at birth

* *A* = autograft; *H* = homograft; n.r. = not removed.

tion of the gravid uterus in ewes between the 110th and 143rd days of gestation (normal period 149 days) and to have normal lambs delivered at term. While it was considered desirable to extend the observations into earlier stages of pregnancy in the current series, we were unaware of the surgical risks involved and decided not to attempt transplantation before the 80th day.

In order to obtain information on the histological changes associated with the grafts, five ewes were killed at post-operative intervals of 9, 15 (2 ewes), 21, and 22 days. The first autopsy in this series was later than that used by Medewar (1944, 1945) in adult rabbits (4 days) but the decision to commence at 9 days was based on the hypothesis that if a homograft reaction was to be encountered it would probably be of a lower order than that noted by Medewar. We now know this to be incorrect and feel sure that a greater amount of information would have been gained had autopsy examinations commenced much earlier.

The remaining ewes were allowed to proceed to term. Soon after parturition each lamb was closely inspected, the operation site located, and observations recorded on the condition of the grafts. Some were removed at this time and others allowed to remain for further observations at later periods. In one lamb (38S7 from ewe 20G210) the grafts were not located until the lamb was killed at 5 months of age and the entire skin thoroughly examined.

Where possible, reciprocal homografts were repeated between available lambs during postnatal life. The details of these transplants are presented later.

(*b*) *Surgical Procedure*

Anaesthesia was induced and maintained throughout the operations with either "Kemithal" (I.C.I.) or "Nembutal" (Abbott) administered intravenously. All incisions were made with the use of a diathermy unit.

The abdominal wall was prepared with 1 per cent. aqueous "Cetavlon" (I.C.I.) after close clipping and opened with a 4-5 in. paramedian incision, commencing about 2 in. in front of the mammary gland. The uterus was readily located and brought to the site of the abdominal incision, but it was not exteriorized. By gentle manipulation and the use of sterilized packing towels (soaked in sterile isotonic saline), it was possible to bring the foetus to lie just inside that area of the uterine wall exposed by the abdominal incision. The uterus was incised for a distance of about 2 in. in a suitable intercotyledonary area, care being taken to avoid large blood vessels. The ends of the incision were identified with haemostats. The membranes, which tended to bulge through the uterine incision, were picked up with haemostats about 1½ in. apart and opened with scissors. In the sheep, the allantois does not separate the amnion and chorion completely, but usually only for about one-half the transverse circumference of the amnion. It is thus possible to enter the amniotic cavity without penetration of the allantois, provided that a chorio-amniotic and not a chorio-allantoic region is presented at the uterine incision. By making the uterine incision along the greater curvature, a chorio-amniotic area was usually presented in this series. On two occasions the margin of the allantois passed across the field but it was possible to avoid penetration of it.

Because of the delicate nature of the membranes, the edges were clamped to the margins of the uterine incision. On opening the membranes, some loss of amniotic fluid occurred. By appropriate manipulation of the foetus prior to opening the uterus it was found possible to keep this loss to less than 100 ml in most cases, and usually less than 50 ml.

An area of skin of the foetus was grasped with forceps and withdrawn above the level of the amniotic fluid while transplantation was carried out. Previous preliminary observations suggested that implantation of the grafts on the surface ("fitted" grafts) might not be successful in the foetus because of the difficulty in applying the necessary vertical pressure (Billingham and Medewar 1951). The great majority of grafts were therefore transplanted subcutaneously by the method shown in Figure 1. Two full dermal thickness specimens of skin, each about 10 by 3 mm, were removed and placed in a small blood clot at some convenient point in the operation field, care being taken to prevent dehydration of the specimen. Two small incisions (5 mm) were made about 1 in. apart and a small subcutaneous pocket effected with a probe. Into one of these was placed the autograft in the manner illustrated, and into the other the homograft from the pair mate. In the early cases, silk suture material was used to effect anchorage of the graft and to close the skin incision, but in later cases black braided nylon was adopted with a view to facilitating subsequent location. Prior to closing the membranes the area was mapped for record purposes and 300,000 units of penicillin in saline were placed in the amniotic cavity.

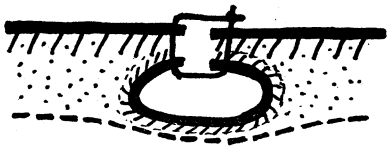

Fig. 1.—Sectional view of the method of making subcutaneous transplants.

Where fitted grafts were carried out, these were about 7 mm square and were anchored by means of a mattress suture on each of the four sides and a single suture in each corner. Contrary to expectation, they proved to be quite satisfactory as judged from subsequent observations. When the ewe was used as a homograft donor, the graft specimen was placed in aqueous penicillin (30,000 units per ml) for 5-10 min prior to grafting.

Owing to their extremely delicate nature, it was impossible to suture the membranes and the incision was therefore closed by gathering up the edges and tying off the incised area. As is indicated later, this did not prove to be an affective method of obtaining permanent closure.

Finally, the uterus was closed with a single layer of interrupted gut sutures, the abdominal wall with one or two series of gut sutures (mattress pattern), and the skin with a single layer of nylon sutures (mattress pattern). No dressings of any kind were applied to the abdominal incision, but each ewe received 10 mg progesterone and 300,000 units procaine penicillin intramuscularly immediately after the operation. Rectal temperatures were taken daily and any sheep showing a rise above 103.5°F was treated with procaine penicillin until after return to normal.

(c) *Histological Procedures*

All sections were fixed in formol-saline for a minimum period of 24 hr. Routine methods of dehydration of specimens were adopted and all specimens embedded in paraffin for section cutting. The blocks were cut vertically and serially, alternating 15 sections at 8 μ and 15 sections at 15 μ until exhaustion. Every fourth section only was mounted. Masson's tri-chromic stain was adopted as a standard for the majority of slides, but some slides in each case were also stained with Anderson's haematoxylin and eosin, phosphotungstic acid haematoxylin, and Giemsa.

III. Results

(a) *Surgical*

We are unaware of any previous attempts to make a surgical interference with the foetus in a recovery type operation as opposed to one in which the animal is killed. In fact, it seems to be commonly believed that such procedures are well-nigh impossible. This is erroneous if applied to the ewe in the last half of pregnancy. Of the 16 ewes operated on, five of which were destroyed from 9 to 22 days later, one ewe died 6 days and one aborted 11 days after operation. The remainder carried their lambs to full term. In the previous series of observations (unpublished data) in which the object was to develop the surgical technique, one of the four ewes operated on aborted 21 days later whereas the remaining three carried through to term.

Dissection of the uterus, in the five ewes on which autopsies were performed, revealed that the purse string suture applied to the membranes during the operation had become detached, leaving a hole through the chorio-amnion some 4 in. in diameter. As a result most of the amniotic fluid had escaped and only a small quantity of mucoid material remained in the amnion. Additionally, six of the nine ewes allowed to proceed to term developed herniae at the operation site, presumably due to the relatively avascular nature of the abdominal wall in this region and the increasing pressure on the ventral wall with advancing pregnancy. It seems possible that these factors, either singly or in combination, may have been responsible for the fact that five of the nine ewes had to be assisted at lambing. In all cases assisted, the lamb was normally presented but the genital tract appeared dry.

(b) *Grafting*

By the nature of the experiment, it was difficult to place more than one homograft and one autograft on each animal during the prenatal grafting. Only one graft of each type from each animal was therefore available for subsequent visual and histological examination. The results of the grafts have been assessed on the basis of visual and histological examinations, and are presented here in four sections:

(i) Histological examination of grafts removed from the foetuses at various post-operative intervals;

(ii) Visual examination of the operation field of the lambs at birth and during postnatal life;

(iii) Histological examination of prenatal grafts removed in postnatal life; and

(iv) Histological examination of second-set postnatal homografts.

(i) *Post-operative Histological Examination*

The sequence of histological events following skin autografts and homografts in rabbits has been described in detail (Medewar 1944) at 4-day intervals to the 24th day. The essential differences in the reaction to the two types of graft are seen in the activity and fate of the graft epithelium, the vascular reaction, and the cellular activity within the graft dermis. In *autografts* the epithelium undergoes vertical thickening and differentiation and then returns to normal thickness and morphology; the follicles regress, the hair is shed, and a new population of follicles develops. The vascular and cellular reactions of the dermis are of a relatively mild nature similar to that associated with primary healing and a mild inflammatory reaction. In *homografts*, primary healing takes place, but is followed by a series of events which probably result in the removal of the entire graft. The epithelium is undermined by a process of "blistering" and is finally shed; there is marked invasion of the dermis by capillaries which eventually stagnate and rupture, and finally, in addition to considerable fibroblast activity, there is a massive cellular invasion of the graft by lymphocytes and a few monocytes.

The current series of observations, which are based on five individuals at three post-operative stages, does not differ in any essential feature from that described by Medewar (1944) and strongly indicates the incompatibility of homografts in the foetal lamb at 110-117 days of age.

Autografts.—The single case at 9 days showed considerable increase in the vertical thickness of the epidermis, but little lateral outgrowth. There was a moderate increase in the number of dermal capillaries and a moderate oedema of the dermal collagen with new collagen being laid down: cellular elements of the graft dermis comprised mostly fibroblasts with a few lymphocytes, monocytes, and odd polymorphs in the dermal papillae (Plate 1, Fig. 1; Plate 2, Fig. 3). Generally speaking, the inflammatory reaction was negligible. Degeneration of the inner root sheath of the follicles was evident. The two cases at 15 days showed persisting thickness of the graft epithelium and its lateral outgrowth, which had progressed to the point of coalescence with the host epithelium. The follicles and their accessory structures had degenerated, leaving a mild inflammatory reaction around those fibres remaining embedded in the graft dermis. A few tortuous capillaries persisted and the graft dermis still showed some increase in the cellular population together with persisting oedema and collagen replacement. There was, however, an absence of any marked inflammatory reaction. In the 21- and 22-day cases, one had formed a complete subcutaneous cyst (Plate 3, Fig. 9), while in the other the epithelium of the graft and host had established continuity. Capillary vessels were still present, although a little reduced as compared with the 15-day cases; the cellular population of the graft dermis had practically returned to normal. Active deposition of new collagen was taking place.

Homografts.—The histological picture in the single case at 9 days was typical of the classical homograft reaction in rabbits at 8 days as described by Medewar (1944) for violent breakdown. The majority of the cells of the epidermis had lost all morphological detail and, being strongly acidophilic, stained with the light green component of Masson's stain. A few very small groups of homograft epithelial cells still showed a basophilic cytoplasm and could not be classed as dead with certainty. Additionally the epidermis had become separated from the dermis by "blistering" (Plate 1, Fig. 2). As compared with the autograft of the same animal the vascular and cellular response was strikingly greater (cf. Plate 1, Figs. 1 and 2). There was an extreme proliferation of capillaries in the dermis which had proceeded to the stage of stagnation with rupture of the endothelial lining and escape of red cells to the surrounding tissue. Not only were these vessels present in vastly greater numbers than in the autograft, but their diameter was from two to five times as great. There was a massive invasion of the dermis by lymphocytes, together with many fibroblasts. All the follicular elements were degenerate and the host epithelium was commencing to undermine the entire graft. In this 9-day case the grafts were located on the left shoulder. The prescapular lymph node draining the region was markedly enlarged, being nearly twice the diameter of the right prescapular node, and on being cut showed the characteristic glistening associated with glands draining regions in which an inflammatory reaction is taking place. Histologically the gland was apparently normal, save for considerable oedema and some congestion.

Both the 15-day cases showed a moderately violent homograft reaction, only slightly reduced from that seen in the 9-day specimen. In one case the native (host) epithelium had almost completely undermined the entire graft, whereas in the other the host epithelium had bitten deeply into the graft at about the level of the base of the follicles. The grafts showed typical dermo-epidermal blistering and disengagement. It is possible that a few homograft epithelial cells were still surviving in both cases, but they showed pronounced pathological change (Plate 2, Fig. 5). The graft dermis showed heavy lymphocyte infiltration and vascular engorgement with breakdown of the capillary endothelium and haemorrhage.

In the 21- and 22-day cases there was considerable reduction of the activity seen in the earlier homografts. The host epithelium had penetrated under the homograft from all sides, with the result that the graft was in the last stages of being shelled out (Plate 2, Fig. 6; Plate 3, Figs. 7 and 8). The fragments of necrotic graft epithelium were, at this stage, so acidophilic as to stain bright green with the tri-chromic stain. There was no trace of viable graft epithelium and both cases showed long-standing degeneration of the graft.

That the homografts had "taken" initially in all cases and become vascularized was indicated by the fact that the dermis was always extensively invaded by native mesenchyme cells, and blood capillaries which were seen in this series in varying degrees of stagnation and breakdown according to age.

(ii) *Visual Examination at Birth*

All grafts were examined, after close clipping, within 24 hr of birth. In most cases the site was readily located with the aid of the map constructed at the time of operation, and by means of the sutures where these were still present. In one case (12H75) the autograft only was located; in another (20G210) the grafts were not located until the lamb was destroyed at 5 months of age.

There was a marked difference in the nature of the autografts and homografts, irrespective of the age at which they were examined. The *autografts* (Plate 4, Fig. 11) consistently formed either a small crater about 3 mm in diameter and 4-6 mm deep with a slightly raised margin, or a subcutaneous pocket 3-5 mm in diameter and maintaining a smaller opening to the surface. In all cases the graft was producing wool fibre. The *homografts*, on the other hand, had apparently been rejected and only a small scar about 2 mm in diameter remained. It was frequently raised a little above the general skin level. Occasionally a small piece of yellow material, which was readily dislodged, was present over the graft site.

In one case in which a "fitted" autograft had been used, the graft was raised above general skin level and covered with a quantity of exfoliated material. The graft was producing normal wool fibre. One subcutaneous autograft (lamb 38S6) transplanted from the dorsal shoulder region to the anterior surface of the right metacarpus was left in situ until the lamb was 4 months old. By this time it had formed a subcutaneous 'cyst' 1.0 cm diameter and 3.0 cm long, but maintaining an opening to the surface about 3 mm diameter. The centre of the 'cyst' was filled with wool fibre and a mass of white creamy material.

(iii) *Postnatal Histology of Prenatal Grafts*

In general, postnatal histological examination confirmed the expectations from visual examination of the graft sites. As a group the autografts formed an entirely different histological series from the homografts, where the two were compared on a within-animal basis. The general picture in the autografts was one which showed a complete epithelium with a reduced follicle population, as compared with adjacent native epithelium, but accompanied by normal sebaceous glands and producing fibre (Plate 4, Fig. 11). This was so even in the youngest specimens taken at birth, when the transplants were 33 days of age. Some fibroblast activity may have remained in the dermis, but this was much reduced and disappeared rapidly. In summary, the autografts were little different from normal skin.

Scars one-quarter to one-tenth the diameter of the autografts occupied the homograft sites. Considerably increased numbers of fibroblasts and tortuous capillaries suggested a continuing reaction to, and dissolution of, parts of the graft enclosed by the host epithelium. The epithelium had returned to nearly normal thickness in the youngest specimen examined (33 days postoperative), but was devoid of follicle development. The youngest homograft site to show signs of a regenerating follicle population was examined 52 days after the

original transplantation. All cases were consistent with a picture of "shelling out" and complete rejection of the homografts and subsequent repair of the graft site (Plate 3, Fig. 10). The homografts in which the ewe was used as donor were rejected in the same fashion as the foetal homografts.

As an incidental observation to the main series it is of interest to record that the regenerated follicle population in the autografts was composed entirely of secondary follicles. It has been shown (Medewar 1944, 1945) and is confirmed here, that all the follicles of an autograft degenerate soon after transplantation and are succeeded by a new follicle population developing from the basal layer of the epidermis. Two types of follicles in the skin of sheep have been demonstrated (Carter 1943); primary follicles which develop between the 30th and 80th or 85th day of prenatal life, and secondary follicles which develop thereafter. Of the four cases in this series in which it was possible to make a careful study of the regenerated follicle population, all show that this population is made up solely of secondary follicles. It is to be noted that all these grafts were carried out when secondary follicles only were being formed. It seems that the skin has lost entirely the morphogenic stimulus necessary for the production of primary follicles, even in a regenerating population.

(iv) *"Second-Set" Postnatal Homografts*

Only two pairs of lambs on which reciprocal transplants had been made during the foetal stage were available for second-set homografts during postnatal life. These comprised lamb numbers 38S1 and 38S2 (pair No. 3 of Table 1) and 38S3 and 38S4 (pair No. 4 of Table 1).

Reciprocal homograft transplants were performed between S1 and S2, under local anaesthesia, when the lambs were 13 and 12 days old respectively, i.e. 54 days after the original foetal homograft operation. Two grafts, each 1 cm by 3 mm, were reciprocally transferred from one lamb to the other, using the subcutaneous method employed earlier save that the graft was not sutured with the host skin. No control autografts were done at this stage. One homograft was removed from each animal after 5 days and the second after 8 days.

A similar technique was adopted with lambs S3 and S4, save that three reciprocal transfers were made. The first graft was removed from each animal at 4 days, the second at 9 days, and the third at 16 days from S4 and at 21 days from S3.

The major weakness of this plan is the time lag between the foetal (first-set) homografts and the postnatal (second-set) homografts. This was unavoidable because, in view of the failure to obtain closure of the amnion originally, we considered the surgical risks attached to a second penetration of the uterus and membranes to be too great.

The fate of the second-set homografts.—The main features of the breakdown of second-set homografts (Medewar 1944) are:

(*a*) The precocious invasion by, and breakdown of the primary vascular system, and as a consequence of this, the feeble cellular activity.

(*b*) The failure of the graft epidermis to undergo general vertical thickening and lateral outgrowth.

(*c*) The reduced survival time of the epidermis as compared with first-set grafts.

The current series of observations showed no appreciable variation from these criteria. In the 4- and 5-day specimens, only one of the four cases showed marked capillary invasion and engorgement. All 8- and 9-day cases showed typical second-set reactions. Blood vessels had passed through the stage of engorgement to one of complete stagnation. The lymphocytic invasion of the dermis, which is much less than that seen in the first-set grafts, had reached the point where the leucocytes had fragmented and were now dead. In only one case was there a suggestion of a "black band" (Plate 4, Fig. 13). There was no sign of hyperplasia of the graft epithelium, which gave every indication of having survived in a "vegetative" state only (Medewar 1946) (Plate 4, Fig. 13). Around the margins of the graft, proliferating epithelium of native origin had commenced to undermine either the entire graft or the graft epithelium (Plate 4, Fig. 12). The 16- and 21-day cases showed a logical progression of the above sequence of events. In each case the native epithelium had progressed to a stage of forming a complete cyst, which had shelled out the graft into the cavity of the cyst (Plate 4, Fig. 14).

The survival time of the second-set homografts was estimated as being less than 9 days, whereas that of the first-set grafts was of the order of 15-16 days.

It is generally recognized that the second-dose response, which is a characteristic of actively acquired immune reactions, is best observed if the second dose is applied when the reaction to the first is at its height, or soon after. Despite the relatively long delay between first- and second-set grafts in this series, it is quite clear that a second-dose response has been observed. These results are thus in agreement with earlier observations on older animals which have shown quite clearly (Gibson and Medewar 1943; Medewar 1944) that the incompatibility normally seen following homograft operations belongs to the general class of actively acquired immune reactions, and that *innate* immunity is insufficient to account for the observed facts.

IV. Discussion

The object of these observations was to determine if skin homografts would succeed at the foetal stage in sheep. From the results obtained there seems to be no possible doubt that they do not succeed. The evidence for this comprises firstly, the contrasting histological picture of autografts and homografts seen after transplantation; secondly, the obvious macroscopic differences between the two graft types seen in lambs after birth; and thirdly, the failure to establish second-set homografts in postnatal life, using the same donor and recipients as were used for the prenatal transplants. Within the present series, age had no obvious effect on the reaction.

There is no doubt that the foetal reaction to the skin homografts in this series is one of active rejection by the foetus; it is not merely a failure of the graft to "take" due to inadequate vascularization. That the reaction is of foetal and not maternal origin is indicated by the fact that, in both cases

in which maternal skin was grafted to the foetus, the homografts were rejected. It must be concluded therefore that the foetus is able to make an immune response. This appears to be a contradiction of such statements as "foetal mammals and chick embryos are incapable of producing antibody" (Burnet and Fenner 1949, p. 102) and "the embryo is immunologically inactive" (Anderson *et al.* 1951).

We are unaware of any previous work which takes the form of a direct study of the ability or otherwise of foetal mammals to produce an immune response. The fact that the foetal lamb is born with no circulating antibody following the introduction of bacterial antigens into the ewe (Mason, Dalling, and Gordon 1930) cannot be considered evidence of the failure of the foetus to produce antibody. There is considerable evidence that such antigens do not cross the placental barrier in sheep, thus the lamb has no opportunity to produce a response.

The main studies of the ability of embryos to produce antibodies have been carried out in the developing chick (Grasset 1929; Burnet 1941). Grasset has shown that no immunity to diphtheria toxin was developed in the chick following injection of the egg with the corresponding toxoid on the 7-18th day of incubation. Similarly, Burnet showed that influenza virus, which is a potent antigen in adult fowls, failed to stimulate antibody production in the developing chick when inoculated on the twelfth day of incubation.

Two possibilities are suggested to account for the discrepancy between the results of observations recorded in this paper and current concepts of the immunological behaviour of embryos. Firstly, it may be that the foetal lamb is "abnormal" in the sense that it acquires an antibody-producing mechanism at an early age. In an immunological sense, the new-born lamb does appear to be more mature than, for example, the new-born mouse. The latter has neither red-cell antigens nor the capacity to produce antibody at birth. On the other hand, Ycas (1949) has shown that the lamb at birth possesses nine red-cell antigens capable of detection with prepared antisera, although two further antigens and the corresponding naturally occurring iso-antibodies are not present until 2-3 weeks of age. Secondly, it is possible that the foetus is unable to respond immunologically to antigens which have a determinant pattern for antibody production widely different from its own "marker" pattern, using the term "marker" in the concept of Burnet and Fenner (1949). If this were so it would account for the failure to observe antibody production in chick embryos in response to the bacterial and viral antigens. At the same time the hypothesis allows for the development of an immune response, as recorded here, where the antigen is not of greatly different genetic makeup from the recipient. This requires that the foetus has the ability to respond to "individuality differentials" (Loeb 1945) of small degree (as occurs in homografts), but not to those of extreme differences (heterografts and bacterial and viral antigens). Although there is little direct evidence to support such a hypothesis, it may be recalled that the cellular reaction varies according to the degree of genetic difference, or "individuality differential," of donor and recipient. Loeb (1945) has drawn attention to the fact that the reaction to homografts is essentially one of lympho-

cytes, fibroblasts, and a few monocytes, whereas the reaction to antigens of wider differentials is largely of polymorph leucocytes. The predominant part played by lymphocytes in the homograft reaction has been amply demonstrated by Medewar (1944, 1945) and has been observed consistently in these observations.

In dizygotic cattle twins, each member receives cellular components from its opposite number at a very early age, whereas in these observations the earliest homografts did not take place until the 80th day of prenatal life. There must be a zero point in the ability of the embryos to recognize an antigen as "non-self." This point must be between fertilization and the 80th day of prenatal life in the sheep.

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VI. References

Anderson, D., Billingham, R. E., Lampkin, G. H., and Medewar, P. B. (1951).—*Heredity* **5**: 379.

Billingham, R. E., and Medewar, P. B. (1951).—*J. Exp. Biol.* **28**: 385.

Burnet F. M. (1941).—*Aust. J. Exp. Biol. Med. Sci.* **19**: 291.

Burnet, F. M., and Fenner, F. (1949).—"The Production of Antibodies." (Macmillan: Melbourne.)

Carter, H. B. (1943).—Coun. Sci. Industr. Res. Aust. Bull. No. 164.

Ferguson, L. C., Stormont, C., and Irwin, M. R. (1942).—*J. Immunol.* 44: 147.

Gibson, T., and Medewar, P. B. (1943).—*J. Anat., Lond.* **77**: 299.

Grasset, E. (1929).—*Publ. S. Afr. Inst. Med. Res.* **4**: 171.

Loeb L. (1945).—"The Biological Basis of Individuality." (Thomas: Springfield, Ill.)

Mason, J. H., Dalling, T., and Gordon, W. S. (1930).—*J. Path. Bact.* **33**: 783.

Medewar, P. B. (1944).—*J. Anat., Lond.* **78**: 176.

Medewar, P. B. (1945).—*J. Anat. Lond.* **79**: 157.

Medewar, P. B. (1946).—*Brit. J. Exp. Path.* **27**: 9.

Owen, R. D. (1945).—*Science* **102**: 400.

Owen, R. D., Davis, H. P., and Morgan, R. F. (1946).—*J. Hered.* **37**: 291.

Stormont, C., and Cumley, R. W. (1943).—*J. Hered.* **34**: 35.

Ycas, M. K. W. (1949).—*J. Immunol.* **61**: 327.

Explanation of Plates 1-4

Plate 1

Fig. 1.—Autograft, 9 days, showing vertical thickening of the epithelium and early stages of follicle degeneration. Masson's tri-chromic.

Fig. 2.—Homograft, 9 days, from same animal as autograft in Figure 1. Note dermo-epidermal "blistering" and disengagement of the epidermis and vascular and cellular reaction in graft dermis. Masson's tri-chromic.

Plate 2

Fig. 3.—Autograft, 9 days, Masson's tri-chromic.

Fig. 4.—Homograft, 9 days, Masson's tri-chromic.

Fig. 5.—Homograft, 15 days, showing "blistering" of the epithelium, which is not certifiably dead, but shows pronounced pathological changes. Note lymphocyte invasion of dermis. Masson's.

Fig. 6.—Homograft (fitted), 22 days. The graft has been almost completely undermined by native epithelium growing in from each side. Note persisting reaction in graft dermis. Masson's.

Plate 3

Fig. 7.—Homograft, 21 days, showing the last stage of the undermining process by the native epithelium. H & E.

Fig. 8.—Homograft, 21 days, in the last stages of being "shelled-out" by native epithelium. The dark mass of the centre comprises partly new cuticle formed by the new epithelium and partly the necrotic graft. Masson's.

Fig. 9.—Autograft, 21 days. The graft has become completely encysted at this stage, the centre of the cyst being filled with wool fibres and a mass of exfoliated cuticle. H & E.

Fig. 10.—Homograft, 33 days. The graft has been completely undermined by native epithelium to form a cyst similar to that in Figure 9, except that the centre of the cyst is now filled with the necrotic remnants of the graft together with fragmented mesenchyme cells. Masson's.

Plate 4

Fig. 11.—Autograft, 82 days, showing the 'pit-like' form and the development of follicles. Masson's.

Fig. 12.—Second-set homograft, 8 days, showing the development of native epithelium, which is commencing to undermine the entire graft. H & E.

Fig. 13.—Second-set homograft, 8 days, showing cellular invasion of the graft dermis, reminiscent of the "black band" observed by Medewar only in first-set grafts. Note the "vegetative" condition of the graft epithelium. H & E.

Fig. 14.—Second-set homograft, 21 days. The graft has been completely "shelled-out" and lies in the cavity of the cyst formed by native epithelium. H & E.

SKIN TRANSPLANTATION IN FOETAL LAMBS

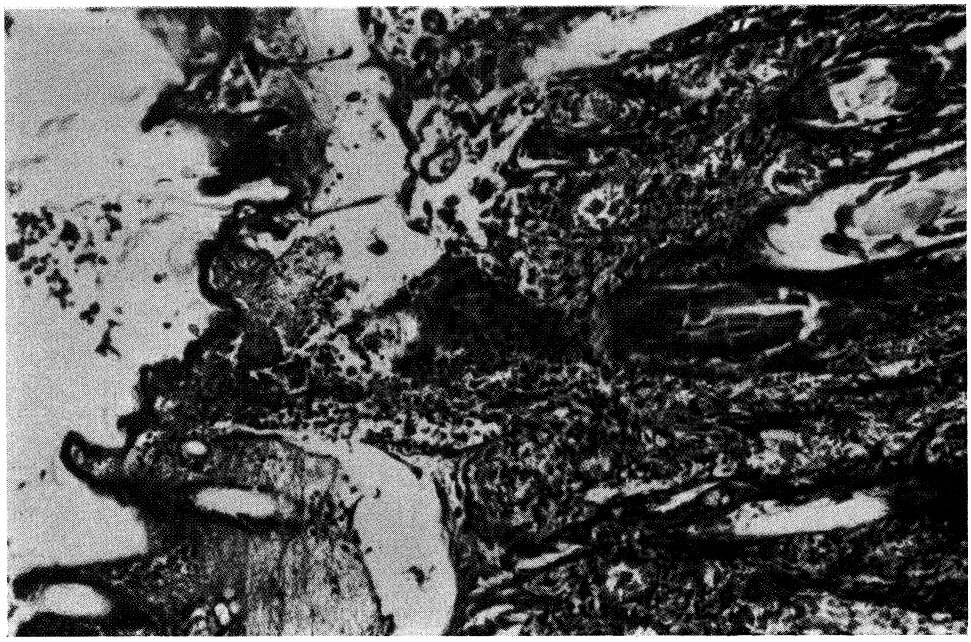


Fig. 2

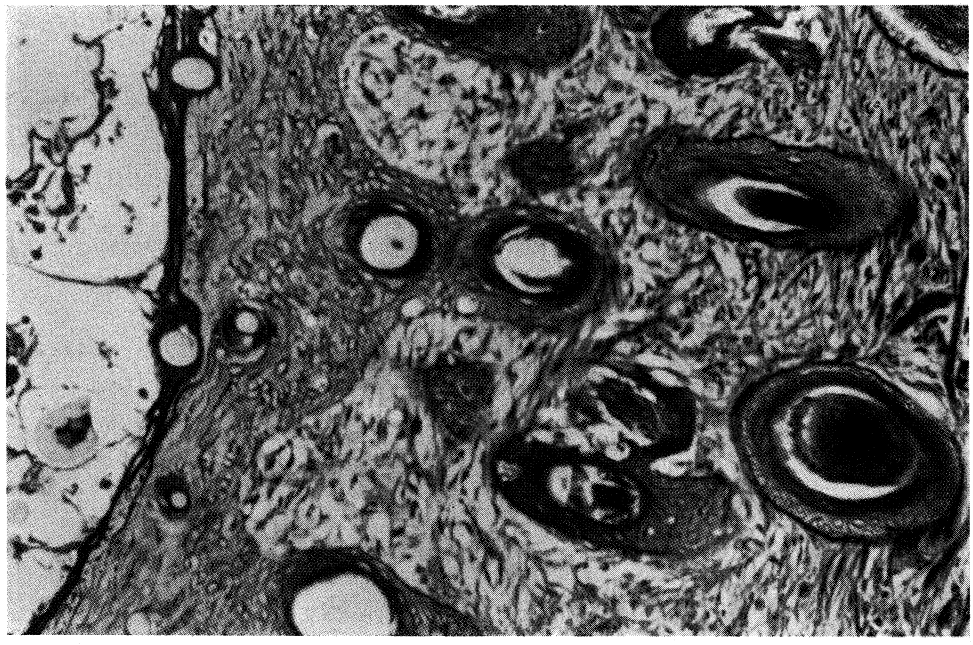
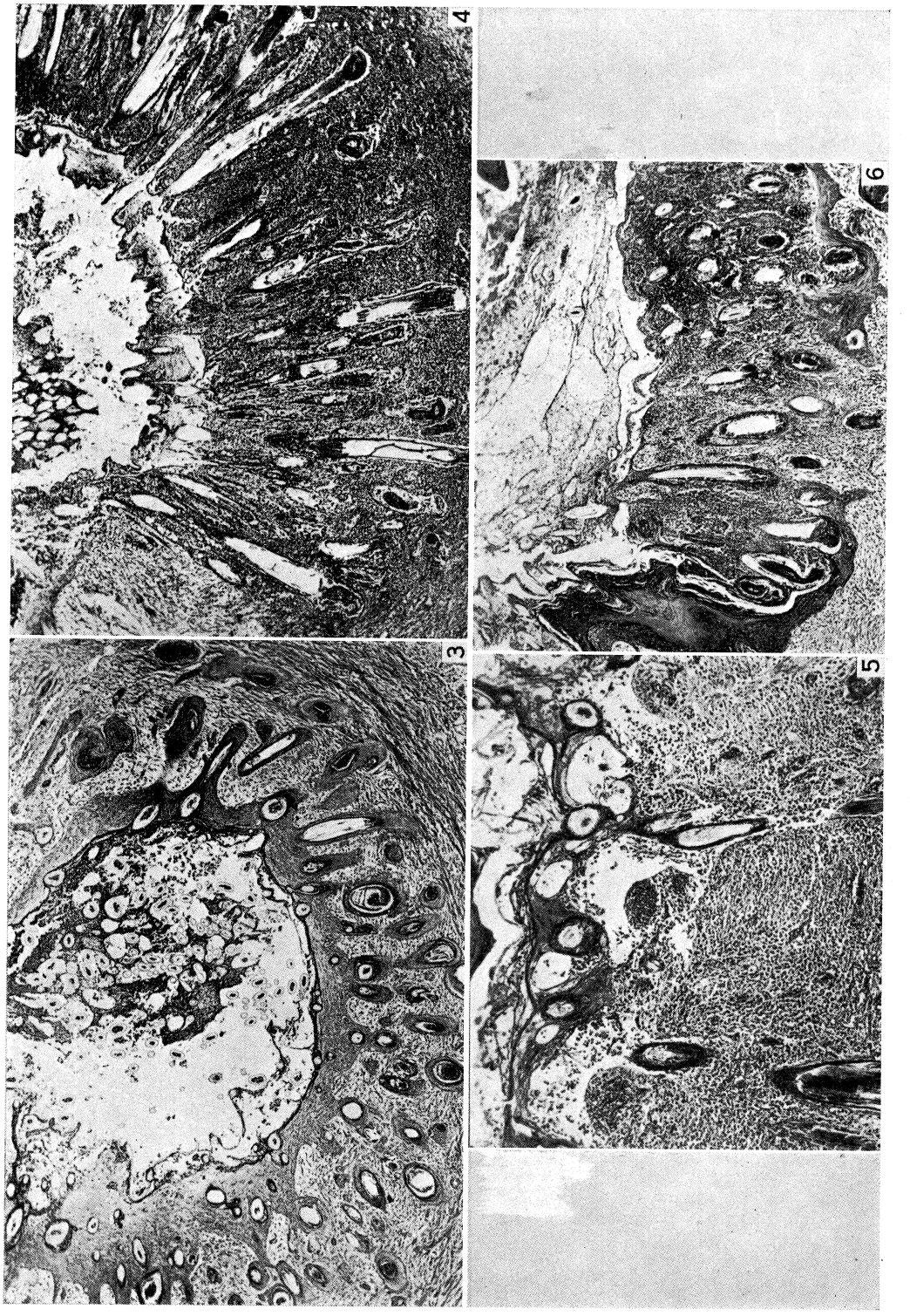


Fig. 1

SKIN TRANSPLANTATION IN FOETAL LAMBS



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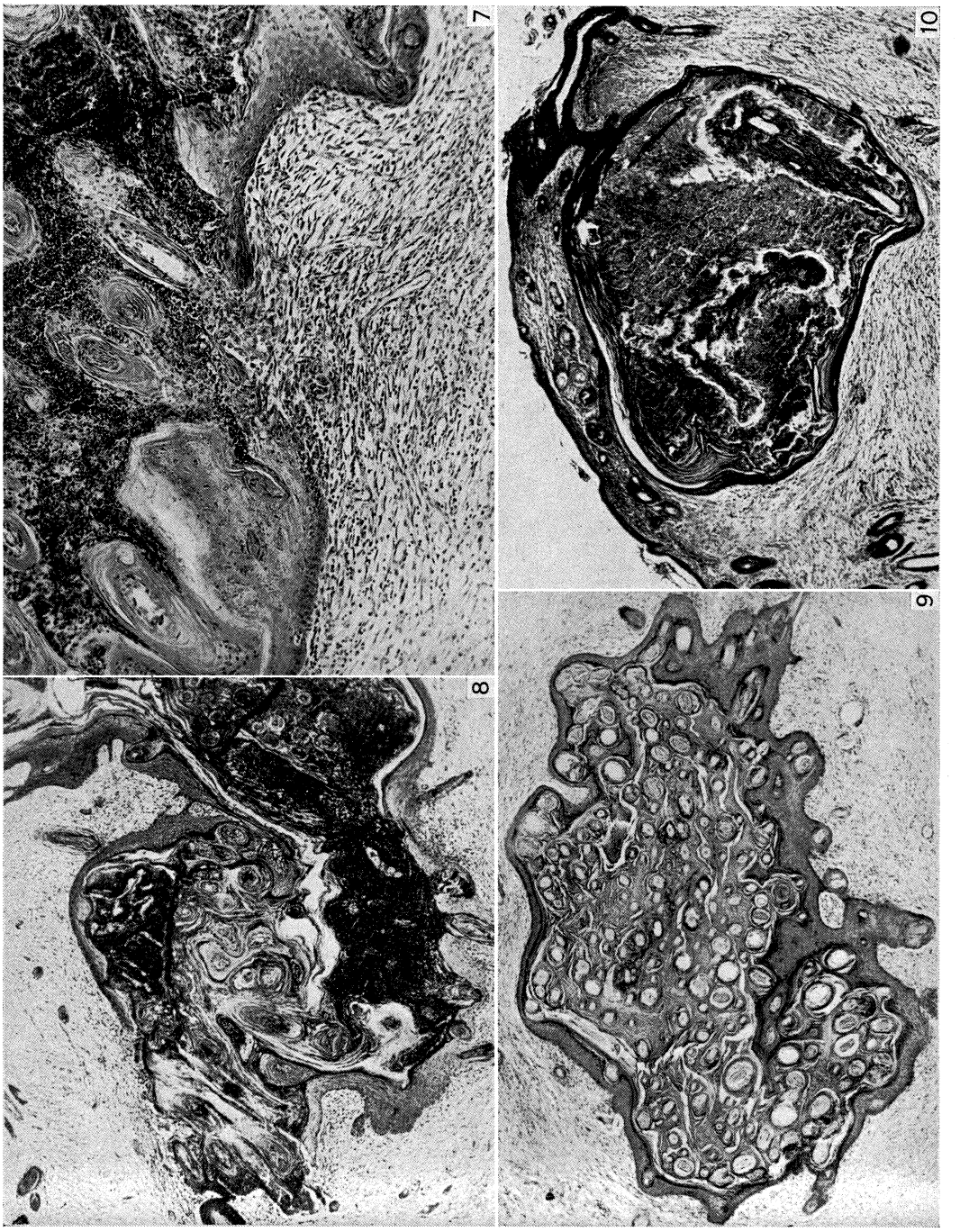

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