XLIV. THE EFFECT OF IRRADIATION OF THE ENVIRONMENT WITH ULTRA-VIOLET LIGHT UPON THE GROWTH AND CALCIFICATION OF RATS, FED ON A DIET DEFICIENT IN FAT-SOLUBLE VITAMINS. THE PART PLAYED BY IRRADIATED SAWDUST, II

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INTRODUCTION.

IN a paper published in 1924 [Hume and Smith, 1924], the writers showed that when young rats, fed on a diet deficient in fat-soluble vitamins, are kept in glass jars containing sawdust which has been irradiated with the mercury vapour quartz lamp, they grow and their bones calcify, as is the case with rats which are irradiated directly. The present work was then undertaken to ascertain in what way the sawdust acts, whether the rats have to be in actual contact with it, so that they can consume some of it, which an examination of their faeces shows that they do when they have the opportunity, or whether direct contact is not necessary in order that the effect shall be produced. If the former alternative be the correct one, the work falls into line with that of Steenbock and Black [1924] and of Hess and Weinstock [1924, 1], who showed that oils, inactive in preventing rickets in rats, when fed to them, can be rendered active by irradiation with ultra-violet light. Since then evidence with regard to the capacity of substances, including cholesterol [Drummond *et al.*, 1925; Hess *et al.*, 1925] and phytosterol [Hess *et al.*, 1925], to be activated has accumulated.

On the other hand, though it seemed probable that direct contact between the rat and the irradiated sawdust was necessary in order that the effect might be obtained, there existed a certain amount of evidence showing that irradiated bodies can exercise an action upon a photographic plate, and that they therefore give off some sort of radiation or emanation. Kugelmass and McQuarrie [1924], for example, published experiments which seemed to show that irradiated bodies give off something which is able to fog a photographic plate, even through a transparent quartz screen. Papers published a good many years ago by Russell [1905] showed that many substances, and the wood of conifers in particular, after exposure to a strong light, give off an

emanation which acts strongly on a photographic plate and which he proved was a vapour and not a radiation.

Nelson and Steenbock [1925] found that rats living on wire screens, soiled with their urine and faeces, receive powerful protection from rickets if the screens are irradiated and if the rats are in direct contact with them, so that irradiated excreta are conveyed by the rat's paws to its mouth. But if the screens are suspended 6 inches above the rats' heads, where the animals cannot touch them, no anti-rachitic effect can be demonstrated.

The present experiments were therefore devised to test firstly whether anti-rachitic radiations of any kind are given off from irradiated sawdust, and secondly whether any volatile substance having anti-rachitic potency is given off. The rats in these experiments were separated from the irradiated sawdust by a transparent quartz plate and by wire grids respectively. At the same time the possibility that emanations or radiations, capable of acting on a photographic plate, are given off by irradiated sawdust was examined by Dr N. S. Lucas, who concluded [1926] that nothing is given off, which can pass a transparent quartz screen sealed with airtight joints, but that a vapour is given off which can act at short range on a photographic plate. Ultra-violet radiations are not given off, but some volatile substance is; this substance Lucas held to be hydrogen peroxide, as did Russell, or else ozone.

TECHNIQUE.

The rats used were all white or black and white, of about 50 g. in weight, and were bred in the Lister Institute. Rats belonging to the same litter were distributed equally, as far as possible, among the various experimental groups to be compared.

The diet used was deficient only in fat-soluble vitamins; full details are set out in the writers' note in the current number of this journal and in former papers [Hume and Smith, 1926].

A mercury vapour quartz lamp, of the Hewitt Electric Co., with straight burner, was used. All irradiations were carried out six times a week. Rats irradiated directly were treated for 10 minutes at a distance of 50 cm. Irradiation of sawdust and of cotton wool in the various experiments took place at distances of from 30–70 cm. and for 10–30 minutes.

The rats were kept singly in cylindrical 8-litre glass jars, except those which were separated from the irradiated sawdust by a quartz plate. The latter were housed in specially constructed, rectangular glass vessels of smaller size.

The sawdust used was ordinary pine sawdust from a sawmill. It was changed every time it was irradiated, so that particles of food or excreta were not irradiated.

The experiments were continued as long as the rats showed any growth, *i.e.* from 10–16 weeks; they were then killed and the bones of the hind legs were cleaned and dried, and calcium estimations were made by the method of Aron and Sebaauer [1910].

EXPERIMENTAL.

Four experimental groups were arranged, as follows:

Group 1 consisted of 14 rats, which inhabited glass jars with sawdust at the bottom, but the sawdust was not irradiated.

Group 2 consisted of 14 rats, which inhabited glass jars with irradiated sawdust at the bottom.

Group 3 contained 13 rats, which inhabited glass jars with irradiated sawdust at the bottom, but the rats were separated from the sawdust by a wire grid, of tinned iron wire, 10 mesh. The distance between the rats and the sawdust was not more than an inch. The grids were never irradiated.

Group 4 comprised only 4 rats. Each rat inhabited a square glass box, into which, at about an inch from the bottom, a transparent quartz plate was fixed so as to be airtight. The quartz plate, 0.2 cm. thick, formed the floor on which the rats lived; underneath, at a distance of about an inch, was a layer of irradiated sawdust. The excreta were cleaned up and a small piece of cotton wool was given towards evening, as bedding, otherwise the rats became cold and suffered.

Table I. *Total growth during period of experiment and percentage of calcium in the dry bones of rats placed on a diet deficient in fat-soluble vitamins and treated in various ways with irradiated and non-irradiated sawdust.*

(For the growth the averages for males and females are given separately; for the calcification all the animals in each group are averaged together. Groups 5-7 are comparable with one another, but not with groups 1-4.)

Treatment		Total increase in weight in period of exp. Average g.	No. of animals averaged	% Calcium in dry bones. Average
<i>Group 1.</i>	Rats in jars on sawdust not irradiated	♂ 66	8	18.2
		♀ 52	6	
<i>Group 2.</i>	Rats in jars on irradiated sawdust	♂ 92	8	19.4
		♀ 66	6	
<i>Group 3.</i>	Rats in jars on wire grid over irradiated sawdust	♂ 91	9	19.3
		♀ 71	4	
<i>Group 4.</i>	Rats separated from irradiated sawdust by quartz plate	♂ 58	2	18.0
		♀ 57	2	
<i>Group 5.</i>	Rats in jars on wire grid over sawdust not irradiated	♂ 43	3	17.2
<i>Group 6.</i>	Rats in jars on wire grid over irradiated sawdust	♂ 53	3	18.5
<i>Group 7.</i>	Rats in jars on wire grid over non-irradiated sawdust, themselves irradiated directly	♂ 78	2	20.3

In Table I, groups 1-4, the average growth made by the male and by the female rats in each group, together with the average percentages of calcium in the bones of all the rats in each group, are set out. The rats of the control group (1), on non-irradiated sawdust and the rats separated from irradiated sawdust by a quartz plate (group 4), give figures which both for growth and calcification are very near to one another, the result of group 4 must therefore

be taken to be negative. Groups 2 and 3, on the other hand, containing respectively rats in contact with irradiated sawdust and rats only separated from irradiated sawdust by a wire grid, also give results which are very close together, and which both for growth and calcification are definitely, though not very greatly, higher than those of the controls. The results therefore indicate that rats living on a wire grid, adjacent to irradiated sawdust, are subject to some influence, which promotes growth and calcification to a greater degree than occurs with control animals, living in direct contact with sawdust, which has not been irradiated. It was observed in the course of the foregoing experiments that the rats living on the wire grids consumed much more of their own faeces than did those of any of the other groups. This was recognised as a possible source of error. Steenbock, Sell and Nelson [1923], and Dutcher and Francis [1924] have observed that rats on a diet deficient in vitamin B can resist the deficiency much longer, if they have access to their own faeces than if they have not. A fresh set of experiments was therefore set up, in which the controls were arranged somewhat differently.

Three experimental groups were made, in all of which the rats inhabited a glass jar, with sawdust in the bottom, over which was placed a wire grid, as already described.

Group 5 contained 3 controls, which lived on wire trays over non-irradiated sawdust, instead of in direct contact with it, as did the previous set of controls (group 1).

Group 6 was the same as group 5, but the sawdust below the wire grid was irradiated.

Group 7. In group 7 two rats living on wire trays, over non-irradiated sawdust, were themselves irradiated directly.

In Table I, groups 5-7, the results of the three last experiments are set out in the same way as was done for groups 1-4. The rats irradiated directly show a marked superiority, both in growth and calcification, over either of the other groups. The rats on the wire grids over irradiated sawdust, though markedly inferior to the rats irradiated directly, still show a small superiority, both in growth and calcification, to the rats on wire grids over non-irradiated sawdust.

In the course of the above investigation experiments were also made in which rats were kept in contact with (1) sawdust irradiated in a shallow glass tray, instead of at the bottom of a deep jar; (2) sawdust allowed to stand for 2 hours after irradiation before the rats had access to it; and (3) irradiated cotton wool. The percentage of calcium in the bones for a corresponding group of control rats on non-irradiated sawdust was 18.9, and for a corresponding group of rats on irradiated sawdust 20.3; when the sawdust was irradiated in a shallow glass tray it was 20.7; when the sawdust was allowed to stand for 2 hours it was 20.5; when cotton wool was irradiated it was 18.4. The first two experiments give a clear positive result, the activity of the irradiated sawdust is in no way diminished, either by standing for 2 hours, or by irradiating-

tion in a shallow glass dish and subsequent removal uncovered to another room. Irradiated cotton wool, on the other hand, is entirely negative, although it was freely eaten by the rats.

SUMMARY AND CONCLUSIONS.

The clearly negative result obtained when a quartz plate was interposed between the rats and the irradiated sawdust disposes of the possibility that the sawdust exercises its action by means of any secondary radiations which it gives off and which can penetrate quartz. This result agrees with that of Lucas [1926], who found that irradiated sawdust does not give off anything which is able to fog a photographic plate through a transparent quartz screen.

The other results which the writers have obtained are, however, not so easy of interpretation. Even when the experiments were all made under conditions equally favourable to the consumption of their own faeces by the rats, and thereby favourable to a conservation of vitamin reserves, rats living on wire grids over irradiated sawdust still grew and their bones calcified somewhat better than was the case with rats on wire grids over sawdust which had not been irradiated. The effect, however, was small and not sufficient to justify the conclusion that irradiated sawdust gives off a volatile substance, which is able to promote growth and calcification in rats. The only vapour which Lucas could detect coming from irradiated sawdust was hydrogen peroxide or some other peroxide, and Hess [Hess, 1924; Hess and Weinstock, 1924, 2] found that neither hydrogen peroxide nor ozonised water, fed to rats, had any anti-rachitic action. The only conclusion for which the evidence is satisfactory is that irradiated sawdust exercises a beneficial action on growth and calcification when consumed by the rats.

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