Forgotten Mysteries in the Early History of Vitamin D

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ABSTRACT In the early 1920s, workers in both England and the US had discovered that rats on a rachitic diet would remain healthy if irradiated with ultraviolet light. However, they also found, to their surprise, that “control” rats too would recover if either their jar was irradiated without the rat in it or if a cage-mate was removed for irradiation and then returned. The ideas that either air or material objects that had been irradiated continued to convey healthful secondary radiations were investigated but not confirmed. There was then the commercially important finding that with irradiation, some rachitic diets would become anti-rachitic. However, this effect did not explain all the previous findings. Consumption of either small irradiated fecal particles or of feces from irradiated rats was the likely explanation for the recovery of nonirradiated rats, but this was not tested by direct experiment, and it now appears unlikely that feces from irradiated rats would show significant antirachitic activity. It is suggested that an alternative possibility—activity of grease from irradiated fur—deserves investigation. J. Nutr. 129: 923–927, 1999.

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The “mysteries” that we refer to consist of some results reported by British and American workers in the period from 1922 to 1924. This was a period of intensive research into the cause of rickets and the possible role of the newly discovered fat-soluble vitamin(s).

THE BACKGROUND

By 1900, it was well-accepted that the bones of a rachitic child had an abnormally low mineral content, and specifically of calcium and phosphate, but that giving calcium salts did not cure or prevent the condition. One physician wrote that: “it looks as if the blood lacks something that would allow the salts to deposit themselves in the cartilage of the growing bone” (Parry 1872).

It was agreed that the disease occurred mostly in large cities in Northern latitudes (Owen 1889; Snow 1895). One explanation put forward for this was the lack of exposure to sunlight in the areas of the disease (Palm, 1890 and also Mozolowski, 1939 citing a publication in 1822). There was also a long folk tradition in some areas that cod liver oil was a specific, potent preventive, and this was endorsed by many physicians (e.g., Trousseau 1873). Others thought of it merely as a source of animal fat, deficient in the typical diet of weaned infants (e.g., Cheadle 1888, Hutchison 1907).

The development of x-ray photography after 1900 gave investigators a more objective measure of the effect of different treatments on bone calcification. The curative value of both direct sunlight and of artificial ultraviolet (UV) light was reported (Huldschinsky 1919, 1920, Raczynski 1912/13), and a careful controlled study in Vienna after World War I confirmed that the same curative effects could be obtained by either sunlight or cod liver oil (Chick et al. 1922). It was, of course, a puzzle as to how two such different stimuli could have the same effect.

Until that time, dogs had been the only species that had been found useful as a model for human rickets. With hindsight, it is understandable that rats, which were convenient for nutritional studies, should be more resistant since they had evolved the ability to live out of sunlight and to thrive on a diet consisting almost entirely of seeds, so that they had no source of the antirachitic vitamin. However, E. V. McCollum and his colleagues discovered that young rats would develop a similar condition if their diet contained an excess of either calcium or phosphorus and a low intake of the other element (McCollum et al. 1921). This led to a burst of experimentation with rats and confirmation of the value of both UV light and cod liver oil in treating this condition (Park 1923). The active factor in cod liver oil was not vitamin A, but another fat-soluble factor, soon named vitamin D (McCollum et al. 1922).

THE MYSTERIES

In London, two scientists who had been part of the team working in Vienna used a new type of “control” group in their radiation experiments. In addition to irradiating one group of rats each housed in its individual glass jar, they had another group where the rat was removed before its jar was taken to be irradiated, and then replaced afterward. To their surprise, those whose empty jar had been irradiated did nearly as well as those directly irradiated, and better than other controls where there had been no irradiation (Hume and Smith 1923). The procedure is shown diagramatically in Figure 1. The glass lid

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2 Abbreviation used: UV, ultraviolet.

Figure 1 Schematic diagram of the experiment reported by both Hume and Smith (1924) and Nelson and Steenbock (1925a), using three treatments but the same rachitic diet. I: a rat that is not irradiated and becomes rachitic; II: a rat irradiated in its jar and remains healthy; III: a rat that is removed from its jar before the jar is irradiated and then replaced, but also remains healthy.

to each had been removed during irradiation and then immediately replaced.

Their provisional conclusion was that the “irradiated air” in the jars had been responsible for the rats’ response. They tested this idea in a further trial by instructing their assistant to blow out the air in each empty jar immediately after it had been irradiated, and then to replace the lid. When that was done, the rats did not show improvement. This appeared to confirm their first conclusion, and they referred to an earlier German report that “irradiated air” had proved as helpful as irradiation itself in stimulating the recovery of dogs from anemia (Kestner 1921).

This was an extraordinary and exciting finding. Another group in London immediately tried to replicate the effect but failed to obtain it (Webster and Hill 1924). The original workers then re-studied their procedures and realized that their assistant, before blowing air out of an irradiated jar, had tipped out the sawdust which provided bedding for the rats, so as not to have it blowing around the room, and then added fresh material after the “blow out.” In a later trial, they irradiated just fresh sawdust and found that rats responded to having this added to their jar. They observed wood fragments in the rats’ feces and concluded that the response came from their having obtained something useful from the sawdust that they had eaten as well as rested on (Hume and Smith, 1924, 1926).

Meanwhile, Steenbock and his colleagues at the University of Wisconsin-Madison also had taken notice of Hume and Smith’s first paper (De Kruijff 1928). They were interested because they, too, had obtained a mysterious result with a control group in an irradiation experiment. They had housed two rats per cage so that their conditions were identical, including the supply of a rickets-producing diet, except that one of the two in each cage was periodically removed for a few minutes of irradiation. The irradiated rats responded as expected but did so did their unirradiated cage-mates (Jones et al. 1924, Steenbock and Black 1924). At first it was suspected that there had been an error in the make-up of the diet, but then, in further trials, the same effect was seen, while further controls, separately housed, did become rachitic (Nelson and Steenbock, 1925a).

Steenbock’s group then repeated the original British “empty jar” radiation experiment, except that their jars differed in having a raised wire screen on the floor so that the rat feces would fall through. Again, rats receiving no direct radiation but replaced into irradiated jars showed a response! However, if the screen previously in use was replaced by a new (or newly scrubbed) screen before the rat was returned, there was no response. The authors reported that the “used” screens appeared to be clean, so that they presumably had no adhering feces, but that they felt slightly “unclean” to the touch (Nelson and Steenbock 1925a). We interpret this as meaning that the screens felt “sticky” or “greasy.”

THE PRACTICALLY IMPORTANT OUTCOME

The group at Madison also commented that the response to the irradiated empty jar in London perhaps was due to the irradiation of spilt food particles among the sawdust, that the rats later ate, having in some way been activated. They therefore tried irradiating their own rachitic diet and discovered that indeed rats did respond after eating it (Steenbock and Black 1924, Steenbock and Nelson 1924–25). From that beginning, they and others studied what fraction of the diet was responsible for the response (e.g. Hess and Weinstock 1924). Soon it was traced to the sterol fraction, and it was then found that the activated factor produced both by irradiation of sterols or of the skin of living animals was indeed the same (or at least very similar) to that present in cod liver oil.

The paradox of two such apparently different treatments having the same effect was therefore resolved (Holick 1989). Steenbock himself was able to obtain a patent for the commercial radiation of materials to produce vitamin D, with most of the multimillion dollar proceeds over the following decade going to finance further research through the Wisconsin Alumni Research Foundation (Schneider 1973).

However, that the stimulus to Steenbock’s irradiation of foods came from investigating Hume and Smith’s “irradiated air” work has been largely forgotten. Of the many reviews of the history of vitamin D that we have seen, it is mentioned only in two (Hede 1975, Jukes 1983).

EXPLANATIONS OF THE REMAINING MYSTERIES

Although the activation of sterols in food or wood particles by UV light was interesting, and to prove of great practical value, it could not explain everything reported from the two laboratories at this point, particularly why a nonirradiated rat got better when caged with an irradiated one. Other possibilities will be considered under three headings.

Secondary radiations. At this time a group at Yale reported that all substances (such as cod liver oil, egg yolk and bile) which cured rickets, when oxidized, emitted UV rays (Kugelmars and McQuarrie 1924). These were detected by their fogging a photographic plate when screened with quartz but not when screened with glass. The authors suggested that the common mechanism for the cure of rickets by such different measures as UV irradiation and giving cod liver oil was that the latter also emitted UV rays when being metabolized in the body. Nothing further was to be heard of this idea but, at
itself been directly irradiated. We will return later to this material coming from irradiated rats, but their excreta had not been irradiated while on the screens; in the later experiment it was may contaminate the screens are entirely sufficient."

These activated compounds which have to be consumed to empty cages, the authors wrote that "evidently the amounts of ferring back to their earlier experiment with irradiation of falling down from the upper cage, i.e., "certain compounds and Steenbock 1925b). The authors concluded that it could not be "rays" from the irradiated rats that were helping the lower cage remained healthy. But when, in another pair of cages, the rats in the lower cage were irradiated, those in the upper cage did not benefit and became rachitic.

The time, it suggested to both the British and American investigators that they needed to test whether either activated objects or irradiated rats were themselves irradiating health-giving rays, rather than passing on some activated material.

In London, rats did not respond to the presence of irradiated sawdust if a quartz screen was placed between (Hume and Smith 1926). In Madison also, irradiated screens no longer had any effect when placed near to the rats, but out of their reach (Nelson and Steenbock 1925a).

Steenbock's group then devised a test as to whether non-irradiated rats would benefit from being near irradiated ones, but out of touch with them. Four special cages only two inches high were used that could be stacked one on top of another with only the wire grid at the bottom of one, and that at the top of the other separating the animals in the two cages, as shown in Fig. 2. The wire grids (three mesh to the inch) allowed the small fecal pellets to fall through, though a small amount of sticky residue remained attached. Altogether 16 rats were used. When a cage of four irradiated rats was kept on top of a cage of four nonirradiated rats, the latter remained free from rickets. In the other pair of cages the nonirradiated animals were on top, and they did develop signs of deficiency despite the presence of irradiated rats beneath them (Nelson and Steenbock 1925b). The authors concluded that it could not be "rays" from the irradiated rats that were helping the nonirradiated ones because these would presumably act both upward and downward.

Consumption of excreta. The only alternative explanation for the results just described appeared to be something material falling down from the upper cage, i.e., "certain compounds excreted or secreted by the irradiated animals" and then referring back to their earlier experiment with irradiation of empty cages, the authors wrote that "evidently the amounts of these activated compounds which have to be consumed to produce an effect are almost infinitesimal. Such amounts as may contaminate the screens are entirely sufficient."

The authors here were comparing two different things. In the earlier experiment it was material that had been itself irradiated while on the screens; in the later experiment it was material coming from irradiated rats, but their excreta had not itself been directly irradiated. We will return later to this point. Nelson and Steenbock (1925b) concluded specifically that nonirradiated rats benefited from their association with irradiated animals housed above them by being able to consume some of the latter's excreta, that could presumably include urine dried onto the screens. They did not suggest a deliberate consumption, only that rats would obtain it incidentally when cleaning their paws that had been contaminated with sticky residues on the screens.

In the same period, Steenbock and his colleagues studied the condition under which rats would become deficient in "vitamin B"—later to be recognized as a complex of several factors. They had observed that rats kept on wire screens would fail to grow while others, on the same marginal diet, thrived when living on a bed of infrequently changed wood-shavings so that they had: "access to accumulated excreta."

They concluded that these rats did better because they obtained significant quantities of vitamin from eating some of the excreta. They added that: "putting our animals on screens does not entirely solve the problem [when group-feeding] as some rats... will seize the feces of their companions as soon as excreted. We are inclined to believe, however, that the amount so obtained is a factor of but little importance in determining the final result" (Steenbock, et al 1923). By "the final result" they meant the inferior performance of the rats housed on the screens, which was explained by their access to a much smaller supply of fecal material.

Under the conditions of the final experiment where a rat in the lower cage would have to act as a "catcher" waiting for anything dropping from above, one can understand that the authors would have thought only of the animals getting any sticky residues that adhered when fecal pellets fell through the lower screen bottom. They do not say so specifically, but they presumably felt that the same mechanism explained the findings in the first Madison experiments where rats were taken from their cage to be irradiated, and then put back with their nonirradiated cage-mate. In other words, they expected that the nonirradiated animal in each cage would get some fecal material from its companion on its paws and might also obtain some actual fecal pellets, though not in a large quantity.

With hindsight, it appears the proportion of fecal pellets consumed by rats living on screens can be ~50%, i.e., much greater than had been envisioned in 1925 (Barnes et al. 1957; Kon 1962). However it has also been suggested that rats vary in the extent to which they do this, and the Steenbock rats may not have engaged in it to a large extent when living on screens in view of the results in the "vitamin B" study referred to above. And, of course, one would expect the lower rats in the two-tier trial to have found it more difficult to "catch" fecal pellets falling from above, before they fell further through the screen in the lower cage.

In any case, the irradiated rats would have had to excrete, in total, many times more than enough vitamin D (or bioactive derivative) to provide for the needs of a second animal, considering that only a small proportion would be consumed by its cage-mate or one in another cage below. If, for example, a rat were to excrete each day, in active forms, as much as 5% of the vitamin D synthesized as a result of irradiation, and if another rat were to consume 20% of these excreta, and thus remained healthy, it would mean that the irradiation of the first rat had caused the production of 100 times its own need for the vitamin, which seems extraordinarily high.

It is now realized that the vitamin D formed by irradiation of naturally occurring sterols still has to be metabolized in two stages to form the highly active "hormone" and that it is also degraded by body tissues into inactive metabolites (De Luca 1997). This makes it more difficult to assess the biological

![Figure 2](https://example.com/image2.png)
value of material with a possible mixture of several forms. It is known, from work with isotopically labeled "25-hydroxy D" that rats excrete a considerable proportion of the label in their bile and thus into the feces, but the molecule appears then largely to have been metabolized to inactive forms (Bolt, et al. 1992). This also appears to be the case in humans (Clements et al. 1984). Rats also secrete some of the "label" from labeled vitamin D (or its active metabolites) into their urine but it then again appears to be in inactive, polar breakdown products (Reddy and Tseng 1989).

After a very large dose of vitamin D was given to rats by mouth, rat assays of the feces produced over the next 4 d gave responses equivalent to 18% of the dose having been excreted in this way (Kodicek 1956). However, when a dose of vitamin D is given in the physiological level, was given by intravenous injection, rat assay of the feces showed no significant excretion of biologically active material by this route (Lund and De Luca 1966).

Body grease. In the Madison "empty cage" experiment, the use of wire screens that proved to be a source of antirachitic activity after irradiation were described as being "apparently clean, but feeling unclean to the touch." One would think that even traces of adhering rat feces would, because of their dark color, be obvious. A possible alternative is that the "unclean feel" of the screens came from lipids on the coat of the rats that had been lying on them, and that these contained sterols which resulted in vitamin D being formed on UV irradiation. To benefit from this, the rat would then have either to lick the screen directly or, as a result of its lying on the screen, for some of the irradiated grease to be transferred back onto its coat, and then being licked off during normal grooming.

In the first Madison trials, where cage-mates benefited from the irradiation of the other rat, they would have to lick the other's coat, or for there to be some transfer of "irradiated grease" to its own coat when they were lying together for them to benefit in this way as well as from ingestion of the cage-mate's feces.

These ideas go against current thinking. It has, at one time, been thought that chickens obtained their vitamin D from the activation of material from preen glands that the birds had distributed onto their feathers and later consumed (Hou 1928,1929). However, it now appears that they obtain the vitamin directly from synthesis under the skin of their legs which are not obscured by feathers (Knowles et al. 1935, Koch and Koch 1941, Tian et al. 1994). Their legs were deliberately exposed to irradiation in the tests but would receive it naturally when outdoors with the sunlight striking at an angle.

Direct irradiation of a rat's shaved skin also results in vitamin D synthesis (Estvelt et al. 1978, Holick et al. 1979). But this does not, of course, rule out their having an alternative route for obtaining the vitamin since their skin is naturally covered with fur. In both the London and Madison experiments, the UV radiation of the rats came directly from above, and the rats were in glass jars that they could not climb, so that they were almost certainly in their usual resting position with only their backs exposed to the radiation. It seems at least possible therefore that it was the grease on the animal's fur which was activated in these tests, and from which they benefited as a result of their "grooming" behavior. Analysis of rat hairs did not show the presence of vitamin D activity, but the sample did not, presumably, come from an animal irradiated with UV light (Holick 1989).

It would be of interest now that sensitive analytical methods are available, for some of these key experiments in the early history of vitamin D to be repeated so as to clarify how far the results are to be explained by coprophagy and/or consumption of irradiated grease by grooming. We have no direct evidence either to support or negate this possible alternative mechanism.

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LITERATURE CITED


