TOLERANCE TO HOMOGRAFTS, TWIN DIAGNOSIS, AND THE FREEMARTIN CONDITION IN CATTLE

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Received 16.i.52

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1. INTRODUCTION

THE present paper is a sequel to our first published enquiry (Anderson, Billingham, Lampkin, and Medawar, 1951) into the use of skin grafting as a means for distinguishing between one-egg and two-egg twins in cattle. Experience with laboratory mammals has shown that skin does not long survive transplantation between animals having any lesser degree of affinity than may be achieved by upwards of a dozen consecutive generations of strict inbreeding; failing this, skin so grafted will elicit an immune response from its host—the " homograft reaction "—and succumb to it after a period which rarely exceeds two weeks. Since two-egg twins are no more closely related to each other genetically than are ordinary full siblings, it was expected that skin grafts exchanged between them would be no exception to the rule. On the other hand, skin grafts exchanged between one-egg twins should, like autografts, survive indefinitely.

In practice it turned out otherwise. Skin exchanged between what were presumed to be monozygotic twins did indeed survive indefinitely; but the great majority of dizygotic twins also proved to be tolerant to grafts of each other's skin, even when they were of unlike sex. Their tolerance was thrown into high relief by the fact that grafts exchanged between ordinary siblings provoked homograft reactions of great violence, and no such graft lived longer than 15 days.

Owen (1945; Owen, Davis and Morgan, 1946) has already demonstrated the tolerance of two-egg twin cattle to genetically foreign tissue by other means, for each member of such a pair contains not only the red blood cells that derive from its own zygote division lineage, but also those that derive from the zygote lineage of the other member of the pair. In effect, therefore, the twins have the same blood groups, at least during their youth. Since red cells are continuously manufactured anew throughout life, this can only mean that the two-egg twin calf contains living cells of two zygote lineages of different genotypes and is therefore a genetical chimaera. It has presumably become so as a result of the mutual exchange of red cell precursors during embryonic life that is made possible by the embryos' having confluent blood circulations. Another presumed consequence of the synchorial condition is the notorious fact that the female member of approximately 90 per cent of two-egg twin pairs of unlike sex is sterile.

The failure of our attempts to identify two-egg twin calves by mutual exchange of grafts led us to pursue and extend our work on the following lines:

(i) An extension of our series of graft interchanges between cattle classified by orthodox methods as two-egg twins. We have already shown that not all two-egg twins are fully tolerant to grafts of each other's skin, and we were interested in finding out whether the proportion of twins in which exchanged grafts were not acceptable was approximately the same as the proportion of normal or almost normal females to be found in twin pairs of unlike sex. If the synchorial condition is a necessary prerequisite both for mutual graft tolerance and for the infertility of the female in twin pairs of unlike sex, then the proportions should be fairly similar.

(ii) An extension of our "control" experiments to include exchanges of skin between dam and offspring of both single and two-egg twin birth. These complete the grafting tests that are necessary to demonstrate the uniquely anomalous behaviour of two-egg twins towards genetically foreign tissue.

(iii) An examination of the possibility of using other specialised methods of grafting for distinguishing between one-egg and two-egg twins.

2. METHODS

The practice of transplanting ear skin grafts to the "withers" has continued to give satisfactory results, and only one technical improvement has been made. Instead of relying upon a Hessian truss around the thorax (1951, plate 1, figs. 7, 8, 9) to protect the grafts and bring adequate perpendicular pressure to bear upon them, we now pass a strip of 3 in. wide Lastonet bandage round the entire thorax, the free ends being brought together under tension and secured to each other temporarily with Michel's suture clips. The Lastonet bandage is then stuck down to the skin by a thin film of Copydex. (Lastonet is an open mesh unmedicated elastic bandage, and "Copydex" a watery solution of rubber latex.) The Lastonet prevents side slip, and, being applied under tension, provides all the perpendicular pressure that is necessary. The immediate graft dressings—tulle gras, a layer of surgical gauze, and a thick rectangular pad of cotton wool now so shaped as to overlap the graft-bearing area by about an inch all round—remain unchanged, and the dressings are completed by fitting a loose Hessian blanket to give added protection.

This variant of our technique has given particularly satisfactory results.

3. RESULTS

(i) *Further Grafting Experiments on two-egg Twins*

The results of our new tests on the transplantation of skin from one member of a two-egg twin pair to the other are summarised by table 1. They relate to 10 pairs of female twins, 5 pairs of male twins, and one group of triplets consisting of a male and two (almost certainly) monozygotic females. The animals we used were not of course always known with complete certainty to be two-egg twins, though there was no reason to question the accuracy of the diagnosis. Twins of dubious classification were not used.

We have already published the results of exchanging grafts reciprocally between ten pairs of two-egg twins of which three pairs were of unlike sex (1951, tables 3 and 5). These earlier results had made it clear that twins are by no means necessarily symmetrical in their responses: grafts transplanted from one twin to the other might be tolerated during the entire period of observation in spite of the fact that grafts of the reciprocal transplantation were eventually destroyed. In our present experiments we used the limited time at our disposal by grafting the majority of our twin pairs " one-way " only—each member being either a donor or a recipient, but not both—instead of grafting a smaller number reciprocally. Nothing is lost by this procedure if there is no internal correlation between the responses of the twin pairs. But there must surely be *some* such correlation, even if it falls far short of complete symmetry of response, and if this is so, our sampling net is cast so much the wider by the procedure we have actually adopted.

Either three or four grafts were transplanted from one twin to the other. Two animals, DZ 28A and DZ 29A, were grafted on a second occasion because the grafts originally transplanted to them showed signs of weakness and inflammation. The behaviour of the second set of grafts showed that these pathological changes were the consequence of faulty healing and not of a homograft reaction.

Taken as a whole, our present results and those already published give evidence on 42 animals which received grafts from their respective two-egg twins. All animals displayed a high degree of tolerance to homografts from their twins, for no graft lived for less than 70 days after transplantation; only six animals showed a degree of intolerance that ultimately led to complete graft breakdown. We may therefore take it that 36 animals in 42, about 86%, were completely tolerant within the time limit of the experiment, which on only two occasions was less than 100 days.

TABLE 1

Donor	Recipient	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Observation period (days)	Notes
D 4B	D 4A	Sh.	Sh. × H.	240	408	131	
D 10B	D 10A	H.	Fr.	126	148	131	
D 12B	D 12A	,,	Sh.	119	180	131	
D 13B	D 13A	,,	Sh.	97	172	114	Breakdown almost complete at 74 days; see also Table 2B and plate 1, figs. 1, 2
D 14B	D 14A	,,	A.	70	130	131	See also table 2B
DZ 18A	DZ 18B	,,	Sh.	209	325	131	
DZ 19B	DZ 19A	,,	A.	223	338	95	Grafts normal at 40th day, broken down by 95th day
DZ20B	DZ20A	,,	Sh.	174	267	131	
DZ 21A	DZ 21B	,,	Sh.	102	160	91	See also tables 2B, 3B
DZ 21B	DZ 21A				159	129	
DZ 24A	DZ 24B	,,	Fr.	93	176	119	Breakdown in progress in 24A at 119 days; cf. tables 3A, 3B
DZ 24B	DZ 24A				171		
DZ 27B	DZ 27A	,,	Fr. cross	96	147	131	See also table 3B
DZ 28B	DZ 28A	,,	Fr. cross	94	159	36+95	See also tables 2B, 3B
DZ 28A	DZ 28B	,,		130	204	95	
DZ 29B	DZ 29A	,,	Sh.	91	191	64+67	
DZ 29A	DZ 29B			155	269	67	
DZ 30B	DZ 30A	,,	Sh.	65	120	131	
DZ 31B	DZ 31A	,,	Sh.	65	112	131	
B 61	MZ 61A	Fr.	Fr.	52	109	103	Triplets. See also table 3A, and plate 1, figs. 6 and 7
MZ 61A	B 61				116		
B 61	MZ 61B				114		
MZ 61B	B 61				116		

TABLE 1.—Transplantation of grafts between twin pairs classified as two-egg. All homografts remained normal to outward appearance over the stated period of observation unless the contrary is stated. Entries for weight, age, observation period, etc., relate to the recipient animals entered in each row. Animals numbered with Z in the prefix are female; all others are male.

Note.—Abbreviations of Breed Names: Sh., Shorthorn; H., Hereford; Fr., Friesian; A., Ayrshire.

(ii) *Transplantation of skin from mothers to their calves*

We have already described the consequences of exchanging skin grafts between (*a*) unrelated cattle, individually of twin birth (1951, table 1); (*b*) a set of four pedigree Ayrshire cows, individually of separate birth (1951, table 1); and (*c*) full siblings of separate birth (1951, table 4). Every such homograft was destroyed by the 15th day following its transplantation, and the majority were completely necrotic by the 9th day.

These control experiments do not quite suffice to show that the behaviour of two-egg twins is unique, because anomalous tolerance to homografts might conceivably extend to skin transplanted from dam to calf. We therefore extended our survey to include homografts transplanted (*a*) from dam to calf of single birth, and vice versa; (*b*) from dam to calves of two-egg twin birth; the reciprocal transplantations could not be done. The results are summarised by tables 2A and 2B respectively.

Homografts transplanted from dam to calf of single birth survive no longer than they do when transplanted between full siblings of separate

TABLE 2A

Donor	Recipient	Sire	Dam	At operation:		Homograft survival time (days)
				Age (days)	Wt. (lbs.)	
Swain's Whim (dam)	Abgro Ruby (calf)	A.	A.	519	780	>7⩽15
Abgro Ruby (calf)	Swain's Whim (dam)	A.	A.	1989	1200	<7
Staverton M. (dam)	P4 (calf)	Fr.	Fr.	292	498	>7<10
P4 (calf)	Staverton M. (dam)	Fr.	Fr.	1283	1300	Grafts accidentally lost
Swain's Violet (dam)	AF4 (calf)	Fr.	A.	54	124	>7⩽15
AF4 (calf)	Swain's Violet (dam)	A.	A.	2087	1150	<7

TABLE 2A.—Reciprocal exchange of grafts between dam and calf of single birth. The three calves were females.

birth. Evidently the calves are in no appreciable degree tolerant to their mothers' cells. Owing to a technical fault, only two of the three dam/calf pairs gave evidence of the fate of homografts in the reciprocal transplantation, i.e. from calves to dam. It is noteworthy that in these two pairs the homografts on the dam broke down very rapidly indeed, the process being complete by the 7th day. Histological examination of the graft at the 7th day showed that there had been no proliferation of the skin epithelium; the graft vessels were grossly dilated and engorged with red cells, and their endothelial linings had long since been disrupted; and cellular infiltration of the dermis by leucocytes was comparatively slight, no doubt because of the precocious breakdown of the blood vessels (cf. plate 1, fig. 8). In common laboratory animals, this combination of properties is distinctive of " immune " breakdown, i.e. of the behaviour of homografts transplanted to animals that have been immunised by an earlier grafting of skin from the same donor. The evidence, though slight, hints at the possibility that the mother had been in some degree immunised against (and had therefore become specially intolerant to) her offspring's cells. It cannot be said whether or not the immunisation was concomitant with the pregnancy of the calves studied here, for all the mothers were multiparous.

TABLE 2B

Donor (Dam)	Recipients (calves)	Sire	At operation:		Homograft survival time (days)	See also:
			Age (days)	Wt. (lbs.)		
Sh.	D 13A	H.	133	228	>20	Table 1; plate 1, figs. 4, 5, 6
	D 13B			231	>13<16	
A.	D 14A	H.	106	196	>13<16	Table 1
	D 14B			164	>11<13	
Sh.	DZ 21A	H.	221	333	>10⩽14	Tables 1, 3B
	DZ 21B			332	⩽10	
Fr. cross	DZ 28A	H.	158	256	>10⩽14	Tables 1, 3B
	DZ 28B			249	<10	

TABLE 2B.—Transplantation of grafts from dam to twin calves classified as dizygotic. The first two pairs of calves are males, the others females.

Table 2B shows that homografts transplanted from a dam to each of her two-egg twin calves also broke down. This fact settles the main question at issue by showing that grafts exchanged between dam and calves of two-egg twin birth are not exempt from the consequences of transplantation immunity. Two other facts, however, require an explanation: (i) although the homografts eventually broke down, they lived longer (cf. plate 1, fig. 3) than homografts transplanted from dam to calves of single birth (table 2A) or between full siblings of separate birth (1951, table 4); and (ii) that the homografts survived for different lengths of time on the two members of the twin pair (contrast plate 1, fig. 3 with plate 1, figs. 4, 5). These apparent anomalies will be discussed in section 4 (ii).

(iii) *Other skin grafting methods for distinguishing between one-egg and two-egg twins*

It has now been clearly shown that the mutual exchange of skin grafts between twin cattle cannot be relied upon to distinguish their modes of origin. Grafts exchanged between two-egg twins *sometimes* break down, but very much more often they do not. Any incompatibility that may be revealed is, moreover, of very low degree, for grafts which break down at all usually live for two or three months before finally doing so.

We have therefore tested a method of twin diagnosis based on different principles. The two-egg twin calf, although a genetical chimaera, must surely consist of cells that are *predominantly* of one genotype; in particular, the skin of such a calf contains cells of which upwards of 99% presumably derive from one zygote lineage, and it may be that only a very few red cells and leucocytes derive from the other. Grafts

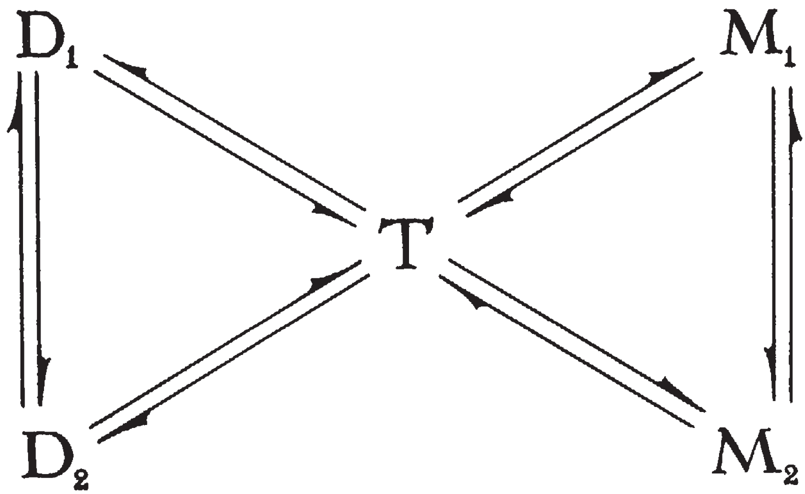


FIG. 1.—The style of grafting operation used in tests 1 and 2 (table 3A). Each arrow represents the transplantation of three grafts. D1, D2 and M1, M2 are two-egg and one-egg twins respectively; T is a test animal unrelated to either. Each animal received three autografts in addition to the 12 homografts indicated by arrows.

from two-egg twins should therefore be antigenically distinguishable, and if grafts from them are transplanted on the same occasion to a third and unrelated " test animal ", their survival times should differ perceptibly (Medawar, 1945; Dempster and Lennox, 1951; Billingham and Medawar, 1951).

With this possibility in mind, we therefore conducted two tests in the grafting pattern illustrated by text-fig. 1 and a third test in the pattern illustrated by text-fig. 2. Each test should make it possible

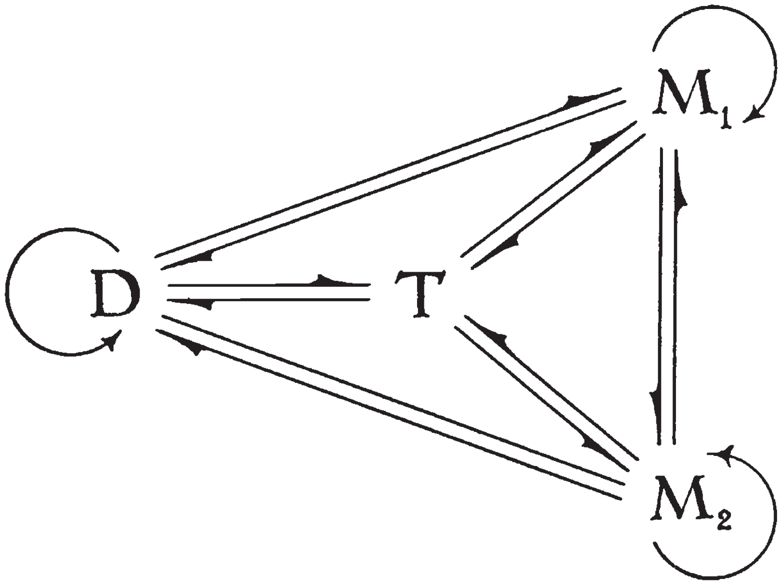


FIG. 2.—The pattern of grafting operation adopted for test 3 (table 3A). D, M1, M2 are triplets of which M1, M2 are almost certainly one-egg twins. The principle of the test is the same as that illustrated by text-fig. 1.

(*a*) to decide whether grafts from two-egg twins D1 and D2 elicit reactions of different intensity after transplantation to an unrelated test animal T; (*b*) conversely, to decide whether grafts from a test animal T elicit distinguishable reactions from D1 and D2; (*c*) to compare two-egg twins in each such respect with one-egg twins M1 and M2; and (*d*) to compare differences of genuinely immunological origin with any which, being revealed in grafts from the same donor to the same recipient, must have arisen in some other way. No appreciable differences of this last sort were in fact revealed.

Interpretation was based almost wholly upon the histological analysis of biopsy specimens, for the outward appearance of homografts can be most misleading. In the first test, biopsy specimens were taken at the 7th and 10th days; in the second test, at the 9th day; and in the third test at the 9th and 12th days.

Animal D in test 3 was twin brother to the monozygotic female twins M1 and M2: as table 1 shows, each animal was tolerant for at least 103 days to skin from its two-egg twin (see plate 1, figs. 6, 7).

On the other hand, table 1 shows that one of the two-egg twins in test 2, DZ 24A, eventually reacted against grafts from its twin DZ 24B.

In summary of an exhaustive series of comparisons between grafts, it can be said that the only distinct differences that were revealed were between the grafts from T on D1 and on D2 in test 2, and between the grafts from T on D and on M1 (or M2) in test 3. Otherwise the results were negative: differences to be expected theoretically were not revealed. There is no reason to doubt that this is because the reaction against foreign grafts in the cow is so rapid and violent as to obscure subtle differences of reaction intensity. Breakdown was invariably in progress so soon as 7 days after transplantation, and was almost invariably long complete by the 9th day. The results are therefore more properly described as uninformative rather than negative.

It later turned out that grafts from a dam to her two-egg twin offspring (section 3 (ii)) did quite unexpectedly what tests 1-3 were expected, but failed, to do, viz. to discriminate between the responses of two-egg twins to homografts. Not knowing this at the time, we adopted a variant of tests 1-3 in an attempt to accentuate any difference there might be between the survival times of grafts transplanted from the members of a two-egg twin pair (D1 and D2) to a test animal T. The modified operation was done in two stages. In the first stage, 4 grafts were transplanted to T from one only (D1) of the twin pair. Twelve days later, when these grafts of first planting were wholly necrotic, 4 grafts were transplanted to T from both D1 and D2. Biopsy-specimens of both sets of grafts were removed seven days later.

This procedure was expected to magnify the difference between the survival times of the grafts from D1 and D2 for the following reason. Although D1 and D2 certainly share many of their antigens in common, it was to be expected that D1 should contain some antigens absent from (or present in negligible amounts in) D2. The immunity developed in T as a consequence of the earlier grafting of skin from D1 must therefore be directed more strongly against D1 than against D2.

Three sets of tests were done. The results showed that a high degree of immunity was directed against grafts from both D1 and D2, for grafts from both donors showed the accelerated breakdown, suppressed epithelial proliferation, precocious vascular stagnation and relatively sparse leucocyte infiltration that are characteristic of homografts transplanted to animals already immunised against them (plate 1, fig. 8; contrast plate 1, figs. 9, 10). In all three tests, the grafts from D1 showed these characteristics more strongly. The distinction was not sharp enough to make the basis of a workable routine test, but sharp enough to justify the hypothesis that D1 and D2 do not share all their antigens in common. The mistake was to choose as a test animal a cow too distantly related to either twin: the parallax of the comparison was too fine.

Information relating to the animals used in the two classes of tests described in this section is contained in table 3.

TABLE 3A

Test	Animal No.	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Notes
1	T=P10	Fr.	Fr.	140	296	Test animal; plate 1, figs.9,10
1	M1=MZ 56A M2=MZ 56B	H.	Sh.	100	169 180	One-egg twins
1	D1=DZ 21A D2=DZ 21B	H.	Sh.	102	159 160	Two-egg twins
2	T=FA4	A.	Fr.	108	218	Test animal
2	M1=MZ 53A M2=MZ 53B	Fr.	A.	162	225 217	One-egg twins
2	D1=DZ 24A D2=DZ 24B	H.	Fr.	93	171 176	Two-egg twins
3	T=AF3	Fr.	A.	150	277	Test animal
3	M1=MZ 61A M2=MZ 61B D=B61	Fr.	Fr.	52	109 114 116	One-egg twins (M1, M2); Triplets (M1, M2, D)

TABLE 3A.—Information about the animals used in the experiments illustrated by text-fig. 1 (Tests 1 and 2) and by text-fig. 2 (Test 3). Animal B61 was male; the others are females.

TABLE 3B

Test	Animal No.	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Notes
1	T=JF4	Fr.	Jersey	232	370	Test animal
1	D1=DZ 28A D2=DZ 28B	H.	Fr. cross	115	206 204	Two-egg twins
2	T=P5	Fr.	Fr.	280	571	Test animal; see plate 1, fig. 8
2	D1=DZ 24A D2=DZ 24B	H.	Fr.	162	278 291	Two-egg twins
3	T=P6	Fr.	Fr.	274	501	Test animal
3	D1=DZ 27A D3=DZ 27B	H.	Fr. cross	117	181 187	Two-egg twins

TABLE 3B.—Information about the animals used in the second series of tests described in section (iii), in which skin was transplanted from both members of a pair of supposedly two-egg twins to an unrelated test animal that had already been immunised against skin from only one of them. All animals were females.

4. DISCUSSION

The results described here confirm and extend those of our earlier report. All cattle we tested were in some degree tolerant to homografts from their two-egg twins, even when the twins were of unlike sex; the majority were, within the terms of our experiments, completely tolerant.

The mutual exchange of skin grafts therefore failed, in the majority of trials, to distinguish between one-egg and two-egg twins. Tolerance, however, is not symmetrical: if D1 and D2 are dizygotic twins, grafts from D1 may survive on D2 although those from D2 eventually break down after transplantation to D1. The toleration of genetically dissimilar grafts is peculiar, in cattle, to two-egg twins; it extends neither to ordinary siblings nor to grafts exchanged between dam and calf.

(i) *Anomalous graft tolerance in relation to the freemartin condition*

The work of Owen (*loc. cit.*) justifies the strong presumption that tolerance to homografts exchanged between two-egg twins is a consequence of the same peculiarity of embryonic development as that which leads to sexual abnormality in the female member of two-egg twin pairs of unlike sex: the anastomosis of the foetal circulations. If this is so, the proportion of animals in which vascular anastomosis is so complete (or has happened so early?) as to lead to female sterility (the freemartin state) should be correlated with the proportion of animals in which it has made possible the development of tolerance to homografts. Exact correspondence is not to be expected: sexual abnormality and homograft tolerance may provide measures of different sensitivity: there are degrees of both, and there is no knowing what degree of abnormality corresponds to any particular degree of tolerance.

Of 42 individual cattle of either sex that received homografts from their respective two-egg twins, 36 (*i.e.* about 86%) proved to be completely tolerant within the time limits of observation. Swett, Matthews and Graves (1940) give 11 out of 12 as the approximate proportion of freemartins to be found in the female members of dizygotic twin pairs of unlike sex—*i.e.* about 92% of such females have achieved a degree of vascular anastomosis that is made evident by some degree of infertility. The correspondence is close enough to be regarded as strong evidence that homograft tolerance and female sterility share at least one necessary causal condition in common.

(ii) *Asymmetry of reaction to skin homografts in dizygotic twins*

The experiments of section 3 (iii) gave weak evidence that when skin is transplanted from an unrelated test animal to each member of a two-egg twin pair, the two members respond to the grafts by reactions of slightly different intensity. When, however, grafts are transplanted from a dam to each of her two-egg twin offspring, the distinction becomes very clear indeed, as may be seen from the entries in table 2B.

The distinction may be more conspicuous merely because homografts from dam to two-egg twin offspring last longer than homografts between unrelated animals. They also last longer than grafts transplanted between full siblings or from dam to calves of separate birth. Such a prolongation of survival is to be expected, because each individual, being unresponsive to its twin's antigens as well as to its own, is clearly unresponsive to a wider range of antigens than a calf of single

FIG. 1.—Appearance of homograft from D 13B 74 days after transplantation to its two-egg twin D 13A. Breakdown is very nearly complete, and only traces of much eroded surface epithelium remain. The vascular system of the graft is no longer functional. Contrast with plate 1, fig. 2, showing a graft from the same donor to the same recipient 8 days after transplantation. ×58.

FIG. 2.—Appearance of homograft from D 13B 8 days after transplantation to its two-egg twin D 13A. The graft shows some degree of traumatic inflammation, but no specific pathological change. Note the hyperplastic condition of the epidermis. Contrast with plate 1, fig. 1. ×58.

FIG. 3.—Illustrating the perfectly normal appearance of a homograft 20 days after its transplantation from a dam to one of its two-egg twin calves (D 13A). New hairs have begun to form and glandular epithelium is well differentiated. Contrast with plate 1, figs. 4, 5, which illustrate the grafts transplanted from the dam to the other member of the twin pair. ×58.

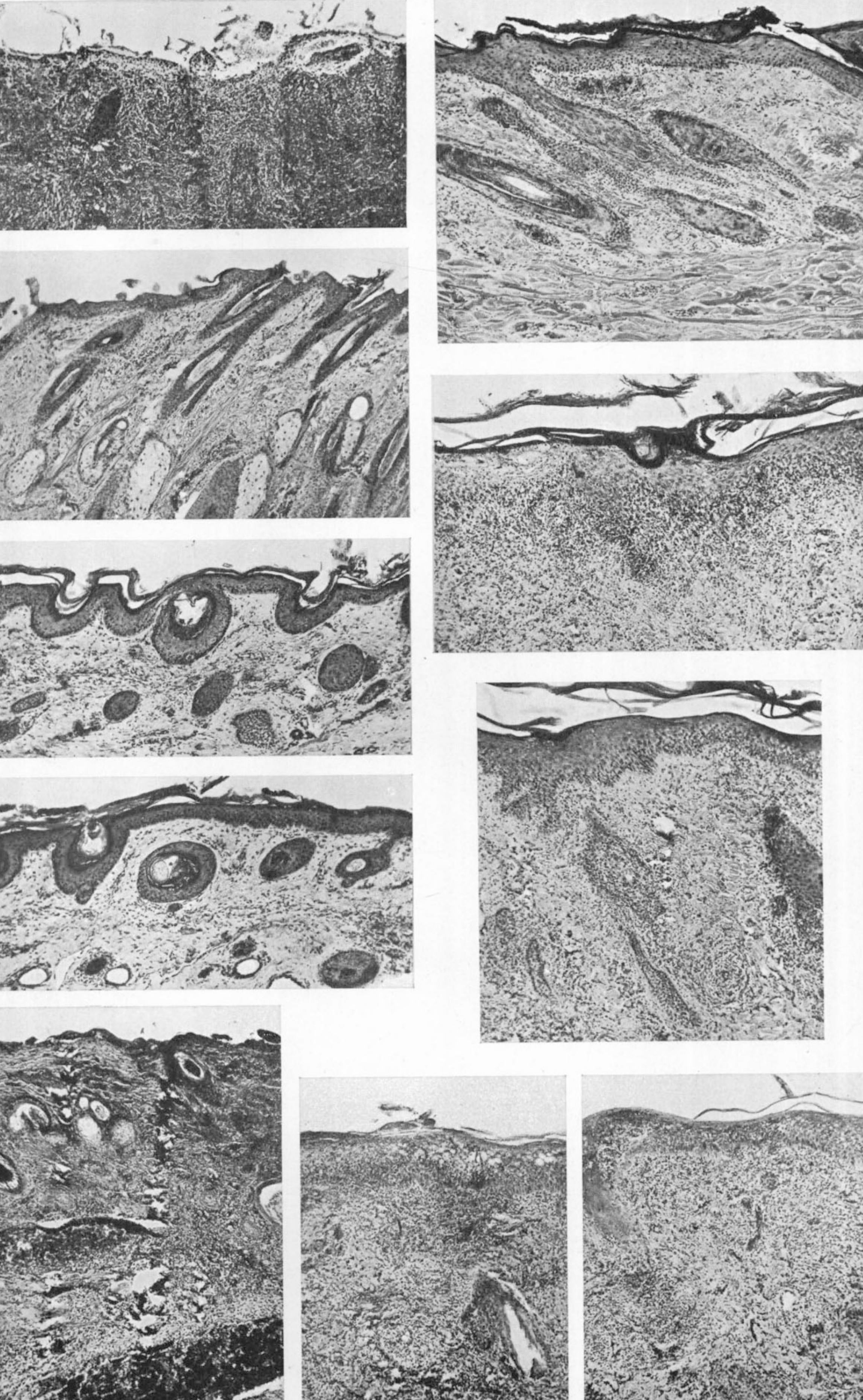
FIGS. 4, 5.—Contrast with fig. 3 : illustrating the beginnings of breakdown in a homograft 11 days after its transplantation from a dam to the other (D 13B) of its dizygotic twin calves. The cellular reaction in the graft dermis is not very intense (contrast, for example, figs. 9, 10) but erosion of the follicle and surface epithelium has clearly begun. Breakdown was complete by the 16th day. Figs. 3, 4, 5 illustrate both the prolongation of survival to be expected in grafts transplanted from dam to dizygotic twin calves and the differences between the responses of the calves themselves. ×58.

FIGS. 6, 7.—Transverse sections through an 8-day homograft transplanted from the male (B 61) to a female (MZ 61B) member of a set of triplets (table 1). The graft is in every respect autograft-like, and continued to be so over the period of observation (103 days). ×58.

FIG. 8.—Illustrating "immune" breakdown : a homograft from DZ 24A seven days after transplantation to a recipient, P5, which had already received and reacted against grafts from the same donor source (table 3B). Breakdown is complete : note the enoromusly dilated and now stagnant blood vessels and the complete absence of any sign of epithelial proliferation. Contrast with figs. 9, 10. ×58.

FIGS. 9, 10.—Illustrating an " acute " homograft reaction : a graft from DZ 21A seven days after transplantation to an unrelated test animal P10 (table 3A). Gross cellular infiltration of the dermis; maceration and vacuolation of the strongly hyperplastic epidermis. Contrast with the " immune " type of breakdown illustrated by fig. 8. ×58.

All histological specimens have been stained with Ehrlich's haemotoxylin and eosin.



birth. (Dissimilar triplets should be still less responsive than dissimilar twins, and so on; it would not be surprising if grafts from a dam to any one of a group of dissimilar quintuplets lasted for several months.) What *is* surprising is not that two-egg twins should react weakly to skin from their mother, but that they should react differently. By the ordinary luck of segregation, a dam may well contain antigens present in neither of her two-egg twin offspring; but an antigen conferred upon either one is, according to our interpretation, effectively conferred upon the other as well; for the one twin does not in fact react against antigens which are present in the other though absent from itself. In short, if twins are tolerant to grafts from each other, they should be to an equal degree intolerant to grafts from their mother.

There may be two sorts of explanation for the fact that grafts from the dam survive for different lengths of time. The twins may be expected to differ in respect of genetical factors that influence their capacity to react against foreign tissue. Alternatively, it may be that foetal cell interchange is rarely if ever a symmetrical process, so that one member of the pair becomes more or less tolerant than the other. The second possibility must surely account for part of the inequality of reaction, for it is a well established empirical fact that the responses of twins to grafts from each other do indeed differ widely wherever some degree of intolerance allows it to be seen that they differ at all.

We have not yet studied the responses of one-egg twins to grafts from their mothers, though it is in the highest degree unlikely that they would differ to any appreciable degree. Nor have we done sufficient experiments to say whether the grafting of skin from a dam to her two-egg twins is a uniformly reliable method of revealing their two-egg origin, though of all the methods we have so far tested, it is the only one that looks hopeful.

5. SUMMARY

1. Thirty-six out of 42 cattle of two-egg twin birth were found to be completely tolerant to skin homografts transplanted from their respective twins. Homografts between full siblings or from dam to offspring of separate or twin birth are not tolerated.

2. All two-egg twins show some degree of tolerance to homografts transplanted from one to the other, and the degree of tolerance is widely variable.

3. Two-egg twins are not symmetrical in their responses: grafts from one twin to the other may be tolerated although grafts of the reciprocal transplantation are eventually destroyed.

4. The proportion of cattle of two-egg twin birth that show complete tolerance to grafts from their respective twins corresponds closely with the proportion of females in two-egg twin pairs of unlike sex that reveal some degree of infertility or sexual abnormality. It is therefore argued that homograft tolerance and the freemartin state share at least

one necessary causal condition in common. This condition is presumed to be the confluence of the foetal circulations of cattle twins.

5. Mutual graft exchange cannot be used as a method of distinguishing one-egg from two-egg twins in cattle.

6. In each of four trials, grafts from a dam elicited significantly different reactions after transplantation to each of her two-egg twin calves. Later experiments may reveal that this asymmetry of reponse provides the basis of a reliable method of identifying twins of two-egg origin.

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Acknowledgments.—The work described in this paper was done at Cold Norton Farm (Stone, Staffordshire), one of the experimental farms of the Agricultural Research Council's Animal Breeding and Genetics Research Organisation. Miss Jean Morpeth gave the most valuable technical assistance on all occasions, and Mr J. S. Tavernor put every facility at our disposal. We have had very helpful discussions of the work in all its stages with Dr H. P. Donald and have received valuable advice and practical help from Dr P. L. Krohn.