LII. ON THE ABSORPTION OF VITAMIN D FROM THE SKIN.

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An animal can either obtain the necessary supply of vitamin D for skeletal growth from its food or it can make it for itself from some precursor, if its surface is sufficiently exposed to sunlight or ultra-violet rays.

The principle which is activated to form vitamin D has hitherto been found associated with cholesterol, but from observations of Rosenheim and Webster [1926; 1927, 1, 2] and Heilbron, Kamm and Morton [1927] the precursor of vitamin D is not ordinary cholesterol, although it clings to it during successive recrystallisations. Rosenheim and Webster [1927, 2] adduce good reasons for supposing that it is ergosterol. They find that ergosterol is 2000 times as rich in the provitamin as brain cholesterol. Ergosterol unlike ordinary cholesterol has three double bonds and has three absorption bands in the ultra-violet, the strongest being at a wave length of 280 $\mu\mu$, accompanied by two others of less intensity, at wave lengths of about 270 $\mu\mu$ and 295 $\mu\mu$. Hess and Weinstock [1925] have shown that "cholesterol" irradiated by ultra-violet rays exerts its anti-rachitic action when injected subcutaneously as well as when taken by the mouth. They also found [Hess, 1925; Hess and Weinstock, 1925] that pieces of irradiated skin from infants and calves exercised an anti-rachitic action when eaten by rats. Hess, Weinstock and Helman [1925] showed that lanolin contains the precursor of vitamin D and suggested that it is by the activation of the cholesterol in the layers of the epidermis and its subsequent absorption that animals are protected from rickets. Yet another possibility is that the precursor is activated whilst coursing through the superficial capillaries of the skin, but for this to occur, sufficient radiant energy of the required wave length must penetrate the 0·1 to 0·2 mm. of the epidermis covering the capillaries. The possession by ergosterol of a strong absorption band about the wave length of 280 $\mu\mu$ [Rosenheim and Webster, 1926] indicates that light of this wave length would be particularly effective in activating it. The epidermis, however, is very opaque to this wave length. One of us (N. S. L.) has not succeeded in obtaining any indication of the 280 $\mu\mu$ line in a spectrogram of a quartz mercury vapour lamp, distant 2 feet, after 3 hours' exposure, through 0·1 mm. of epidermis when using an Ilford Process plate (H. and D. = 50).

Radiations of wave length 280 $\mu\mu$ are not present to a significant extent in sunlight which has passed through the upper atmosphere [Cornu, 1878, 1879, 1880, 1890; Miethe and Lehman, 1909; Dember, 1912; Wiegand, 1913] so that the precursor of vitamin D must be capable of being activated, if more slowly, by ultra-violet light of greater wave length impinging on the body. For ultra-violet light of slightly greater wave length, the epidermis is much less opaque. Calculating from Hasselbalch's [1911] observations, 1/56 of the incident light of a wave length of 297 $\mu\mu$ penetrates 0·1 mm. and 1/3000 penetrates 0·2 mm. of epidermis. At present we are ignorant of the amount of radiant energy required to activate sufficient of the precursor to provide an adequate supply of vitamin D for an animal, but it would appear to be very small, when of the appropriate wave length; for 1½ minutes' daily exposure to a mercury vapour quartz lamp is sufficient to prevent rickets in an albino rat, for the most part covered with fur, and fed on a rickets-producing diet [Hess, 1922]. It was observed by Chick and co-workers [1923] that rickets in a child was healed when the hand and fore-arm only were exposed to ultra-violet irradiation. Hess and Unger [1921] made a similar observation, but in this case the exposure was to the rays of the sun.

The accompanying experiments were designed to test whether irradiated cholesterol, applied to the external surface, could exercise its anti-rachitic action through the skin.

Technique.

Two methods were used for testing the potency of the various anti-rachitic measures tried. For the first method, young rats were used, which at about 50 g. weight had been placed upon a diet deficient in both fat-soluble vitamins; the method, which has been described before [Hume and Smith, 1926], takes advantage of the fact that the animal's reserve of vitamin D is exhausted before that of vitamin A. After the rats had been on this diet for 4 weeks the treatment to be tested was applied, and the degree to which growth was restored was taken as a measure of the anti-rachitic potency of the treatment.

For the second method, young rabbits, between 300 and 500 g. weight, were used and they were fed on the diet No. 3143 of McCollum [1921], which is low in phosphorus. They also received on week days 5 cc. of orange juice, given with a pipette; it could not be mixed with the diet, as this was given dry. On this diet rabbits develop gross lesions of rickets, unless anti-rachitic treatment of some sort is applied. In the first experiment the rabbits were kept on the diet for 8 weeks and in the second for 5 weeks. At the end of the experiment a femur from each rabbit was ashed, after extraction with ether and alcohol. The histology of the rib junctions was also studied.

The part of the body chosen, as most inaccessible to the animal itself, for the application of irradiated cholesterol was the middle of the chest in rats and the back of the neck in rabbits. The skin was kept as clear of hair as

possible by depilation and cutting of the hair. Depilation was at first carried out with barium sulphide, but in the experiment with rats and in the first experiment with rabbits, it caused slight soreness, so the experiment with rabbits was repeated using toilet "Veet," in which the barium sulphide is diluted with a cream; no soreness then resulted.

About 0·2 g. of cholesterol was dissolved in ether and evaporated on a glass slide in order to provide a thin layer. This was irradiated for 10 minutes, at a distance of 16 inches from a mercury vapour quartz lamp (Ulviarc), freshly for every application, and the application was made three times a week. The cholesterol was gently applied to the skin with the point of a finger, over an area of about one square inch, and a little hardened cotton-seed oil was afterwards applied to make the cholesterol adhere. The animals were dressed in chamois-leather jackets, fastening down the back, which prevented them from gaining access in any way to the treated patch of skin and each animal was kept in a separate cage. It was found extremely difficult to be perfectly certain that no particle of irradiated cholesterol should accidentally reach the animals' mouths, and the experiment was performed three times, each time with added precautions, but each time with the same result.

Table I. *Growth of rats on a diet deficient in fat-soluble vitamins; irradiated cholesterol applied to the skin from 27th–47th day.*

Treatment	No. of rat	Sex	No. of litter	Weight on 27th day g.	Weight on 47th day g.	Total increase g.
Control	L 1	♀	310	52	60	8
	L 3	♂	,,	68	78	10
	L 4	♂	,,	71	84	13
Irradiated cholesterol applied to skin	L 2	♀	310	51	73	22
	L 5	♂	,,	67	86	19
	L 6	♂	,,	66	81	15

Exp. 1. In the first experiment, six white rats of the Wistar Institute breed, all belonging to the same litter, were used. They all received the diet, deficient in fat-soluble vitamins, already referred to. After 27 days on the diet, irradiated cholesterol was applied to a depilated patch on the chests of three of the rats, three times a week for 20 days. The remaining three were left untreated as controls. Table I shows the growth of the two sets of animals, over the twenty days of treatment. The three treated animals show growth which is about double that of the untreated animals and it would therefore appear that by inunction the irradiated cholesterol has been able to exercise a definite effect. It was however felt that by using a larger animal it would be considerably easier to take the necessary precautions against the entry of the anti-rachitic agent, by any other channel than through the intact skin. It was therefore decided to use rabbits.

Exp. 2. Five rabbits, all stated to belong to one litter, were used. They all received the diet, McCollum 3143. Two received no further treatment; irradiated cholesterol was rubbed into the shoulders of two 3 times a week,

and one was irradiated directly, with a mercury vapour quartz lamp for 10 minutes daily, at a distance of 46 cm. Treatment lasted for 8 weeks. Table II A, which gives the results, shows that both histologically and in their percentage ash the bones of the animals which had had cholesterol applied to their skins approached those of the animal irradiated directly, while the controls showed rickets histologically and a considerably lower percentage of ash.

The two animals which received cholesterol, were, however, at the end of the experiment, slightly sore and it was also felt that in order to imitate any natural absorption which might take place, the irradiated cholesterol should have been gently stroked on the skin, and not rubbed in as had so far been done. The next experiment was also planned to include rabbits irradiated directly, over a small area of the body only.

Exp. 3. In Exp. 3, seven rabbits, stated to belong to two litters, were used. Depilation was carried out by means of "Veet." The diet was the same as in the last experiment, but treatment was only continued for 5 weeks. Two of the rabbits were used as untreated controls. On two, a patch was depilated at the back of the neck, and three times a week irradiated cholesterol was very gently stroked on to the skin and a little hardened cotton-seed oil was lightly smeared over it. The application was only made to a small area of about one square inch and it was felt during the experiment that, if the treatment in so slight a form could produce an effect, it must be very potent. From Table II B it will be seen that a very definite effect was produced and though the results obtained are not quite as good as in the case of animals irradiated directly, yet they are very much better than the results yielded by the controls, one of which showed an extraordinarily low ash in the bone.

Table II. *Histological report on the rib junctions and percentage ash in the extracted bone of rabbits, fed on diet* 3143 *of McCollum, and treated with irradiated cholesterol applied to the skin, or with direct irradiation of whole or part of the body.*

Treatment	No. of rabbit	Sex	No. of litter	% ash in extracted bone	Histological report
A. Control	2	♀	1	46·8	Severe rickets
	5	♀	,,	48·1	,,
Irradiated cholesterol applied to skin	3	♀	,,	56·3	No rickets
	6	♂♀	,,	55·7	,,
Irradiated directly ...	4	♀	,,	58·0	,,
B. Control	8	♂	2	46·2	Moderate rickets
	13	♂♀	3	37·1	Very severe rickets
Irradiated cholesterol applied to skin	7	♀	2	52·9	No rickets
	10	♂	3	52·5	Slight rickets
Irradiated through hole 2·5 × 3·5 cm.	9	♂	2	56·4	—
	11	♂	3	55·6	—
	12	♂	3	57·0	—

The three remaining rabbits were irradiated directly through a small hole in a black cloth. The cloth was of black sateen doubled. Penetration of light

through the cloth was tested by means of a spectrograph; the spectrogram showed no penetration by ultra-violet rays, after half an hour's exposure to a mercury vapour arc. The hole in the cloth measured 2·5 by 3·5 cm. The rabbit was rolled in the black cloth and a depilated area on its back was exposed directly through the hole, at a distance of 50 cm. to a mercury vapour quartz lamp. The exposure lasted 10 minutes and took place three times a week. Approximately, but not quite, the same area of skin was irradiated at each exposure. With one rabbit, it was attempted to cleanse the depilated area with ether and free it from cholesterol, but this was found to be quite impossible and the rabbit, No. 12, is therefore included with Nos. 9 and 11, in the group of those irradiated directly through a hole.

All the rabbits in Exp. 3 were killed when they were slightly younger than the rabbits in Exp. 2, so that a slightly lower range of values for the percentage ash might be expected in Exp. 3. Allowing for this it is apparent that calcification in an animal, of which only a small area has been irradiated periodically for 10 minutes, is only slightly inferior to that of an animal whose whole body has been irradiated for the same time.

Conclusions.

1. Vitamin D in irradiated cholesterol can be absorbed from a small area of undamaged skin in sufficient amount to supply the needs of the animal.

2. The suggestion of Hess that activation of lanolin by sunlight and its subsequent absorption through the skin is a possible source of this vitamin is supported by experiment.

3. Rickets was prevented in rabbits fed upon a rickets-producing diet, and almost normal calcification of the bones produced, when an area of skin 2·5 × 3·5 cm. was irradiated for ten minutes three times a week.

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