THE ANTIRACHITIC EFFECTIVENESS OF VITAMIN D FROM VARIOUS SOURCES*

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The proper evaluation of antirachitic agents for human use as well as for animals of economic importance is a problem of immediate concern to all interested in rational therapeutics. Chemical assays are as yet out of the question and animal assays reveal a species variation in the degree of reaction to different forms of vitamin D which does not parallel that of the human. Unfortunately as only the vitamin D produced from ergosterol is known in pure form, the difficulties of proper evaluation of an antirachitic agent may be complicated by the presence of unknown forms of vitamin D, the presence of synergistic substances, or the presence of both. It is accordingly important to know how many forms of vitamin D must be recognized, to what extent each occurs in nature or is produced artificially by irradiation, what factors influence the activity of each, and how each may be evaluated properly by laboratory assays with animals.

That irradiated ergosterol was not identical with the vitamin D of cod liver oil was indicated by the work of Massengale and Nussmeier (1) who found that on the same rat unit basis irradiated ergosterol had less antirachitic effect than cod liver oil when tested on chickens. Steenbock and coworkers (2), recognizing the possible synergistic action of other substances in cod liver oil, fed irradiated ergosterol with small amounts of cod liver oil and carotene and obtained no better results than with ergosterol alone. They concluded in consequence that the vitamin D produced by irradiating ergosterol is different from that found in cod liver oil.

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Waddell (3) reported that irradiated cholesterol unit for unit, unlike irradiated ergosterol, was as effective an antirachitic agent for chicks as cod liver oil. This observation has been confirmed (4). He also concluded that the provitamin D of cholesterol was not ergosterol present as a contaminant. Tisdall and collaborators (5) found irradiated cholesterol to be an effective antirachitic for infants. However, Koch and collaborators (6) showed that highly purified non-activatable cholesterol could be converted to a provitamin D which was activatable by ultra-violet radiations. Heating converted “purified” non-activatable cholesterol into a substance activatable with ultra-violet light. These tests were made with rats. Koch and Koch (7) and Hathaway and Lobb (8) recently have also shown that, unit for unit, such cholesterol when irradiated and fed to chicks was more nearly as effective as crude irradiated cholesterol than as irradiated ergosterol. It is not excluded, therefore, that the provitamin D in heated cholesterol is identical with that present in crude cholesterol.

Difficulties in proper evaluation of potency arise even with the naturally occurring vitamins D. Bills and collaborators (9), from studies with chicks, have concluded that the vitamin D activity of fish liver oils may not be restricted to one form of vitamin D. For example, a sample of tuna liver oil was found to be approximately one-sixth to one-eighth as effective with chicks as cod liver oil on an equal unitary basis. Halibut liver oil was found to have an efficiency intermediate between tuna liver oil and cod liver oil.

A great deal of confusion also exists in the assessment of the proper vitamin D efficiency of various vitamin D milks. We (4) have recently reported the relative ineffectiveness of the vitamin D from yeast milk as compared with irradiated milk. These results were obtained with chicks. Similar data for chicks have been presented by Bethke et al. (10). However, clinical results have suggested an antirachitic effectiveness for vitamin D milks of a higher order than that shown for cod liver oil even as given with milk (11).

1 A term used for milk enriched with vitamin D.
2 Vitamin D milk produced by feeding irradiated yeast to lactating cows.
That the medium in which vitamin D is administered may be a factor in producing different results is indicated by the work of Lewis (12). He reported from a clinical study that calciferol added in propylene glycol to milk was more effective than calciferol in corn oil. More recently Lewis (13) has reported that for the infant crystalline vitamin D incorporated in the daily ration of milk is more effective on a comparable rat unit basis than crystalline vitamin D administered in corn oil or propylene glycol. He has concluded that milk as a medium for the administration of vitamin D increases its effectiveness, and believes that the observed clinical effectiveness of vitamin D milks can be explained on this basis.

The efficiency of synthetic antirachitic agents has also received some attention. Windaus (14) found 22-dihydroergosterol and a 7-dehydrocholesterol prepared in his laboratory activatable by exposure to ultra-violet light. Tested with rats these compounds were approximately one twenty-fifth to one thirtieth as active as irradiated ergosterol. Very recently McDonald (15) has reported that rat unit for rat unit irradiated 22-dihydroergosterol had an efficiency for chicks which was intermediate between irradiated ergosterol and cod liver oil. It actually approached the efficiency of cod liver oil more than irradiated ergosterol. Yoder, Thomas, and Lyons (16) have reported the synthesis of a cholesterol sulfonic acid which is antirachitically active without irradiation with approximately the same degree of effectiveness for the chick as irradiated cholesterol.

EXPERIMENTAL

The experiments reported in this paper represent a miscellaneous study including a determination of the antirachitic efficiency of vitamin D from various sources, the effect of certain modifications in the technique of irradiation, an investigation of the alleged beneficial effects of propylene glycol as a carrier for vitamin D, and a correlation of the appearance of certain absorption bands in cholesterol with its activatability.

Parallel assays with rats and chicks were made to compare the antirachitic effectiveness of the various preparations. In our assays with rats we used the curative technique of the 10 day line test. The rats were given Steenbock and Black's (17) basal
rachitogenic Ration 2965. The chick assays were made according to the prophylactic technique with the chick on the basal rachitogenic ration of Hart, Kline, and Keenan (18). The chicks used were white Leghorns, 1 day old, as obtained from commercial hatcheries. They were kept in groups of fifteen in electrically heated brooders with screen bottoms. At the end of 5 weeks their tibias were analyzed for ash after extraction with alcohol.

Series I—In the light of the work of Waddell (3) on cholesterol with chicks, it became of interest to determine the relative effectiveness of irradiated oils of plant and animal origin, since the sterol fractions are credited with containing ergosterol and cholesterol respectively. Of further experimental interest was the fact that while fish oils contain large quantities of cholesterol, yet of these at least one, viz. cod liver oil, is well known not to have its vitamin D activity increased by irradiation (19–21).

The following oils or fats were irradiated: soy bean oil, coconut oil, red palm oil, wheat germ oil (hot pressed, cold pressed, and alkali-refined), corn oil, cottonseed oil, peanut oil, sesame oil, lard, chicken fat, cod liver oil, burbot liver oil, halibut liver oil, sardine oil, and tuna liver oil. The chicken fat was rendered and filtered in the laboratory; all the others were obtained from commercial sources. The tuna and halibut liver oils were commercial products sold under the trade names of tuniver oil and haliver oil (Abbott Laboratories) respectively. The former was known to carry a minor admixture of the oils of related species.

The oils were treated as follows: 100 cc. were exposed in a porcelain, flat bottomed dish, with an area of 72 sq. inches, to the radiations from a Hanovia Alpine Sun lamp at a distance of 18 inches for 1 hour. They were stirred every 5 to 10 minutes. Fats solid at room temperature were irradiated at a temperature of approximately 42°. Two of the fish oils, viz. commercial tuna liver oil and halibut liver oil, were irradiated in smaller quantities, but the amount of oil per sq. inch was kept constant.

For feeding to our chicks the oils were diluted with Wesson oil so that the requisite dose of vitamin D was incorporated in the rachitogenic diet with the addition of 2 per cent of the diluted oil. The irradiated lard and chicken fat, because of their low activity, were fed undiluted at a somewhat higher level, viz. 3.75 per cent. Only four of the irradiated plant oils and one irradiated fish oil,
namely cod liver oil, were included because we felt that sufficient representative data could be obtained from them.

The data obtained with rats are not presented in detail in order to conserve space. In general, however, it may be said that the vitamin D activity of the fish oil failed to be increased by irradiation, while all the others were found to be activatable. It re-

**TABLE I**

*Series 1. Ash in Tibia of Chicks Fed Vitamin D from Irradiated Plant and Animal Oils*

<table>
<thead>
<tr>
<th>Additions to basal ration</th>
<th>International units per 100 gm. ration</th>
<th>No. of chicks</th>
<th>Average weight of chicks</th>
<th>Average weight of ash</th>
<th>Average ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>14</td>
<td>170</td>
<td>0.1534</td>
<td>30.58</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>8.25</td>
<td>14</td>
<td>240</td>
<td>0.2808</td>
<td>38.35</td>
</tr>
<tr>
<td>Irradiated cod liver oil</td>
<td>8.25</td>
<td>15</td>
<td>210</td>
<td>0.2551</td>
<td>37.44</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>16.5</td>
<td>15</td>
<td>275</td>
<td>0.3785</td>
<td>46.40</td>
</tr>
<tr>
<td>Sardine liver oil</td>
<td>16.5</td>
<td>15</td>
<td>241</td>
<td>0.2950</td>
<td>43.27</td>
</tr>
<tr>
<td>Burbot</td>
<td>16.5</td>
<td>15</td>
<td>250</td>
<td>0.3335</td>
<td>44.69</td>
</tr>
<tr>
<td>Halibut</td>
<td>16.5</td>
<td>14</td>
<td>218</td>
<td>0.2514</td>
<td>42.20</td>
</tr>
<tr>
<td>Tuna</td>
<td>16.5</td>
<td>13</td>
<td>228</td>
<td>0.2789</td>
<td>40.34</td>
</tr>
<tr>
<td>Irradiated chicken fat</td>
<td>16.5</td>
<td>15</td>
<td>258</td>
<td>0.3328</td>
<td>44.37</td>
</tr>
<tr>
<td>&quot; lard</td>
<td>16.5</td>
<td>13</td>
<td>218</td>
<td>0.2436</td>
<td>38.82</td>
</tr>
<tr>
<td>&quot; coconut oil</td>
<td>82.5</td>
<td>14</td>
<td>174</td>
<td>0.1681</td>
<td>34.71</td>
</tr>
<tr>
<td>&quot; wheat germ oil</td>
<td>82.5</td>
<td>14</td>
<td>212</td>
<td>0.2157</td>
<td>35.20</td>
</tr>
<tr>
<td>&quot; peanut oil</td>
<td>82.5</td>
<td>14</td>
<td>186</td>
<td>0.1867</td>
<td>34.57</td>
</tr>
</tbody>
</table>

The difference in effectiveness between irradiated chicken fat and irradiated lard was explained by our final rat assays wherein it was found that our preliminary assays were in error. It was found that the irradiated chicken fat was approximately twice as effective as we had believed.

*Originally determined by assay with rats as Steenbock units and converted to international units by the factor 3.3.

required from 15 mg. (coconut oil) to 750 mg. (soy bean oil and lard) to give an antirachitic activity equivalent to 1 Steenbock unit (3.3 international units) of vitamin D.

The data obtained with chicks are presented in Table I. They show, first, that the vitamin D activity of cod liver oil for the chick was not increased by irradiation; secondly, that the effectiveness of the vitamin D in various fish oils for chicks was approximately
the same as for rats, unit for unit. Commercial tuna liver oil and
pure halibut liver oil, unit for unit, were somewhat less effective
than the other oils. The differences were, however, not so great
as those reported by Bills, Massengale, and Imboden (9). They
investigated the liver oil from the blue fin tuna and reported a
material difference in the unit for unit effectiveness between it
and cod liver oil. Very recently Bills (22) has reported that for
the chick the antirachitic effectiveness of tuna liver oils was de-
termined by species. That from one species, the blue fin tuna,
was approximately one-eighth as effective as cod liver oil, whereas
that from other species was found to be more effective than cod
liver oil on an equivalent rat unit basis. Whether species differ-
ences account for the discrepancy between our results and the
carly results of Bills and collaborators we, unfortunately, could not
determine. Thirdly, our results show that the provitamin D of
animal fats is different from that in plant oils, the former resem-
bling cholesterol and the latter ergosterol with respect to their anti-
rachitic effect on chicks. Bethke et al. (23) in a recent paper have
reported similar results.

Series 2—That the solvent in which ergosterol is dissolved in-
fluences the rate and efficiency of vitamin D synthesis with ex-
posure to ultra-violet light was reported by Bills et al. (24) in 1931.
He presented time-activation curves for ethyl alcohol, ethyl ether,
and cyclohexane as determined with rats by the line test. We
questioned whether the products produced were identical
forms of vitamin D. We consequently irradiated cholesterol and
ergosterol in ethyl ether and ethyl alcohol solution (0.1 per cent)
and in addition in crystalline form. The irradiation was carried
out with a Hanovia Alpine Sun lamp at a distance of 18 inches for
1 hour. The crystals were stirred every 5 to 10 minutes during
the irradiation period. The resultant products were fed to chicks
on an equal unitary basis, as determined with rats. Table II
shows that the solvents had no effect upon the character of the
vitamin D produced.

We have recently reported that skim milk fed with various
sources of vitamin D had no effect on their comparative antirachi-
tic effectiveness for chicks. Lewis (12), however, has reported
that calciferol added to milk in propylene glycol solution was
approximately twice as effective for the infant as calciferol ad-
ministered in corn oil solution. We anticipated that propylene glycol might have an effect in the absence of milk. We accordingly fed calciferol and irradiated cholesterol both in propylene glycol and Wesson oil solutions. The propylene glycol solutions were incorporated into the basal chick rations to the extent of 1

Table II

Series 2. Ash in Tibia of Chicks Fed Vitamin D from Cod Liver Oil, Irradiated Cholesterol, Irradiated Ergosterol, and Calciferol

<table>
<thead>
<tr>
<th>Additions to basal ration</th>
<th>International units per 100 gm. ration</th>
<th>No. of chicks</th>
<th>Average weight of chicks</th>
<th>Average weight of ash</th>
<th>Average ash per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0</td>
<td>12</td>
<td>168</td>
<td>0.1738</td>
</tr>
<tr>
<td>Cod liver oil diluted with Wesson oil</td>
<td>16.5</td>
<td>14</td>
<td>265</td>
<td>0.0609</td>
<td>43.84</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td></td>
<td>16.5</td>
<td>15</td>
<td>279</td>
<td>0.3764</td>
</tr>
<tr>
<td>Cholesterol irradiated as crystals and fed in Wesson oil solution</td>
<td>16.5</td>
<td>15</td>
<td>255</td>
<td>0.3593</td>
<td>45.88</td>
</tr>
<tr>
<td>Cholesterol irradiated as crystals and fed in propylene glycol solution</td>
<td>16.5</td>
<td>16</td>
<td>245</td>
<td>0.3301</td>
<td>43.88</td>
</tr>
<tr>
<td>Cholesterol irradiated in alcohol and fed in Wesson oil solution</td>
<td>16.5</td>
<td>15</td>
<td>255</td>
<td>0.3574</td>
<td>45.12</td>
</tr>
<tr>
<td>Heated purified cholesterol irradiated as crystals and fed in Wesson oil solution</td>
<td>16.5</td>
<td>15</td>
<td>257</td>
<td>0.3617</td>
<td>45.24</td>
</tr>
<tr>
<td>Ergosterol irradiated in alcohol and fed in Wesson oil solution</td>
<td>165</td>
<td>15</td>
<td>224</td>
<td>0.2538</td>
<td>37.67</td>
</tr>
<tr>
<td>Ergosterol irradiated as crystals and fed in Wesson oil solution</td>
<td>165</td>
<td>14</td>
<td>230</td>
<td>0.2655</td>
<td>38.32</td>
</tr>
<tr>
<td>Ergosterol irradiated in ether and fed in Wesson oil solution</td>
<td>165</td>
<td>15</td>
<td>217</td>
<td>0.2498</td>
<td>39.08</td>
</tr>
<tr>
<td>Calciferol fed in propylene glycol solution</td>
<td>165</td>
<td>15</td>
<td>210</td>
<td>0.2260</td>
<td>36.57</td>
</tr>
<tr>
<td>Calciferol fed in Wesson oil solution</td>
<td>165</td>
<td>13</td>
<td>232</td>
<td>0.2761</td>
<td>39.30</td>
</tr>
</tbody>
</table>

per cent of the diet in the same manner as our oil solutions. Cod liver oil emulsified with propylene glycol instead of the usual Wesson oil was also included in this series. Table II shows that propylene glycol was no more efficient carrier for vitamin D than Wesson oil.

Koch and his collaborators (6, 25) have reported that the pro-
vitamin D content of cholesterol purified by bromination could be increased by heating it above its melting point. Their data on absorption spectra revealed that no spectral absorption characteristic of ergosterol, in fact, no banded absorption, was produced in the purified and heated preparation. We repeated Koch's experiments using chicks as well as rats to determine whether the vitamin D produced from heated purified cholesterol was identical with that produced from crude cholesterol. Although Koch et al. (6, 25) failed to find absorption bands in his heated purified cholesterol, we (Chart 1) found four bands spaced similarly to those of crude cholesterol but shifted somewhat to the red end of the spectrum. The possibility was not excluded, therefore, that irradiation of these cholesterols might have produced a different vitamin D. A purified cholesterol therefore was
prepared according to the bromination technique of Bills et al. (26). After irradiation this was found to produce no response with the line test, when fed at a 50 mg. level. However, after we had heated and irradiated the preparation according to Koch's (6, 25) technique, 1 mg. of the irradiated preparation was found to have a potency of 3.3 international units. This preparation when fed to chicks (Table II) produced calcification more closely resembling in amount that induced by irradiated crude cholesterol than ergosterol. These results confirmed those of Hathaway and Lobb (8) which appeared after our experiments were completed.

SUMMARY

1. The antirachitic activity of certain fish oils, viz. cod liver oil, halibut liver oil, tuna liver oil, burbot oil, and sardine oil, was not increased by irradiation.

2. The comparative antirachitic effectiveness of the fish oils, as determined with chicks and rats, was approximately the same. However, the commercial tuna liver oil that we fed was somewhat less effective than the other oils studied.

3. Irradiated plant oils, viz. coconut oil, wheat germ oil, and peanut oil, were less effective antirachitically for chicks than irradiated animal fats, viz. lard and chicken fat, on an equivalent unitary basis.

4. Propylene glycol as a solvent for vitamin D did not increase its effectiveness.

5. The solvent, in which ergosterol or cholesterol was dissolved during irradiation, had no influence upon the relative antirachitic effectiveness of the resultant vitamins D as determined with chicks.

6. Purified cholesterol, heated and then irradiated, produced a response in chicks more like that produced by irradiated crude cholesterol than irradiated ergosterol.

7. Absorption spectra are presented which show the presence of absorption bands in heated purified cholesterol.

BIBLIOGRAPHY

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