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EFFECT OF CORTISONE ON SURVIVAL OF SKIN HOMOGRAFTS IN RABBITS

BY

R. E. BILLINGHAM, M.A., D.Phil.*

P. L. KROHN, B.A., B.M., B.Ch.

AND

P. B. MEDAWAR, M.A., D.Sc., F.R.S.

(From the Departments of Zoology and Anatomy, University of Birmingham)

The experiments to be described in this paper were done with two purposes in mind: (a) to find out if the administration of cortisone prolongs the life of skin transplanted from one rabbit to another; and (b) to find out in what manner the administration of cortisone influences the healing, growth, and differentiation of skin grafts as such—whether transplanted from one rabbit to another or from one part to another of the same rabbit. Cortisone is known to have a profound influence on the activity of all mesenchymal tissues, and to this family belong not only the cells that mediate immunological responses, but also those that are universally and intimately concerned in the processes of repair.

There is no acceptable evidence that the epithelium of skin grafts long survives transplantation from one human being to another, and in rabbits, mice, and guinea-pigs there is fully documented evidence that it does not. This general rule forms the baseline of the present investigation.

It is known, however, to require at least three qualifying clauses. First, it has repeatedly been shown—most recently by McIndoe and Franceschetti (1950)—that skin may be successfully interchanged between human twins of monozygotic origin, and also between mice or guinea-pigs of populations whose members, by very prolonged and strict inbreeding, have come to resemble each other almost as closely as if they were monozygotic twins (Billingham and Medawar, unpublished). It would be an almost inexplicable anomaly if this were not indeed the case. Secondly, skin may also be interchanged between twin cattle of unquestionably dizygotic origin (Anderson, Billingham, Lampkin, and Medawar, 1951). The majority of dizygotic cattle twins are known to have confluent foetal circulations, and to exchange haematopoietic cells which, although of genetically foreign origin, nevertheless endure into adult life (Owen, 1945). Such twins are therefore already genetical chimeras or "graft hybrids," so it is hardly surprising that they are tolerant to grafts of each other's skin. This dispensation is not known to have any bearing on the fate of homografts in rabbits, mice, or men.

The third qualification is of equally specialized import. The rule that skin cannot be interchanged between mammals of ordinary genetic diversity need only apply to *orthotopic* grafts of skin—that is, to skin grafts transplanted to posi-

tions formerly occupied by skin. Certain positions in the body (like the brain) are known to be immunologically privileged, in the sense that homografts transplanted to such positions either cannot elicit an immune response from their hosts, or, if they do so, are protected from its consequences. This exception likewise has no practical bearing on the clinical uses of homografts of skin.

The rule formulated above related only to the behaviour of the cellular constituents of skin grafts. The fibrous elements of the corium are known to be very much more resistant to effects of homoplastic grafting, although they are in due course replaced by fibres of native (that is, autogenous) origin. In certain circumstances the epithelium of skin homografts may be surreptitiously replaced by epithelium of native origin. The appearance so produced is sometimes hard to distinguish from that of a genuinely surviving homograft, and it is to this fact that claims in the literature for the occasional success of skin homografts can probably be ascribed.

A full report has already been given of the behaviour and fate of skin homografts in rabbits (Medawar, 1944, 1945, 1946a, 1946b), and these papers form the basis of several generalizations made below about the behaviour of skin grafts in normal rabbits. The only earlier work known to us which has an immediate bearing on the present is that of Whitelaw (1951), who has described the unexpectedly long survival of skin homografts in a patient under treatment with A.C.T.H. On the other hand, Baxter, Schiller, and Whiteside (1951) have described a patient on whom the administration of A.C.T.H. had no such effect.

Methods

Material.—Adult rabbits of both sexes (see Table), weighing 2.2 to 2.9 kg., were used in the experiments. Cortisone was given by a single daily subcutaneous injection of 10 mg. of a suspension of crystalline cortisone acetate ("cortone," Merck). The cortisone was provided from a generous gift made jointly to the Medical Research Council and the Nuffield Foundation by Merck & Co., Inc. The rabbits were fed *ad lib.* on the pellet Diet 18 (Bruce and Parkes, 1946), with occasional supplements of raw cabbage.

*British Empire Cancer Campaign Fellow.

Details of Experiments

Exp.	Donor	Recipients	Autografts at 6 Days		Graft Bed (12 Days) Percentage of Area on Day of Operation	Homograft Survival Time (Days)		Day of Second- stage Operation	Period of Cortisone Treatment (Days)
			Thickness of Surface Epi- thelium (mm.)	Mean Mitoses per mm., $\pm$ Standard Error		First-stage Operation	Second-stage Operation		
1	Co-4 M	{ Co-3 M Co-5 M	0.010-0.020 0.050-0.095	1.7 $\pm$ 0.2 10.6 $\pm$ 0.6	82 49	>24 <27 7	$\sim$ 12	17	0-21 13-32
2	Co-8 M	{ Co-6 M Co-7 M	0.015-0.020 0.040-0.060	0.8 $\pm$ 0.1 11.4 $\pm$ 0.4	72 36	>20 <25 9	$\sim$ 12	21	0-25 17-39
3	Co-11 F	{ Co-9 F Co-10 F	0.008-0.012 0.040-0.045	0.3 $\pm$ 0.1 5.2 $\pm$ 0.2	63 61	27 7	$\sim$ 15	18	0-21 14-33
4	Co-14 M	{ Co-12 M Co-13 M	0.008-0.012 0.040-0.045	0.7 $\pm$ 0.1 4.6 $\pm$ 0.4	73 37	>21 <24 7	>9 <12	17	0-21 13-31
5	Co-15 F	{ Co-16 F Co-17 M			55 44	16 9			4-22 —
6	Co-18 M	{ Co-19 M Co-20 M			54 44	18 7			4-22 —
7	Co-2 F	Co-1 F				>15 <20			6-20
8	Co-1 F	Co-2 F				>40 <49			0-40

Entries in italics relate to data obtained from rabbits under cortisone treatment. Animal Co-2 received only 5 mg. cortisone per day from the 20th day onwards.

Plan of the Experiments

Each of the principal experiments (Table, Nos. 1 to 4) made use of three rabbits: two recipients, R_1 and R_2 , and their common donor D. Each experiment was done in two stages according to the following common plan (Fig. 1):

In the first stage, R_1 and R_2 each received eight skin homografts from D, and eight skin autografts as well. R_1 received the first injection of cortisone on the day before operation and was injected daily for 21-25 days thereafter. R_2 received no cortisone to begin with, and the homografts carried by it were completely destroyed within 10 days of their transplantation. R_2 being thus thoroughly immunized, daily administration of cortisone was started 13-17 days after the first operation. Four days later R_2 was made the subject of a second operation—a replica of the first, as the diagram shows, except that the eight homografts and eight autografts were transplanted to the opposite side of the body. The administration of cortisone was maintained for from 14 to 18 days after the second-stage operation. (In these apparently arbitrary choices of times we were guided by pilot experiments entered in the Table as experiments 7 and 8.)

Each such experiment made possible a controlled appraisal of the influence of cortisone on the behaviour of homografts both in a "virgin" animal, R_1 , which had received no homografts before, and in an animal, R_2 , which was specifically immune in the sense that it had already received and reacted against homografts from the same donor.

The adequacy of this experimental scheme is obvious except in one particular—the genetical relationship between animals R_1 and R_2 .

Ideally, R_1 and R_2 should be identical twins, or should be rabbits of the same age and sex from a very highly inbred line. If cortisone were without appreciable action, the homografts transplanted in the first-stage operation from D would then be expected to live exactly as long on R_1 as on R_2 .

Unfortunately, no such rabbits were available to us. Since the inheritance of the antigens controlling transplantation immunity is highly polygenic, the correct alternative was to choose three animals of great genetic disparity; in fact, in each experiment rabbits D, R_1 , and R_2 were of different breeds. In practice this provided a high degree of uniformity of response, for so great was the disparity between the donors and the R_2 recipients that the homografts broke down in all of them about as fast as homografts in rabbits are ever known to do. An added advantage of this scheme was to make the test on the effect of cortisone a particularly stringent one. It must, however, be made clear that the "null hypothesis" appropriate to such a choice of animals is, not that homografts should survive as long on R_1 as on R_2 , but simply that the homografts on R_2 should outlive those on R_1 as often as not. As it turned out, the effect of cortisone was so obvious as not to call for the refinements of statistical appraisal.

Besides the four experiments done in the manner just described, two others (Table, Nos. 5 and 6) were done in the same way except for (a) the fact that the administration of cortisone to the R_1 recipients was not started until the fourth day after operation, and (b) the omission of the second-stage operation on R_2 . In addition, certain useful information was obtained from two pilot experiments (Table Nos. 7 and 8) of simpler but less satisfactory design. These made use of two rabbits, Co-1 and Co-2: each one received eight autografts and eight homografts from the other. Co-2 received the full dosage of cortisone (10 mg./day) for the first 20 days, beginning on the day before operation, and half this dosage for a further 20 days. Cortisone treatment was not given to Co-1 until the sixth day after operation, and was continued until the 20th day.

Operative Methods

The grafts were cut in the manner of pinch grafts from the shaved skin of the dorsum of the ears: each was a thin, flat, circular disk of skin comprising the epidermis and the full thickness of the corium, and so anatomically best described as a thin Wolfe graft. Uniformity of size was achieved by defining the

outline of each graft immediately before its removal with a sharpened cork-borer of 9 mm. bore.

The grafts were transplanted to rectangular raw areas (6×7 cm.) prepared by removing the skin down to the fascia overlying the panniculus carnosus muscle on one side of the chest. Each animal received eight autografts and eight homografts, evenly spaced in a 4×4 pattern and alternating with each other in chessboard fashion. The grafts were thus transplanted in "open" style—that is, in such a manner that each could enlarge its epithelial surface by outgrowth over the raw area separating it from its neighbour or from the margin of the operation field. Dressings were applied in the way described by Medawar (1944).

The grafts were first examined six days after operation and thereafter at three-day intervals. Biopsy specimens were removed at 6, 9, 12, and 15 days after operation, and then as often as proved necessary. Measurements of the area of the operation field and (at later inspections) of the autografts were made at each inspection. Total and differential leucocyte counts were started the day before operation, and were continued for the duration of each experiment—at first daily, and latterly, when it had proved sufficient, at two- or three-day intervals.

Operations and inspections were done under pentobarbitone sodium analgesia, supplemented, if necessary, by open ether or ethyl chloride.

Computing the Survival Time of Homografts

The breakdown of homografts is very conspicuous to the naked eye (see below), but the *accurate* timing of their survival can be based only on the microscopical evidence provided by regular graft biopsies taken over the period of breakdown. A three-day serial sampling interval was adopted, and the chosen measure of "survival time" (see Table) is that time at which the breakdown of the homograft epithelium is *just* complete. An estimate of, for example, seven days' survival is to be taken to mean that breakdown was seen to be in progress and far advanced in the six-day biopsy specimen and to be long complete in that taken at the ninth day. With most of the cortisone-treated animals, however, the process of breakdown was too vacillating and long-drawn-out to justify this sort of interpolation: an entry in the Table such as " $>24 < 27$ days" simply means that there was some surviving epithelium in the sample removed at the 24th day and none in that removed at the 27th, so that breakdown must have been complete at some time between the two.

The validity of this technique of serial graft sampling has been demonstrated by Medawar (1944, 1945). It turns upon (a) the fact that epithelial breakdown occurs simultaneously and to the same degree in all the orthotopic skin grafts transplanted on one occasion from one donor to one recipient, so that (accidents of healing apart) one homograft is truly representative of the population as a whole; and (b) the fact that breakdown is a progressive and never a cyclical or vacillating process in skin homografts transplanted between animals of ordinary genetic disparity. Breakdown times can therefore be reliably computed by interpolation. Even so, the discrimination between "surviving" and "dead" epithelium is a purely histological one: how accurate it can be was shown by comparing the histological diagnosis of survival with a biological test, in which homografts at various stages of breakdown were transplanted back to their original donors and so given an opportunity to recover.

Results : (1) The Healing and Histological Reorganization of Grafts and Raw Areas

(a) The Operation Field

As earlier work (Ragan, Howes, Plotz, Meyer, and Blunt, 1949) had led us to expect, cortisone conspicuously retarded the development of granulation tissue—particularly after the second-stage operation on the R_2 animals, in which treatment had started four days before the raw area forming the graft bed was prepared. The tracks of even the finest vessels of the raw surface remained visible for as long as 12 days after operation, and sections showed that the surface of the raw area consisted of the scarcely modified fascia of the graft bed as it was first prepared. Beyond the 12th day granulation tissue began to build up very slowly, and, correspondingly, the raw surface began to accept the imprint of its immediate dressing, tulle gras.

It will be clear from the entries in the Table that cortisone treatment also retarded the contracture of the operation field: column six shows, for both R_1 and R_2 animals, the area of the general graft bed at the 12th day post-operatively, expressed as a percentage of its area on the day of operation.

(b) Autografts: Healing and Histological Reorganization

The primary healing of the grafts in the cortisone-treated animals was much weaker than in their controls. After it has been in place for six days quite a brisk tug is needed to strip a normal autograft or homograft from its bed, but under cortisone treatment a six-day graft can be removed by an action more aptly described as peeling it away. Grafts peeled off at the sixth day could simply be replaced in their beds, whereupon they healed into place and continued their development without suffering a serious setback. If this is done with normal grafts of six days' standing they usually die. What seems to be a sufficient explanation of this difference will be given below.

Weakness of healing was more obvious after the second-stage operation on the R_2 animals because, by the sixth day post-operatively, they had been under cortisone treatment for 10 days. It was often found at primary inspection that the grafts had slipped or slid from the positions to which they had originally been transplanted. On the other hand, the grafts on animals Co-16 and Co-19 had healed very little less firmly than on their respective controls, presumably because the first injection of cortisone had been delayed until the fourth day post-operatively.

The administration of cortisone suppressed the traumatic inflammation that accompanies the healing process. The blood vessels and lymphatics that penetrated the grafts on cortisone-treated animals were fewer in number and individually much smaller than in their controls. Indeed, the vascular supply of the more superficial layers of the corium was conspicuously inadequate even so late as the ninth day after transplantation. The degree of proliferation of the graft epithelium makes a very sensitive measure of the adequacy of vascularization. Normal autografts and homografts have an abnormally rich blood supply at the sixth day, and the autografts retain it for six to nine days longer or even more. At the sixth day the graft epithelium displays a tremendous outburst of cellular proliferation, which subsides slowly as the vascularization returns to normal. The epidermis of the

grafts, 15–20 μ thick on first planting and containing either one or two layers of living cells, thickens to between 40 μ and 100 μ by the sixth day and acquires the differentiation into strata that is to be seen, for example, in human skin.

It is quite otherwise with grafts from the cortisone-treated animals, which at the sixth day may still be described as indolent or vegetative. As the Table shows, mitotic activity is negligible and the epidermis is no thicker than on the day of grafting (cf. Green and Ghadially, 1951).

Beyond the sixth day the difference becomes smaller, because mitotic activity in the control grafts slowly subsides and that in the cortisone-treated grafts slowly gains momentum.

The mitotic indolence of cortisone-treated grafts explains well enough why, if they are peeled off at the sixth day, they will develop without a severe setback upon replacement. There is no less reason why they should do so on this occasion than upon first grafting. A normal autograft cannot be so treated: as Peyton Rous first showed (1946), and we have repeatedly confirmed, an "active" graft with rapidly proliferating epithelium has particularly exacting metabolic requirements, and it will not withstand prolonged interruption of its blood supply.

Although the inhibitory effect of cortisone on epithelial mitosis might be due to a direct action, our own results can be equally well explained as being a secondary consequence of the effect of cortisone on the dermal blood supply. Mitotic activity in the epithelium of the graft seems to be unawakened rather than suppressed, and when in the ordinary course of growth the epithelium spreads from the graft over the surrounding raw surface it promptly thickens and shows brisk mitotic activity. Yet in this situation, because of its better blood supply, cortisone should have more immediate access to the epithelium. Moreover, the epithelium of the grafts on animals Co-16 and Co-19 was vigorously hyperplastic at the sixth and ninth days: cortisone treatment had not been started until the fourth day after grafting, so that the vascularization of the grafts was normal. This is telling evidence that the indolence of the graft epithelium in cortisone-treated animals is principally the consequence of an inadequate blood supply.

As the foregoing observations might lead one to expect, the administration of cortisone retarded the normal processes of histological reorganization within grafts. The hairs transplanted with normal grafts are thrown off in the course of the proliferation of the epithelium of the follicles, which become cystically dilated and subsequently open out widely on to the graft surface. The primordia of new hairs are normally first visible in a graft of 10 to 12 days' standing, and a fine new pelt of hairs will have pierced the graft surface by the 20th day. In grafts on the cortisone-treated animals the original hair shafts remained in place for longer and the primordia of new follicles first appeared between the 20th and 25th days. A cortisone-treated graft thus always looks more backward and immature than its normal contemporaries.

Normal autografts, if pigmented to begin with, invariably show a transient depigmentation during their proliferative phase. This is known to be causally connected with their high rate of epithelial proliferation, and is due either to the inability of epidermal melanophores to keep pace in division with their Malpighian-cell neighbours, or to the temporary diminution of pigmentary activity in rapidly dividing melanophores, or probably to both causes. Either explanation is con-

sistent with the fact that grafts on cortisone-treated animals show little if any depigmentation and are thus more deeply pigmented than their controls. Evidently the administration of cortisone does not *inhibit* pigmentary activity, and its enhancement may be a secondary consequence of their subdued mitotic activity.

(c) Autografts: Outgrowth and the Properties of Intergraft Epithelium

By the sixth day from planting, both normal and cortisone-treated autografts had given rise to a 0.75- to 1.25-mm.-wide annulus of migratory skin epithelium, though, being at this stage poorly keratinized, it was hardly visible to the naked eye. Beyond the sixth day, outgrowth from the cortisone-treated grafts fell much behind. It often happened that outgrowth from the grafts, having extended so far as 3–4 mm. from the perimeter, made little further progress for the subsequent week or more. The perimeter of this stationary ring of epithelium was bluntly rounded, so giving the impression that the epithelium was physically unable to make progress over an unsuitable substratum.

It was the rule with cortisone-treated animals that, although vascular and fibroblastic activity and the laying down of new fibrous tissue were retarded in that part of the general graft bed that was still exposed and raw, capillary and fibroblastic proliferation developed promptly under epithelial cover. Thus the epithelium spreading from the grafts never more than briefly lay upon the almost unmodified fascia that forms the floor of the operation area, and came to be separated from it by a wide stratum of young fibrous tissue, which collagenized in the usual way, if perhaps more slowly. This effect was most conspicuous whenever epithelial migration was retarded in the way that has just been described: the building up of fibrous tissue under the stationary ring of epithelial outgrowth caused the graft to be surrounded by a raised-up, thickened tyre.

Everything that has been said of epithelial outgrowth from the grafts applies also, of course, to epithelial ingrowth from the margins of the wound. The combination of the two processes eventually provides the entire field with epithelial cover. The cover consists in part of intergraft epithelium (i.e., that which bridges the grafts) and in part of the epithelium of the grafts themselves. The intergraft epithelium is unstable, and the area it occupies slowly disappears; when the process is complete the autografts, variously changed in shape, are approximated edge to edge and to the edges of the original operation field, which is now no larger than can just accommodate them. The entire process is usually referred to as "contracture," but measurements have shown that the loss of intergraft epithelium is concurrently made good by the *expansion* of the grafts as well: expansions of area between threefold and fourfold have been recorded even in animals that have received autografts alone, so it clearly does not depend upon the alternation of autografts with homografts, as in our normal experimental design. Expanded grafts are thinner and more transparent than on first planting; during expansion the mouths of the hair follicles become separated to an extent proportional to the degree of linear magnification, and the hair pelt becomes correspondingly more sparse.

The operation field in cortisone-treated animals underwent the same sequence of changes, though so retarded as to take two or three times as long. The retardation of contracture that has already been

referred to temporarily stabilizes the intergraft epithelium. The grafts therefore remain separated for 20 days or longer by an epithelium which is flat, shiny, and (because of the development of fine cuticular fissures in an epithelium unwaxed by sebaceous secretion) of somewhat crackled or marbled appearance.

The results of sections (b) and (c) may be summarized by saying that the administration of cortisone retards the sequence of processes consequent upon grafting and the healing of raw areas without either modifying them in kind or affecting their final outcome.

(d) Graft Donor Areas

The ears of the untreated rabbits showed the expected sequence of changes. Within 24 hours they were somewhat swollen at the base and hyperaemic, and each graft donor area was seen by transmitted light to be surrounded by a halo of dilated vessels. Thick and rather nobbly scabs formed on them and their edges became puckered. The scabs could be picked off—or they dropped off—by the 10th or 12th day to reveal a fully epithelized surface, sometimes with a small central fibrous knob. The knob was larger in those donor areas from which scabs had been prematurely picked away.

The ears of cortisone-treated rabbits were at no time swollen or unduly warm, and the donor areas showed no inflammatory halation at any stage. The raw surfaces remained damp with serous exudate for one or two days until a thin, flat, parchment-like scab formed over them; their edges remained unpuckered. The scabs were attached more strongly and remained in place for longer than on the untreated animals. When epithelization was complete (we have no accurate information about its rate of progress) the donor areas proved to be uncontracted and, so far from showing any central fibrous nodules, were recessed below the plane of the surrounding skin.

(e) Striae

Typical white striae were found on the 25th day after operation in the skin surrounding the operation field on animal Co-2. Striae were found on no other rabbit.

(f) The Blood Picture

The immediate effect of the grafting operation alone is a rise in the total white cell count made up of a small increase in the number of granulocytes and a much larger increase in the number of lymphocytes (Fig. 2). When cortisone treatment is combined with the operation the number of lymphocytes, instead of rising, falls, owing to the lympholytic action of the cortisone, while the number of granulocytes increases (Fig. 3). The level of the overall white cell count varies according to the way in which these

two effects balance out. The lymphocyte count remains low throughout the treatment with cortisone, and the normal blood picture is restored about 10 to 14 days after the last injection.

Results: (2) The Behaviour of Homografts

Controls

Homografts exchanged between animals of remote genetical affinity, such as those deliberately chosen for the experiments described here, elicit a rapid and violent reaction. Their primary healing is as sound as with autografts, and, like autografts, they enter a period of vigorous cellular hyperplasia. The changes they then undergo are briefly as follows. The grafts swell and harden; instead of being supple and yielding, they move as a whole, when poked, if they move at all. Meanwhile their colour changes from the delicate pink that is evidence of vascularization to brick-red and then to various shades of dirty yellow, green, or brown. The surface of the graft, instead of being white, firm, and dry, becomes damp and pasty, and the epidermis may then be peeled off to reveal the

FIG. 2.—Rabbit Co-5. Total white cell, granulocyte, and lymphocyte counts.

FIG. 3.—Rabbit Co-2. Total white cell, granulocyte, and lymphocyte counts.

haemorrhagic and exuding surface of the corium below. The perimeter of the graft, at first firmly united to the graft bed, becomes free all round. In the course of a few days the graft turns into a hard, dry scab; a tide of autogenous epithelium flows round it which, by biting into the deeper layers of the corium or into the surface of contact between graft and bed, gives the graft the appearance of a miniature mushroom attached to the bed by a stout fibrous stalk. When this is finally cut through by the further advance of native epithelium the mummified remains of the homograft simply drop away, leaving a small, smoothly rounded fibrous scar as evidence of its original position.

Internally, the process of breakdown is accompanied by a very violent inflammatory reaction in which the lymphocyte is the most abundant member of the invading mass of leucocytes. Destruction of the graft is accompanied by the stagnation and disruption of its grossly dilated and engorged blood vessels, the choking of lymphatics with miscellaneous cellular debris, and, finally, the breakdown of every living element in the graft, whether of native or foreign origin. In spite of the violence of the reaction, two to three days may pass between the first signs of epithelial erosion and the disappearance of the last traces of surviving epithelium. It is this latter time that has been used for computing the homograft survival time (see above).

Reference to the Table will show that no homografts in rabbits untreated with cortisone survived their transplantation for longer than nine days. (The data of Medawar (1944) gave a median survival time of 10.4 ± 1.1 days for skin homografts exchanged in about the same dosage between rabbits of a *randomly* chosen heterogeneous stock.)

Homografts in Animals Under Cortisone Treatment

The deliberate choice of animals of wide genetic disparity has subjected the effect of cortisone to a very exacting test. To translate our figures for survival times to those more appropriate to the human being (cf. Padgett, 1932; Gibson and Medawar, 1943; Baxter and Entin, 1951) they should be multiplied by a factor of three or four.

The results of the first-stage operations upon the cortisone-treated animals in experiments 1 to 4 will be considered first: in these animals the administration of cortisone began on the day before operation and continued for 21 to 25 days thereafter.

The administration of cortisone converted the acute and rapidly progressive reaction shown by the controls into a feeble, chronic reaction of delayed onset and cyclical or intermittent progress. The survival time of the homografts was prolonged by a factor of three or four (see Table). It will be seen that in animal Co-6 (as also in animals Co-16 and Co-19 of a different series) the homografts finally "died" within the term of cortisone administration, and that in no animal did the homografts live more than a few days after the last injection of the daily series. The vacillating nature of the reaction was made particularly clear by animal Co-9, in which the 21-day homograft showed a higher degree of survival than the homografts removed at the 18th and 15th days.

The inflammatory processes that accompany the normal homograft reaction were also proportionately subdued; oedematous swelling was delayed and

lessened, and the lymphocytic infiltration, instead of being massive, was merely sparse and spotty. In general, the reaction was diminished in intensity without being altered in kind.

In experiments 5 and 6 the administration of cortisone began on the fourth day after operation, and in experiment 7 on the sixth. The six-day biopsy specimens from these three animals showed that a specific reaction had already begun. Nevertheless, the administration of cortisone approximately doubled their expected survival times.

The prolongation of the life of homografts by cortisone might be due to one, or a combination, of the following causes: (a) the homografts' diminished ability to *elicit* a reaction, as a result of the general retardation of healing, vascularization, and proliferative activity that has been seen to apply to autografts as well. That this plays an appreciable part can hardly be doubted (see below). There are several reasons why it cannot be the whole explanation, the most obvious being the prolongation of the life of the homografts on animals Co-1, Co-16, and Co-19: in these animals delay in the first administration of cortisone allowed the grafts to become as fully vascularized and as "active" as their controls. (b) It might be due to the partial suppression of the recipient's systemic immunological response, or (c) to the thwarting by cortisone of the local inflammatory and other cellular activities that accompany the breakdown of foreign epithelium and may be causally connected with it.

The second-stage operation on animals Co-5, Co-7, Co-10, and Co-13 makes possible a distinction between causes (b) and (c): these rabbits were already highly immune when cortisone treatment was started and when they received their second set of homografts. Skin homografts transplanted to an untreated rabbit that has been immunized by an earlier grafting from the same donor are known to respond in a highly modified way: epithelial proliferation is suppressed, vascular stagnation is precocious, and breakdown is very much accelerated. Upon this "normal" response must be superimposed the non-specific effect of cortisone that has already been mentioned—the retardation of healing and proliferative activity that affects autografts as well.

Homografts transplanted to pre-immunized but cortisone-treated animals remained indolent throughout their lives; they were always partially broken down by the ninth day, and in only one animal, Co-10, could it be said that there was a trace of surviving foreign epithelium at the 15th day. The response was long-drawn-out and the timing of its completion proportionately difficult and uncertain; the figures entered in column 8 of the Table are therefore much rougher approximations than those entered for the first-stage operation (column 7). Histologically, the grafts resembled "normal" second-stage homografts very closely. If some allowance is made for the delay in vascularization produced non-specifically by cortisone (that is, for reasons unconnected with the behaviour of homografts as such) then it can be fairly inferred that cortisone did not significantly prolong their lives.

Our provisional conclusion is therefore that cause (b) is the principal one, that cortisone prolongs the life of homografts by delaying the development of the systemic immune state rather than by preventing its local fulfilment. This is confirmed by the reduced

effectiveness of cortisone treatment in those animals (Co-1, Co-16, Co-19) in which its administration was not started until some degree of immunity had already been provoked.

The fact that cortisone prolongs the life of homografts and diminishes the inflammatory reaction that accompanies their breakdown has one rather deceptive secondary consequence: it facilitates the insidious creeping replacement of the homograft epidermis by epithelium of native origin. It was the general rule in our experiments that the fibrous elements of homografts in rabbits under cortisone treatment were progressively resurfaced by native epidermis. The appearance so produced was sometimes very difficult to distinguish by naked eye from that of a surviving homograft. In our control animals, the homografts broke down too quickly for the tide of native epithelium to have reached their margins, and the corium was in any case so poorly vascular (if vascular at all) as to make a physically unsuitable substratum for epithelial migration. Such homografts were invariably undermined, not overgrown.

Clinical Bearing

It need hardly be said that the response of the rabbit to cortisone, as we have described it above, may be very different from that of the human being. Species differences are to be expected, and our colleague Miss E. M. Sparrow finds that relatively higher doses of cortisone (5 mg./day) administered to guinea-pigs prolong the life of homografts to a somewhat less degree. With this qualification in mind we may now single out certain consequences of the administration of cortisone that may prove to have a specially direct bearing on the surgery of skin repair. The principal effect of cortisone in the experiments we have described has been to *delay* the onset and progress of certain processes mediated by mesenchymal cells; its principal benefaction in the surgery of repair is therefore likely to be to give the surgeon extra *time*, or to bring the tempo of certain naturally occurring processes under more immediate control.

Prolongation of the Life of Homografts.—The possible usefulness of this requires no comment. One of its secondary consequences is the special ease with which autogenous epithelium will overgrow (instead of undermining and throwing off) the homograft dermal fibres. Mere epithelial cover is well known to be insufficient to arrest wound contracture. It may be that epithelium is stabilized for a worth-while extra time by being caused to overgrow what becomes, in effect, a homograft of dermal fibres. To bring this about, auto-grafts and homografts should obviously be so grafted as to lie in alternation. This is the pattern of grafting adopted in the experiments we have described and already made use of, where it is appropriate, in clinical practice (Mowlem, 1951, personal communication).

Retardation of Wound Contracture.—Fibrous contraction of the wound takes place more slowly. More time is therefore available to avoid the cosmetic difficulties due to dense scar formation.

Delay of Healing.—The weakness of the primary union of graft to bed under cortisone treatment might be thought to be a serious disability, by no means wholly compensated for by the fact that a displaced graft can be made to resume its growth and histological reorganization by merely reapposing it to its bed. One solution for this difficulty might be to allow

three or four days to pass between grafting and the first administration of cortisone. It is true that four days' delay curtailed the life of homografts on rabbits (experiments 5 and 6 by contrast to experiments 1 to 4), because it allowed the building up of an effectively high degree of systemic immunity. In the common run of human surgical practice, however, the homograft reaction is slower to begin, though the rate of healing of grafts is much the same. Four days' delay between grafting and the first administration of cortisone might therefore make sound healing possible without at the same time curtailing the life of homografts.

Granulation Tissue.—The "raw area" that forms the general graft bed in cortisone-treated rabbits granulates very slowly and makes a physically poor substratum for the migratory outgrowth of epithelium.

Summary

Experiments have been designed to examine the effect of the administration of cortisone upon the healing, growth, and histological reorganization of skin autografts and upon the survival time of skin homografts in rabbits.

The daily subcutaneous administration of 10 mg. cortisone acetate to adult rabbits of either sex weakens the primary healing of grafts, delays their vascularization and the outburst of cellular proliferation consequent upon it, and retards their histological reorganization.

The contracture of graft-bearing areas is retarded by cortisone treatment, but proceeds eventually to the same degree as in untreated animals.

The administration of cortisone lengthens the life of skin homografts in rabbits by a factor of three or four.

The prolongation of the life of homografts is thought to be partly due to a non-specific reduction of the graft's own ability to elicit an immune response, but to be mainly due to the reduction of the response itself.

The survival time of homografts in rabbits already made specifically immune by an earlier grafting from the same donor is little lengthened by the administration of cortisone. It is therefore argued that cortisone affects the systemic development rather than the local fulfilment of the immune response.

[The numerical data that have been compiled, and a fuller description of our histological findings, will be included in another publication.]

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A Reuter report from Kharkov, in the Ukraine, states that 30,000 Soviet citizens are over 100 years old. "There are very few examples of such longevity in Capitalist lands," the message adds.