THE RELATIVE VALUE OF IRRADIATED YEAST AND IRRADIATED ERGOSTEROL IN THE PRODUCTION OF VITAMIN D MILK

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In 1924 Luce (1) demonstrated that the diet of the cows was the principal factor which influenced the antirachitic value of the milk. This observation was soon after confirmed by Golding, Soames and Zilva (2) who showed further that high doses of cod-liver oil reduced the percentage of fat in the milk. Later studies by Hart, Steenbock, Teut, and Humphrey (3) indicated that the vitamin D of cod-liver oil was poorly, if at all, absorbed from the intestinal tract of the cow.

The availability of substances made highly antirachitic by exposure to ultra-violet rays has made possible a further attack of the problem of increasing the antirachitic potency of cow’s milk. Thus Wachtel (4) and Steenbock and associates (5) have shown that the feeding of irradiated yeast caused a definite increase in antirachitic potency and a similar response was noted by Krauss, Bethke and Monroe (6) when irradiated ergosterol was employed.

In 1931 Thomas and MacLeod (7) in a preliminary report of a comparison of the efficiency of the two irradiated products, yeast and ergosterol, showed that 1.5 to 3 times as much irradiated ergosterol was necessary for the production of a milk of the same potency as that obtained when irradiated yeast was used. This investigation was continued and in a later report by Hess, Lewis, MacLeod and Thomas (8) it was concluded that irradiated yeast was 3 times as effective as irradiated ergosterol in the production of vitamin D milk. This difference in the efficiency of the two sources of the factor is quite striking when it is considered that isolated ergosterol is activated in one case and presumably it is the same substance which is activated in the yeast. A critical examination of the paper by

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Hess, Lewis, MacLeod and Thomas (8) revealed that the line test ratings used in estimating the potency of the butterfats were 3+ and 4+ grade. It has been our experience, as well as that of Bills and associates (9) and Dyer (10), that a considerable difference in supplement levels is required to cause a difference in rat responses of the 3+ and 4+ grade, but that when supplements are fed which produce a narrow continuous line, 1+ grade, the animal response is more sensitive to smaller differences in amounts of supplement and a more quantitative estimate of potency is possible. Hence it was considered that the type of response used by Hess and associates did not allow as quantitative an assay as was desirable and it was deemed advisable to determine again the relative efficiency of the two products in the production of vitamin D milk at various levels of feeding. Furthermore, such a study might reveal facts which would lead to a better understanding of the nature of the actions of these substances and, if a marked difference in efficiency actually existed, to methods of increasing the efficiency of the irradiated ergosterol. It was recognized that the factor developed in the yeast cell might be protected in some manner, possibly against oxidation, and therefore the use of hydroquinone, as an antioxidant, in some of the solutions of irradiated ergosterol was suggested. Consequently a series of experiments was conducted in which accurately assayed irradiated yeast and solutions of irradiated ergosterol, with and without hydroquinone, were fed to cows at various intervals, and the butterfat assayed for antirachitic potency.

THE DETERMINATION OF ANTIRACHITIC POTENCY

Young rats 24 to 28 days of age and weighing 55 to 60 grams were depleted of their stores of the factor on the Steenbock 2965 rachitogenic diet in 23 or 24 days. The amount of material to be tested, mixed with 50 grams of the rachitogenic diet, was consumed during the first 6 or 7 days of the test period. During the remainder of the 10 day test period the unsupplemented diet was fed. When it was not feasible to mix the supplement with the diet, the amount to be tested was fed separately in daily portions, during the first 6 or 7 days of the test period.

The degree of calcification was observed in the split radius and ulna essentially according to the Shipley technique (11). The following signs were used in expressing the extent of calcification:

No calcification in the metaphysis ........................................................... -
Calcification just beginning .............................................................................. -(±)
Narrow broken line of calcification ............................................................ ±
Better than ± but not + .................................................................................... ±(+)
Narrow continuous line of calcification ................................................ +
Better than + but not ++ .................................................................................... +(++)
Medium line of calcification .......................................................................... ++
Wide line of calcification or narrow epiphyseal cartilage ... +++
Very narrow epiphyseal cartilage or complete healing ...... ++++

When the responses of + grade, including one-half of those of ± (+) grade, constituted 60 per cent of the total number of responses one rat unit of the antirachitic factor was said to be present in the total amount of material fed. In order to determine accurately the unit amount, an attempt was made to feed a level to which the response was slightly less than that considered to be the unit amount and one to which it was slightly greater. When a unit response was not obtained with one of the levels fed, an interpolation was made between a level which gave too great a response and one to which the response was not sufficient.

ANTIRACHITIC POTENCY OF THE IRRADIATED YEAST AND IRRADIATED ERGOSTEROL

An amount of yeast sufficient for both series was reserved. The potency at the beginning of Series 1 and at the end of Series 2 was 542 units per gram. Several lots of irradiated ergosterol were prepared from a stock solution and assayed. The potency of the solutions in corn oil used in the feeding trials varied from 13,300 to 16,000 units per cc. These values were the result of a quantitative assay in which a number of levels of the products were fed. The levels differed from each other, near the unit level, by 10 to 15 per cent and therefore the potency was within 15 per cent of the actual value and was not a minimum value.

FEEDING PLAN

The experimental groups consisted of 4 cows each from the herd of the Walker-Gordon Laboratory Co., Inc., Plainsboro, New Jersey, used in the production of certified milk. The groups were constituted so that the individuals of each had essentially the same milk and butterfat production records as those of the other groups and were in practically the same stage of lactation. The breed distribution was 2 Holsteins, 1 Guernsey, and 1 Jersey in each group. The ration consisted of a grain mixture, beet pulp, silage, and alfalfa with which a milk of very low vitamin D potency was produced as demonstrated by the no supplement sample, Table 1. During the course of the experiment the cows were not permitted to come into contact with direct sunlight. The two irradiated products were incorporated in the grain mixture and an amount of the mixture fed daily which supplied the number of rat units indicated in Table 1 for each group.

COLLECTION OF SAMPLES

In the case of Series 1, after 6 weeks of the experimental régime, 3 samplings were made a week apart. The cows were milked 3 times in 24 hours and therefore a sample was composited of the cream from each milking
upon the basis of production and fat content, so that the resulting sample represented the fat content of the 24-hour sample. Butter was prepared from the cream of each of the 3 samples, taken at weekly intervals, and the water and curd removed by centrifuging the liquid butter. A composite sample of the butterfats of the three 24-hour samples was then made, the amount of each of the samples used being determined upon a fat production basis. After the completion of Series 1 a sample of butterfat was obtained from the general herd which did not receive an antirachitic supplement but which received the same ration as the cows of the experiment. This sample was considered representative of a negative control group. The samples of Series 2 were collected and prepared as just described for Series 1, after the supplements had been fed for 5 weeks.

RESULTS AND DISCUSSION

The butterfats, as well as the supplies of irradiated ergosterol and irradiated yeast were assayed in two laboratories, by two groups of workers, essentially by the method described above. Different breeding rations were used in the two colonies and in Laboratory A a slightly higher percentage of calcium carbonate was employed than is prescribed for the Steenbock 2965 diet. On several occasions check readings of the line tests of one laboratory by a worker from the other showed the same conception of value for the scorings listed above. At times Laboratory A supplied animals to Laboratory B and in a few instances those of the latter laboratory were used by the former but the line test readings did not reveal any marked difference between the animals of a laboratory and those brought into it. The results of one laboratory were not consistently higher than those of the other for the two series, but in Series 1 those of Laboratory B averaged about 25 per cent higher than those of Laboratory A, whereas in Series 2 the reverse was true.

In Series 1 a smaller number of test rats per level was used than in Series 2, because as soon as it became evident that the milks were considerably below 160 units per quart, which had been mentioned as a therapeutic standard (8), Series 2 was begun and the work of the first series abandoned. However the results in Series 1 have considerable quantitative significance because a series of feeding levels was used and it was possible to designate certain levels as meeting the requirements of a unit level with a level immediately below to which the response was less and one immediately above to which the response was greater. Furthermore if the rats of both laboratories are considered as a single group the results in most instances do not differ greatly from the average for the two laboratories as presented in Table I.

In the case of Series 2, the first assay was made in July, 1932. Laboratory A used at least 6 and usually 8 or more animals at and near the unit
levels whereas Laboratory B used only 4 to 6. In November, 1932 the latter laboratory used enough additional test animals to bring the total at the important levels to 10 except in one instance when only 6 animals were used. Although the number of animals used in July or in November in Laboratory B was not sufficient for a quantitative estimate, the responses in each set of tests were of the same order and it is justifiable to combine the two sets. For Series 1, 151 test animals were employed and for Series 2, 396. Limitations of space permit the presentation of only a summary of the unit levels.

As the first step in the reinvestigation of the comparative effectiveness of irradiated yeast and irradiated ergosterol the two sources of the factor were tested as the 60,000 unit level already reported to be effective in the case of yeast (8) and in the case of irradiated ergosterol at twice this level. On account of differences in interpretation of the results of the rat assay, it is not possible to make an accurate comparison of the results of this

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>SUMMARY OF DAILY SUPPLEMENTS TO THE COWS AND THE ANTIRACHITIC VALUE OF THE MILKS PRODUCED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DAILY SUPPLEMENT TO COWS</strong></td>
<td><strong>LABORATORY A</strong></td>
</tr>
<tr>
<td>Unit supplement</td>
<td>Test rats used at unit level</td>
</tr>
<tr>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td><strong>Series 1</strong></td>
<td></td>
</tr>
<tr>
<td>60,000 rat units irradiated yeast</td>
<td>1200</td>
</tr>
<tr>
<td>60,000 rat units irradiated ergosterol</td>
<td>1100</td>
</tr>
<tr>
<td>60,000 rat units irradiated ergosterol with hydroquinone</td>
<td>600</td>
</tr>
<tr>
<td>120,000 rat units irradiated ergosterol</td>
<td>800</td>
</tr>
<tr>
<td>No supplement</td>
<td>(Used 8 test animals)</td>
</tr>
<tr>
<td><strong>Series 2</strong></td>
<td></td>
</tr>
<tr>
<td>120,000 rat units irradiated ergosterol with hydroquinone</td>
<td>375</td>
</tr>
<tr>
<td>180,000 rat units irradiated ergosterol</td>
<td>275</td>
</tr>
<tr>
<td>180,000 rat units irradiated ergosterol with hydroquinone</td>
<td>275</td>
</tr>
<tr>
<td>180,000 rat units irradiated yeast</td>
<td>225</td>
</tr>
<tr>
<td>300,000 rat units irradiated ergosterol</td>
<td>275</td>
</tr>
</tbody>
</table>

* Refers to number of rats used on a level above and a level below that selected as a unit level.
investigation with those reported by other workers, but approximate comparisons can be made. As indicated in Table I the potencies of the butterfats produced when 60,000 units each of irradiated yeast and irradiated ergosterol were fed are essentially the same, and that of the yeast milk, 35 units per quart, less than that reported by Hess and associates (8) at the 60,000 unit level, namely 160 units per quart. It is possible that these workers used an irradiated yeast which was known to contain at least 60,000 units per daily portion yet its potency may have been actually much higher. At the 100,000 and 200,000 unit levels of irradiated ergosterol the potencies of the butterfats reported by these investigators are in only rough agreement with those obtained in this laboratory at the 120,000 and 180,000 unit levels. For the 180,000 unit level of irradiated ergosterol the results are of approximately the same order as those reported by Krauss, Bethke and Monroe (6) when 200,000 units were fed.

A graphic representation, Fig. 1, of the results displayed in Table I, shows the relationship between the daily supplement to the cow and the potency of the milk when the daily allowance of the irradiated substances was increased. Although only two points are available for the irradiated yeast curve, the slope of this curve as compared with that for irradiated
ergosterol shows that the potency of the milk increases more rapidly with an increase in the amount of irradiated yeast fed than in the case of an increased allowance of irradiated ergosterol. The direction of the curve for irradiated yeast feeding also shows that a maximum milk potency is not being approached. Furthermore some commercial vitamin D milks, produced by the feeding of irradiated yeast, when assayed in this laboratory have shown a potency of as much as 200 units per quart. The general trend of the curve for irradiated ergosterol is parabolic in character and indicates that a further increase in the number of units of this product fed to the cow would not be accompanied by a marked increase in the potency of the milk.

When Hess, Light, Frey, and Gross (12) fed 450,000 units of irradiated yeast per day the potency of the milk, calculated from their data, was 975 units per quart and when the daily supplement was 1,500,000 units of irradiated ergosterol the calculated potency of the milk was 1950 units per quart. These values are considerably higher than those which would be expected if the ratio of the largest number of units fed to the cow to the number produced per quart in the present experiment prevailed at the higher levels used by Hess and associates. This ratio would predict 390 units per quart in the case of irradiated yeast and 710 units for irradiated ergosterol if the amounts used by Hess and associates were fed. The cause of this lack of agreement is not known.

It was considered possible that the greater effectiveness ascribed to irradiated yeast by Hess and co-workers (8) might be due to the protective influence of some natural antioxidant in the yeast cell. In an attempt to simulate such a condition in the use of irradiated ergosterol, 0.75 per cent of hydroquinone was introduced into the oil solution. The use of this antioxidant has been studied by Huston and Hoppert (13). The feeding of this solution at the same levels as the irradiated sterol without an added antioxidant resulted in a milk somewhat more potent at the 60,000 and 120,000 unit levels but the difference was only slight at the 180,000 unit level. Since the general trend of the curves for irradiated ergosterol without hydroquinone and of the extrapolation of the curve for irradiated ergosterol with the antioxidant indicate that a maximum is being approached, it is possible that any antioxidant properties are less effective at higher levels because other limiting factors come into play. It is of interest, Fig. 1, that the efficiency of irradiated ergosterol with hydroquinone in the production of vitamin D milk is greater at the 60,000 unit level than either irradiated yeast or irradiated ergosterol alone. At higher levels it is also more effective than irradiated ergosterol alone but less effective than irradiated yeast.

Thomas and MacLeod (7) reported irradiated yeast to be 1.5 to 3 times as effective as irradiated ergosterol and Hess and co-workers (8) placed the
effectiveness at 3 times that of the irradiated sterol in the production of vitamin D milk. In the present investigation the use of 180,000 units of irradiated ergosterol produced a milk whose potency was slightly less than that produced when 180,000 units of irradiated yeast were fed, and the ratio of the effectiveness of irradiated yeast to that of the irradiated sterol is about 1.25 : 1.0. Essentially the same response was obtained with 180,000 units of yeast as with 300,000 units of the sterol and the ratio of the effectiveness in this case is of the order of 2 : 1.

Upon the basis of an average daily production per cow of 16 quarts of milk, when 180,000 units of the antirachitic factor were consumed in the form of irradiated yeast 1.7 per cent of the factor appeared in the milk; when 300,000 rat units of irradiated ergosterol were consumed 0.7 per cent of the factor was found in the milk. For none of the other feeding levels studied was the transfer to the milk greater than 1.7 per cent.

**SUMMARY**

When 60,000 units were fed per cow per day, the efficiency of irradiated ergosterol in the production of vitamin D milk is approximately the same as that of irradiated yeast. The potency of the milk was 35 to 40 units per quart. At higher levels, 180,000 units per cow per day, irradiated yeast is the more efficient product, the potency of the yeast milk being of the order of 150 to 160 rat units per quart and that of the irradiated ergosterol milk 120 to 130 units. A further increase in the daily allowance of irradiated ergosterol from 180,000 to 300,000 units caused only a slight increase in the potency of the milk.

The addition of hydroquinone increases the effectiveness of irradiated ergosterol, but to a greater extent at the lower than at the higher levels of feeding.

In the case of both products less than 2.0 per cent of the units of the antirachitic factor ingested appeared in the milk.

**BIBLIOGRAPHY**

(1) **Luce, E. M.** The influence of diet and sunlight upon the growth-promoting and antirachitic properties of the milk afforded by a cow. Biochem. J. 18: 716. 1924.


(4) **Wachtel, M.** The increase in volume and vitamin content of human and cow milk by means of irradiated yeast. Münch. med. Wochenschr. 76: 1513. 1929.


(7) **Thomas, B. H., and MacLeod, Florence L.** Increasing the vitamin D potency of cow's milk by the daily feeding of irradiated yeast or irradiated ergosterol. *Sci.* 73: 618. 1931.


