choice can lie in only one direction if the profession is to retain an honoured place in the community. The British Medical Association, with its far-flung interests and its considerable influence, is well fitted and equipped to meet any challenge which threatens the rectitude of the profession. With unity of purpose, unshakable confidence, and sound medical statesmanship, success can be assured, for we yield to none in our devotion to our profession. Meanwhile

"Let us, then, be up and doing.
With a heart for any fate;
Still achieving, still pursuing,
Learn to labour and to wait."

FAILURE OF AN ANTIHISTAMINE DRUG TO INFLUENCE THE COURSE OF EXPERIMENTAL HUMAN BURNS

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In the *British Medical Journal* of July and August, 1951, a correspondence took place on the value of antihistamine drugs in the treatment of burns (Milne, Balfour, and Yeoman, 1951; Shubsachs, 1951; MacPherson, 1951; Levine, 1951; Slack, Boesen, and Husain, 1951; Laborit, 1951). Some of the writers gave the drugs by mouth, others as local applications in the form of creams. The favourable effects claimed included the relief of pain within a few hours of burning, the prevention or reduction of blistering and oedema, the prevention or reduction of infection of the burns, and an increase in their rate of healing.

If these claims are correct, antihistamine drugs should play an important part in the treatment of burns. However, we questioned their validity, since it had previously been demonstrated in this laboratory that various drugs used to protect guinea-pigs against the action of histamine had no detectable effect on experimental burns of various degrees of severity. The changes investigated in the guinea-pigs included erythema; capillary permeability; qualitative blood-flow changes in the skin, including stagnation and stasis of the blood flow; the clinical degree of oedema formation; skin sensibility; and the subsequent clinical courses of the burns (Sevitt, 1949a). Similarly, Weeks and Gunnar (1949), using rabbits, showed that pyribenzamine hydrochloride failed to alter either the increase of capillary permeability or the histological inflammatory picture after burning, although they claimed a reduction in the area of the post-burn erythema.

Nevertheless, negative results with animals do not rule out the possibility that antihistamine drugs may influence the course of human burns. We decided, therefore, to experiment on a group of human volunteers, each of whom would receive burns of the same severity and half of whom would take an antihistamine drug. By carefully observing the changes taking place in the burns, using clinical, photographic, and other methods, any significant differences between the two groups, such as those claimed in the *Journal* correspondence, should be noted. No such differences were found.

The Experiment

Burning.—Each of eight healthy male volunteers (aged between 27 and 41 years) received two small circular burns on the lateral surface of the left upper arm over the triceps muscle. The burns were spaced about 2 in. (5 cm.) apart, one above the other. They were inflicted by applying, with moderate pressure, the heated surface of a constant-temperature burning-iron on to the skin, which had been shaved the previous day. The iron was essentially a hollow brass cylinder closed at one end, which was the burning surface; it was similar to that previously described (Sevitt, 1949b) except that the burning surface was ½ in. (1.3 cm.) in diameter. A current of hot water, the temperature of which could be regulated and recorded, circulated through the iron. During burning the temperature of the iron was maintained at 60° C. and each period of application was 15 seconds. The temperature and duration of burning were selected on the basis of previous experience to give partial skin-loss burns (Bull, unpublished observations; Sevitt, 1949b). In this way two small circular burns of the same severity were inflicted on similar sites on each of eight volunteers.

Analgesia.—A few minutes before burning, the outline of a diamond-shaped area of skin on the outer surface of the upper arm was infiltrated with 1% procaine (without adrenaline), using about 25 ml. of solution for each person. By this means an area of anaesthetic or nearly anaesthetic skin was produced on which the burns were inflicted. Each subject found that the burning produced a little discomfort, particularly during the infliction of the lower burn.

Antazoline and Dummy Tablets.—The antihistamine drug used was antazoline (2-phenyl-benzyl-aminomethyl-imidazoline methane hydrochloride; "antistin"). This was chosen because it is readily available to the medical profession, has an efficient antihistamine action, and produces minimal side-effects. Antazoline (100 mg.) and dummy tablets were obtained in eight bottles, numbered 1 to 8, each containing 40 tablets. Four of the bottles contained antazoline and the others dummy tablets. The latter were similar in size, colour, shape, and taste to the former, but contained no antihistamine drug.* No one taking part in the experiment knew which of the bottles contained the antazoline tablets, and the bottles were allocated to the eight volunteers (which included the authors) by the drawing of numbered lots. In this way an attempt was made to avoid bias in the observations. Fourteen days after burning, when the main observations were completed, the key to the identity of the tablets was obtained from the manufacturers. The tablets were taken by mouth, the first one immediately after burning. The dosage was four tablets on the day of burning, and thereafter one tablet three times a day for the next eight days.

Confirmation of Antihistamine Action.—Skin histamine tests were performed on the seventh day, when the tablets were being taken, and on the twelfth day, three days after stopping the tablets. The tests were performed by pricking histamine acid phosphate, 1 g.% and 0.1 g.%, into the skin (Squire, 1950). Two pricks through each dilution were made on each forearm. The diameters of the wheals and flares produced were measured, and, by comparing the measurements obtained on the two occasions, three of the four subjects taking antazoline and three of the four con-

*We are informed that the composition of the dummy tablets was: quinine sulphate, 2.68 mg., lactose, 53.6 mg., starch, 83.6 mg., talc, 8.04 mg., and gelatin, 2.08 mg.

trols were correctly identified. The nature of six of the tablets taken was later tested by guinea-pig "protection" experiments. Half a tablet was ground up in saline and injected intraperitoneally, and an hour later 1 ml. of 1% aqueous Evans blue was injected intracardially. Skin histamine tests were then performed by injecting 0.05 ml. of histamine (1 in 1,000) intradermally. By comparing the sizes of the blue-stained areas around the injection sites it was possible to identify correctly five of the six tablets tested, three of which were antazoline and the remainder dummy tablets.

Dressings.—After each of the three observations in the first 24 hours each burn was covered with a 2 by 2 in. (5 by 5 cm.) sterile sheet of nylon, which was attached to the skin by strapping and then covered by a crêpe bandage. On the second day after burning this dressing was discontinued, and the daily dressing for the next seven days was penicillin cream on gauze covered with cotton-wool and secured by a crêpe bandage. This "closed treatment" of burns with penicillin cream (1,000 units/g. in "lanette wax SX") had the advantage of preventing the scabs from drying, becoming hard and adherent, and so interfering with the observations. Instead, the fibrinous exudate, called "pellicle" in this unit, remained moist and gelatinous and easily removable. When it was removed the underlying burn could be easily observed and photographed. During this time examinations and dressings were carried out with sterile precautions. From the ninth day onwards dressings were discontinued.

Recording and Examinations

The findings were recorded serially on eight charts, one for each subject. The chart headings included all the macroscopic changes which we have found may occur in burns. Those observed will be described later. The observations were made by two of us and often checked by others.

The burns were first examined 25 to 40 minutes after burning, when the skin anaesthesia had not yet worn off, and at three and a half to four and a half hours, when the skin sensibility had returned to normal. Bacteriological swabs were taken. The burns were photographed and covered with nylon dressings as previously described.

Next morning, 24 hours after burning, the burns were again examined, swabbed, and photographed. Blisters had now formed and blister fluid was removed by heparinized capillary pipettes from the burns of five of the volunteers. Total protein estimations were made by the micro-Kjeldahl method, and filter paper electrophoresis was performed to establish the albumin–globulin proportions.

The blister tops from all of the burns* were then cut away with sterile scissors and fixed in formol–saline for histological section. The exposed erythematous oozing bases of the burns were then examined, swabbed, photographed, and covered with nylon dressings.

Subsequent observations were made daily, except on the fourth day, until the ninth day after burning, when the penicillin dressings were discontinued. Photography and bacteriological swabbing were also done daily. Later examinations and photography were carried out on the twelfth, fourteenth, twenty-first, and twenty-eighth days.

Photography.—A special photographic bench was arranged to enable photographs on different days to be taken at the same distance and with the same lighting each time. In this way comparable photographs, using orthochromatic film and 35-mm. coloured transparencies (Kodachrome), were taken of the burns at each examination. In addition a few special "close-ups" were taken. Each photograph included a millimetre rule and serial identification number. To facilitate comparison of the photographs they were all enlarged to natural size.

Comparison of Burns on Subjects Taking Antazoline With Those Taking Dummy Tablets During the Stages of Acute Inflammation and Healing

Tablets Taken	Subject	Stage of Acute Inflammation					Stage of Healing			
		Clinical Oedema After Burning		% of Burn Area Distended with Blister Fluid 24 Hours After Burning	Blister Fluid Protein (g./100 ml.)	Time of Onset of Capillary Stasis (No Blanching)	Vascularization from the Periphery	Epithelization from the Edge and Base		
		½ hr.	4 hr.				Width of Peripheral Red Ring* (in mm.), 14th Day	Day Edge Epithelium First Noted (mm.)	Width of Edge Epithelium, 7th Day (mm.)	Day Burn Floor First Found Covered by Epithelium
Antazoline ..	A	+/+	++/++	60/60	Insufficient available	24 to 48 hours after burning	2·7/5·2	3/3	2/3	8/7
,, ..	B	+/+	+/+	80/80	5·8	,,	4·0/3·5	3/3	3/2	7/7
,, ..	C	+/+	+/+	100/100	5·8	,,	3·5/3·5	3/3	2/3	7/7
,, ..	D	+/+	±/±	30/60	5·3	,,	4·0/5·0	4/4	3/3	8/7
Dummy ..	E	+/+	++/++	100/100	5·0	,,	3·7/4·2	3/3	3/3	7/8
,, ..	F	+/+	+/++	20/10	Insufficient available	,,	4·7/3·7	3/3	3/3	8/8
,, ..	G	/+	/+	/20	,,	,,	/4·0	/3	/3	/7
,, ..	H	+/+	+/+	80/60	6·0	,,	3·5/3·7	3/3	3/3	7/8

NOTE.—(1) Numerals or signs to the left of the oblique strokes refer to the upper burns, those on the right refer to the lower burns. (2) The protein value from subjects B, C, E, and H are from the pooled blister fluids, that from subject D is from the lower blister. (3) Day 0 = day of burning; days 1, 2, etc. = following days. (4) ±, +, and ++ are degrees of visible burn oedema or blister distension.
* Calculated from the diameters across the inner and outer edges of the red ring.

Results

Before it was known which of the volunteers were taking antazoline tablets it was found impossible to divide the subjects into two groups according to the pathological and clinical findings. Some variations and differences in the burns were noted, but when the subjects were finally classified into antazoline and control groups the similarities and differences within each of the groups were much the same as the similarities and differences between the groups. The more important findings are summarized in the accompanying Table.

We have divided the changes in the burns into those which occur during the first 48 hours and those which occur later. The first 48 hours is the period of the acute inflammatory change, and, by inference from the H-substance theory of Lewis (1927), it is during this period that antihistamine therapy would most likely have beneficial effects. The later changes, including those of healing, will then be described, possibly in more detail than is necessary

*In one subject (G in the Table), later found to have been taking dummy tablets, the upper burn did not produce oedema and failed to blister. Only a localized area of blanching erythema developed, which faded in a few days without scabbing or ulceration. When this burn was being inflicted the temperature of the burning-iron fell from 60° to 59° C. (this was the only burn in which this happened), but whether the different features of this burn were due to this is difficult to say. This obviously superficial burn will be omitted from the analysis.

for the purpose of this paper. We feel, however, that the findings will be of interest to those concerned with the healing of burns.

Period of Acute Inflammation (First 48 Hours)

Pain.—As procaine-induced regional analgesia, which lasted about an hour, was used for the burning, early post-burn pain was not experienced by any volunteer. Pain was absent when the analgesia had worn off about an hour after burning. However, the early spontaneous pain after any burn usually disappears within a few hours, and the claims made that antihistamine drugs relieve burn pain may be due to lack of appreciation of this fact.

Flare and Oedema.—Half an hour after burning and after the first tablet had been swallowed—that is, before the effects of antazoline could be considered to have taken place—three of the antazoline and two of the control group showed well-marked "flares" extending 10 to 15 mm. beyond the edge of the burns. These had disappeared at four hours. Definite and approximately equal degrees of oedema were present in all the burns half an hour after burning. At four hours (Fig. 1) the oedema had increased in both burns of one of the antazoline subjects (A) and had decreased in both burns of another (D). Increase in oedema also occurred in both burns of one of the controls (E) and in the lower burn of another (F) (Fig. 2). In the remainder of the burns in both groups the clinical degree of oedema remained unchanged from half to four hours. Next morning, when the blistering was maximal, oedema of the dermis was slight or absent in all of the burns.

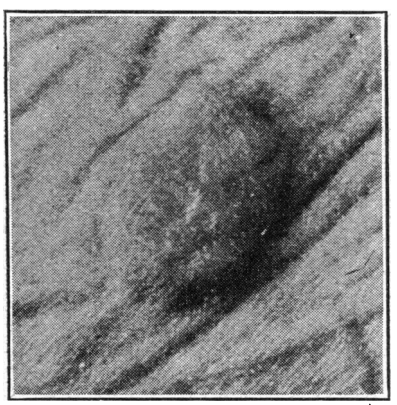

FIG. 1.—Subject A, four hours after burning and after beginning antazoline. The lower burn, showing considerable oedema. (Note: The irregular skin folds in Figs. 1 to 5 were due to the dressing. Figs. 1 to 7 are twice actual size, and Fig. 8 four times.)

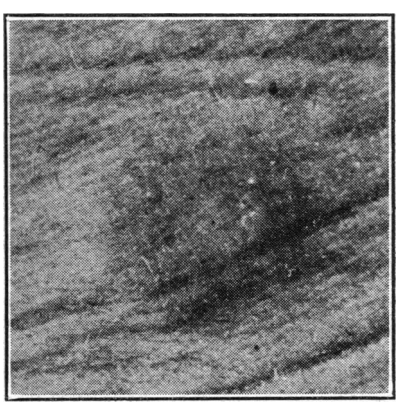

FIG. 2.—Subject F, control, four hours after burning. The lower burn, showing a well-marked oedema.

Blistering. — At four hours after burning, blistering was noted and was more definite in two of the antazoline subjects than in the remaining six volunteers. At 24 hours, blister distension involving 60% or more (Fig. 3) of the areas of both burns was present in three of the four antazoline subjects and in the lower burn of the fourth. Blister distension of similar extent (Fig. 4) was present in two of the four controls. In the remainder blistering was present, but the distended area involved 30% or less of the area burned (Fig. 5).

Blister Fluid Protein. — Sufficient fluid was aspirated from the blisters of three antazoline subjects and of two controls for protein estimations to be carried out. The protein content of blister fluid is a rough index of the permeability of the vessels in the underlying dermis. All of the fluids examined contained a high protein content, indicating a high permeability. For three of the antazoline group the values were 5.8, 5.8, and 5.3 g. protein per 100 ml. of fluid, and in the control group the two values available were 5 and 6 g. per 100 ml. Filter-paper electrophoresis showed that most of the protein was albumin, that α and β globulins were present, and that the proportions were similar to those found in normal serum. These findings do not suggest any difference in the degree of vascular permeability between the groups.

Histology of the Blister Tops.— Nearly all of the epidermis in every blister top was completely necrotic, and most of the blister tops contained the superficial thirds or so of a few hair follicles. Since either the whole thickness of the epidermis or the epidermis down to the deeper part of the Malpighian layer was present in each section, the site of blistering was either at the dermo-epidermal junction or through the deeper third of the Malpighian layer, or at both levels, in every burn. Differential white-cell counts were made of the cells in the exudate adherent to the deep surface of the blister tops. The haematoxylin and eosin sections were supplemented by sections stained with chromotrope 2R for eosinophil cells. The percentage of polymorphonuclear leucocytes was very high in all the exudates, varying between 91% and 95% of the white cells, the remainder being lymphocytes. In two of the antazoline subjects 1% of eosinophils was found: no eosinophils were seen in the other sections.

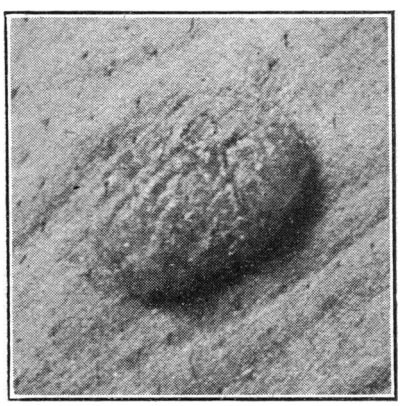

FIG. 3.—Subject B, taking antazoline. The upper burn, 24 hours after burning; showing a large distended blister. (Note: Blister formation varied within the antazoline and control groups. In each group large well-distended blisters (Figs. 3 and 4) and small often poorly distended blisters (Fig. 5) were formed.)

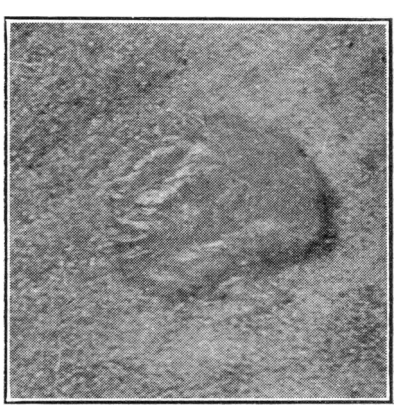

FIG. 4.—Subject E, control. The upper burn, 24 hours after burning; showing a large distended blister.

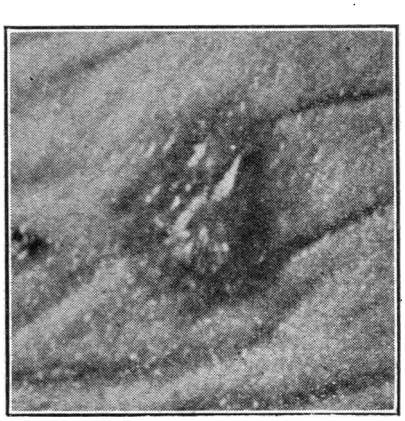

FIG. 5.—Subject D, taking antazoline. In the upper burn, 24 hours after burning, blister formation involved only about 30% of the burned area.

Erythema; Stagnation and Stasis of the Burned Dermis (see Table).—The blister tops were removed 24 hours after burning and the underlying dermis was exposed. In both antazoline and control groups the burns now appeared as localized pink or red raw areas oozing some fluid. When they were pressed with the edge of a glass slide the erythema blanched temporarily. However, the degree of blanching varied: in only one of the four volunteers in the antazoline group (both burns) was the blanching definite; in the remainder the blanching was slight. In two of the control group fairly good blanching was obtained; in the others the blanching was slight. At 48 hours after burning the erythema was still present in each burn, but blanching was now unobtainable—that is, stasis of the blood flow had set in. From these observations we may conclude that, 24 hours after burning, stagnation or retardation of the blood flow in the vessels of the upper part of the burned dermis had occurred in many of both groups of burns: at 48 hours the superficial local blood flow had ceased in all the burns.

Petechiae.—Small superficial petechial haemorrhages were seen in all the burns, and lasted a week or longer.

Peripheral Red Ring.—At 24 hours after burning a very narrow red ring was present at the periphery of the burned area in many of the burns. At 48 hours the red rings were quite distinct in all the burns, although only 0.5 mm. or less in width. Each one blanched well on pressure. These early rings probably indicate a lesser degree of vascular damage at the edges of the burns characterized by vascular dilatation without subsequent stasis.

Later Changes

Exudation

The gross appearance of an exudate from a burn depends on whether it is allowed to dry or not. If no dressing is used drying occurs and the familiar scab forms. If penicillin dressings are used and the burns are adequately covered, as they were from the second to the ninth day after burning in our series, any exudate formed appears as a gelatinous greyish layer lying on but not adherent to the burns. This wet form of burn exudate, called "pellicle" in this unit, consists histologically of a fibrin network containing fluid and numerous polymorphs. Pellicle was found in both groups of burns on the second and third days after burning. Thereafter the amount of pellicle was reduced: on the seventh, eighth, and ninth days either none was found or the amount was recorded as slight.

Healing

Healing of a partial skin-loss burn involved sloughing of the dead dermis or its invasion by a vascular granulation tissue, or both processes, and epithelization of the surface. It will be convenient to describe vascularization, epithelization, and sloughing separately.

Vascularization took place from the edge and from the base of the burns. Edge vascularization appeared early, beginning and progressing from the narrow peripheral red rings seen at 24 and 48 hours after burning. These rings progressively increased in width, reaching a mean diameter of 1.5 mm. in the 16 burns of the antazoline group and 1.4 mm. in the 15 burns of the control group on the seventh day (Figs. 6 and 7). The inner edges of the rings continued to move centrally, often irregularly in different sectors, so that the widths varied a little from sector to sector. In both groups the mean width of the rings was 2.7 mm. on the ninth day and 3.9 mm. on the fourteenth day, and the variations in width from burn to burn were similar in both series. After this the redness of the outer part of the rings began to fade and visible vascularization of the bases of these burns which did not slough (see below) appeared. By the fourth week the peripherally vascularized area had become normal in colour and the centres of the healed burns were erythematous.

Epithelization occurred both from the edge and from the base of the burns.

Edge.—In every burn on the third day a thin, *dry*, semi-transparent, whitish ring appeared at the inner edge of the peripheral red ring and measured about 0.5 mm. in diameter. That this was the first sign of epithelization was shown by its inward spread and thickening on successive days. On the seventh day the peripheral epithelial ring was about 3 mm. in width in every burn (Figs. 6 and 7). At first the outer part and later the inner part of this new epidermis became thicker, keratinized, and wrinkled when the skin was gently pinched. On the ninth day the edge epidermis reached a maximum width of about 4 mm. in diameter in most of the burns. By then, however, the remainder of each burn area had become covered by a thin sheet of epithelium which had presumably spread from epithelial elements in the base. On the fifth to the seventh day after burning careful examination of the central parts of the burns with a hand lens revealed the presence of tiny, whitish, less translucent foci slightly raised above the general level of the central area (Fig. 8). From these foci, often at the sites of regenerating hair follicles and presumably arising from them, the central burn area became covered with epithelium.

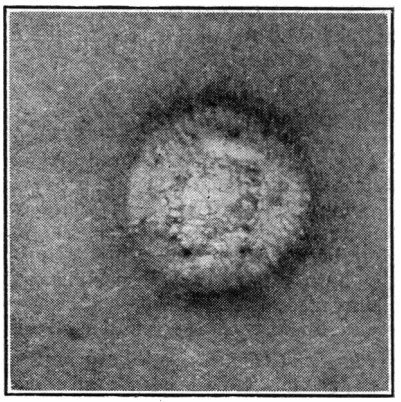

FIG. 6.—Subject D, taking antazoline. The lower burn, seven days after burning. (Note: In Figs. 6 and 7 each burn is surrounded by a narrow (1 to 2 mm. wide) red ring, which appears black in the photographs. This ring is due to vascularization at the edge of the burn, and within it is a ring of newly formed epidermis about 3 mm. wide. This, in turn, surrounds a central slightly depressed area containing tiny epithelial foci which can just be recognized in the photographs. The degree of edge vascularization and epithelization is about equal in the two groups.)

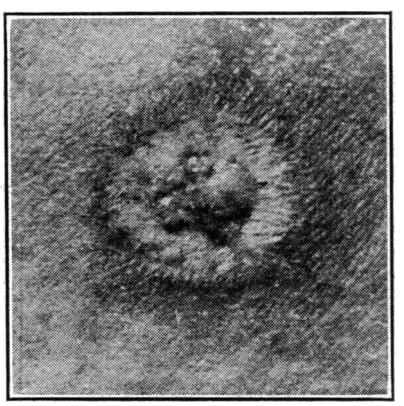

FIG. 7.—Subject E, control. The lower burn, seven days after burning.

Base.—On the seventh or eighth day the whole central area of each burn was seen to be completely covered by a thin transparent layer of epithelium drying after exposure to the air for a few minutes. Next day, or within the next few days, short hairs made their appearance, often in the centre of the epithelial foci. No difference was noted between the two groups in the times of first appearance of epithelial foci in the bases of the burns.

Sloughing and Re-epithelization of Epithelized Skin.—Within 48 hours of burning, the blood supply of the superficial dermis had ceased. The reddened base faded in colour and became whitish by about the fifth day after burning. This avascular and presumably *necrotic* superficial dermis was vascularized from the edge of the burn and was covered by epithelium arising from the edge and from hair follicles in the base. This was the situation on the ninth day, when the penicillin cream and dressings were discontinued and the burns were exposed. After this any exudate oozing through the thin unkeratinized epithelium

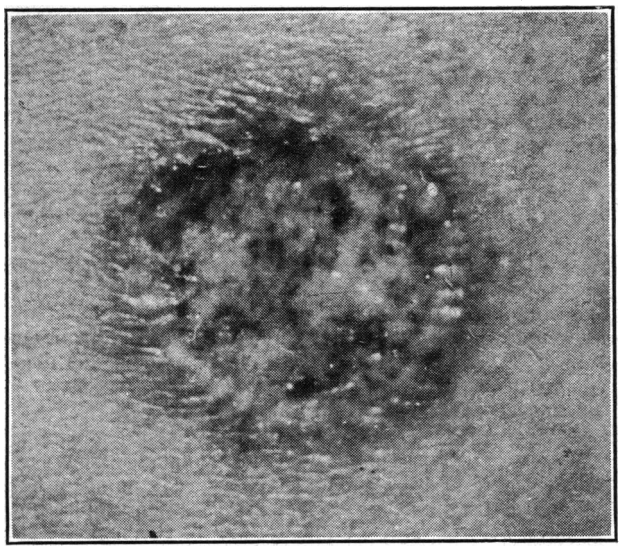

FIG. 8.—Subject D, taking antazoline. Lower burn, five days after burning. A number of tiny epithelial foci are present in the central two-thirds of the burn. For example, three epithelial foci are near the white reflection dots at the 4 o'clock position.

over the central part of the burns was allowed to dry and scab. In some of the burns in both groups, blood vessels invaded the superficial necrotic dermis and the surface epithelium continued to mature and to become keratinized. In more than half of the burns in both groups another process took place. In these, the white avascular superficial dermis dried into a brown scab about 1 mm. thick adherent to the base of the burn. Later, usually in the third or fourth week, the sloughs fell off or were easily removed, revealing red well-epithelized surfaces. One slough was sectioned and showed necrotic collagen with epidermis on both surfaces. It is probable that this sloughing was due to *drying* of the avascular dermis; that, instead of the necrotic dermis becoming incorporated into the healing burn as it commonly does (unpublished observations), a level of demarcation between the deep vascular and the superficial avascular dermis took place, and the latter sloughed. Epithelium spread once more at the line of demarcation, which became the new skin surface. Both processes occurred equally in the two groups, but in some subjects the two burns were affected in different ways.

Bacteriology.—Swabs of the cleansed skin taken just before burning produced a few micrococci from seven of the eight subjects and a few coliform bacilli from the other. Next day all the 15 blister tops were sterile, and swabs taken of both upper and lower burns during the next five days either produced no bacterial growth or contained only a few micrococci or diphtheroid bacilli. No difference was noted between the groups. The absence of colonization of the burns was probably due in part to the use of penicillin cream during this period.

Discussion

We have shown that there is no essential difference in the behaviour of small experimental burns of the same severity inflicted on four volunteers who took antazoline tablets and in four volunteers who took dummy tablets. During the acute inflammatory period of the first 48 hours both groups of burns gave rise to oedema of similar degree and duration; in both groups the area and distension of the blisters varied in a similar fashion; in both the histological level of blister separation, the concentration and nature of the blister fluid proteins, and the nature of the cellular exudate were very similar; in both groups stagnation and stasis of the blood flow in the vessels of the superficial dermis set in at about the same time. Spontaneous pain was not noted by any subject. The duration and, so far as could be judged clinically, the amount of exudate forming were the same in both groups. No real difference between the groups in the rate and manner of visible vascularization of the burns was found, and the epithelization from the edges and from the bases of the burns also occurred in a similar fashion in the two groups. Daily culture of the burns showed either no bacterial growth or very small numbers of saprophytic bacteria.

We can therefore conclude that the taking of antazoline within a few minutes of burning and its continuation in the dosage of one 100-mg. tablet three times a day had no effect either on the acute inflammatory changes during the first 48 hours or on the mode and rate of healing.

Procaine, however, was injected into every subject, and, since this drug has antihistamine properties, the possibility arises that the vascular changes might have been affected in *both* groups of burns and thus have weakened our conclusions. We believe this to be unlikely, because the antihistamine activity of procaine is weak and, if it took place, must have been short-lived in our experiment. This is supported by the observation that in five subjects well-marked flares developed around the burns within half an hour of the procaine injection and the burning.

Our observations demonstrate some of the variations which may occur even when burns are inflicted with a burning-iron at the same temperature and applied to similar skin areas for the same period. These variations may occur not only in different people but even in the same subject. Variations commonly occurred in the area and distension of the blisters and sometimes in the clinical degree of oedema. Later, rates of vascularization and epithelization also varied a little. If such variations can happen with controlled burns of the *same* severity, one can appreciate how difficult it is to draw conclusions from the behaviour of a series of accidental burns *varying* in severity, some treated with an antihistamine drug and others not so treated acting as "controls." Unless there is a great difference in the results of the treated and untreated groups no valid conclusions can be drawn.

Summary

Two small "partial-thickness skin-loss" burns were inflicted on similar skin sites on each of eight human volunteers. Each burning was carried out with a special burning iron at 60° C. applied for fifteen seconds. Four volunteers took antazoline tablets, starting immediately after the burns were inflicted and continuing (one tablet three times a day) for nine days. The other four took dummy tablets at the same time. The identity of the tablets was not known until the fourteenth day.

Observations during the first 48 hours were made on the clinical degree of oedema and its onset and disappearance; on the area and distension of the blister and the histological level of separation; on the concentration and nature of the blister fluid protein; on the nature of the cellular exudate; and on the time of onset of stagnation and stasis of the blood flow in the superficial dermis. Later, special attention was paid to the time of commencement, the rate, and the mode of development of vascularization and epithelization of the burns.

Although some minor variations and differences in the burns were found, the similarities and differences within both the antazoline and control groups were the same as the similarities and differences between the groups. We therefore conclude that the antazoline tablets failed to influence the course of the burns.

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CONVERSION OF FOLIC ACID TO THE CITROVORUM FACTOR IN HEALTH AND PERNICIOUS ANAEMIA

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In this paper the name "folic acid" is used collectively for all the substances in urine which stimulate the growth of *Lactobacillus casei* in microbiological assays; pteroylglutamic acid (P.G.A.) is used only for the pure substance. Citrovorum factor (C.F.) is synonymous with folinic acid, or leucovorin. The relation between these substances themselves, and between them and vitamin B_{12}, has been ably reviewed by Welch and Heinle (1951), and the reader is referred to their paper for more detailed references. There is reason to believe that in the body folic acid is converted to folinic acid, and that the latter is the form in which it acts. In the megaloblastic anaemia which develops in monkeys placed on a scorbutic diet the conversion of folic to folinic acid is impaired, and folinic acid is much more effective than folic acid in repairing the anaemia; the inability to activate folic acid in scurvy can be overcome by administering vitamin C (May, Sundberg, and Schaar, 1950). Callender and Lajtha (1951) found that folinic acid was more effective than folic acid in ripening human megaloblasts in marrow cultures, but the evidence collected by Welch and Heinle suggests that folic and folinic acids may be equally effective in the treatment of pernicious anaemia in man. It would therefore seem unlikely that pernicious anaemia is due to a failure to convert folic to folinic acid; but to obtain further evidence on this matter we have studied the excretion of folinic acid in the urine in health and in pernicious anaemia. It was already known that the oral administration of P.G.A. to normal men is followed by an increased excretion of C.F. in the urine (Broquist, Stokstad, and Jukes, 1951).

Methods

The subjects were 11 normal people, 8 patients with untreated pernicious anaemia, and 5 of the same group of patients after the anaemia had been treated. We measured the excretion of folic acid and C.F. in the urine during the first five hours after a dose of 5 mg. of P.G.A. by mouth. This period was chosen because earlier work (Spray and Witts, 1952) showed that the urinary excretion of folic acid after a dose of 1 mg. of P.G.A. by mouth was virtually complete in five hours. The excretion of folinic acid was also found to be largely completed in five hours in normal subjects after doses of both 1 mg. and 5 mg. of P.G.A. by mouth. The normal subjects were members of the department who were doing their usual work at the time of the test. The patients were confined to bed when untreated, but when the tests were repeated after treatment they were following their normal routine. Since neither age nor degree of activity appeared to affect the excretion of folic acid after oral doses of 1 mg. of P.G.A. in normal subjects (Spray and Witts, 1952), it was felt that the differences between the conditions in the different groups would not affect the results.

Urine for the 12 hours immediately before giving the P.G.A. was collected into a vessel containing toluene. All subjects were allowed a light breakfast, and about an hour afterwards they were given 5 mg. of P.G.A. in tablet form ("folvite") by mouth. Urine for the following five hours was collected into a fresh vessel. The volumes of urine were recorded, and samples were stored at 0° C. under toluene.

Folic acid in the urine was assayed with *L. casei*, using the same technique as previously (Spray, Fourman, and Witts, 1951). C.F. was assayed with *Leuconostoc citrovorum* in a medium containing acid-hydrolysed casein supplemented with glucose and various amino-acids, purines, pyrimidines, vitamins, and salts. The standards used for this assay were prepared by diluting a solution of the barium salt of the synthetic C.F. kindly sent to us by Dr. T. H. Jukes, of the Lederle Laboratories Division of the American Cyanamid Company. For both organisms the urine was diluted with water and added to the basal culture medium. Data for the recovery of added P.G.A. obtained from normal urine by this method have already been given (Spray and Witts, 1952), and Table I shows the recoveries of added C.F.

TABLE I.—*Recovery of Added C.F. from Normal Human Urine*

Urine from Subject No.	C.F. Added (mμg. per ml. Urine)			
	0·5	1·0	1·5	2·0
	C.F. Activity Recovered (mμg per ml. Urine)			
1	0·48	0·96	1·5	2·3
2	0·41	0·72	1·2	1·5
3	0·49	0·88	1·3	1·7
4	0·43	0·85	1·3	1·8
5	0·33	0·65	1·1	1·5
6	0·68	1·2	1·8	2·3
7	0·71	1·2	1·8	2·2
8	0·56	0·70	1·4	2·1
9	0·44	0·89	1·2	1·7
Means	0·50	0·89	1·4	1·9

TABLE II.—*Excretion of Folic Acid and C.F. in the Urine by Normal Subjects After 5,000 μg. of P.G.A. by Mouth*

Subject	Sex	Age	Folic Acid (μg. Excreted in 5 Hours)		C.F. (μg. Excreted in 5 Hours)	
			Before Dose*	After Dose	Before Dose*	After Dose
1	F	26	0·70	1,700	0·37	5·5
2	M	31	0·39	1,500	0·26	5·0
3	M	31	0·43	1,100	0·36	5·2
4	F	35	0·34	910	0·26	3·9
5	M	40	0·30	980	0·42	12
6	M	32	0·54	1,200	0·42	12
7	F	37	0·22	430	0·21	1·3
8	M	39	0·88	1,100	1·6	8·4
9	M	31	0·40	440	0·39	6·3
10	M	32	0·58	1,700	0·30	11
11	F	28	0·34	1,900	0·24	5·8
Means			0·46	1,180	0·44	6·9

* The values before the dose have been calculated from the excretion during the 12 hours immediately before the dose.