CLXVII. NOTE ON THE ANTI-RACHITIC ACTION OF IRRADIATED SAWDUST.

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(Received October 27th, 1926.)

No adequate experimental explanation has as yet been offered in interpretation of the results recorded by Hume and Smith [1923] in which increased growth was observed when rats on a diet deficient in fat-soluble vitamins inhabited glass jars previously irradiated. The suggestion that the effect might be due to the ionisation of the irradiated air was soon disproved [Webster and Hill, 1924; Hume and Smith, 1924; Nelson and Steenbock, 1925; Hughes, Nitcher and Titus, 1925] and it was shown to be connected with irradiation of the sawdust, used as bedding in the original experiments. In their last communication on this subject, Hume and Smith [1926], whilst eliminating the possibility of any kind of secondary radiations from the sawdust having an anti-rachitic effect, find their other results "not so easy of interpretation." In these experiments, the rats were kept in glass jars, separated from the irradiated sawdust by a wire grid. The authors state, however, that "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."

The fact discovered independently by Hess, Weinstock and Helman [1925], Steenbock and Black [1925] and by ourselves [1925] that sterols acquire anti-rachitic properties on irradiation, suggested to us that the effect of irradiated sawdust on growth might be due to the presence in it of sterols. The occurrence of these substances in all vegetable tissues is well known, and crystallised phytosterols have been isolated from wood, bark, cork and resins, all of which may form the constituents of mixed sawdust.

We tested this suggestion experimentally by extracting with chloroform 250 g. of mixed sawdust, as used for bedding in our animal experiments, until Liebermann's sterol reaction was no longer given by the extract. The chloroform extract was concentrated *in vacuo* and left a yellowish resin (3.2 %), possessing the characteristic sawdust odour. No attempt was made to isolate the sterol (or terpene?) in a pure condition, since the purpose of the experiment was to test the anti-rachitic action of the whole extract. The resin was irradiated for half an hour (40 cm. distant from a Cooper-Hewitt mercury-vapour lamp) and administered, dissolved in olive oil, to rats kept on Sherman-Pappen-

heimer's diet No. 84. The control rats developed typical rickets, whilst the animals receiving 5 mg. *per diem* of irradiated extract of sawdust were entirely protected, as proved by radiographic and chemical evidence.

The extracted sawdust no longer acquired, when irradiated, the power of fogging a photographic plate, but the resinous extract showed this property even when exposed for a short time only to ordinary white light. The action on a photographic plate of cod-liver oil, irradiated vegetable oils and sterols has led many recent writers to assume a connection between anti-rachitic and photochemical activity (see especially Vollmer and Serebrijski [1926]). The experimental results obtained by Kugelmass and McQuarrie [1924] in particular, were adduced in explanation of the anti-rachitic action of irradiated sawdust, although these authors [1925] soon withdrew their original claims. Their results were explained by themselves and others as being due, not to radiations, but to volatile "peroxides" (the "Russell effect"; for a review of the literature see Niederhoff [1926]). The latter substances are, however, not produced when sterols are irradiated *in vacuo* or in hydrogen and the effect on the photographic plate is absent [Hamano, 1925]. On the other hand, the anti-rachitic activity of cholesterol, when irradiated under similar conditions (nitrogen atmosphere), is not only more pronounced but also more stable than that of air-irradiated cholesterol [Rosenheim and Webster, 1926]. This evidence together with other facts which we have obtained appears to be definitely against any association between anti-rachitic activity and the chemical action on a photographic plate of volatile "peroxides."

In explanation of the growth effect observed by Hume and Smith in their original experiments, we may now exclude the effect of (1) ionisation of irradiated air, (2) secondary radiations, and (3) volatile "peroxides." It seems justifiable to conclude, from the results of the above-described experiment, that the increased growth under the experimental conditions of Hume and Smith is due to the ingestion by the rats of sawdust, the sterols of which had become anti-rachitic by irradiation.

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