CXLI. THE ACTION OF X-RADIATION UPON VITAMIN D IN ACTIVATED ERGOSTEROL.

BY ROBERT ROWLEY MORRISON, PHILIP REDINGTON PEACOCK
AND SAMSON WRIGHT.

From the Middlesex Hospital, London.

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OUR interest in the possibility that X-rays may have an effect upon vitamin D was aroused by the paper of Hopf and Herman [1926] in which they described a pathological condition produced in rats fed on a diet which had been submitted to the action of X-rays. As this condition bore a striking resemblance to experimental rickets, it was decided to re-investigate the problem and to determine whether the result was due to the direct destruction of the vitamin or to the formation of a substance or substances in the irradiated foodstuffs which might either bring about a pathological condition resembling rickets or indirectly destroy the vitamin. The matter could be decided by submitting activated ergosterol [Rosenheim and Webster, 1927] to the action of X-rays and then examining the antirachitic properties of the product by biological methods.

METHODS.

Rats were used and fed on a modified, No. 3143, McCollum diet consisting of

Whole wheat coarsely ground or crushed	33
Whole maize coarsely ground or crushed	33
Wheat gluten	15
Gelatin powdered	15
Calcium carbonate	3
Sodium chloride	1

Lard 1 % and marmite 0.1 % by weight of the dry mixture were added, the food was mixed with distilled water and cooked for 5 minutes. The allowance was 10 g. of the dry mixture per rat per day and unlimited supplies of water. The food was stored dry and cooked fresh each day. The animals did very well on the diet, the cooked food being in our opinion an improvement on the dry mixture.

In a preliminary experiment with eight young rats on this modified diet, it was found that rickets resulted in every case by the end of the 4th week without the general health being adversely affected to any appreciable extent.

The following terms are used to designate the different solutions to be tested for antirachitic value:

- PVD (Pro-vitamin D) = Pure unactivated ergosterol.
- VD (Vitamin D) = Ergosterol activated by ultra-violet rays.
- VDX (Vitamin DX) = VD exposed to X-rays.

Exp. 1. The pure non-activated ergosterol (kindly supplied by Dr Rosenheim) was dissolved in absolute alcohol to form a 0.1 % solution; 1 cc. of this solution was transferred to a 30 cc. quartz flask which was then filled with nitrogen and closed with a glass stopper lubricated with rubber grease. The flask was half submerged in tap water at 20°, rotated at 180 r.p.m. and irradiated for 1 hour, the burner consuming 316—311 watts (79—76 volts, 4—4.1 amps).

The activated ergosterol was then transferred to a measuring cylinder, the flask washed out with alcohol and the washings added to the solution in the measuring cylinder, diluting the original to 10 cc. (= 0.01 % solution VD). 5 cc. of this solution was allowed to evaporate to dryness in a watch glass which was then covered with tissue paper and exposed to X-rays. The X-ray exposure, which was started 5 hours after the termination of the ultra-violet irradiation, proceeded for 18 minutes at a distance of 20 cm. from a 10 ma. radiator Coolidge tube excited by alternating current at 65 kv. and 5.6 ma. This turned a Sabouraud pastille lying on metal at the distance of the material under exposure to tint B, *i.e.* a dose of 4 PD. After exposure, the sample was redissolved in absolute alcohol to give a 0.01 % solution (VDX). The alcoholic solution was added to pure olive oil to give a dilution of 0.0004 mg. VDX per cc. Similar solutions of PVD and VD were also prepared.

Three samples of olive oil were thus obtained containing respectively pure ergosterol (PVD), activated ergosterol (VD) and activated ergosterol which had received a known dose of X-rays (VDX). 0.25 cc. of oil containing 0.0001 mg. of the substance to be tested was administered daily to each animal by means of a graduated pipette, except on Sundays. The Sunday dose was added to the Saturday and Monday doses. In the first experiment, 36 young rats of mixed stock and of approximately the same age were used, the weight varying from 31 g. to 48 g. and averaging 40 g. They were divided into three groups and were put on the diet 20 days before oil feeding commenced, in order to diminish their store of vitamin D. Group I received the activated ergosterol (VD), Group II received the activated ergosterol plus X-rays (VDX), Group III received the pure ergosterol, pro-vitamin D (PVD).

RESULTS.

Three days after oil administration had commenced, three animals died, one from each group and, on examination, two showed slight rickets and one no rickets.

Nine animals were killed after 26 days of oil administration; seven after 37 days and thirteen after 43 days. All showed signs of rickets but of varying severity. The degree of rickets was estimated by examining the costo-chondral junctions of the animals. Those with only a slight beading of the junctions were labelled slight, those in addition with a bending of the rib in one plane, moderate, and those with bending in two planes as well as beading were labelled severe.

On arranging all in order of severity and dividing into thirds, the group incidence was as follows:

Table I.

	1st third. Slight rickets	2nd third. Medium rickets	3rd third. Severe rickets
I. VD	8	1	1
II. VDX	0	6	4
III. PVD	2	3	4

This experiment, while in keeping with the findings of Hopf and Herman, was open to the objection that the number of animals was small and the dose of VD was not fully curative.

Exp. 2. 60 young rats of approximately the same age and from the same mixed stock were used; this experiment was similar to the first except that the dose of the activated and non-activated ergosterol was doubled, namely, 0.0002 mg. per rat per day in 0.1 cc. olive oil. The dose of X-rays was also doubled; the dose of ultra-violet was the same in both experiments, but in this experiment all samples of ergosterol solution were evaporated to dryness before being dissolved in the olive oil; this controlled the possible effect of evaporation on the X-rayed sample in the previous experiment. The X-ray exposure in this case was started $1\frac{1}{2}$ hours after termination of ultra-violet irradiation. The animals were put on to rachitic diet only 2 days before oil-feeding started. Twelve animals died or were killed before the experiment had been in progress 3 weeks and are not included in the results. The remaining 48 were killed 6 weeks after the experiment had commenced.

The results are expressed below in a similar manner to the first experiment:

Table II.

	Slight	Medium	Severe
I. VD	11	5	2
II. VDX	4	6	3
III. PVD	1	4	12

It is clear that as a result of radiation by X-rays the vitamin D activity of the substance is reduced. Spectroscopic observations have been made and show that, whereas the spectrum of pro-vitamin D is markedly changed after irradiation by ultra-violet light, no change occurs after pro-vitamin D has been submitted to heavy doses of X-rays.

So far as our experiments have gone, they indicate, however, that the spectrum of activated ergosterol is altered when this substance is irradiated with X-rays; the spectrum of the X-irradiated material extending further into the ultra-violet region. Thus it appears that X-rays produce a change in vitamin D which biological experiments show to be of a destructive nature.

The destruction observed in our experiments is only partial but this is not surprising in view of the fact that (1) the degree of activation of the ergosterol used is unknown, (2) the dose of X-rays necessary to destroy the vitamin completely still remains to be established.

SUMMARY.

1. A dose of 0.0002 mg. of ergosterol activated under the conditions described will partially protect against, and cure, experimental rickets.

2. X-rays when applied to a dry sample of activated ergosterol exposed to the air exert a destructive effect upon vitamin D. This destruction appears to require large doses of X-rays.

REFERENCES.

- Hopf and Herman (1926). *Sweiz. med. Woch.* **1**, 373.
Rosenheim and Webster (1927). *Biochem. J.* **21**, 389.