VASCULAR PERMEABILITY CHANGES IN INFLAMMATION:

I. The Role of Endogenous Permeability Factors in Ultraviolet Injury

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Received for publication December 10, 1965

Previously Logan and Wilhelm (1966) reported an investigation of the time-course of erythema, increased vascular permeability and tissue leucocytosis in the skin of guinea-pigs, rats and rabbits injured by local ultraviolet irradiation. The results formed a base-line for the present investigation of the role of various endogenous substances that have been proposed as mediators of the permeability changes in inflammation (Wilhelm, 1962; Spector and Willoughby, 1963).

The identification of mediators of the permeability changes in inflammation has been previously discussed by Miles and Wilhelm (1960) and Wilhelm and Mason (1960). Briefly, the attempted isolation of various permeability factors (PFs) is complicated by the ubiquity and ready activation of most known factors that occur in both normal and inflamed tissues. Depletion of some substances, *e.g.* histamine, may have non-specific effects (Wilhelm, Mill, Sparrow, Mackay and Miles, 1958) while for others it is impractical—*e.g.* kallikrein or globulin PF (Wilhelm *et al.*, 1958; Miles and Wilhelm, 1960).

Accordingly, we have investigated the mediation of the permeability responses in ultraviolet (UV) injury by testing the effects of antagonists of various PFs on the early and late permeability responses that characterize this and other types of injury.

MATERIALS AND METHODS

Animals.—The methods of preparing guinea-pigs, rats and rabbits for tests with UV injury or permeability factors were described in a previous paper (Logan and Wilhelm, 1966). Guinea-pigs weighing 600–800 g. were used for tests with UV injury, but smaller animals (450–600 g.) were used for tests with permeability factors.

Ultraviolet injury was induced with a Kromayer lamp according to Logan and Wilhelm (1966).

Drugs.—Histamine was used as the acid phosphate (British Drug Houses) and 5-hydroxytryptamine (5-HT) as the creatinine sulphate (Upjohn Co., Kalamazoo, U.S.A.). The antihistamines, triprolidine (Burroughs Wellcome) and promethazine (May and Baker), were used as hydrochloride, chlorprophenpyridamine (Allen and Hanburys) and mepyramine (May and Baker) as maleate.

The weights of histamine, 5-HT and antihistamines are cited as base. The 5-HT antagonist, 2-brom-D-lysergic acid diethylamide (BOL 148), was supplied by Sandoz Ltd., Sydney; compound 48/80 by the Wellcome Research Laboratories, Beckenham, England; and polymyxin B sulphate by Burroughs Wellcome and Co.

Globulin permeability factor (globulin PF) from guinea-pig serum was a pooled preparation containing fractions G_2 and G_2/1R prepared according to Mackay (1955) and Wilhelm, Mill and Miles (1957) at the Lister Institute of Preventive Medicine, London.

Kallikrein prepared from hog pancreas was supplied by Winthrop Laboratories, Sydney; crystallized trypsin (salt free, 2500 Armour units per mg.) prepared from bovine pancreas

by Armour Pharmaceutical Co. Ltd., England; streptokinase by Burroughs Wellcome and Co.; and synthetic bradykinin by Sandoz Ltd., Sydney. A preparation of human fibrinolysin ("Actase") was obtained from the Ortho Pharmaceutical Corp., Raritan, New Jersey, U.S.A. Preparations of streptokinase were supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York, U.S.A. ("Varidase") and by A. B. Kabi, Stockholm, Sweden ("Kabikinase").

Soya bean trypsin inhibitor (SBTI) was supplied by Worthington Biochemical Sales Co., U.S.A. A stock solution was prepared by dissolving approx. 5 mg. in 1 ml. phosphate buffer pH 8, $I = 0{\cdot}2$, and then diluting to the required concentration in 0·85 per cent saline. All other preparations for tests in guinea-pigs and rabbits were dissolved in saline, for tests in rats in Locke's solution (see Wilhelm *et al.*, 1958). Lima bean trypsin inhibitor (LBTI) was supplied by the Nutritional Biochemical Corp., U.S.A., potato trypsin inhibitor (PoTI.; fraction $4/ITS_1$) by Dr. J. Hladovec, Pharmaceutical and Biochemical Research Institute, Prague, Czechoslovakia. The trypsin kallikrein inactivator from bovine parotid gland ("Trasylol"—1000 KIU/ml.) was supplied by Farbenfabriken Bayer, Leverkusen, Germany.

Ovomucoid trypsin inhibitor was prepared by Dr. P. J. Mill at the Lister Institute of Preventive Medicine, London, according to Lineweaver and Murray (1947). ε-Amino caproic acid and 2-deoxy-D-glucose were supplied by the California Corp. for Biochemical Research, U.S.A.; α-amylase by Rystan Co., Mt. Vernon, N.Y., U.S.A. through Winthrop Laboratories, Sydney; and sodium α-naphthyl-acetate by Carnegies of Welwyn Ltd., Herts., England.

All cited doses of PFs or antagonists, given intracutaneously, refer to the amounts in the standard injection volume of 0·1 ml.

RESULTS

In guinea-pigs, rats and rabbits, UV irradiation evokes a diphasic permeability response (Logan and Wilhelm, 1966). The first or early phase of the response quickly follows irradiation (see below) and appears to be mediated by histamine in guinea-pigs, and 5-hydroxytryptamine (5-HT) in rats; its mediator in rabbits has not been identified. The second or late phase of the permeability response is prolonged in duration, but in none of the 3 test species has its mediator been identified (*cf.* thermal injury—Wilhelm and Mason, 1960).

Ultraviolet Injury in Guinea-pigs

Early permeability response

In guinea-pigs, 120 sec. irradiation induces an early response that appears in the first minute, is maximal in 7–8 min., and lasts only 15–20 min. (Logan and Wilhelm, 1966).

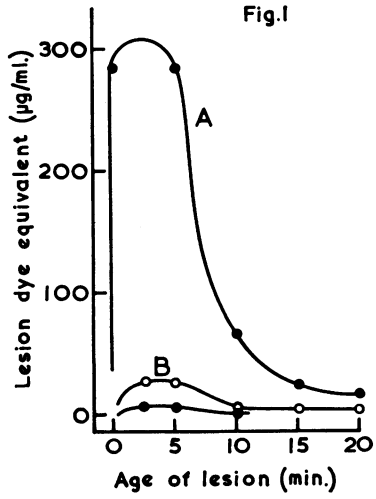
The early response in UV injury, like that in thermal injury (Wilhelm and Mason, 1960) is highly susceptible to antihistamine (Fig. 1). In untreated animals, the intensity of colour (approx. ++) of the early response corresponds to a dye-equivalent (Logan and Wilhelm, 1966) of 220–350 μg./ml. of Evans blue. In animals given intravenous triprolidine, 0·01 mg./kg., the colour intensity is only ± to + (dye-equiv. = 42 μg./ml.) and after 0·1 mg./kg. the lesions are barely identifiable (colour-intensity = trace or ±; dye-equiv. = 10 μg./ml.). The high susceptibility of the early response to antihistamine strongly suggests that the response is mediated by histamine (Wilhelm and Mason, 1960).

Late permeability response

Whereas the induction of the early response requires relatively long exposures of 120 sec., the late response is consistently evoked by only 15 sec. irradiation (Logan and Wilhelm, 1963 and 1966). The early response is succeeded by a

latent interval of 2–3 hr., after which the late response gradually appears, becoming maximal in 19–20 hr. and then steadily declining. Normal low permeability is restored in lesions about 72 hr. old (Fig. 3, Logan and Wilhelm, 1966).

Effects of antihistamines.—The relatively slow maturation of the late response in UV injury suggests that appropriate pharmacological antagonists may be more effective than in thermal injury, in which the permeability events develop relatively rapidly. On the other hand, most of the antagonists themselves have rather short term effects. For example, the duration of effect of various antihistamines was tested by giving each in a dose of 0·1 mg./kg. i.v. to batches of 3–4 guinea-pigs,



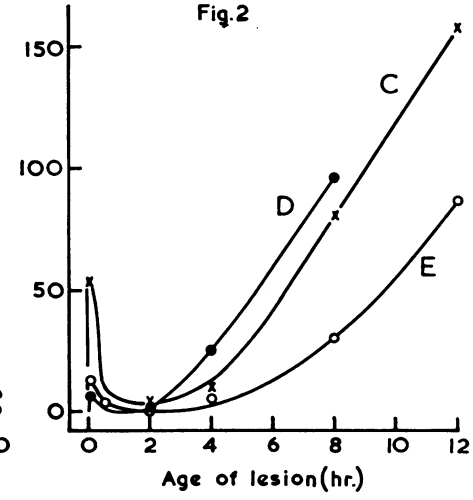


FIG. 1.—The suppression by intravenous triprolidine of the early permeability response to ultraviolet injury in the guinea-pig. A = control; B = triprolidine, 0·01 mg./kg.; the bottom response-line = triprolidine, 0·1 mg./kg.

FIG. 2.—The effect of the protease antagonist "Trasylol" (see text) on the early and late permeability responses in ultraviolet injury in the guinea-pig. C = control; D = single intravenous dose of Trasylol, 500 units/kg.; E = repeated systemic doses of Trasylol each of 500 units/kg (see text).

and testing the PF response to graded doses of histamine i.c. after intervals of ½–6 hr. (Fig. 3). The results, summarized in Table I express antihistamine activity of the test drugs as the factor by which the PF potency of histamine in untreated control guinea-pigs exceeds that in treated animals—*i.e.*, the factor of inhibition.

TABLE I.—*Suppression of the PF Response to Injected Histamine at Varying Intervals after Various Antihistamines* (0·1 *mg./kg. i.v.*)

	Factor of inhibition after (hr.)					
Antihistamine	1/4–1/2	1	2	3	4	6
Triprolidine . . .	1880	2425	235	7	5	1·0
Chlorprophenpyridamine .	1373	2218	nt	13	nt	4
Mepyramine . . .	66	66	45	8	nt	1·0
Promethazine . .	12	28	12	8	nt	11

nt = not tested

Despite the strong antihistaminic potency in the first hour of triprolidine, chlorprophenpyridamine, and to a lesser extent mepyramine, the effects of all 3 drugs largely subside in 3 hr. (Fig. 3).

To test the effects of antihistamine on the late UV response, systemic triprolidine, 0·1 mg./kg., was given ¼ hr. before irradiation and repeated when the lesions were 3 and 21 hr. old. The first dose was given to provide an antihistamine " cover " for the first 3 hr., as well as to abolish the early permeability response. The effect was extended by a second dose in 3 hr. Although repeated 3-hourly doses would have been preferred for the whole test period, this régime was considered likely to produce non-specific effects; and a third and final dose was

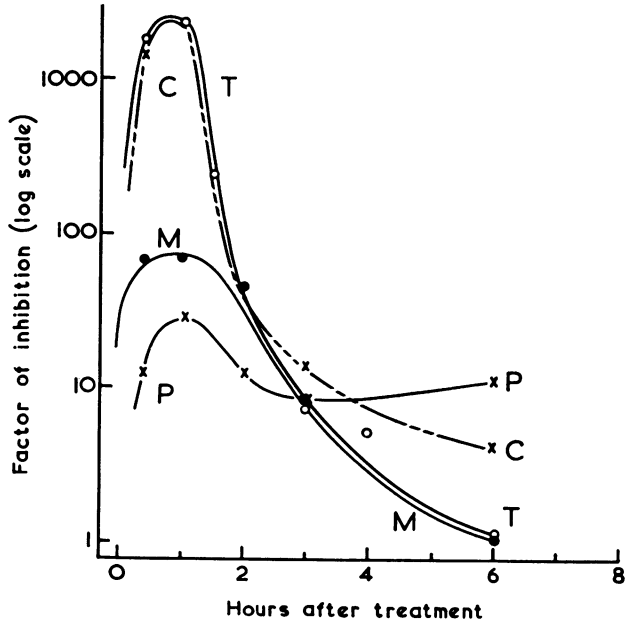


FIG. 3.—The potency and duration of action of various antihistamines (0·1 mg./kg., intravenously) in the guinea-pig. T = Triprolidine; C = Chlorprophenpyridamine; M = Mepyramine; P = Promethazine.

therefore given 1 hr. before blueing. In one experiment, the first dose was given i.v. and the second and third i.p.; in another, the first and second doses were given i.p., and the third i.v.

All animals were given dye i.v. or " blued " at 22 hr. and the lesions inspected ½ hr. later. In each case, the lesions in animals treated with antihistamine were similar in colour-intensity to those in controls treated with saline.

The effect of local i.c. antihistamine was also tested at similar intervals. The details of the experimental plan are illustrated in Fig. 4. A row of 6 sites was irradiated on either side of the back. For convenience, the left side in Fig. 4 is referred to as side A, the right as B. Sites A1, A6, B1 and B6 were left as untreated controls. Triprolidine, 10 μg. in 0·1 ml., was injected 5–15 min. before irradiation in A2; and 3, 20 and 21½ hr. after irradiation in sites A3, A4 and A5 respectively. At corresponding intervals, saline was injected in sites B2–5. The

guinea-pigs were blued at 22 hr. and the lesions examined ½ hr. later. Variation in PF response over the test area was minimized by using groups of 3–4 animals in each experiment, by using alternate sides of the animal for test and control injections, and partly randomizing the individual sites injected at the various intervals after injury. Neither triprolidine nor mepyramine affected the late response.

In summary, the late response is unaffected by systemic or local antihistamines in doses that strongly suppress the early response to histamine, or decrease the PF response to injected histamine 2000-fold. Furthermore, suppression of the

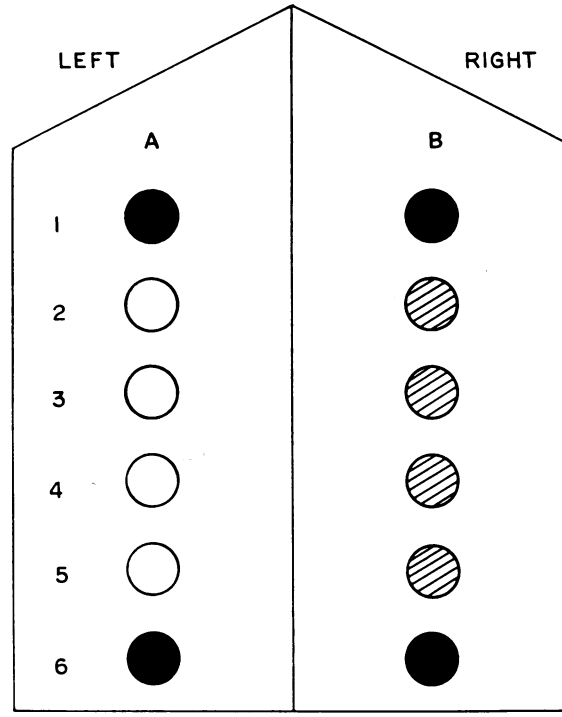


FIG. 4.—Distribution of sites on the dorsal trunk of the guinea-pig for tests with antihistamines and other inhibitors, given by local intracutaneous injection (see text).

early response by antihistamine in no way affected the development of the late response (Wilhelm and Mason, 1960).

Effects of protease inhibitors.—The activation of proteolytic enzymes and the appearance of polypeptides in injured tissues during the late phase of increased permeability suggest that proteolytic activity may be responsible for the permeability changes. In the absence of specific antagonists of proteases like plasmin, kallikrein or globulin PF, we had to rely on the trypsin and protease inhibitors such as those from soya bean, lima bean, potato and ovomucoid.

As in the earlier work on thermal injury (Wilhelm and Mason, 1960), the effects of the above inhibitors were tested on the late permeability in UV injury. Given i.v. to guinea-pigs in doses up to 3·3 mg./kg., the trypsin inhibitor from soya bean does not substantially suppress the permeability changes induced by globulin PF ;

and larger doses appear to have non-specific effects in that both globulin PF and histamine are antagonized (Wilhelm and Mason, 1960). Effective doses of systemic soya inhibitor are also toxic in rabbits (Zweifach, Nagler and Troll, 1961).

Accordingly, the effects of trypsin inhibitors were tested only by local i.c. injection in the irradiated sites, preparations from soya bean, lima bean, potato and ovomucoid each being injected in doses of 10 μg. in 0·1 ml. according to the schedule for antihistamines (Fig. 4). Preliminary tests demonstrated that 10 μg. of each inhibitor suppressed the permeability effects of guinea-pig globulin PF and trypsin as follows :

	Number of EBD* inhibited	
Inhibitor	Globulin PF	Trypsin
Soya bean	10	10
Lima bean	0·3	nt
Potato	10	3
Ovomucoid	0	nt

nt = not tested.

* EBD = effective blueing dose; see Wilhelm *et al.* (1958).

None of the above protease antagonists consistently antagonizes the late response. In 2 experiments, mild suppression was obtained with the 3 hr. and 20 hr. doses of soya inhibitor, but the results were still within the range of variation exhibited by control sites injected with saline.

Effects of ε-amino caproic acid.—Although plasmin has been found to have little "short-term" PF activity in guinea-pigs (Wilhelm, Miles and Mackay, 1955), its ability to form kinins from plasma globulins (Lewis, 1960) suggests that it may nevertheless play a role in mediating the late permeability response in inflammation. The effects of ε-amino caproic acid were therefore tested on the late ultraviolet response.

In concentrations of 10^{-4} to 10^{-2} M, ε-amino caproic acid is a potent *in vitro* antagonist of the activation of plasminogen to plasmin (Ablondi, Hagan, Philips and de Renzo, 1959; Alkjaersig, Fletcher and Sherry, 1959; Fukutake, Shida, Arakawa and Kato, 1960), but has little effect on pre-formed plasmin. However, in concentrations exceeding 10^{-2} M, ε-amino caproic acid enhances the proteolytic activity of plasmin (Alkjaersig *et al.*, 1959).

Given systemically, ε-amino caproic acid is rapidly absorbed and excreted, 75 per cent of a single i.v. dose of 1·0 mg./kg. being cleared from the blood in rabbits in 15 min. (Flaum, 1960). Accordingly, its effects were tested by local i.c. injection.

The maximum non-blueing dose of ε-amino caproic acid is 16·2 μg. Injected in irradiated sites according to the schedule in Fig. 4, ε-amino caproic acid, 10^{-1}–10^{-3} M, suppresses neither erythema nor the late permeability response, compared with untreated control lesions or sites injected with physiological saline. In fact, the exudation of dye seems slightly enhanced by treatment with ε-amino caproic acid.

In other tests, local ε-amino caproic acid decreased the permeability effects of neither globulin PF, nor the histamine liberator, compound 48/80, but suppressed those of histamine 3-fold.

The results with histamine suggested that ε-amino caproic acid might suppress the early response in ultraviolet injury. However, the effects of the antagonist

are only slight. An i.v. dose of 13 mg./kg. immediately before irradiation only mildly suppresses the early response, and local i.c. doses of 10^{-3}–10^{-1} M have no effect.

Effects of "Trasylol".—This preparation is a polypeptide inhibitor of kallikrein and trypsin isolated from the parotid gland of cattle (Frey, Kraut and Werle, 1950; Werle and Appel, 1958). In relatively small intravenous doses (500 units/kg.), Trasylol inhibits neither histamine, globulin PF, trypsin, kallikrein nor bradykinin; in fact, the effects of all these PFs are slightly enhanced. The dosage needs to be increased 10-fold before a consistent suppression is obtained, and even then the maximal inhibition of trypsin or globulin PF is only 2- to 3-fold.

The effect of locally injected Trasylol (10 units) was tested according to the scheme indicated in Fig. 4. Compared with sites injected with saline, Trasylol treatment slightly decreased both erythema and the late permeability response, but the effects on permeability were only slight (dye-equiv. of control lesions, 90–138 μg./ml.; of treated lesions, 66 μg./ml.). Suppression of the permeability response was greatest in lesions injected up to 2 hr. before "blueing"—*i.e.* 18 and 19½ hr. after irradiation; but in any case, the permeability responses in the treated sites still fell within the range of response in untreated control lesions in other experiments.

In further experiments, the effects of systemic Trasylol were tested in the first 12 hr. after irradiation—*i.e.*, during the development of the late response. In 3 batches of guinea-pigs, skin sites were irradiated for 20 sec. so that each animal bore 2 lesions aged 12, 8, 4, 2, 1 and 0 hr. when "blued" 12 hr. after irradiation.

The first group received a single intravenous dose of Trasylol, 500 units/kg., immediately before the first lesion was applied. The second group was given Trasylol i.v., 500 units/kg., immediately before the first exposures and further intraperitoneal doses in 3½ hr., 7½ hr. and 11½ hr. The third or control group was given saline at the same intervals as the second group.

All animals were blued 12 hr. after the initial lesion was applied (Fig. 2). In the first batch of animals given a single dose of Trasylol, the development of the late response was somewhat enhanced, compared with the saline-treated controls. In the second batch given repeated doses, the late response was less prominent than in the controls, but the differences appeared insignificant, compared with the variation in response in control animals in other experiments. In neither group was there any effect on erythema.

Single or repeated doses of Trasylol also decreased the early response to 20 sec., irradiation, the dye-equivalent being reduced from 56 to 8–14 μg./ml. (Fig. 2); but even 5000 units/kg. of Trasylol did not affect the early response induced by 120 sec. irradiation.

Effects of guinea-pig IPF.—The natural inhibitor (IPF) in serum of globulin PF, is isolated in a serum fraction G1S/P (Wilhelm, Miles and Mackay, 1955). When mixed with globulin PF for 90 min. before intracutaneous injection, IPF in a "non-blueing" concentration of 0·05 per cent moderately inhibits the effects of globulin PF. Tested by i.c. injection according to the scheme in Fig. 4, IPF slightly suppresses the late response, but the order of suppression is similar to that with Trasylol and the results are correspondingly inconclusive.

Summary.—The late permeability response was mildly suppressed by various protease inhibitors, particularly soya bean inhibitor, the polypeptide preparation Trasylol, and the natural IPF in guinea-pig serum. The suppression was always

slight, but occurred repeatedly with various preparations. The results were difficult to evaluate, not only because of the low order of effect, but also because the results still fell within the overall range of response in other control animals. The latter factor suggests that the mild suppression may be insignificant, but the results seem to warrant further investigation with more specific inhibitors of proteases when these become available.

Insusceptibility to 2-deoxy-(D)-glucose.—Little information is available concerning the role of carbohydrate metabolites in the permeability changes in inflammation. One of the few reports is that of Goth (1959) who observed that a glucose analogue, *viz.*, 2-deoxy-(D)-glucose, suppressed the anaphylactoid reaction of rats to intravenous dextran or ovomucoid, but not the reaction to compound 48/80.

In preliminary experiments, the responses in our guinea-pigs to intracutaneous histamine, globulin PF and compound 48/80 were unaffected by glucose analogue i.v., 200 mg./kg., given ½–2 hr. earlier (Goth, 1959). Although the above PFs have short-term permeability effects, the longer response to *Clostridium welchii* α-toxin (Elder and Miles, 1957) was similarly unaffected.

The effect of 2-deoxy-(D)-glucose on the permeability response to ultraviolet injury was tested in 2 ways. The first was similar to that already outlined for tests with systemic antihistamine. Two groups of guinea-pigs received glucose analogue, 200 mg./kg., ¼ hr. before irradiation and again 3 hr. and 21 hr. after irradiation. In one batch of animals, the first and second injections were given i.p. and the third i.v. In another batch, the first injection was i.v. and the next two i.p. A third batch of control animals was treated with saline as in the second group given analogue.

On each animal, 10 sites were irradiated ¼ hr. after the first injection of analogue and given i.v. dye 22 hr. later (*i.e.* 1 hr. after the last dose of glucose analogue). In both treated groups, the mean dye-equiv. of the lesions in the treated groups (125 and 72 μg./ml. respectively) exceeded that in the control animals (55 μg./ml.).

In a second experiment, the effect of analogue was tested during the first 12 hr. of the time-course of increased permeability as with repeated doses of Trasylol. Skin sites were irradiated for 20 sec. at various intervals so that when the animals were blued, they bore lesions 0–12 hr. old. Glucose analogue (200 mg./kg.) was given i.v. immediately before the initial lesions were applied, and further doses given i.p. 3, 7, and 11 hr. later. A second group of control animals was injected at similar intervals with saline; and both groups of animals blued 12 hr. after irradiation and examined ½ hr. later. The response-lines for both the early response and the development of the late response in the treated animals were almost identical with those in the control group.

Insusceptibility to α-amylase.—The buccal application of α-amylase has been claimed to decrease post-traumatic oedema in orthopaedic injuries (Thompson, Glick and Silverstein, 1960).

In our animals, the PF response to histamine, histamine liberators (48/80 and polymyxin B), globulin PF or bradykinin was unaffected by α-amylase s.c., 10 mg./kg.; but in other tests (Jean Carr, unpublished) the response to these PFs was suppressed by 1 mg./kg. i.p. 1 hr. before testing.

However, the late permeability response in ultraviolet injury was unaffected by doses of 1 mg./kg. i.p. 1 hr. before irradiation, again 2½ hr. after injury and

finally, i.v. 21 hr. after exposure, the animals being blued at 22 hr. and examined ½ hr. later.

Insusceptibility to sodium α-naphthyl acetate.—This preparation has been reported to decrease oedema in formalin injury of rats' paws (Holler, Lindner and Stoklaska, 1958). In earlier work, however, the preparation failed to suppress the permeability changes that occur in thermal injury (Wilhelm and Mason, 1960). Accordingly, the effects of the preparation were now tested by local i.c. injection in irradiated sites. Given according to the schedule in Fig. 4 sodium α-naphthyl acetate had no effect on the late permeability response in ultraviolet injury.

Insusceptibility of erythema

In the above experiments on the permeability response, the intensity of erythema in the irradiated sites was usually estimated before the animals were given dye i.v. This was done for all test preparations, except the trypsin inhibitors from lima bean and potato. None of the preparations seemed to affect erythema at any stage of the response.

Ultraviolet Injury in Rats

The early permeability response in rats is consistently induced by 30 sec. irradiation, the response having a short time-course similar to that in guinea-pigs. The late response in rats is evoked by a shorter exposure of 10 sec. but whereas the late response in guinea-pigs is maximal in 18–21 hr., its peak development in rats takes 48–54 hr. (Logan and Wilhelm, 1966).

For both early and late responses in the rat, the work was restricted to tests with 5-HT and histamine antagonists.

Early permeabilitty response

The early response in rats is strongly suppressed by the 5-HT antagonist, BOL-148. Given i.v. immediately prior to 30 sec. irradiation, BOL-148, 1 mg./kg., decreases the dye-equiv. of the lesions in the early response from 60–120 μg./ml. (in untreated controls) to less than 10 μg./ml. (Fig. 5). Even a dose of 0·1 mg./kg. is sufficient to decrease the dye-equiv. to 16–38 μg./ml. This high order of susceptibility of the early response to BOL-148 strongly suggests that the response in the rat is mediated by 5-HT.

Although doses of 1·0 mg./kg. i.v. of the antihistamine, triprolidine decrease the PF response to histamine 30- to 50-fold, it has no effect on the early UV response or the PF effects of injected 5-HT.

Late permeability response

The effects of BOL-148 and triprolidine were each tested by giving a single injection i.v., or repeated injections i.p.

In the first experiment, skin sites were irradiated for 10 sec. at intervals 0–72 hr. in 3 batches of animals. The first batch received BOL-148, 2 mg./kg., in Locke's solution i.v., the second batch the same dose of triprolidine, and the third batch given Lock's solution alone served as controls. The results are recorded in Fig. 6. The 5-HT antagonist mildly suppressed the late response, the dye-equivalent of the peak response being decreased from 550 μg./ml. in the control

animals to 435 μg./ml. (Fig. 6). With triprolidine the suppression was somewhat stronger, the dye-equiv. being decreased to 300 μg./ml. (Fig. 6).

In a second experiment, 8 sites were irradiated in each of 9 rats, which were then divided into 3 batches of animals. The first batch received BOL-148, 2 mg./kg. i.p., immediately before irradiation, and again 5, 24 and finally 51 hr. later. The second batch was similarly treated with triprolidine, the third with Locke's solution. All animals were blued immediately after the last dose of test preparation. Estimated $\frac{1}{2}$ hr. after blueing, the mean dye-equiv. of the lesions in the control rats was 250 μg./ml. ; in the BOL-treated rats, 275 μg./ml. ; in the triprolidine-treated rats, 120 μg./ml.

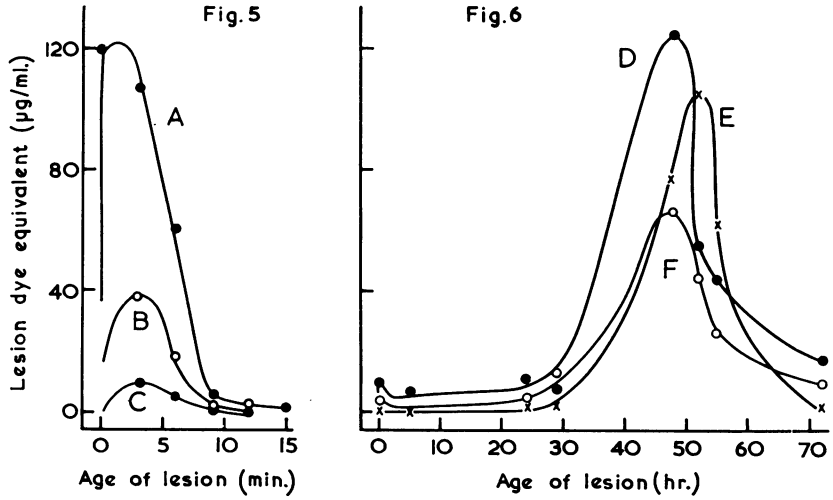


FIG. 5.—The suppression of the early permeability response to ultraviolet injury in the rat by the 5-HT antagonist, BOL-148, given intravenously. A = control ; B = BOL-148, 0·1 mg./kg. ; C = BOL-148, 1·0 mg./kg.

FIG. 6.—The effects of intravenous BOL-148 and triprolidine on the late permeability response to ultraviolet injury in the rat. D = control ; E = BOL-148, 2 mg./kg. ; F = triprolidine, 2 mg./kg.

In summary, the early permeability response in rats is highly susceptible to a 5-HT antagonist but insusceptible to antihistamine. On the other hand, the late response is never more than slightly suppressed by the 5-HT antagonist but moderately so by an antihistamine.

Ultraviolet Injury in Rabbits

In the rabbit, as in the guinea-pig, histamine has high and 5-HT relatively low PF activity (Sparrow and Wilhelm, 1957). These results were confirmed in the present work ; and hence only the effects of the antihistamine, triprolidine, were tested on the UV lesions. Furthermore, the time-course of both the early and late permeability responses induced by UV injury in rabbits closely resembles that in guinea-pigs. Since the rather extensive tests in guinea-pigs gave no clue to the mediation of the late response in that species, the investigations in rabbits were restricted to the early response alone.

The early response to 120 sec. irradiation in rabbits differs from that in the guinea-pig and rat in being more prolonged (Fig. 7). Maximal permeability is attained in 25–30 min., and then declines rather slowly, a low level of permeability being reached in about 60 min. Although the permeability effects resemble those induced by histamine i.c. in the rabbit (Wilhelm *et al.*, 1958), the failure to inhibit the response by antihistamine treatment suggests that histamine is not the mediator of the early response.

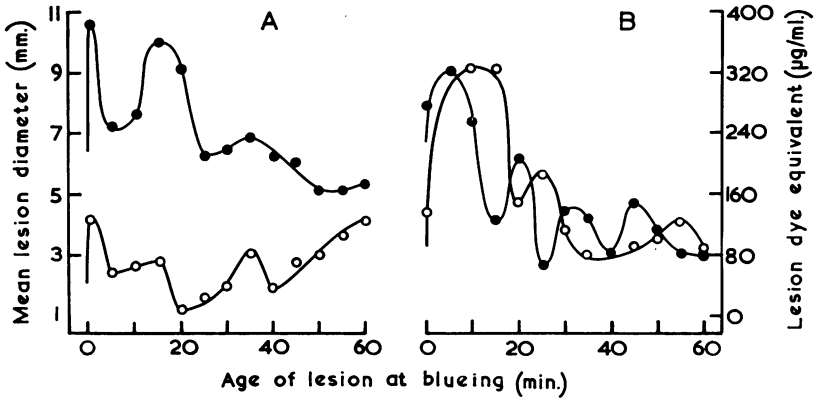


FIG. 7.—The susceptibility to intravenous triprolidine (0·1 mg./kg.) of the PF response to intracutaneous histamine (A) in the rabbit, and the insusceptibility of the early permeability response in ultraviolet injury (B). Control animals are indicated by solid circles (●——●), treated animals by open circles (○——○).

Triprolidine, 0·1 mg./kg i.v., suppressed the dosage-response line to injected histamine approx. 35-fold; and correspondingly suppressed the 60-min. time-course of the PF changes induced by 0·6 µg. of histamine i.c. (Fig. 7, A). The same dose, however, had no effect on the early permeability response to UV injury in the rabbit, whether given before 120 sec. irradiation (Fig. 7, B), or immediately before blueing animals bearing lesions 60 min. old.

The insusceptibility of the early response to a histamine antagonist, and the relative inactivity of 5-HT as a PF in the rabbit (see Sparrow and Wilhelm, 1957) suggest that neither factor is the main mediator of the early permeability response in UV injury in this species.

DISCUSSION

The work reported in this and the preceding papers (Logan and Wilhelm, 1963, 1966) was undertaken as an extension of an earlier and similar study in moderate thermal injury (Wilhelm and Mason, 1958, 1960). It has demonstrated that in UV, as in thermal injury, the pattern of increased permeability is diphasic in the guinea-pig, rat and rabbit, the 2 permeability responses—early and late—being quite distinct.

The early response

The early response has much in common for both types of injury. In UV lesions in guinea-pigs, the early response closely resembles the picture in thermal

injury, running its whole course in 15–20 min. and being mediated by histamine. In rats, a similar early response occurs in UV injury, the mediator in this case being 5-HT. On the other hand, the early response is minimal or absent in thermal injury in the rat: and judged by tests on the early oedema in unblued animals, histamine is at least partly responsible for the early thermal response in this species.

In the rabbit, the early response in UV injury is less clear-cut than in the guinea-pig or rat. It lasts at least an hour in the rabbit, and varies in intensity during this period.

Although the time-course of the early UV response in the rabbit resembles that for injected histamine in this species (Fig. 7), it is unaffected by antihistamine. Earlier work (Wilhelm and Mason, 1960) suggested that the early UV response in the rabbit might be mediated by histamine: but this has not proved to be the case, and the mediator remains unidentified. On the other hand, histamine appears to play the main role in evoking the early response in thermal injury in the rabbit.

Two other features of the UV response seem worthy of mention. Firstly, the suppression of the early response with pharmacological antagonists in no way seems to affect the development of the late phase of increased permeability. This feature is common to both UV and thermal injury: and it seems that the early response in inflammation may simply reflect the ease with which amines, such as histamine and 5-HT, are liberated by various types of injury and hence induce a transient increased permeability of venules in the injured site (Wells and Miles, 1963; Cotran and Majno, 1964).

A second feature of the UV lesions concerns the duration of radiation required to evoke the early response—120 sec. in guinea-pigs and rabbits, and 30 sec. in rats, whereas in all 3 species, the late response is induced by shorter periods of radiation. In contrast, the late response in thermal injury requires stronger stimulation than the early one.

The late response

The late UV response, like that in thermal injury, is preceded by an interval of " normal " or low permeability. It then matures more slowly than in thermal injury, maximal UV responses developing in 19–20 hr. in the guinea-pig, 48–54 hr. in the rat and 23–26 hr. in the rabbit. In both types of injury, however, the maturation of the late response is accelerated as the stimulus is increased in intensity or duration.

As in thermal injury, the mediator of the late response in UV injury has not been identified. Miles (1958–59) suggested that the emigration of leucocytes into injured tissues may be related to the late phase of increased permeability. But the late UV response seems unrelated to the emigration of neutrophils into the damaged skin and is not consistently related in time-course to the wave of tissue leucocytosis in either rats or rabbits (Logan and Wilhelm, 1966; also see Hurley, 1963; Grant, 1965).

Conclusion

The shortcomings of tests to identify natural permeability factors by antagonism have been discussed elsewhere (Wilhelm and Mason, 1960; Wilhelm, 1962, 1965; Miles, 1963). However, it seems that the early permeability response in

both UV and thermal injury is usually mediated by amines, such as histamine and 5-HT. This conclusion is further supported by recent work of Wells and Miles (1963) as well as Cotran and Majno (1964) who all demonstrated that, in thermal injury, the early permeability response involves venules, thus resembling the effects of factors like histamine, 5-HT, bradykinin, and guinea-pig globulin PF (Miles, 1963). On the other hand, the late phase is a capillary response, although venules and even arterioles may be involved in relatively strong thermal injury (Cotran and Majno, 1964; Hurley and Spector, 1965).

A somewhat puzzling feature of the overall diphasic picture of increased permeability has been the restricted duration of the early response and the occurrence of the subsequent latent interval of low permeability. However, the short early phase may simply reflect the readiness with which venules respond to a wide range of permeability factors (Majno, Palade and Schoefl, 1961; Peterson and Good, 1962; Movat *et al.*, 1963). If increased permeability in venules can be sustained for only short periods of about 10–20 min., and subsequently the affected venules are unable to respond for some hours (Miles and Miles, 1952), then we have a reasonable explanation for the early permeability response with its short duration, as well as for the succeeding interval of low permeability.

Nevertheless, the identification of the mediators of the late permeability response in inflammation remains a major problem. It seems that the prolonged late response may be induced by factors which act mainly on capillaries. The existence of such factors is illustrated by the ability of the toxins of various clostridia and *Vibrio cholerae* to elicit a delayed-type permeability response (Elder and Miles, 1957; Craig and Miles, 1961; Craig, 1965); and the demonstration that clostridial toxins mainly exert their permeability effect on capillaries (Miles, 1963). The next stage in the unravelling of the permeability events in inflammation may well be the identification of endogenous factors that induce a prolonged increase in the permeability of capillaries rather than of venules.

SUMMARY

The mediation of the early and late permeability responses induced by moderate UV injury in the skin of guinea-pigs, rats and rabbits, has been investigated by testing the effects of various pharmacological antagonists. Permeability changes were indicated by the exudation of circulating Evans blue in the injured skin sites.

The early response appears to be mediated by histamine in the guinea-pig, by 5-HT in the rat, the response being strongly suppressed by corresponding antagonists in both species. In the rabbit, the early response is insusceptible to antihistamine, and its mediator has not been identified.

In none of the test species has the mediator of the late response been established. In the guinea-pig, antihistamines i.v. strongly suppress the permeability effects of histamine i.c. for at least 2 hr., but repeated systemic or local doses do not decrease the late ultraviolet response. Suppression of the early response by antihistamine also does not affect the late phase of increased permeability.

The late response in guinea-pigs is also unaffected by local doses i.c. of the trypsin inhibitors from soya bean, lima bean, potato and ovomucoid given before ultraviolet irradiation or in lesions 3–21½ hr. old. In 2 experiments, mild suppression was obtained with soya trypsin inhibitor, but the results were inconsistent.

Other preparations which had no substantial effect on the late response included ϵ-amino caproic acid; a glucose analogue—*viz.*, 2-deoxy-(D)-glucose; α-amylase; sodium α-naphthyl acetate; and a bovine polypeptide preparation "Trasylol" which antagonizes both kallikrein and trypsin.

In the rat, the investigation of the late response was confined to the possible role of histamine and 5-HT. The response was unaffected by a 5-HT antagonist, but moderately suppressed by antihistamine. The late response was not investigated in the rabbit.

Of all the preparations tested in guinea-pigs, none appeared to affect erythema in the irradiated sites.

We are indebted to Upjohn Co., Kalamazoo, U.S.A. for supplies of 5-hydroxytryptamine creatinine sulphate; to Sandoz Ltd., Sydney for BOL-148 and synthetic bradykinin; to the Wellcome Research Laboratories Beckenham, England, for compound 48/80; to Professor Sir Ashley Miles of the Lister Institute of Preventive Medicine, London, for globulin permeability factor; to Winthrop Laboratories, Sydney for kallikrein and α-amylase; to the Ortho Pharmaceutical Corp., Raritan, New Jersey, U.S.A. for "Actase"; to A. B. Kabi, Stockholm, for "Kabikinase"; to Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York, U.S.A. for "Varidase", to Farbenfabriken Bayer, Leverkusen, West Germany for "Trasylol"; to Dr. J. Hladovec, Pharmaceutical and Biochemical Research Institute, Prague, for potato trypsin inhibitor; and to Carnegies of Welwyn Ltd., Herts., England, for sodium α-naphthylacetate.

Much of this work has been reported by one of us (G. L.) in a thesis accepted by the University of New South Wales for the degree of Doctor of Medicine.

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