IX. THE PERMEABILITY OF HUMAN EPIDERMIS TO ULTRA-VIOLET IRRADIATION.

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THE work of Rosenheim and Webster [1927] and Windaus and Hess [1927] has shown that the irradiation of ergosterol with ultra-violet rays produces vitamin D. Since Hess, Weinstock and Helman [1925] found irradiated lanolin to be antirachitic, it may be concluded that ergosterol or a similar sterol is associated with cholesterol in the sebaceous material of the skin.

It has been shown by Hume, Lucas and Smith [1927] that ordinary impure cholesterol after irradiation, if gently rubbed into the intact shaven skin, can protect rabbits on a rachitogenic diet from rickets. The cholesterol was dissolved in ether and the solution poured on to a glass plate, and irradiated under a mercury vapour quartz lamp. The irradiated cholesterol was mixed with cottonseed oil to facilitate inunction, and rubbed into shaved areas on the backs of rabbits, these areas being covered with wash-leather jackets to prevent ingestion by the mouth.

Absorption through the skin of vitamin D, produced in skin fat, may not however be the only, or the usual, way in which the antirachitic effect of ultra-violet light is communicated to the animal body. Rosenheim and Webster have shown that only 0.0001 mg. of irradiated ergosterol daily is required to protect a rat from rickets or even to cure it. Moreover the amount of ultra-violet irradiation which reaches the surface of the rat is very small, since, with the exception of the ears, feet and horny tail, the rest of the rat is covered with fur; yet it is enough to protect the rat from rickets. It may be that enough ultra-violet rays of sufficiently short wave-length to activate the ergosterol may penetrate the epidermis and reach the ergosterol contained in the capillaries lying just below. The epidermis on the parts of the human body other than the palms of the hands and soles of the feet, is approximately 50μ thick at the peaks of the dermis where the network of capillaries is thickest.

PREVIOUS OBSERVATIONS.

Our knowledge of the permeability of skin and epidermis to ultra-violet light of different wave-lengths largely depends on the observations of Hasselbalch [1911] made 20 years ago, using light of wave-lengths ranging between 437μ and 289μ . His conclusions are those commonly referred to in the

literature. Hasselbalch's method was to use skin from a corpse, placing it between two discs of optically flat clear quartz so fitted into a short brass cylinder as to form an air-tight chamber in which the skin was pressed flat between the discs. He used a Kromayer quartz mercury vapour arc lamp and a quartz spectrograph. The skin was placed 5 cm. in front of the slit of the spectrograph which was widely open. To receive the photographic image he used Solio white matt printing-out paper. The time necessary to produce a suitable image, through the skin was recorded. The dark-slide was then lowered by the rack and pinion and the illumination repeated through the quartz discs only, the image being received immediately above the preceding one and the exposure being continued until the two images of a selected mercury vapour band were of equal density. Owing to the use of Solio paper it was possible, without spoiling the image produced, to interrupt the exposure from time to time, to examine both photographs and stop the exposure at the desired point. When two images of equal density of one of the mercury-vapour bands of longer wave-length had been obtained and the respective times recorded, the same piece of paper could be replaced and the exposures continued for bands of shorter wave-length.

In the calculations which were the outcome of his experiments, Hasselbalch made use of absorption coefficients, calculating these according to the formula

$$\frac{J}{J_0} = 10^{-\epsilon' d} \quad \text{or} \quad \frac{-\log \frac{J}{J_0}}{d} = \epsilon'$$

where ϵ' is the coefficient of absorption, J is intensity of the transmitted light, J_0 that of the incident light, and d is the thickness of the skin in millimetres. The modern practice is to use centimetres, but Hasselbalch used millimetres and to render comparison easier his practice has been followed in presenting my results. The values for the transmitted and incident light are inversely proportional to the times of exposure required to produce an image of the same density. Thus

$$\frac{J}{J_0} = \frac{t'}{t}$$

where t and t' are the times required to produce images of the same density from transmitted and incident light respectively.

The absorption coefficients for skin, calculated by Hasselbalch, varied from 2.3 for a wave-length of $437 \mu\mu$ to 5.2 for a wave-length of $313 \mu\mu$ which was the shortest he was able to examine. For epidermis his figures were:

Wave-length	404	360	334	313	302	294	289 $\mu\mu$
ϵ'	2.3	4.2	6.6	8.5	12.4	17.5	39

Anderson and Macht [1928, 1, 2] made two series of experiments on the penetration of ultra-violet rays through living skin. In their first series they irradiated the skin of a living anaesthetised rabbit, reflected from the body, with monochromatic light from a mercury vapour arc lamp. They recorded

the energy transmitted through the skin by means of a thermopile and galvanometer. The result showed that between the wave-lengths $360\mu\mu$ and $280\mu\mu$ the percentage transmission increased from 11.4 % to 56.3 % and that there was then a drop to 23 % at $265\mu\mu$ with a further rise to 42.8 % at $253\mu\mu$.

This paper was criticised by Hill [1928] and by Bachem [1928]. Hill's criticism is chiefly based on the results of his own physiological experiments. He showed the great protection against ultra-violet irradiation afforded by a layer of omentum which is far thinner than skin, and also the protection to tubercle bacilli afforded by a thin layer of serum. Bachem [1928] drew attention to the possible influence of stray light rays of other wave-lengths due to the imperfect production of monochromatic light, and to the error due to the fluorescence of the skin. These fluorescent waves affected the thermopile, and the energy thereof was registered as energy from ultra-violet waves coming through the monochromator.

Anderson and Macht in their second paper [1928, 2] used a special photometer to control the thermopile method and found that the amount of stray light was negligible. They admitted the error due to the fluorescence of the skin and corrected it. Nevertheless their findings show a rise of transmission from 7.5 % to 9.8 % between the wave-lengths $302\mu\mu$ and $280\mu\mu$. This is contrary to the findings of workers on the ultra-violet absorption of other protein substances.

It is not out of place at this juncture to point out that the error due to the inclusion of fluorescent rays can almost certainly be excluded when the transmitted rays are analysed by the spectroscopic method instead of a thermopile. In this method any fluorescent light from the skin is recorded on the photographic plate as visible light and not as ultra-violet. A source of error would only be possible if a proportion of the incident light were converted into light of a shorter wave-length by fluorescence. This form of conversion has not yet been established.

METHODS USED IN THE PRESENT WORK.

Two years ago I repeated Hasselbalch's experiments with human skin, but using parallel light. The results obtained, expressed as absorption coefficients per millimetre, indicated that the skin used was 100 to 10,000 (according to the wave-length) times more opaque than that used by him. In searching for a solution of this discrepancy the conclusion was reached that it was due to the loss of intensity of the issuing light caused by scattering, owing to the different conditions of our experiments.

Skin is not optically homogeneous. Incident light, in addition to being diffusely reflected at the surface, is irregularly refracted as it passes through the layers of cells which compose it. When therefore skin is interposed between the light-source and the slit of the spectrograph, many of the rays are reflected and the remainder so much bent that they miss the slit altogether, while others pass through at such an angle that they impinge on the wall of the collimator

tube and not on the prism. The result is that only a fraction of the incident light which is unabsorbed by the skin is recorded and measured against the incident light. If the incident rays are parallel the effect of scattering is maximal, and this is the reason why my early figures are higher than those of Hasselbalch, who apparently did not use parallel light.

When light impinges on the skin of a living animal scattering is not of such great importance, because the transmitted light does not subsequently encounter a narrow slit. Although much diffuse reflection occurs at the surface, rays of light striking the skin surface at one point ultimately reach the blood vessels, though possibly at positions widely separated from the point of incidence.

Another explanation of the exaggerated value for the absorption of skin obtained in experiments of this type is that the light traverses a devious path, the length of which is not necessarily represented by the thickness of the skin, which is the measure used for the calculation of absorption coefficients. It is not therefore possible to determine the absorption coefficient, which depends for its estimation on the thickness of the layer actually traversed. For this reason the expression of the absorption of light by the skin by means of absorption coefficients has been abandoned in these experiments, and the degree of absorption for light of different wave-lengths is expressed in terms of relation between the logarithms of incident and transmitted light respectively, $(\log \frac{J}{J_0})$, as measured by the respective times of exposure needed to obtain images of equal density after passage through the particular thickness of skin or epidermis used. As was stated previously the times of exposure are inversely proportional to the respective intensities of the light,

$$\left(\log \frac{J}{J_0} = \log \frac{t'}{t}\right).$$

In order to minimise the effect of scattering of light, it was decided to insert scattering surfaces in front of the light-source in the form of ground quartz discs, through which the light would pass in tests both with, and without, the skin. The skin was placed between two clear quartz discs as before (Fig. 1, 1 and 1'). One of these was recessed into an outer casing (2), with a fine screw thread on the outer side, while the other disc was similarly recessed into a thick ring of metal (3), which slid into (2), and fitted sufficiently well to make the cavity air-tight. The whole of this was then screwed into an outer casing (4), with a thread on the inner side. The scattering of the incident light was produced by two quartz discs each ground on both surfaces (5 and 5') fixed with plasticene on to the inner and outer surfaces of the front surface of (4). The whole then slid into the hood of the spectroscope from which the front cap had been unscrewed.

The light-source was a Hewittic quartz mercury vapour lamp working at 2.25 amperes. It was enclosed in a metal case and the light emerged through an aperture $1\frac{1}{2}$ inches in diameter.

The introduction of the ground quartz discs reduced the intensity of the light incident on the slit to such a degree that considerably longer exposures were needed. For this purpose a photographic shutter was made of a piece of flat metal blackened on both sides attached to the long arm of a lever, the short arm of which was operated by the armature of an electromagnet. This was placed in front of the cell holding the epidermis. When an electric current passed through the magnet the armature was pulled down, and the shutter on the long arm raised, thus making the exposure. The duration of the exposure depended on the length of time the current flowed. The timing apparatus consisted of a wooden pulley geared to a gramophone motor in a ratio

Fig. 1. Air-tight cell arranged to hold epidermis.

which produced one revolution in 5 seconds. A series of wooden discs into each of which was recessed a brass sector was attached to the face of the pulley. Two strips of springy brass were fixed so as to wipe against the face of the disc on the pulley and complete the circuit during the time they passed over the brass sector. The ends of the strips were in the line of the radius of the pulley face, and therefore reached the edge of the brass sector simultaneously. The shutter by its own weight fell back in front of the cell. Sectors were made to give exposures of from $1/10$ sec. to 3 sec., varying by $1/10$ sec. Instead of Solio paper, used by Hasselbalch, Ilford "Process" plates (H & D = 50) were employed.

The constancy of the source of illumination was examined by taking a series of spectrographs with the same period of exposure, one below the other, on the same plate. It was found that after the lamp had been lit for 30 minutes the light was so steady that there was no discernible difference of intensity between any of the spectrographs.

With a piece of human skin in position a series of exposures was made on a plate, varying between 1 second for the long wave-lengths and 30 minutes for the short ones. These provided the measure of the light transmitted through the skin or epidermis, which was then removed. With the cell otherwise the same, shorter exposures were made on further plates by means of the electromagnet shutter. These gave the measure of the incident light.

The two series of plates were developed for an equal time with developer at the same temperature. Then for each wave-length the intensity of a line in the series obtained from the transmitted light was matched with that of the same line in the series obtained from the incident light. The times of exposure of the two were then noted. If no two images showed exact equality of intensity, the average was taken between the two exposures of incident light, which gave images of the selected line of intensity respectively greater and less than the intensity of that from the transmitted light.

A trial of this apparatus showed that the diminution of the intensity of the incident light by scattering at the ground quartz surface was so great that no image of any line of shorter wave-length than $313\mu\mu$ could be obtained through skin of 0.2 mm. thickness except by exposures of 24 hours and longer.

Skin was therefore abandoned in favour of epidermis, a change the more justifiable, because it is only the thin covering of epidermis (of thickness about 0.05 mm.) which in the living body is interposed between the dermal capillaries and the source of illumination. Epidermis was obtained from the front of the fore-arm by means of a blister produced with chloroform vapour. Transverse sections, appropriately stained, showed that the stratum granulosum was included in the separated portion. These pieces of epidermis were stored in a refrigerator in tap-water, and when placed in the cell were moistened with tap-water.

EXPERIMENTS WITH HUMAN EPIDERMIS.

1. *Diminution of error due to scattering of light by interposing ground quartz discs.*

An experiment was made with a specimen of human epidermis 0.08 mm. thick and values for $\log \frac{J_0}{J}$ were obtained for wave-lengths corresponding to the chief lines in the violet and ultra-violet parts of the emission spectrum of the mercury vapour arc, *i.e.* from wave-length $437\mu\mu$ to $256\mu\mu$. In the first experiment (*a*) the incident light was previously scattered by the insertion of ground quartz discs as described above. A second experiment (*b*) was then performed with the light made approximately parallel. In order that images of submaximal intensity should be obtained for long wave-lengths of the incident light, the lamp was placed at a distance of 11 feet from the spectrograph. The resulting figures are shown in Table I, cols. *a* and *b*, and are reproduced graphically in Fig. 2, *a* and *b*, where the ordinates are values of $\log \frac{J_0}{J}$ and the abscissae are wave-lengths expressed in millimicrons.

Table I. *Absorption of violet and ultra-violet radiations by human epidermis 0.08 mm. thick.*

Wave-length in $\mu\mu$	Values of $\log J_0/J$	
	(a) Using light scattered by ground quartz discs	(b) Using parallel light
437	0.55	1.68
404	0.62	1.82
360	0.76	2.14
334	1.02	2.52
313	1.14	2.93
302	1.40	3.28
294	1.66	3.55
289	2.44	—
264	2.80	—
256	2.66	—

Fig. 2. Effect of using incident light scattered by ground quartz surfaces. Absorption curves of human epidermis (0.08 mm. thick).

a. Incident light scattered. b. Incident light parallel.

The apparent increase in the transmission through the epidermis when the incident light was previously scattered was unexpectedly great, being from 13 (for wave-length $437\mu\mu$) to 78 (for wave-length $294\mu\mu$) times greater than in Exp. b, where parallel light was used. As the same piece of epidermis was used in both cases, this difference is only an apparent one, due not to the epidermis, but to the method of illumination, the only thing changed in the two experiments. For wave-lengths less than $294\mu\mu$ the scattering by the epidermis becomes so great, when parallel light is used, that the light entering the slit is insufficient to produce an image with the exposure used, and the rest of the graph is unobtainable.

Using the ground quartz discs experiments were made with specimens of

epidermis from two other people. These specimens (Fig. 3, *a* and *b*) were thinner than those used in the above experiment being 0.065 mm. and 0.05 mm. in thickness respectively. The values for $\log \frac{J_0}{J}$ were therefore lower and readings were obtained for radiations of the wave-length 246 $\mu\mu$ with both specimens and for wave-length 240 $\mu\mu$ for one (Fig. 3 *b*). These

Fig. 3. Absorption curves of epidermis from two different individuals, using scattered incident light.

a. Thickness 0.065 mm.

b. Thickness 0.05 mm.

graphs resemble in shape the absorption curves of the amino-acids tryptophan and tyrosine, shown in Fig. 7 [Ward, 1923], of serum-albumin [Smith, 1929] and of caseinogen determined by myself (see Fig. 5).

Tyrosine has been found in epidermis by Buchtala [1912] and tryptophan, though not actually shown to be present in epidermis, has been found in epidermal structures such as wool to the extent of 1.8 to 1.87 % [Marston, 1928].

2. *Diminution of error due to scattering of light by employing clarified specimens of epidermis.*

So far, it has been shown that the apparent opacity of epidermis to ultra-violet light may be diminished nearly a hundredfold by altering the optical conditions of the experiments, but even the method of employing previously scattered light by no means eliminates the disturbing effects of subsequent scattering in the epidermis. Recourse was therefore had to the method of clarification, by immersing the epidermis in some liquid the refractive index of which approached that of the materials of which epidermis is composed.

The choice of clarifying agents was governed by two conditions. The reagents should not alter the chemical composition of the epidermis, nor have of themselves an absorption of ultra-violet light in the region under investigation. For this reason the ordinary clarifying agents of microscopy, xylene, toluene, chloroform, etc., were unsuitable. The reagents chosen were glycerol and glacial acetic acid, both of which fulfilled the necessary conditions. Of

these glacial acetic acid was the better, judging by visible standards. The following experiments showed that these reagents did not permanently change the chemical constitution of the epidermis.

A piece of the same epidermis as was used for the experiment shown in Fig. 2 was placed in tap-water and a photograph taken of the transmitted spectrum using parallel light. The results are shown in Table II, col. 1 and

Table II.

Wave-length in $\mu\mu$	Values of $\log J_0/J$						Percentage of incident light transmitted according to Hasselbalch		
	1	2	3	2'	3'	4	Per- centage of incident light trans- mitted in Exp. 4	Percentage of incident light transmitted according to Hasselbalch	
								Epidermis 0.04 mm. thick	Calcu- lated for epidermis 0.08 mm. thick
437	1.78	0.79	1.70	0.40	1.82	0.02	96	—	—
404	1.95	0.80	1.87	0.40	1.94	0.04	91	81	65
360	2.23	1.00	2.08	0.52	2.18	0.10	81	68	46
334	2.75	1.20	2.57	0.60	2.62	0.19	65	54	29
313	3.10	1.34	3.00	0.71	2.90	0.26	55	46	21
300	3.52	1.61	3.24	0.84	3.19	0.35	44	32	10
294	3.86	2.03	3.54	0.94	3.40	0.58	26	20	4
289	—	3.12	—	1.40	—	1.54	2.9	3	0.09
280	—	3.60	—	1.78	—	1.78	1.7	—	—
270	—	3.05	—	1.81	—	1.83	2.0	—	—
265	—	3.13	—	1.61	—	1.60	2.6	—	—
254	—	3.33	—	1.56	—	1.34	4.6	—	—
246	—	—	—	1.72	—	1.43	1.5	—	—
240	—	—	—	2.71	—	—	—	—	—
238	—	—	—	3.16	—	—	—	—	—

- (1) Epidermis, thickness 0.08 mm. in tap-water, light parallel.
 (2) " " cleared in glycerol, light parallel.
 (3) " " washed and replaced in tap-water, light parallel.
 (2') " " cleared in glacial acetic acid, light parallel.
 (3') " " washed and replaced in tap-water, light parallel.
 (4) " " cleared in acetic acid, incident light scattered with
 ground quartz discs.

in graphic form in Fig. 4, curve 1. The epidermis was then placed in glycerol for about an hour after which no further clearing could be observed. It was then clamped between the quartz discs and a similar series of photographs taken. The values of $\log \frac{J_0}{J}$ are shown in Table II, col. 2 and in graph form in Fig. 4, curve 2. The glycerol was then washed out and the epidermis replaced in tap-water. The series of photographs obtained with this material provided measurements of $\log \frac{J_0}{J}$ reproduced in Table II, col. 3 and in Fig. 4, curve 3, which coincides almost exactly with curve 1. The epidermis when cleared with acetic acid furnished data to produce the figures of Table II, col. 2' and the curve 2', Fig. 4. Once more it was replaced in tap-water and from the figures obtained in this experiment, the curve 3' Fig. 4, was set out and found to be almost identical with curves 1 and 3, showing that the clearing reagents did not permanently alter the constitution of the epidermis.

Finally the epidermis was placed in ether to remove any possible fat and

then cleared in acetic acid, and a series of photographs were taken with the ground quartz discs in position, with the idea of still further reducing any possible scattering. The values for $\log \frac{J_0}{J}$ obtained in this experiment are shown in Table II, col. 4, and the corresponding curve 4 in Fig. 4. In accordance with the naked eye appearance acetic acid proved a better clarifying agent than glycerol for ultra-violet light, for the less degree of absorption measured is due no doubt to a less degree of scattering.

Fig. 4. Absorption curve from epidermis of the same individual as in Fig. 2. Thickness 0.08 mm.

Curve 1.	Epidermis in tap-water.	Light parallel.
" 2.	" glycerol.	Light parallel.
" 3.	" tap-water.	Light parallel.
" 2'.	" glacial acetic acid.	Light parallel.
" 3'.	" tap-water.	Light parallel.
" 4.	" glacial acetic acid.	Light scattered.

In comparing in Fig. 4 the curves 2, 2' and 4, the first thing noticed is that in general shape, the curves are similar to each other, to those in Fig. 3 and to curve *a* in Fig. 2. The actual values of $\log \frac{J_0}{J}$ are not comparable in the different experiments, as the specimen of epidermis used for the tests illustrated in Fig. 4 was thicker than those used for the experiments shown in Fig. 3.

Curve 4 (Fig. 4) showed a very interesting phenomenon. The introduction of ground quartz discs reduced the apparent opacity of epidermis, even when partially cleared, to a marked extent, as far as the wave-length $294 \mu\mu$. After that the curve coincided very closely with curve 2', corresponding to epidermis cleared similarly with acetic acid, but exposed to parallel incident light. This would show that for light of wave-length $294 \mu\mu$ and shorter, the effect of true absorption is so great as to dwarf the effect due to scattering. It is probable therefore that curve 4 shows the best approximation to the true absorption of ultra-violet light by human epidermis.

With the idea of examining in detail that part of the curve in which there is true absorption, a piece of the same epidermis was photographed in water, glycerol and acetic acid in turn. In order to obtain records of the shortest wave-lengths possible, it was decided to use, in addition to the clarifying reagents and the ground quartz discs, a quartz lens placed so as to render the rays, which had previously been allowed to come haphazard from the lamp, parallel before reaching the first scattering surface of the first quartz disc. The intensity of illumination being thereby increased, the time of exposure necessary to obtain records from the shorter wave-lengths was diminished. From these observations values for $\log \frac{J_0}{J}$ were obtained, and curves were drawn to show absorption for lines of the mercury vapour spectrum corresponding to short wave-lengths hitherto unmeasured from lack of intensity of the transmitted light. This gave greater accuracy to the curves.

Fig. 5. Repetition of experiment on epidermis from the same individual as that used for experiments illustrated in Figs. 2 and 4, but with incident light collected on to the ground quartz discs. Thickness of epidermis 0.08 mm.

Continuous Curve 1.	Epidermis in water.
" 2.	" glycerol.
" 3.	" acetic acid.
For comparison:	
Dotted Curve 4.	2.5 % serum-albumin. Thickness of layer 1 mm.
" 5.	2 % caseinogen. Thickness of layer 1 mm.

The curve of absorption by epidermis cleared with glacial acetic acid (Fig. 5, curve 3) showed great similarity to the absorption curve of a 2.5 %

solution of serum-albumin or 2 % caseinogen solution, except that for serum-albumin the drop in the region of wave-lengths $270\mu\mu$ to $250\mu\mu$ is not so great.

Damp epidermis freed from adherent moisture by pressing between filter-paper contains 40.4 % of water, and the absorption curve illustrated in Fig. 5 is not unlike that of a similar thickness of a 2 % solution of caseinogen both qualitatively and quantitatively. The curves shown in Fig. 7 indicate that the

Fig. 6. Curves showing percentage transmission of light through epidermis:
a. Curve derived from Exp. 4. Epidermis 0.08 mm. thick.
b. Curve derived from Hasselbalch's experiment with epidermis 0.04 mm. thick.

opacity of epidermis to ultra-violet rays between the wave-lengths $313\mu\mu$ and $240\mu\mu$ would appear to be principally due to the content of tyrosine and tryptophan, and to be of the same order as that of the proteins, albumin and caseinogen. Cystine does not appear to be responsible for any material part of the opacity of epidermis to ultra-violet rays in the region under consideration, because the great rise in its absorption does not begin until the wave-length $225\mu\mu$.

The best approximation to the true absorption of ultra-violet light by epidermis would appear to have been obtained in Exp. 4 (Table II; curve 4, Fig. 4). Translating the logarithmic figures into arithmetical numbers the percentage of incident light which penetrates and is transmitted through the epidermis and reaches the blood-stream is shown in Fig. 6, curve (a) and in the last column but one of Table II.

It appears then that in the range of wave-lengths of the sun's light arriving at the earth's surface which are active in the cure of rickets amounts varying from 55 % for wave-length $313\mu\mu$ to 26 % for wave-length $294\mu\mu$ may be transmitted to the dermal capillaries.

For comparison a curve illustrating Hasselbalch's experiment with epidermis is included in Fig. 6 (curve *b*). The epidermis which he used was 0.04 mm. thick, whereas the epidermis which provided the material in the present work was 0.08 mm. thick. Nevertheless with the much thicker epidermis the proportion of transmitted light recorded for wave-lengths $404\mu\mu$ to $289\mu\mu$ was greater than that estimated by Hasselbalch. On the basis of his figures the proportion of light transmitted through epidermis of 0.08 mm.

Fig. 7. Absorption curves.

1. Epidermis in water	} from Fig. 5.
2. " " acetic acid	
3. Tryptophan.	
4. Tyrosine.	
5. Cystine.	

thickness has been calculated (see Table II last column). These figures compared with those obtained in the present work show human epidermis to have from 1.5 to 30 times the transparency found by him for radiations of wave-lengths $404\mu\mu$ to $289\mu\mu$ respectively. The reason for this discrepancy is probably due to a failure on his part to differentiate between the loss of transmitted light by scattering, which loss is progressively greater as the wave-length decreases, and the loss by true absorption. It must be emphasised that much more is lost to the spectrograph by scattering when skin is irradiated

under the condition, of these experiments, than would be lost to the animal during irradiation.

SUMMARY.

1. The apparent absorption of ultra-violet light by epidermis as determined by the usual methods of photometry is not entirely due to true absorption, but is in part due to scattering of the incident light by the epidermis, which is not optically homogeneous.

2. This scattering while causing great diminution of the intensity of the light received by the spectrograph causes much less interference in transmission of the light to the underlying tissues when skin is irradiated *in vivo*.

3. For light of wave-length 300μ and less the effect of scattering is dwarfed by true absorption by the epidermis.

4. In shape these absorption curves resemble those of many proteins and amino-acids, *e.g.* serum-albumin, caseinogen, tryptophan and tyrosine.

5. The percentage of ultra-violet light of physiologically active wave-lengths transmitted through the epidermis is higher than previously recorded by Hasselbalch, and is calculated to be about 1.5 to 30 times greater for wave-lengths from 404μ to 289μ respectively.

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REFERENCES.

- Anderson and Macht (1928, 1). *J. Amer. Med. Assoc.* **90**, 161.
—— (1928, 2). *Amer. J. Physiol.* **86**, 320.
Bachem (1928). *J. Amer. Med. Assoc.* **90**, 563.
Buchta (1912). *Z. physiol. Chem.* **78**, 55.
Hasselbalch (1911). *Scand. Arch. Physiol.* **25**, 55.
Hess, Weinstock and Helman (1925). *J. Biol. Chem.* **63**, 305.
Hill (1928). *J. Amer. Med. Assoc.* **90**, 1310.
Hume, Lucas and Smith (1927). *Biochem. J.* **21**, 362.
Marston (1928). *The chemical composition of wool. Council for Sci. and Ind. Research of Australia, Bulletin* 38.
Rosenheim and Webster (1927). *Biochem. J.* **21**, 389.
Smith (1929). *Proc. Roy. Soc. Lond. B* **104**, 198.
Ward (1923). *Biochem. J.* **17**, 898.
Windaus and Hess (1927). *Proc. Soc. Exp. Biol. Med.* **24**, 461.