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Some effects of ultraviolet light on mouse skin

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INTRODUCTION

The effects on skin of exposure to UV light are known to everyone who has experienced sunburn, namely, erythema and edema followed by scaling and changes in pigmentation. The mechanism(s) by which these changes are produced, however, remains unclear.

HISTORY

In 1926 Lewis and Zotterman[12] rendered their classic observations on the progression of changes in human skin following exposure to UV light. They noted that the sharply defined erythema which developed 30 to 60 minutes after irradiation was followed in 4 to 20 hours by a gradual extension of erythema 2 to 3 mm. beyond the irradiated area, the border becoming less distinct. With larger doses of irradiation even greater extension of the erythematous area occurred, and jutting processes could be observed extending away from the irradiated area. These were interpreted as extensions of erythema into lymphatic channels. On the basis of such observations these investigators postulated that UV light caused the liberation of an active vasodilator substance in the skin, and suggested histamine or histamine-like H-substance as the responsible agent. They compared UV light injury to other forms of tissue injury, e.g. freezing or stroking, and considered the relatively long latency of UV damage the only difference.

The role of histamine and histamine-like substances in mediating the effects of tissue injury, including that occurring in skin on exposure to UV light, has since come under extensive investigation. The development of anti-histaminic agents has provided a commonly used means of assaying the

activity of histamine under given conditions. 5-HT (5-hydroxytryptamine) has received increasing attention in recent years as a possible mediator of vascular injury, and 5-HT inhibitors have also been developed. In addition, compounds have been prepared which promote release of both histamine and 5-HT; the best known of these is 48/80.[17,23,35] Propamidine and morphine are also known to release histamine and 5-HT.[34] It has been shown that mast cells are a major source of body histamine,[24,21] and that 48/80 is a mast cell disruptor.[34] Whereas in rats both histamine and 5-HT are released by 48/80, in cats, dogs, and rabbits only histamine is liberated,[34] demonstrating that results obtained in one species do not necessarily apply to other species. Valtonen[25] has carefully studied the effect on the mast cell count in the skin (mouse) following UV irradiation of various wavelengths, and found that irradiation with wavelengths 253.7 mu and 286-350 mu was followed by a continuous rise in the mast cell density, which was corrected for increase in tissue volume due to edema, reaching a maximum after several days. No such change occurred following irradiation with the wavelength 350 mu, consistent with the observation that UV light of wavelength greater than 313 mu does not produce erythema, at least in the human.[4]

The theory that histamine plays an important role in mediating vascular injury following UV irradiation was challenged by the studies of Partington,[19] who showed that in the human UV irradiation did not alter the response to injected histamine, and that, conversely, the injection of histamine or 48/80 did not alter the response of skin to UV irradiation. In addition, it was shown that mepyramine, a histamine inhibitor, did not alter the response of skin to UV irradiation, whereas it significantly diminished the response to the injection of either histamine or 0.9% saline. Similar results were obtained in the rabbit. Partington thus concluded that it is very unlikely that histamine is the mediating agent of the erythema resulting from UV ir-

radiation. Claesson, Juhlin, and Jettermark[7] found that prolonged admini-
stration of 48/80 did not affect the response to UV irradiation in rat
skin, although it has been shown that 48/80 will greatly reduce the skin
content of both histamine and 5-HT.[6,18] However, these investigators
found that the response of skin to UV irradiation could be significantly
diminished by post-irradiative treatment with methotrimeprazine (compound
7044RP), which inhibits both histamine and 5-HT.[18] Coupling their obser-
vations with those of Partington[19] they concluded that 5-HT or an unknown
compound(s) inhibited by 7044RP must be the mediating agent in the reaction
to UV irradiation. A similar conclusion was reached by Parratt and West,[18]
who found that the reaction produced by injection of edema-producing sub-
stances, e.g., 5-HT, histamine, dextran, and 48/80, in the hind paw of a
rat was most inhibited by 5-HT antagonists, e.g., promethazine, and by
Rowley and Benditt,[23] who found that edema following injection of ovomucoid,
48/80, testis extract, or dextran in the rat paw could be partly inhibited
by dibenamine, a 5-HT antagonist, but was not inhibited by pyrilamine, a
histamine antagonist. However, the reaction was best inhibited by both
drugs in combination. The latter investigators also noted that injection
of ovomucoid, 48/80, testis extract or dextran subcutaneously resulted in
damage to mast cells, as well as edema, whereas injection of 5-HT or hista-
mine produced only edema with no damage to mast cells, and that drugs which
inhibit edema do not prevent mast cell damage.

The site of action of UV light in the skin

It is impossible to consider the site at which the photochemical reac-
tions in the skin occasioned by UV irradiation occur separately from a con-
sideration of the absorption spectra of the various layers of the skin for
UV light. Measurements of UV transmission in the skin are considerably com-

plicated by the problems of scattering, the stratification of skin into
layers with different absorption characteristics, and the rapid decrease
in transmission of UV wavelengths which lie in the erythema-producing
range, i.e., generally below 313 mu.[4] One of the most thorough and syste-
matic studies of these problems was made by Bachem[ ] in 1931. Using
fresh dead human skin he cut frozen sections 10-70 u thick perpendicular
to the surface of the skin, enclosed a section between quartz plates 0.2 mm
thick, and placed it in front of the slit of a quartz spectrograph so that
the layers of the skin were at right angles to the length of the slit. He
then directed light from a Kromayer lamp onto the skin, and compared the
transmission of the various layers with spectra obtained without a prepara-
tion in front of the slit.

In separate experiments with dogs and rabbits he found that there were
no remarkable differences between transmission values obtained for living
skin, and those obtained with fresh dead skin which was stretched and kept
moist with Ringer's solution. In a detailed analysis of absorption in hu-
man skin he distinguished 'true absorption' from 'total absorption', i.e.,
true absorption plus scattering. He found that the absorption of UV light
was greater in moist skin than in dry skin, which he ascribed to the greater
scattering effect in moist skin. The sudden increase in absorption of UV
as compared with visible light is mainly due to an increase in true absorp-
tion, the effect of scattering gradually becoming more pronounced towards
the shorter wavelengths.

A compilation of his results from human skin is presented in Fig. 1. The
transmission values are calculated from average values for the thicknesses
of the various layers. It will be noted that at 250 mu 19% and at 300 mu
34% of incident light penetrates the corneum. Bachem concluded that the
antirachitic action of UV light was effected in the stratum corneum or stra-
tum granulosum, whereas the erythema-producing effect was produced in the

HAUT SCHICHT	λ mm	200	250	280	300	400	550	750	1000	(1400)	μ
		100	100	100	100	100	100	100	100	100	Appliziert
CORNEUM	.03	100	81	35	66	20	13	22	29	56	Absorbiert und Reflectiert
		0	19	15	34	80	87	78	71	44	Transmittiert
MALPIGHII	.05	0	8	6	18	23	10	13	6	16	Absorbiert
		0	11	9	16	57	77	65	65	28	Transmittiert
CORIUM	2.0	0	11	9	16	56	72	44	48	20	Absorbiert
		0	0	0	0	1	5	21	17	8	Transmittiert
SUBCUTAN	25	0	0	0	0	1	5	20	17	8	Absorbiert
		0	0	0	0	0	0	0	0	0	Transmittiert

Fig. 1. The Absorption of light in the various layers of human skin (Bachem).

stratum germinativum or corium.

Other investigators have obtained different results. For example,
Blum and Terus,[4] using separated human corneum, obtained transmission
values at the wavelengths 260 mu and 300 mu of 4.1% and 24% respectively,
from which they concluded that the erythema-producing photochemical re-
actions of UV light must occur in the stratum corneum, resulting in dif-
fusion of active vasodilator substance into the papillary layer of the
dermis. This hypothesis was partly based on their observation that UV
light of wavelengths 298 mu-313 mu produces increasing erythema (in the
human) with increase in dose only up to a certain dose, the 'optimal dose',
above which increase in dose results in inhibition of the erythema. No
such inhibition could be demonstrated at the wavelength 253.7 mu. It was
noted, however, that in all cases actual damage to the skin was propor-
tional to dose. They thus postulated the occurrence of a second photo-
chemical reaction in the superficial layer of the dermis in response to
longer wavelength UV light, productive of an erythema inhibiting substance.

Miles and Miles[15] using the intravenous dye (Pontamine sky blue) tech-
nique (see below), noted a similar phenomenon following the intra-dermal
injection of histamine in the guinea pig. Injection in 0.1 ml. of 0.1-0.2 mg
histamine resulted in blueing of the skin within 3 to 5 minutes, reaching
a maximum in 10 to 12 minutes. Doses in 0.1 ml of 1 to 3 mg produced blue-
ing first at the edge of the bleb which gradually progressed to the center.
Doses above 4 mg produced only peripheral blueing, the center remaining un-
colored, and doses of 10-20 mg resulted in a very narrow band of faint blue
at the edge of the bleb. They observed that the capillaries regained their
normal permeability within 10 to 30 minutes after the injection of 2 mg
histamine, and that they are then immune in varying degrees to further injec-
tion of histamine. They considered that the inhibition of increase in

capillary permeability following too large doses might be a result of vasoconstriction lasting until the recovery of normal permeability. That the inhibition was not due to blockage of the vessels with blood corpuscules was shown by the injection of ▬ India-Ink. An alternative explanation was proposed, namely, that the capillary reaction to high concentration of histamine may occur so rapidly that the immune stage is reached before the increased permeability manifests itself.

Claesson, Juhlin, and Wettermark,[8] also using the intravenous dye (Evans blue) technique, demonstrated that the degree of blueing of mouse skin following irradiation with UV light of 280-330 mu increased quite rapidly to a maximum and then declined with increasing doses. Combining results from irradiation with a high intensity flash lamp and a Phillips SP 500W lamp they were also able to demonstrate the validity of the reciprocity law of UV irradiation (minimal effective blueing dose = intensity x irradiation time = constant) over a 10^7 fold range of intensities. They used exposure times of from 35 x 10^{-6} seconds to 500 seconds.

Evidence for the function of the corneum in the elaboration of vasodilator substances following exposure to UV light was presented by Rottier and Mullink[22] (1952). In experiments on the human forearm they removed the horny layer from one area by repeatedly applying and removing adhesive tape (9 - 12 times). They then compared the erythema produced by irradiation of this area with that produced on normal skin, and found that the erythema was augmented by removal of the horny layer following irradiation at 300 mu, but at 250-260 mu it was diminished. From this they concluded that the primary photochemical reaction produced by UV light at 250-260 mu occurred in the stratum corneum, whereas the dose effect at 300 mu is localized to the 'mucous' layer.

Claesson, Juhlin and Wettermark[7] (1959), repeated the experiments of

Rottier and Mullink in humans, rabbits and rats at the wave lengths 253.7 mu
and 280-330 mu. They found that in all cases removal of the horny layer
resulted in an approximately 10-fold increase in sensitivity of the skin to
light in these wavelength regions.

It is apparent that neither the site nor mechanism of action of UV
light in producing its effect in skin is perfectly understood at this time.
The present investigation is a study of the role played by the various skin
layers in mediating the effects of UV light, comparing the effects obtained
at the wavelength 253.7 mu with those obtained using generally longer wave-
lengths. A technique is employed in which irradiation of the underside of
the skin is performed. In addition, a study is made of the doses required
to produce an effect shortly after irradiation as compared with that re-
quired to produce a 'delayed effect'.

The experiments were performed in two parts, the first at the Institute
of Physical Chemistry in Uppsala, Sweden, and the second in the Department
of Pathology of Yale Medical School in New Haven, Connecticut. They will
be described separately.

PART I

Methods

Sources of Ultraviolet light

A commercial, water cooled, high pressure mercury arc (Phillips SP
500 W), equipped with a filter (2 mm. Jena UG 11) provided ultraviolet
light in the wavelength region 250 - 400 mu, with a broad peak between
300 - 350 mu. The transmission curve for this filter is shown in Fig. 2.
It will be noticed that the transmission between 250 - 260 mu is very low;
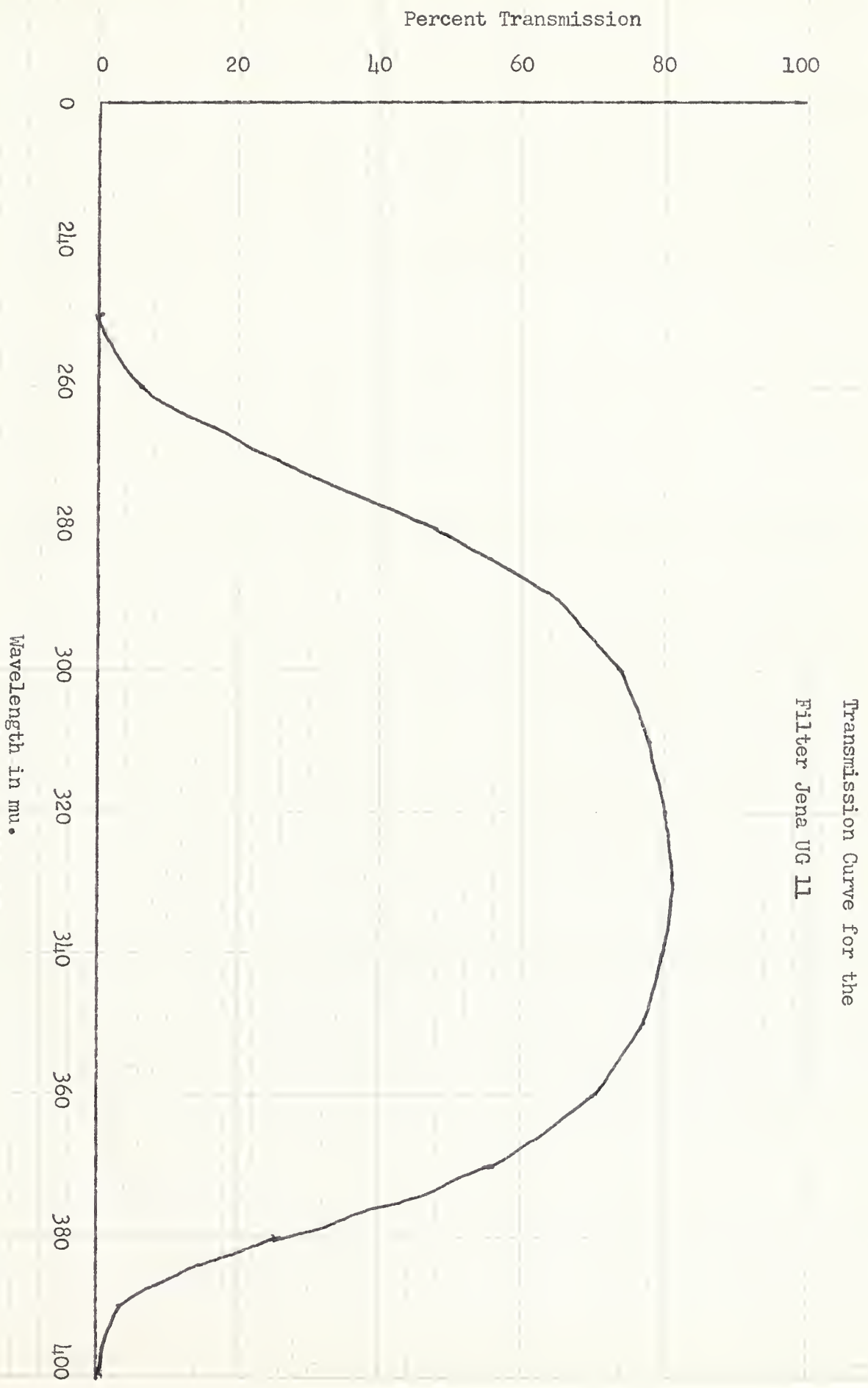the area of the curve bounded between 275 - 375 mu. comprises 92% of the

Transmission Curve for the

Filter Jena UG 11

Percent Transmission

Wavelength in mu.

FIG. 2.

total. The spectral distribution of energy from the lamp has several peaks between the wavelengths 280 - 350 mu,[8] and it may be assumed that the major portion of the effective erythema-producing UV light obtained from this source was in the wavelength region 280 - 350 mu. The filter was built into a brass tube approximately 4 cm. in diameter and 6 cm. in length, which was screwed onto the lamp, and through which part of the exit water from the lamp was diverted to provide cooling for the filter. Mounted vertically, a bakelite tube 7 cm. in diameter and 25 cm. in length was screwed to the underside of the filter enclosure, and was fitted at its lower end with a shutter. The light was thus confined to the irradiation table, a wooden platform kept at 10 cm. beneath the shutter, giving a lamp to animal distance of about 40 cm. The irradiation table could be rotated sideways to allow easy placement of the animal. Measurements of the incident intensity were made at various times during the experiments, employing a ferrioxalate actinometer (Hatchard and Parker, 1956).[10] Seven measurements at irradiation times of 2 to 5 minutes, from the start of the lamp, gave an average value for the intensity of 1.25×10^{16} hν/cm^2 sec., with an average deviation from the mean of 0.19 or 15.2% $\bar{x}$. Experimental irradiation times of less than 2 minutes were made with the shutter 2 - 3 minutes after starting the lamp, to provide this minimal degree of stabilization.

Unfiltered light at the wavelength 253.7 mu. was obtained from a low pressure mercury lamp (Hanovia Sc 2537), described by Moring-Claesson (1956).[16] The irradiation table was kept at 15 cm. from the lamp. Incident intensity, measured as described above, was stable at 5.4×10^{15} hν/cm^{2}sec.

Experimental procedure

Male albino mice of commercial stock weighing between 20 - 30 grams were used in all experiments. The hair on the abdomen was clipped closely, but

gently, with an electric clipper. During early experiments this was followed by complete depilation with a barium sulfide paste, which was abandoned after numerous instances of chemical injury. No marked differences were noted between animals shaved immediately and those shaved 24 hours before experiment; all final experiments were nevertheless done on animals shaved one day earlier.

Ether anesthesia was used during all experimental procedures. For experiments involving long irradiation times a base anesthesia was established with 0.15 ml. intraperitoneal Nembutal (6.0 mg./cc.), which was supplemented with ether. The irradiation diaphragm was a rectangular hole, approximately 1 x 3 cm., cut into a light rubber sheet, pliable enough to drape loosely over the animal. The diaphragm was kept in position over the shaved abdomen by small weights on the sheet at the animal's sides. Care was taken to avoid pressure. For experiments in which the underside of the skin was to be irradiated a longitudinal incision of about 2 cm. was made on the abdominal mid-line. A small flap of skin on one side was then lifted from the muscle by blunt dissection. Very little or no bleeding was encountered. With the animal on its side the edge of the skin flap was grasped by two small surgical clamps, separated by about 1.5 cm., mounted on the sliding portion of a specially constructed metal base, the bottom of which was fitted firmly against the animal. The skin could thus be kept at approximately its normal tension. The diaphragm was placed crosswise, so that about 1 cm.^{2} skin was irradiated on each side of the incision, allowing comparison of the effects from irradiation of the underside and outside on the same animal. Following irradiation the incision was immediately closed with 2 small sutures placed through the skin edges. The skin flap was thus elevated from the muscle for 2 to 5 minutes only. No special measures were taken to prevent drying of the exposed underside during irradiation, and vascular damage, as

judged by leakage of dye, did not occur, except for a narrow strip (1 - 2 mm.) of blueing along the incisional border. About half of the irradiations were made from the outside only, the skin intact.

Tissue damage was judged by leakage of intravenously injected Evans blue dye into the irradiated area, a method first described by Ramsdell (1928),[13] and later by Miles and Miles.[15] Blueing of damaged tissue is considered evidence of an increase in capillary permeability, which allows extravasation of the protein-bound dye. 0.7 ml. of dye in physiological saline (0.3%) was injected. A certain time interval is required after dye injection for blueing of the damaged tissue (see Part II); in these experiments the animal was sacrificed 15 minutes after dye injection, as in previous experiments done at this Institute. After sacrifice, the abdominal skin was removed, fixed with tacks underside-up on cardboard, and allowed to dry. Irradiation damage was judged positive if the irradiated area showed definite blueing with well-marked borders. Determination of minimal dose was made by progressively decreasing irradiation time from an exposure found to produce a strong effect. The 'minimal dose' described here is of course an approximation drawn arbitrarily at the dose below which response is too sporadic to be considered general. Positive responses were occasionally observed at doses lower than the general 'minimum', which were attributed to fluctuations in lamp intensity and normal variations in animal susceptibility. The experiments may be divided primarily into four categories, divisions according to type of UV light used, and direction of its application, i.e., from outside or underside of the skin. Experiments using filtered light (280 - 350 mu) were done on approximately 75 mice; at 253.7 mu, data were collected from 21 mice. In both groups most irradiations were done around the critical dose, a few at doses much higher or much lower. The experiments may further be divided according to time of dye injection.

This was done either just before or after irradiation (it made no dif-
ference), or after an interval of 20 hours. At 280 - 350 mu the exper-
iments were about equally divided between these two conditions; at 253.7
mu, 19 animals were injected immediately after irradiation.

Results

High pressure mercury lamp with filter; 280 - 350 mu.

Immediate effects. Severe vascular damage, as measured by protein-
bound dye extravasation, can be demonstrated within 15 minutes of irradia-
tion, irradiation from either the outside or underside of the skin. In
this time interval the diffusion of the dye appears to be the limiting pro-
cess, as approximately this same interval must be allowed for tissue colora-
tion if the dye is injected 20 hours after irradiation (see Part II). Several
(12) experiments were done, however, in which dye injection preceeded irra-
diation by some minutes, and the animals were sacrificed at very short times
after irradiation. Although maximal coloration was probably not achieved,
several animals (5 - 6) showed damage as early as 5 minutes after a 5 minute
irradiation (3.75×10^{18} hν /cm^2) from the outside on intact skin. Lower
doses were not tried. Minimal dose determinations were made on intact skin
allowing a 15 minute post-irradiation interval (pre-irradiation dye injec-
tion). This dose was found to be about 7.5×10^{17} hν /cm^2 (1 minute expo-
sure). Sensitivity to irradiation from the underside was very nearly the
same, perhaps slightly greater. In these animals the damage to the portion
of skin irradiated from the outside was approximately the same as in the
experiments on intact skin.

Effects after 20 hours. After 20 hours the outside and underside of
the skin showed approximately equal sensitivity to UV irradiation. However,
the minimal dose was much lower, about 5×10^{16} hν /cm^2, or about 15 x less

than that required to produce immediate damage, measured by our criteria. As above, damage to the portion of skin irradiated from the outside adjacent to the incision on operated mice was comparable to that produced on intact skin.

Low pressure mercury lamp: wavelength 253.7 mu.

Experiments at the wavelength 253.7 mu. were done on 21 mice. Eight irradiations were done from the outside on intact skin, followed immediately by dye injection and sacrifice of the animal 15 minutes later. As in the experiments with light of longer wavelength, damage could be shown at this time, with a minimal dose of about 6.5×10^{17} hν/cm^2. With operated animals (10), treated similarly, the underside showed somewhat greater sensitivity. Minimal dose was not determined for a 20 hour effect, but two animals receiving 2.2×10^{16} hν/cm^2 and 1.1×10^{16} hν/cm^2, respectively, showed very marked responses.

A summary of these results is shown in table 1.

Some preliminary experiments with epinephrine.

If the mechanical state of the vessels is important in their susceptibility to UV damage, epinephrine might alter the measured response to irradiation. Experiments on 35 animals were done, varying times for the epinephrine and dye injections with respect to irradiation. Optimal irradiation times were used. Although results were not conclusive, it did not appear in these experiments that irradiation damage as measured after 20 hours could be changed by pre-irradiation injection of epinephrine. 0.2 ml. epinephrine (conc. 0.05%) was injected, as much as possible intracutaneously, the rest sub-cutaneously. On the other hand, in some experiments concluded 4 hours after irradiation the irradiated area was definitely bleached, with only irregular blue patches. This occurred whether the epinephrine was injected before or after the irradiation (at various times

up to shortly before dye injection), so it could not be considered a direct effect on the degree of irradiation damage, but rather only the (normal) constrictor effect of epinephrine, which was, however, sufficiently strong to prevent leakage of the dye. Whether epinephrine injected shortly before dye can prevent leakage of the dye 20 hours after irradiation has not yet been determined.

Incidental skin damage.

Damage to the skin sufficient to cause leakage of dye was very easily produced by injections, shaving, and other handling. The blueing observed after such incidental damage was always patchy, and not easily confused with the well-marked blueing caused by irradiation.

PART II

Methods

The methods used in these experiments were essentially the same as those described in Part 1. A low pressure mercury lamp provided UV light at the wavelength 253.7 mu for all experiments. The lamp was enclosed in a cardboard box, into which a window was cut to allow passage of the animal to be irradiated. A commercial strain of albino mice (female) weighing between 20 and 30 grams, was used. The animals were anesthetized with ether for all experimental procedures, except those involving long irradiation times, which required a base anesthesia established with intraperitoneal nembutal (0.15 ml., 6.0 mg/cc). The abdominal hair was clipped 24 hours before irradiation on all animals, using an electric clipper with a fine head. At the time of irradiation the animal was gently fixed with rubber binders in the supine position on a cork board (10 x 30 cm.) which was fastened rigidly on a ring stand. The whole

assembly was then maneuvered so as to place the animal into the irradiation box for the desired interval. The animal was draped with a pliable material which absorbed completely at the effective wavelength. A rectangular (ca. 0.75 x 2.5 cm.) diaphragm was cut in the cover and placed crosswise on the animal's abdomen, delimiting the area to be irradiated. The lamp to animal distance was kept at 15 cm. At this distance the intensity of the lamp was determined using the ferrioxalate actinometer as described before. Several measurements were done, and the intensity was stable at 6.8×10^{14} hν/cm.^{2} sec.

Intravenous injection of Evans blue dye (0.5 ml., 0.5% in 0.9% saline) was performed either immediately following irradiation or after an interval of 20 hours, as in the earlier experiments. The animals were sacrificed 15 minutes after dye injection (see below). The skin around and including the irradiated area was then removed and fixed with thumb tacks underside-up on cardboard. Irradiation effect was considered positive to the degree that the irradiated area showed well-marked blueing. All experiments were done on intact skin. Determination of the minimal doses required for immediate effect and effect after 20 hours were performed. About 50 animals were used in this group of experiments. In addition, experiments were performed to determine the time following dye injection required for the appearance of blueing in irradiated skin.

Results.

The minimal dose required for blueing 20 hours after irradiation was found to be about 2×10^{16} hν/cm^2, or an irradiation time of 30 sec. For production of blueing with dye injected immediately after irradiation a much longer irradiation time was required, about 20 minutes, or a dose of about 8.2×10^{17} hν/cm^2. Greater variation in the response to a given dose occurred in the latter experiments. This may be partly explained on

the basis of the greater length and depth of anesthesia required during

irradiation which resulted in extension of the anesthetic state into

the interval following injection of the dye, which did not occur when

only sufficient anesthesia was used to immobilize the animal during

the injection of dye. Miles and Miles[15] showed that blueing could be

inhibited in an anesthetized animal. However, the minimal dose deter-

mination here compares favorably with that found for an immediate effect

in the earlier experiments. It will be noted that the dose required for

an effect allowing a 20 hour latent period is about 40 times less than

that required for an immediate effect. This factor is somewhat larger than

that found (15 times) using the filtered light of longer wavelengths in

the earlier experiments. Plates 1 - 12 are photographs of representative

skin specimens obtained in this series of irradiations.

The time required for blueing after dye injection was studied in ex-

periments on 13 animals. The dye (0.5 ml; 0.5% in 0.9% saline) was in-

jected 20 hours after irradiation with 8×10^{16} hν/cm^2 (4x minimal dose),

and the animals were sacrificed at varying intervals thereafter. The skins

were treated as described earlier, and estimations of the degree of blue-

ing were made. The results are presented in Table 2.

It is seen that under these conditions the blueing does not generally

appear before 6 minutes, and reaches a maximum in about 10 minutes. The

one exception occurred in an animal sacrificed only one minute after dye

injection in which definite blueing could be ascertained. These results

agree approximately with those reported by Miles and Miles[15] who injected

histamine intradermally into guinea pigs following the intravenous injec-

tion of pontamine sky blue dye (65 - 75 mg./Kg.). They noted that blueing

appeared in the bleb in 3 to 5 minutes, and reached a maximum in 10 to

12 minutes.

Histological Studies.

The histological changes in human skin following UV irradiation were described by Meirowsky in 1906.[14] He noted no immediate changes, but by 7 hours after irradiation there was dilatation of capillaries, swelling of collagen fibers, and the beginning of a leucocytic infiltration into the irradiated area. The changes progressed to chromatolysis of nuclei, edema, increased pigmentation and an abundant leucocytic infiltration. An increase in the amount of collagen was observed. These changes occurred over a period of several days.

The histology of normal mouse skin has been reviewed by Hansen[9], who quotes Bang[2]. "The epidermis is very thin, and consists in many places of only a single layer of epithelial cells which do not at all show the regular arrangement of cylindrical basal cells, but whose cell walls are not clearly seen; here and there the epidermis is a little thicker and a few epithelial cells are seen lying irregularly superficial to the basal cells. No regular division into strata can be distinguished; there is some desquamation of the epithelial cells on the surface but there is no real marked cornification.

"As in human skin the epidermis is invaginated into the corium to form the openings of the hair follicles which have the same structure as the uninvaginated epidermis; the follicles which run from the depth of these openings pass through the corium and usually pass deep into the subcutaneous layer. The sebaceous glands resemble exactly those in the human skin. Sweat glands appear to be lacking, and I have not seen a definite papillary formation of the corium."

The subcutaneous tissue in the mouse consists of a thick layer of loose areolar tissue interspersed with hair follicles, and a deeper thin layer of striated muscle, the musculus platysma. The histology of normal

mouse skin is illustrated in plate 13.

The changes occurring in mouse skin following UV irradiation have been studied by Blum et al,[5] who irradiated portions of the ears of albino mice with single doses of UV light in the wavelength region 313 mu and shorter. Two doses were used, 2.4×10^6 ergs/cm^2 and 1.2×10^6 ergs/cm^2. The earliest gross change, detectable shortly after the first day (following the higher dose), was the appearance of erythema which usually involved the entire ear. Within a few days the erythema faded on the shielded part but remained over the irradiated part, leaving a line of demarcation. With the lower dose the erythema appeared about the same time but remained more in the exposed area. Microscopically, vascular engorgement was not easily detected, but within three days a leucocytic infiltration of the dermis was noted. A thickening of the epidermis from 2 or 3 cells to 10 or more cells thickness was observed several days after irradiation. At this time the cells of the lowest layer appeared normal, above this layer were cells with swollen or pyknotic nuclei, and more superficially were some cells in which no nuclei were apparent. A lesser thickening of the epidermis on the underside of the irradiated portion of the ear was also seen. Small, sharply localized inflammatory lesions involving both epidermis and dermis appeared within the first few days, eventually becoming observable grossly, associated with crusting and desquamation of the surface. Sebaceous glands and hair follicles decreased in number during this period. The changes observed were similar with the two doses used, but hyperplasia was more pronounced following the high dose. As healing occurred the epidermal hyperplasia and hypertrophy of the cells diminished, the crusted lesions disappeared, and the ear returned to near normal within a few weeks. However, absence of sebaceous glands and hair follicles was observed as late as 39 days following irradiation.

In the present investigation 12 animals were irradiated with an optimal dose of UV light at 253.7 mu (8×10^{16} hv/cm^2) and injected with intravenous Evans blue dye (0.5 ml; 0.05% in 0.9% saline) 20 hours after irradiation. The skins were then removed, fixed, sectioned and stained with hematoxylin and eosin. Microscopic examination of the sections was then done. A control study of sections of normal unirradiated mouse skin was also made.

Histologically, the blueing of the irradiated area, which had been marked on gross observation, was not evident. This may have been partly due to removal of the dye during fixing and staining. In addition, the thinness of the section (10 u) may have made it impossible to identify the blued area. However, certain other changes were noted, as illustrated in Plates 14 through 20. Plate 13 is from a section of normal unirradiated mouse skin. The stratum corneum is seen to be not more than about 25 u in thickness, and occasionally is much thinner. The dermal collagen is well preserved, and is diffusely infiltrated by a population of mononuclear cells, composed chiefly of lymphocytes but containing occasional plasma cells and mast cells, with rare eosinophils and polymorphonuclear leucocytes. In most cases the infiltrate was limited to the dense collagenous layer of the dermis, and usually was present to only a minor degree in the subcutaneous fatty tissue adjacent to the deep hair follicles. The blood vessels are not prominent. Plates 14 through 20 are from sections through irradiated areas of skin. The most common changes noted were marked congestion of capillaries within the dermal papillae (Plate 14), congestion of the capillaries in the subcutaneous tissue (Plate 15), and varying degrees of edema (Plates 14 and 15). Almost all of the irradiated specimens showed some degree of infiltration with polymorphonuclear leucocytes. The overall intensity of the infiltrate when examined at low power

was usually not much greater than that seen in normal skin; however,
the qualitative make-up of the infiltrate was usually quite different
and included a greatly increased proportion of polymorphonuclear leuco-
cytes and mast cells (Plates 16 through 19). The polymorphonuclear in-
filtrate was in almost all cases limited to the irradiated area of the
specimen, although the zone of transition between the irradiated and un-
irradiated areas was gradual, corresponding to the similar observation
made by Blum et al.[5] In two or three specimens the leucocytic infiltrate
reached proportions almost suggesting micro abscess formation, although
no cavity formation was demonstrated (Plates 18 and 19). In one case
such heavy distinct infiltrate appeared to be associated with an over-
lying superficial laceration of the epidermis, indicating that these few
irregular very intense focal infiltrates of polymorphonuclear leucocytes
may have been caused by mechanical trauma to the skin incurred during pre-
paration. Occasionally, small intraepithial infiltrates of mononuclear
cells with a few polymorphonuclear leucocytes were observed in irradiated
areas, which were usually not related to any underlying dermal infiltrate
(Plate 20).

Frozen sections (20 u) of the same skin specimens used in the above
experiments were made in a further attempt to locate the dye in the tissue.
Again, no blue color could be identified. It is probably that a tissue
section thin enough to permit microscopic examination involves a dilution
effect of the dye too great to permit visualization by this method.

Microdissection of the blue area of an irradiated specimen of skin
was then carried out using a Zeiss stereomicroscope with reflected light.
With this technique it was observed that the intensity of the blue color
was increased by removal of the subcutaneous muscle and areolar tissue.
The removed tissue appeared white and without evidence of contained dye.

Dissection of the epidermis from the external aspect produced a similar effect. The blue color appeared deepest in lines between the hairs which were observed in rows. Section and histological examination of the dissected skin showed that the subcutaneous tissue had been removed more or less completely down to the corium. However, much of the epidermis remained intact, although the horny layer had been removed. It was concluded from these observations that most of the dye which extravasates in skin following UV irradiation is confined to the dermis, although the possible presence of smaller concentrations in the subcutaneous tissue, or epidermis, could not be excluded by this method.

Two animals were injected with 0.2 and 0.1 ml., respectively, of concentrated acridine-orange dye in physiologic saline 20 hours after irradiation with 8.0×10^{16} hν/cm^2. The first injection resulted in death of the animal within one minute; the second animal was sacrificed about one hour after injection. Gross and microscopic examination of the irradiated area was performed under UV light. Well demarcated fluorescence of the irradiated area could be observed even in the animal which died one minute after injection of the dye. This fluorescence was noted to persist and remained confined to the irradiated area in the second animal during the hour before it was sacrificed. Frozen sections (20 u) of the skin were then made in an attempt to locate the dye in the tissue. However, the intrinsic fluorescence of the skin was too great, obscuring that of the dye, so that again no localization was possible.

DISCUSSION

On the basis of their observation that UV light of wavelengths
298 mu - 313 mu would inhibit erythema in too-great doses, whereas UV
light at 253.7 mu would not, Blum and Terus[4] hypothesized that the
photolysis or alteration of protein in the epidermis was responsible
for the production of vasodilating substances, which diffused to and
acted on the vessels in the dermis. UV light of longer wavelengths
was supposed to evoke a similar reaction in the epidermis, as well as
an erythema-inhibiting reaction in the superficial layer of the dermis.
Evidence for the partial validity of this hypothesis was provided by
the observation of Rottier and Mullink[22] that removal of the stratum
corneum from human skin resulted in a decrease in its sensitivity to
UV light at the wavelengths 250 - 260 mu. However, a corresponding
increase in sensitivity was found at the wavelength 300 mu. These find-
ings suggested the existence of two separate photochemical reactions in
the skin, one caused by irradiation at 250 - 260 mu occurring in the
stratum corneum, and another caused by irradiation at longer wave-
lengths (specifically 300 mu) occurring in some layer deep to the stra-
tum corneum. However, Juhlin and Wettermark[7] showed increased sensitivity
to UV light in both wavelength regions (253.7 mu and 280 - 330 mu) fol-
lowing removal of the stratum corneum in the human, rabbit, and rat. The
role of the stratum corneum in mediating the effects of UV irradiation
was thus brought into doubt. That photochemical reactions occurring in
the stratum corneum are not solely responsible for the production of va-
sodilating substance following UV irradiation is effectively proven by
the finding that irradiation from the underside of the skin results in
vascular damage, since it must be assumed that essentially all UV light

is absorbed by the subcutaneous tissue and dermis in irradiation from this aspect (See Fig. 1). This becomes increasingly true towards the shorter wavelengths where absorption is more profound. It is noteworthy that at the shorter wavelengths (253.7 mu) irradiation from the underside of the skin is more effective than irradiation from the outside, whereas at the longer wavelengths approximately equal sensitivities were found. This observation could be explained on the basis of the relatively greater absorption of the stratum corneum at 253.7 mu than at e.g. 300 mu, if it is assumed that the stratum corneum exercises only a passive filter effect to UV light.

Complete absorption spectra for the various layers of mouse skin are not available. Hansen[9] studied the transmission of frozen sections of mouse and human skin, cut generally parallel to the skin surface in the wavelength region 300 mu to 500 mu. He then examined the sections microscopically. Using sections of equal thickness (ca. 0.06 mm) which included the epidermis he obtained transmission values at the wavelength 300 mu of about 20% in mouse skin and about 5% in human skin. Somewhat greater transmission through the epidermis of mouse skin might be expected in virtue of its relative thinness. The value obtained by Hansen for the transmission at 300 mu through human epidermis (ca. 5%) is somewhat lower than that obtained by Bachem[1] (16%), although comparison is difficult since they used quite different methods. In addition, the frozen sections used by Hansen did not separate the skin accurately into layers. However, Hansen found that between the wavelengths 300 mu and 500 mu the shapes of the absorption curves obtained from the various layers of mouse skin were similar to those obtained using human skin, with a gradual decrease in transmission towards the shorter wavelengths. It may be assumed that the ratio $\frac{T\ 250}{T\ 300}$ is about the same for mouse skin as

for human skin for any given layer. Using the results of Bachem[1] for human corneum this ratio $\frac{T250}{T300} = \frac{19}{34} = 0.56$. The ratio of the energy per photon at the two wavelengths $\frac{h\nu\ 250}{h\nu\ 300} = 1.2$. If the stratum corneum exercised only a filter effect, then the relative effectiveness of UV light at 250 mu as compared to 300 mu should equal 1.2 x 0.56 = 0.67. The ratio of the minimal doses found at the two wavelengths should then be: $\frac{M.D.\ 250}{M.D.\ 300} = \frac{1}{.67}$ or approximately 1.5. In the experiments on immediate effect, the ratio of the (average) values actually obtained is $\frac{M.D.\ 250}{M.D.\ 300} = \frac{(8.2 + 6.5) \times 10^{17}\ h\nu\ /cm^2}{2 \times 7.5 \times 10^{17}\ h\nu\ /cm^2} = $ approximately 1. A similar ratio calculated for effect after 20 hours is: $\frac{M.D.\ 250}{M.D.\ 300} = \frac{2 \times 10^{16}\ h\nu\ /cm^2}{5 \times 10^{16}\ h\nu\ /cm^2} = 0.4$. The ratio obtained for the doses required for immediate effect is in fair agreement with the predicted value. However, in the experiments in which a 20 hour latent interval is allowed, UV light at 253.7 mu appears to be relatively more effective than it is in producing an early effect. This finding may be more apparent than real, since a factor of only 2.5 is involved, which may reflect experimental error. If valid, the finding could be explained by the hypothesis that UV light at 253.7 mu acts at a locus relatively distant from the vessels which are eventually affected. The vasodilating substance then elaborated would require a longer time to reach an effective concentration at its site of action, namely, the vessels in the dermis. Such a locus of action of UV light at 253.7 mu would probably be in a superficial layer, e.g., a layer between the stratum corneum and the dermis. This interpretation would support the conclusion of Bachem[1] that the erythema producing effect of UV light occurs in the stratum germinativium or corneum. This conclusion applies to the irradiation of skin from the outside only. Since UV irradiation of the underside of the skin at either of the wavelength regions studied is also

effective in producing vascular damage, it must be concluded that photo-
chemical reaction to UV light can occur in deeper layers of the skin as
well. The substance(s) which mediates the vascular damage following UV
irradiation has not yet been definitely identified, although 5-HT has
been implicated by several investigators.[7,18,23] Majno and Palade[13],
studying vessels directly with the electron microscope, have observed
that 5-HT is more potent than histamine in producing vascular damage,
but that their effects are otherwise identical. They observed the leak-
age of intravenously-injected colloidal Hg_5 particles into vessel walls
following local injection of 5-HT or histamine, and demonstrated that
the leaking vessels were not the true capillaries but small vessels
ranging from 7 to 75 u in diameter, which were always found on the venous
side of the circulation. Substances other than 5-HT and histamine may
also be involved in mediating increased vascular permeability. For ex-
ample, Spector[24] has shown that various peptides of chain length 3 - 14
amino acids cause vascular leakage. The release of such substances in
skin by UV irradiation might result from direct effect on tissue protein
or from the activation of certain enzymes which would produce the same
result.

SUMMARY

The effects on the skin of the mouse of UV light at the wavelengths 280 - 350 mu and 253.7 mu have been studied using leakage of intravenous Evans blue dye into the irradiated area as a measure of tissue damage. Irradiations were performed from the underside of the skin as well as from the outside, on the same animal, and on intact skin. A comparison of the results reveals that the skin is about equally sensitive to irradiation from its opposite aspects when UV light in the wavelength region 280 - 350 mu is used. A somewhat greater sensitivity to irradiation from the underside was noted at the wavelength 253.7 mu. It was concluded that photochemical reactions in response to UV light can occur in the deeper layers of the skin.

In other experiments the doses necessary to produce blueing of the irradiated area if the dye was injected immediately after irradiation were determined. It was demonstrated that blueing of the irradiated area could occur as early as 5 minutes after an irradiation time of 5 minutes using UV light in the wavelength region 280 - 350 mu. An analysis of the minimal doses using the two types of UV light suggested that the shorter wavelength (253.7) was more effective if a latent period was allowed, and a possible explanation for this was proposed.

Microscopic dissection and histological examinations of irradiated and blued specimens of skin were performed, and the findings discussed.

Illustrations of some of the results are provided in Plates 1 - 20.

Dye injected after irradiation		Minimal dose in $h\nu/cm^2$	
		280 – 350 mu	253.7 mu
Immediately	Outside	75×10^{16}	65×10^{16}
	Underside	$< 75 \times 10^{16}$	$< 65 \times 10^{16}$
20 hours	Outside	5×10^{16}	$< 11 \times 10^{16}$
	Underside	$< 5 \times 10^{16}$	not determined

Table 1. Results obtained in Part I.

Time in minutes	15	15	15	11	10	10	6	6	3	3	1	1	0	
Effect	+++	+++	++	+++	+++	+++	±	≠	–	–	+	–	–	

Table 2. Degree of blueing observed in animals sacrificed at varying times after dye injection. Dose $\cong 8 \times 10^{16} \, h\nu/cm^2$. Interval $\cong$ 20 hours. See text.

Plate 1. Irradiated with 6 × 10^9 nν/cm^2. Dye injected intravenously 20 hours after irradiation. Markedly increased rectangular area on underside of skin represents when in irradiated portion. Note well-defined borders. See text.

Plate 2. Irradiated with 18 × 10^15 nν/cm^2. Dye injected 20 hours after irradiation. Result similar to that seen in Plate 1.

Plate 3. Irradiated with 1.8 × 10^16 nν/cm^2. Dye injected 20 hours after irradiation. Deep blueing of the irradiated area is observed.

Plate 4. Irradiated with 1.8 × 10^13 nν/cm^2. Dye injected 20 hours after irradiation. Compare with preceeding result.

PLATE 1.

PLATE 2.

PLATE 3

PLATE 4.

PLATE 5.
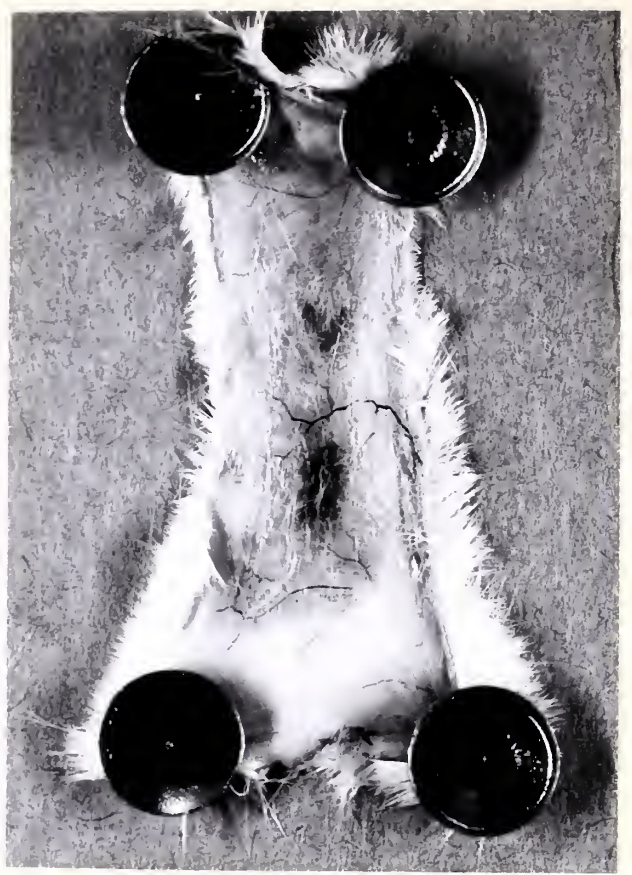
PLATE 6.

PLATE 7.
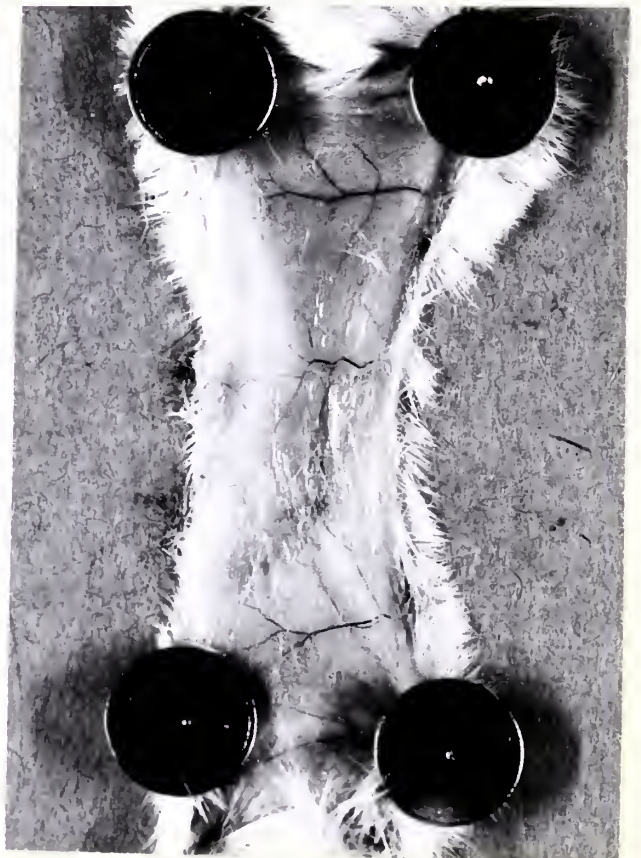
PLATE 8.

Plate 5. Irradiated with 4.1 x 10^{16} hν/cm^2. Dye injected 20
        hours after irradiation. A definite but mild blueing
        of the irradiated area is present.

Plate 6. Irradiated with 2 x 10^{16} hν/cm^2. Dye injected 20 hours
        after irradiation. Somewhat irregular blueing which is
        confined to the irradiated area is observed. This dose
        is considered "minimal".

Plate 7. Irradiated with 1 x 10^{16} hν/cm^2. Dye injected 20 hours
        after irradiation. No blueing is observed.

Plate 8. Irradiated with 1 x 10^{16} hν/cm^2. Dye injected 20 hours
        after irradiation. Again, no blueing is observed.
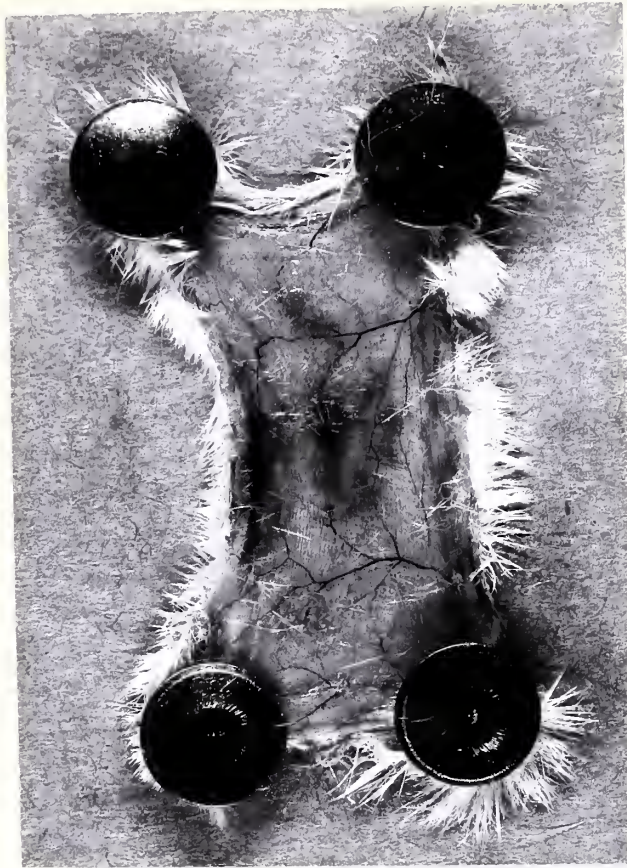
Plate 9. Irradiated with 10×10^{17} hν/cm^2. Dye injected immediately after irradiation. Note irregular weak blueing which occurs in the rectangular irradiated area. The blueing along the edges of the specimen is artefactual.

Plate 10. Irradiated with 8.2×10^{17} hν/cm^2. Dye injected immediately after irradiation. Well-defined blueing occurs in the irradiated area. There is some artefactual blueing as well.

Plate 15. Section of normal unirradiated mouse skin. Note
thinness of epidermis and cellular infiltrate in
the dermis which is chiefly mononuclear. See
text. Magnification x 160.

Plate 16. Section through irradiated skin. Note congested
capillaries in the superficial portion of the
dermis. See text. Magnification x 750.

Plate 17. Section through irradiated skin. Note congested
capillaries in the subcutaneous tissue. Striated
muscle fibers represent the musculus platysma.
See text. Magnification x 1200.

Plate 18. Section through irradiated skin. A low-power view
of diffuse dermal and subcutaneous infiltrate which
contains many polymorphonuclear neutrophils and an
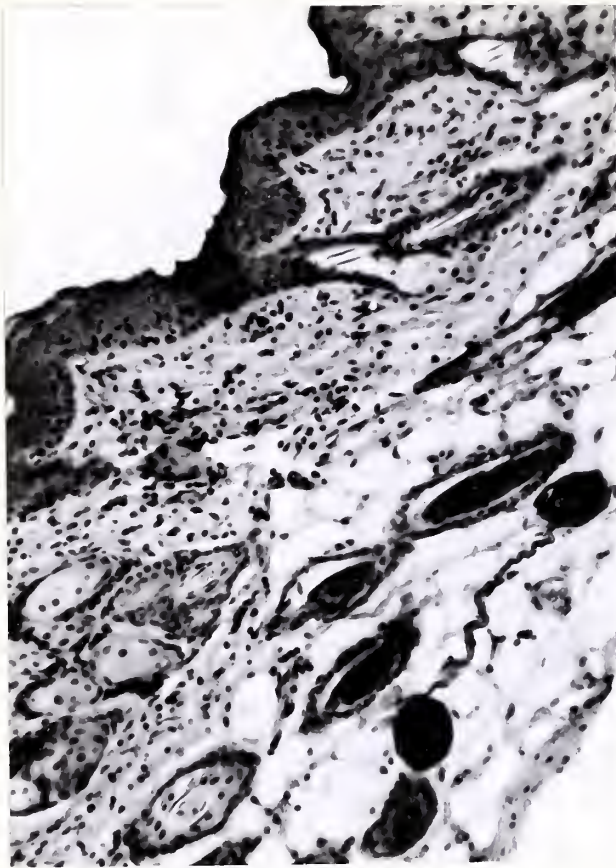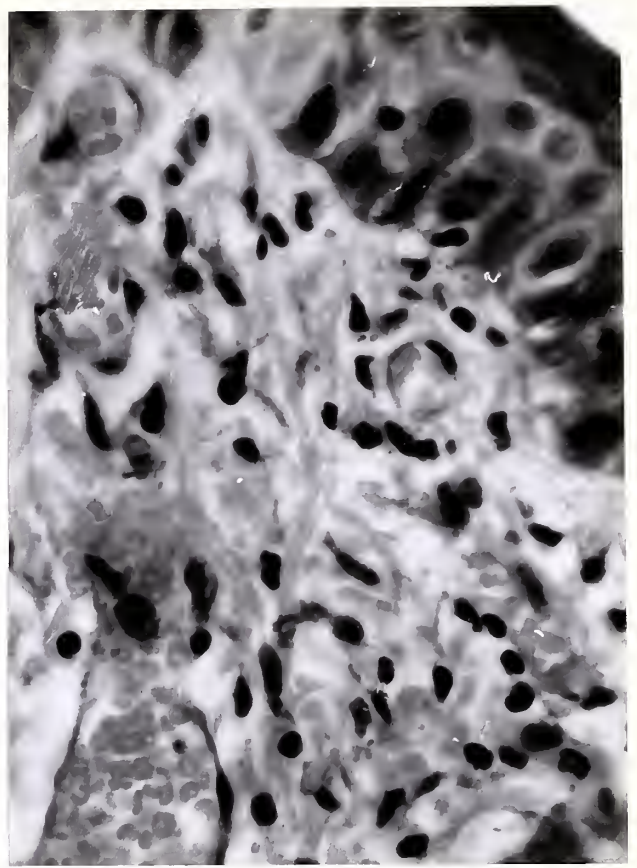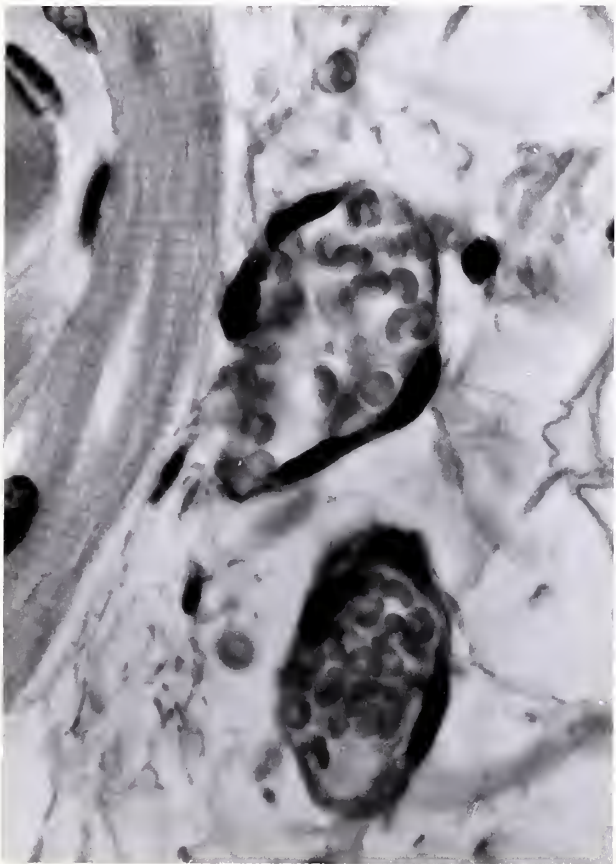increased number of mast cells. See text. Magni-
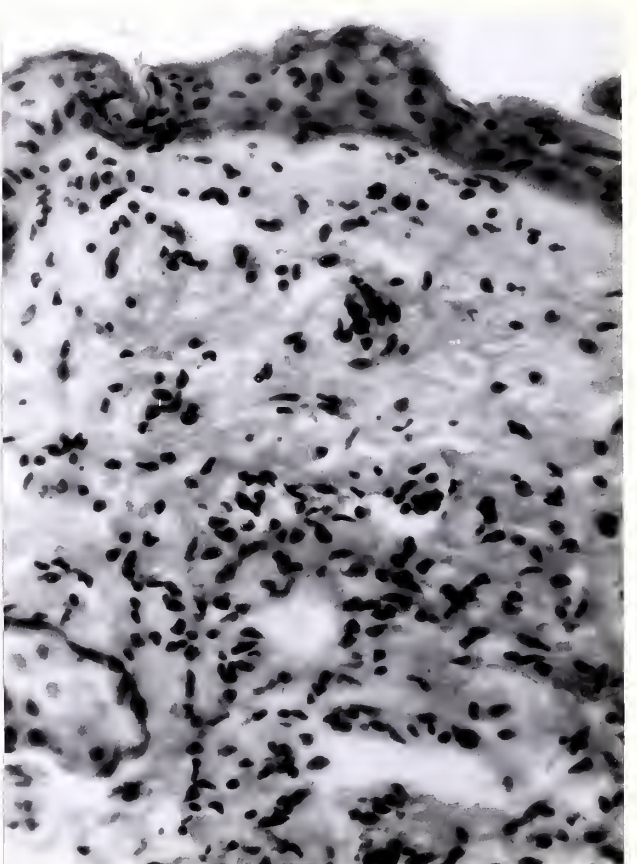fication x 375.
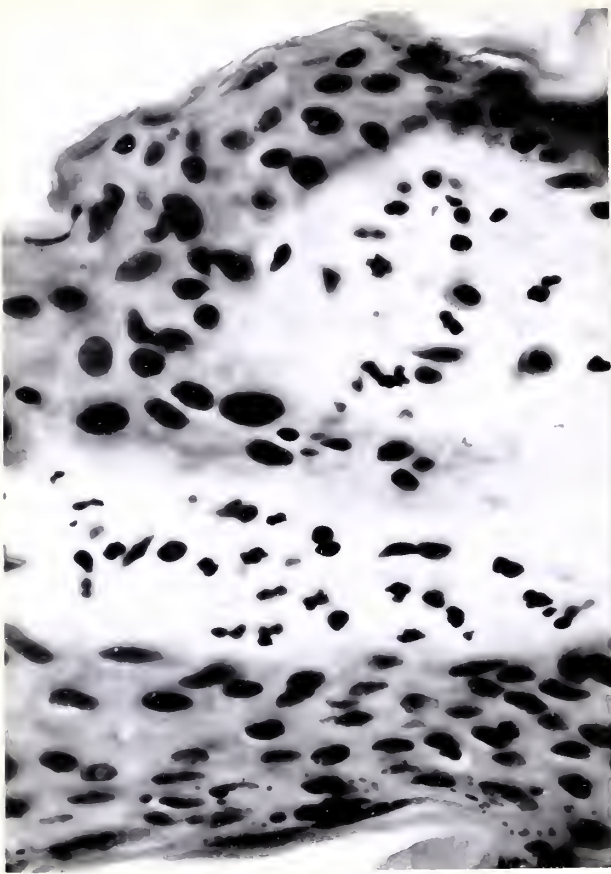
PLATE 13.

PLATE 14.

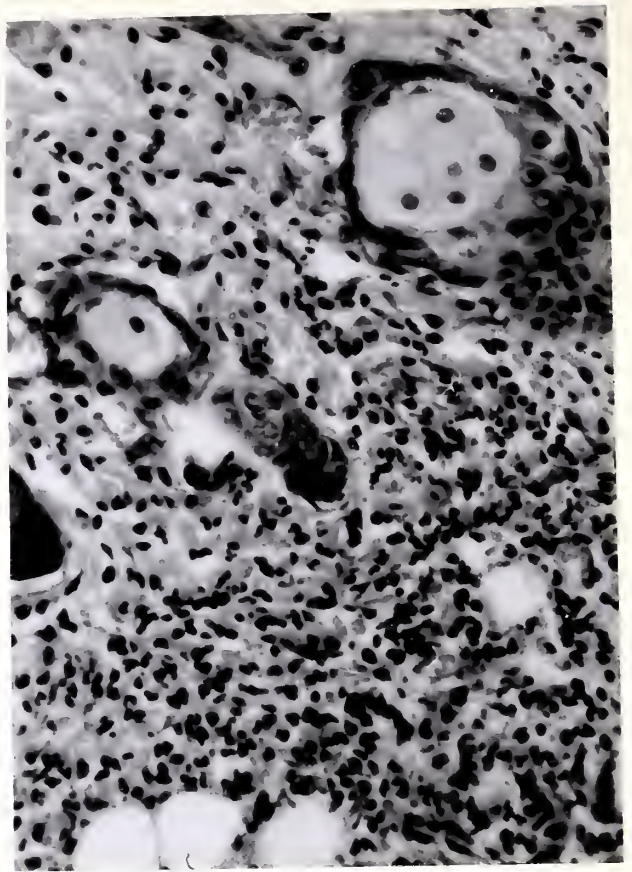
PLATE 15.

PLATE 16.

PLATE 17.
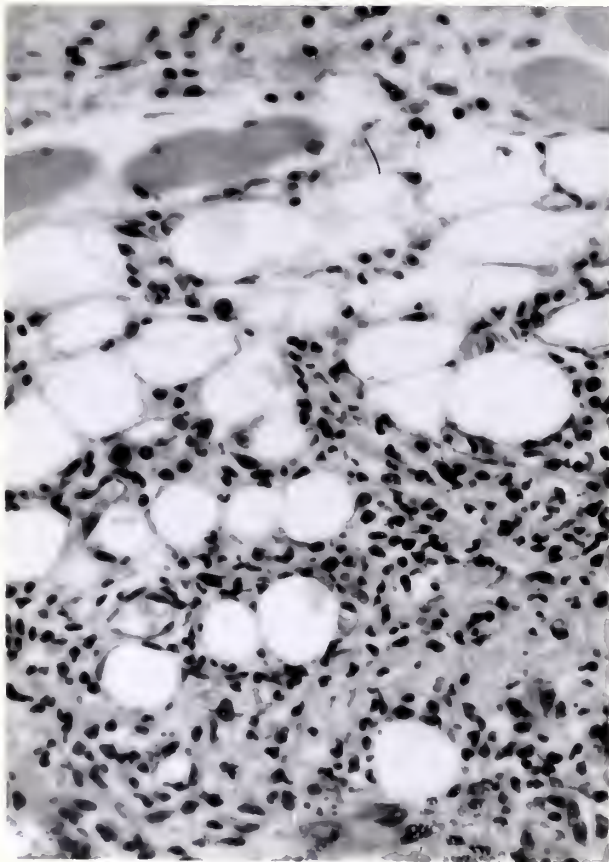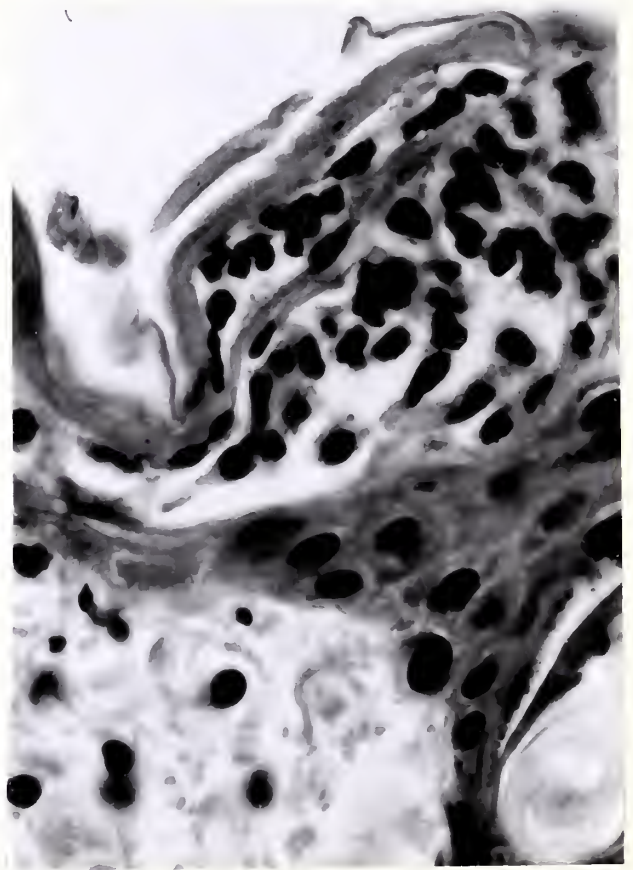
PLATE 18.

PLATE 19.

PLATE 20.

Plate 17. Section through irradiated skin. Note intra-epidermal
          infiltrate. See text. Magnification x 750.

Plate 18. Section through irradiated skin. An intense focal in-
          filtrate is present in the dermis and subcutaneous tissue.
          See text. Magnification x 375.

Plate 19. Section through irradiated skin. A heavy dermal and sub-
          cutaneous infiltrate is seen. See text. Magnification
          x 375.

Plate 20. Section through irradiated skin. A focal intra-epidermal
          infiltrate which is not related to the dermis is present.
          See text. Magnification x 1200.

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