The problem of storage of tissues may demand an elaborate solution.

The future of the homotransplant problem appears to be in the hands of serologists.

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EFFECT OF LOCALLY APPLIED CORTISONE ACETATE ON SURVIVAL OF SKIN HOMOGRAFTS IN RABBITS

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It has already been shown (Billingham, Krohn, and Medawar, 1951) that the daily systemic administration by intramuscular injection of 10 mg. of cortisone acetate to rabbits prolongs threefold or fourfold the lifetime of skin grafts that have been transplanted to them from other rabbits. It will now be shown that the direct local application of no more than 5 mg. of cortisone acetate every third day to such homografts will at least double their expected survival time. This effect has been found to be a genuinely local one—it is not due merely to the absorption of cortisone into the circulation—but to be very much weaker in rabbits that have been made immune by an earlier grafting of skin from the same donor or by a concurrent grafting of skin to which cortisone has not been locally applied.

Cortisone is known to bring about a well-defined eosinopenia after absorption through the skin of adrenalectomized mice (Speirs, 1951) and to cause atrophic changes of the dermis after local application to the skin (Castor and Baker, 1950). The subconjunctival injection of quantities of cortisone far too small to have a significant systemic effect has been used with success in the treatment of inflammatory conditions of the eye (Koff, Rome, Kaspe, Commons, Button, and Starr, 1950; Jones and Meyer, 1950).

Methods

Save where some variant is specially mentioned, we have continued to use the scheme of experiments described in our earlier paper. Each test made use of three rabbits chosen for their genetic disparity; one served as a donor, and eight circular ear skin grafts 9 mm. in diameter were transplanted from it to each of the other two. The homografts were transplanted to a large rectangular raw area stripped down to the level of the panniculus carnosus on the skin of the chest, and were so arranged as to alternate in chessboard pattern with eight autografts cut from the ears of the respective recipients. One recipient acted as control, receiving either no treatment or an injection or local application of the aqueous vehicle ("control fluid") in which the cortisone was suspended. (The cortisone acetate was provided from a generous gift made jointly to the Medical Research Council and the Nuffield Foundation by Merck and Co., Inc. The control fluid was a saline solution containing 1.5% benzyl alcohol and other unspecified suspending agents.)

The survival time of the homografts was estimated, as hitherto, by the histological analysis of biopsy specimens removed at three-day intervals over the period of breakdown: it represents the time at which the destruction of the foreign skin epithelium is just complete. (For

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reasons that will be explained later, a slightly modified scoring system was used in some of the tests comprising experiments 3 and 4.) We have continued to use three subsidiary quantitative measures of the action of cortisone: (a) the degree of contracture of the prepared recipient area, measured by expressing its area at the twelfth day post-operatively as a percentage of its area on the day of operation; (b) the inhibition of mitosis in the epithelium of autografts of six days' standing; and (c) alterations in the total and differential white blood cell counts.

The "mitotic frequency" recorded in the accompanying Tables represents the mean number of mitoses counted in each 1-mm. strip of superficial graft epithelium and in the epithelium of the follicles underlying it. We have no evidence to qualify our belief that the mitotic indolence of the epithelium of grafts transplanted to cortisone-treated animals is a secondary consequence of the diminution of their dermal blood supply.

Controls

The lifetime of homografts transplanted between genetically very dissimilar rabbits and in the dosage we have used lies very uniformly within the range seven to nine days. This is made clear by Table I, which brings together the results recorded in our earlier paper and elsewhere in the present paper. The selection of such genetically dissimilar animals has some disadvantages—for example, the immunity elicited by first-stage homografts causes them to break down so fast that the curtailed lifetime of homografts transplanted to pre-immunized rabbits tends to be concealed numerically; but it has the overriding advantage of making the test on the effect of cortisone as exacting as it can be.

TABLE I.—Summary of Control Rabbits

Donor	Recipient	Mitotic Frequency of Autografts ($\pm$ St. Error)	Graft Bed Area, 12 Days After Operation. (% of Original Area)	Homograft Survival Time (Days)
Co-4 ..	Co-5 M	10.6 $\pm$ 0.6	49	7
Co-8 ..	Co-7 M	11.4 $\pm$ 0.4	36	9
Co-11 ..	Co-10 F	5.2 $\pm$ 0.2	61	7
Co-14 ..	Co-13 M	4.6 $\pm$ 0.4	37	7
Co-15 ..	Co-17 M	Not done	44	9
Co-18 ..	Co-20 M	Not done	44	7
Co-23 ..	Co-25 M	4.4 $\pm$ 0.3	51	8
Co-26 ..	Co-28 F	9.2 $\pm$ 0.5	52	14*
Co-30 ..	Co-31 F	5.8 $\pm$ 0.3	51	8
Co-33 ..	Co-34 F	7.8 $\pm$ 0.3	42	8
Co-35 ..	Co-38 M	6.6 $\pm$ 0.4	31	6
Co-39 ..	Co-42 M	4.4 $\pm$ 0.3	36	7
Co-50 ..	Co-52 F	8.8 $\pm$ 0.6	41	9
Co-53 ..	Co-54 F	3.9 $\pm$ 0.2	52	8
Co-62 ..	Co-64 M	11.9 $\pm$ 0.6	48	7
Co-65 ..	Co-67 M	4.2 $\pm$ 0.2	48	7

* See text for notes on animal Co-28.

Table I contains one conspicuously anomalous result—the homografts transplanted to Co-28 lasted half as long again as on any other control rabbit, and twice as long as on many. Co-28 was the only pregnant rabbit used in our experiments, and it delivered a litter of four normal young on the thirteenth day after operation. Since cortisone prolongs the life of foreign homologous tissue and is thought to be secreted in increased amounts during pregnancy, some increase in survival time of homografts might be expected. This has raised in our minds the possibility that an increased production of cortisone-like adrenal steroids during pregnancy may protect the mother against iso-immunization by the foreign homologous tissue of its foetus. The idea that there is a peculiar connexion between the activity of

the adrenal cortex and the mammalian mode of gestation is not a new one (cf. Elliott and Tuckett, 1906).

The mitotic frequencies of six-day autograft epithelium are clearly very variable; they are distributed, markedly askew, about a modal frequency class of 4-6 and a mean of 7.1 mitoses per mm. Although the range of variation is great, the mitotic frequencies of six-day autografts on many cortisone-treated rabbits fall well below the lowest of them.

The areas of the general graft bed at the twelfth day post-operatively are distributed more or less symmetrically about a mean of 45% of the area of the general graft bed on the day of operation, with a standard deviation of 7.8. An area lying outside the range 30-60% would be expected to turn up by luck of sampling not more often than once in 20 trials.

Experiment 1: Effect of Locally Applied Cortisone

This experiment comprises the four independent tests summarized in Table II. The entries relating to the cortisone-treated animals are in italics and to their controls in roman type.

The first local application of cortisone was made by spreading 5 mg. of cortisone acetate in 0.2 ml. of suspending fluid with a glass rod over the general graft bed, after which the eight homografts and eight autografts were laid upon it. (Animal Co-32 received only 2.5 mg. of cortisone throughout.) Dusting the area with sterile sulphadiazine powder helped to secure an even distribution of cortisone by taking up some of the excess suspending fluid. The usual dressings, including an ordinary fine-meshed tulle gras, were always used. The animals were inspected and re-dressed every third day, when a further dose of 5 mg. (or 2.5 mg.) of cortisone was spread over the operation field and, of necessity, over the healed grafts. Applications were continued until the homografts had finally broken down.

The control recipients were treated in exactly the same way except for the substitution of 0.2 ml. of control fluid for the cortisone.

TABLE II.—Effect of the Local Administration of 5 mg. of Cortisone Acetate Every Third Day (Co-32: 2.5 mg.). The Results Relating to Cortisone-treated Animals are Italicized.

Donor	Recipient	Mitotic Frequency of Autografts ($\pm$ St. Error)	Graft Bed Area, 12 Days After Operation (% of Original Area)	Homograft Survival Time (Days)
Co-30 ..	{ Co-29 F Co-31 F	1.1 $\pm$ 0.2 5.8 $\pm$ 0.3	72 51	>18 <21 8
Co-33 ..	{ Co-32 F Co-34 F	0.4 $\pm$ 0.1 7.8 $\pm$ 0.3	51 42	16 8
Co-50 ..	{ Co-51 F Co-52 F	5.5 $\pm$ 0.5 8.8 $\pm$ 0.6	58 41	>18 <21 9
Co-53 ..	{ Co-55 F Co-54 F	2.9 $\pm$ 0.2 3.9 $\pm$ 0.2	81 52	>18 <21 8

The local application of cortisone depressed the proliferative activity of the autografts (Table II, column 3), retarded the contraction of the general graft bed (column 4), and at least doubled the lifetime of the homografts (column 5). So much the Table makes clear. In addition, the local application of cortisone strongly inhibited the building up of granulation tissue, weakened the primary healing of the grafts, and retarded the outgrowth of epithelium over the general graft bed. (We again observed, however, that fibroblastic proliferation and the laying down of young fibrous tissue started promptly under such epithelium as had

grown outwards from the grafts, although the rest of the raw area, still unsurfaced, failed to build up granulations.) In all these respects the local application of cortisone had the same consequences as the systemic. The autograft donor sites on the ears, however, failed to give evidence of the systemic action of such cortisone as may have been absorbed through its area of local application; as in the control animals, they quickly became puckered and scabbed, and formed nobbly fibrous scars. Nor did the white blood cell picture deviate significantly from normal.

Experiment 2 : Application of the Same Dosage of Cortisone Systemically

It will now be shown that the dosage of cortisone which prolongs the life of homografts when applied to them directly is almost wholly ineffective when allowed to reach the grafts only via the systemic circulation, after absorption from subcutaneous injections or from another prepared raw area.

In tests 1 and 2, symmetrically disposed raw areas were cut from *both* sides of the chest; eight homografts and four autografts were transplanted to one area, and 5 mg. of cortisone was applied every third day to the other. In tests 3 and 4, 5 mg. of cortisone was injected every third day under the skin of the thigh.

TABLE III.—Effect of the Administration of 5 mg. of Cortisone Every Third Day, Either to a Contralateral Raw Area (Tests 1, 2) or Subcutaneously (Tests 3, 4). The Results Relating to Cortisone-treated Animals are Italicized. Controls Received Applications or Injections of 0.2 ml. of Control Fluid

Test	Donor	Recipient	Mitotic Frequency of Auto-grafts ($\pm$ St. Error)	Graft Bed Area, 12 Days After Operation. (% of Original Area)	Homograft Survival Time (Days)
1	Co-35	Co-36 M	2.9 $\pm$ 0.1	37*	7
		Co-38 M	6.6 $\pm$ 0.4	31	6
2	Co-39	Co-40 M	1.1 $\pm$ 0.2	39*	9
		Co-42 M	4.4 $\pm$ 0.3	36	7
3	Co-62	Co-63 M	3.8 $\pm$ 0.1	41	12
		Co-64 M	11.9 $\pm$ 0.6	38	7
4	Co-65	Co-66 M	1.4 $\pm$ 0.2	56	8
		Co-67 M	4.2 $\pm$ 0.2	48	7

* The figures refer to the graft-bearing area—i.e., the one which did not receive cortisone.

The results are summarized by Table III. Comparison of the italicized entries (cortisone-treated animals) with the others show that the systemic or indirect application of 5 mg. of cortisone every third day has had very little effect on the life of the homografts or on the area of the graft bed on the twelfth day after operation. In all four trials, however, the homografts on the cortisone-treated animals outlived their controls, while the raw areas of animals given cortisone contralaterally were also less contracted. Such a result would be reproduced by luck not more often than once in every sixteen such sets of trials, and provides weak evidence that the cortisone had some slight effect. It will also be seen that the cell-division frequency (column 4) in six-day autografts was significantly lower in the cortisone-treated animals than in their controls. Since the white blood cell count revealed no such well-defined difference, the mitotic count appears to be the more sensitive indication of the action of cortisone.

In spite of these minor reservations, comparison of Table II with Table III shows quite clearly that the prolongation of the life of homografts by the local

application of cortisone does not depend on the absorption of cortisone into the systemic circulation. The results of later experiments fully confirm this judgment.

Experiment 3 : Cortisone Locally Applied to Homografts on Specifically Pre-immunized Rabbits

Four control animals (Co-31 and Co-34 from experiment 1, Table II; Co-38 and Co-42 from experiment 2, Table III) were now made the subjects of second-stage operations. Fifteen or seventeen days after their first operation—that is, about a week after the homografts transplanted upon that occasion had finally broken down—eight homografts were again taken from each of their respective donors. Symmetrically disposed raw areas were then cut from both sides of the chest of each recipient and four homografts were transplanted to each side. Cortisone (5 mg. in 0.2 ml. of control fluid) was applied every third day to one of the graft-bearing areas, and 0.2 ml. of control fluid to the other.

The practice of subdividing the homografts into two sets at the second-stage operation and applying cortisone to only one of them made it possible to compare the survival time of cortisone-treated second-stage grafts (the italicized entries of columns 6 and 7, Table IV) not only with that of the first-stage grafts from the same donor (column 3) but also with that of their untreated second-stage contemporaries on the other side of the chest (entries in roman type, columns 6 and 7). This operative procedure is not objectionable on immunological grounds, because the recipients were (and were intended to be) highly immunized, and the untreated second-stage grafts could only have reinforced the immune state already in being.

TABLE IV.—Effect of Locally Applied Cortisone (Italicized Entries) on the Lifetime of Homografts Transplanted to Pre-immunized Rabbits. Each Recipient Bore Four Homografts on Each Side of the Chest; 5 mg. of Cortisone was Applied Every Third Day to One Side Only

Donor	Recipient	1st-stage Survival Time (Days)	Day of 2nd-stage Operation	Side	2nd-stage Survival Time (Days)	90% Breakdown Time (Days)
Co-30	Co-31 F	8	17th	{ Right Left	>9 <12 <6	>6 <9 <6
Co-33	Co-34 F	8	17th	{ Right Left	15 7	>12 <15 7
Co-35	Co-38 M	6	15th	{ Left Right	8 <6	>6 <9 <6
Co-39	Co-42 M	7	15th	{ Right Left	15 7	9 7

The results summarized by Table IV show that the local application of cortisone to grafts on specifically immunized rabbits has much the same degree of effect as the systemic administration of 10 mg. per day, described in our earlier paper (Billingham *et al.*, 1951). It also has the same kind of effect on the histological appearances of the grafts.

Taken at their face value, the numerical results entered in column 6 indicate that cortisone brings about a very well defined prolongation of survival. Unfortunately, the bald numerical results are misleading. Breakdown had started in the cortisone-treated grafts of three animals by the sixth day and was far advanced by the ninth day in all four of them. There was no epithelial outgrowth from any of the homografts. The grafts were flat and diaphanous, and looked quite dead from the ninth day onwards. The graft vessels broke down and their lumina disappeared, leaving the grafts as thin avascular collagenous pads which

could be peeled away from their beds without bleeding. Yet, in spite of these disabilities, the homograft epithelium on three animals survived for many days as a thin, wholly indolent surface film one or two cells thick. The epithelial cells showed the ischaemic changes (swelling and neutrophilic reaction of the cytoplasm and chronically incipient nuclear pyknosis) often to be seen in unvascularized skin grafts lying in the anterior chamber of the eye (Medawar, 1948a) or in skin cultivated *in vitro* under a gas phase insufficiently rich in oxygen (Medawar, 1948b).

To discount this confusing effect, the entries in the last column of Table IV have been computed for a 90% breakdown time instead of for a 100% breakdown time. This minor adjustment of scoring does not influence the estimates of survival for the homografts on the control sides, because their breakdown was both quicker and regularly progressive. (The precocious breakdown of graft vessels and the survival of the epithelium, for as long as it does survive, in a wholly indolent or vegetative state, is the rule in ordinary untreated second-stage homografts; cortisone treatment makes the breakdown a more long-drawn-out process.)

From this supplementary evidence, which the numerical estimates for survival time do not bring to light, it may be argued that locally applied cortisone does *not* prolong the life of homografts on specifically pre-immunized animals either in the same way or to anything like the same degree as it prolongs their life on "virgin" animals. That it has *some* slight local effect on such grafts is made clear by comparing the entries for the left-hand and right-hand sides of the body. These conclusions are reinforced by the results of experiment 4.

Experiment 4: Cortisone Locally Applied to Homografts on Animals Undergoing Independent Concomitant Immunization

In the experiments described in the preceding section cortisone was locally applied to homografts on animals that had been specifically immunized before the grafts were transplanted to them. It now appeared to us important to examine the effect of cortisone locally applied to homografts on "virgin" animals but subjected to a vigorous *concomitant* immunization. In effect, this simply entailed preparing *two* raw areas on a hitherto untreated animal, transplanting homografts to both of them but applying cortisone to only one. This procedure differs in several qualitative ways from that in which homografts are transplanted to pre-immunized animals, one of the most important being

that the cortisone-treated grafts have time to acquire a functionally adequate blood supply before being overwhelmed by the immunity reaction provoked by their untreated contemporaries.

If our general interpretation is correct—that cortisone acts mainly by retarding the development of an immune state rather than by preventing its local fulfilment in the grafts—then (a) the cortisone-treated grafts of this experiment should have much shorter lives than those of experiment 1, in which cortisone was applied to *all* the grafts borne by their recipients; and (b), a corollary of (a), the difference between the survival times of the homografts on the treated and untreated areas should be small, and in any event much smaller than the differences revealed by the experimental and control animals of experiment 1. (As with experiment 3, however, such differences as there are must reveal the effect of cortisone in preventing the local expression of the immune state.) The results of the eight tests summarized by Table V show that these expectations were fulfilled.

The eight tests that comprise experiment 4 were subdivided into two groups of four, according to whether the two raw areas prepared on each animal lay one on each side of the chest ("bilateral operations") or both on one side of the chest ("unilateral operations"). The intention was that the two sets of homografts should have, in the bilateral operation, a completely separate lymphatic drainage, and in the unilateral operation a common drainage. It seemed to us a reasonable guess that the draining lymph nodes play an important part in the building up of immunity against homografts and that cortisone, when locally applied, may act in some degree by incapacitating the draining nodes. If both these guesses were true, the untreated homografts of the unilateral operation should live as long as the cortisone-treated grafts; both sets should live as long, or almost as long, as homografts do when *all* are treated with cortisone, as in experiment 1; and both sets should outlive the homografts of the animals operated upon bilaterally. Since Table V shows that these expectations were not fulfilled, the subdivision into bilateral and unilateral operations may be disregarded.

In practice, as Table V shows, the animals operated upon bilaterally and unilaterally were paired by a common donor. Sixteen grafts were taken from the donor and four grafted to each area on each recipient. The bilateral raw areas were symmetrically disposed on each side of the chest; the unilateral raw areas were rectangles of the same size, elongated dorso-ventrally, and lying symmetrically about the lateral thoracic vessel group. The two unilateral areas were separated from each other by a "vaselined" wall of normal skin not less than 1 cm. wide; cortisone was always applied to the homografts on the anterior area. The dosage of cortisone was the same for all animals—5 mg. applied locally

TABLE V.—Effect of Cortisone Applied Locally to Only One of Two Separate Sets of Homografts Carried by Each Recipient (*Italicized Entries*). Co-61 Died on the Ninth Day after Operation. The Survival Times of the Homografts from Donors Co-44 and Co-47 were Computed on a "90% Breakdown" Basis

Bilateral Operations					Unilateral Operations			
Donor	Recipient	Side	Graft Bed Area 12 Days After Operation. (% of Original Area)	Homograft Survival Time (Days)	Recipient	Area	Graft Bed Area 12 Days After Operation. (% of Original Area)	Homograft Survival Time (Days)
Co-44	Co-45 F	{ Right	80	>15 <18	Co-46 F	{ Anterior	72	>12 <15
		{ Left	54	9		{ Posterior	68	12
Co-47	Co-48 F	{ Right	61	12	Co-49 F	{ Anterior	46	12
		{ Left	48	10		{ Posterior	39	11
Co-56	Co-57 M	{ Right	61	10	Co-58 M	{ Anterior	66	13
		{ Left	34	8		{ Posterior	38	12
Co-59	Co-60 M	{ Right	62	10	Co-61 M	{ Anterior	—	9
		{ Left	38	9		{ Posterior	—	7

every third day. The areas not treated with cortisone received applications of 0.2 ml. of "control fluid" every third day.

The entries in Table V that relate to cortisone-treated areas are italicized. For the reason explained in the description of experiment 3, it was necessary to score the homograft survival times in the first two tests (donors Co-44 and Co-47) on a "90% breakdown" basis. Disregarding the subdivision into unilateral and bilateral operations, we find (a) that locally applied cortisone is as little able to protect homografts against a concomitantly developed immunity as against a pre-existing immune state (experiment 3); and (b) that locally applied cortisone does prolong the lifetime of homografts to some small degree even against a vigorous concomitant immunity, for the eight sets of locally treated homografts outlived their respective controls on every occasion. Since the immune state is systemic, the difference revealed by comparison (b) must be the consequence of cortisone's inhibiting the local expression of the immune state. This experiment, like its predecessor, thus makes it possible to distinguish between the effects of cortisone in preventing the development of an immune state and in preventing its local fulfilment. The former effect greatly outweighs the latter.

The homografts on the untreated areas of animals Co-46, Co-48, Co-49, and Co-58 survived a little longer than ordinary control homografts. This may well have been due to the very weak systemic effect brought about by the absorption of cortisone through its area of local application (see experiment 2). The prolongation of survival is more prominent in the control homografts of the unilateral series, but this is not acceptable evidence of the validity of the hypotheses upon which the subdivision into bilateral and unilateral operations was based (see above).

Discussion

Our results show that the life of homografts on rabbits can be prolonged by the local application of cortisone acetate. As it turned out, the local administration of 5 mg. every third day was somewhat less effective than the systemic administration of 10 mg. every day. It is likely that the local application of smaller quantities at more frequent intervals would have been more effective; and it is possible that cortisone can be better applied than as crystals suspended in a watery base, though the work of Speirs (1951) shows how quickly cortisone is absorbed, even through intact skin, when applied in an ointment base.

The results confirm our earlier interpretation that cortisone acts predominantly by retarding the systemic development of immunity to homografts rather than by preventing its local fulfilment in the grafts. (Its effect upon the "homograft reaction" is in this respect closely analogous to its effect upon the development and local expression of the Arthus reaction in rabbits: Germuth and Ottinger, 1950; and cf. also Harris and Harris, 1950.) This generalization is as valid for homografts transplanted to pre-immunized animals (experiment 3) as for homografts under concomitant immunization (experiment 4). Cortisone does, however, prevent the local expression of the immunity reaction in some small and discernible degree, for, no matter how the immunity had been provoked, the cortisone-treated grafts invariably outlived the untreated homografts borne by the same recipients (experiments 3 and 4). Cortisone may be acting in different ways to bring about these two distinguishable effects: the very slight prolongation of

the life of homografts on concomitantly immunized or pre-immunized animals might be the consequence of an antihistaminic action, an alteration of vascular permeability, or an indirect effect on the division rate of epidermal cells. Whatever its mechanism, this relatively unimportant component of the action of cortisone is better achieved by local application than by systemic administration, for the breakdown of foreign cells in a homograft is accompanied by the congestion and disruption of its blood supply, and, if cortisone has been applied systemically, failure of the blood supply must obviously deny cortisone access to the graft.

The chief clinical advantage of applying cortisone locally is, of course, that it can be used in quantities that do not produce an unwelcome systemic effect. The local dosage of cortisone that doubles the life of homografts has, indeed, a trivial systemic effect. But the results of experiment 4—in which cortisone was applied locally to only one-half of the population of homografts, instead of, as in experiment 1, to them all—enforce one important precaution upon its clinical use: cortisone must be applied locally to *all* the foreign skin transplanted to a patient. This requirement may not always be at all easy to fulfil.

Summary

Skin homografts transplanted in a stated fixed dosage between rabbits of high genetic diversity survive their transplantation for six to nine days.

The local application of 5 mg. of cortisone to such homografts every third day at least doubles their survival time.

The dosage of cortisone that is effective when applied locally is ineffective when administered systemically.

The local application of cortisone to homografts is almost wholly ineffective when they are transplanted to a rabbit pre-immunized by an earlier grafting of skin from the same donor or concomitantly immunized by untreated homografts transplanted on the same occasion.

The principal action of cortisone on homografts is to retard the development of immunity towards them. Cortisone also has a slight but discernible effect in preventing the local expression of the immune state.

If cortisone is to be used by local application to homografts in clinical practice it must be applied to *all* the homografts transplanted to any one patient.

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Writing in the October issue of *Aeronautics*, the chief medical officer of British European Airways remarks that "indigestion and haemorrhoids are so common among aircrew that they may well come to be regarded as occupational ailments."