A NEW VITAMIN D IN COD LIVER OIL

BY CHARLES E. BILLS, O. N. MASSENGALE, K. C. D. HICKMAN, AND E. LEB. GRAY

(From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana, and the Research Laboratory, Eastman Kodak Company, Rochester, New York)

(Received for publication, July 12, 1938)

In previous studies (1) it was shown that the liver oils of different species of fish are unequally effective, per rat unit, for the prevention of rickets in chickens. The experiments indicated the existence of more than one kind of vitamin D in the oils. More recently it was found (2, 3) that commercial cod liver oil, when subjected to molecular distillation, gives physical evidence of containing two principal, and two minor vitamins D, with traces of still two more forms.

The present report covers the bioassay of the most volatile vitamin D of cod liver oil. This is one of the minor forms. It was separated (2, 3) in the Eastman laboratory as the first fraction of the first fraction of the vitamin D-bearing distillate obtained from 2 tons of commercial Norwegian cod liver oil. The bioassay techniques for rats and for chickens, with procedures for estimating errors, are also elsewhere described (4, 5). The rat assay technique is in a somewhat improved form, and includes the use of a reference oil such as is used in the chicken technique.

The cod liver oil was assayed against the U.S.P. reference cod liver oil. 50 rats were used in the final assembly, twenty-five on the test oil and twenty-five on the reference, other rats used in exploratory trial assays not being counted. The oil contained 270 international units of vitamin D per gm. Since the liver oil of the common codfish, Gadus morrhua, contains on the average 100 international units per gm., and rarely more than 150 international units, it is reasonable to suppose that this inordinately potent oil represented some codfishes other than Gadus morrhua.
242 New Vitamin D in Cod Liver Oil

It may be noted that oils from the allied species of Gadus are legally cod liver oil, and in fact are typical of the better medicinal grades of this product. The distillate was assayed similarly, with twenty-three pairs of rats. It contained 2666 international units of vitamin D per gm. Thus it represented a potency concentration of about 10 times, though it was still remote from a pure vitamin D.

The assays with chickens were carried out in two series, which are summarized in Table I. For each test group in each series,
ten chickens were used, and each test group was assayed twice, for better accuracy. Also given in Table I, are the theoretical values, taken from the master curves in the published technique (5), which illustrate the ideal, or expected, femur ash development in response to doses of pure cod liver oil of the same size as the doses of the preparations investigated.

In Series 1 the reference oil gave a response which was somewhat below par, but not significantly so. The commercial oil gave a still lower response, but here again the difference was not significant, if one accepts as the criterion of significance the limit of 3 times the probable error. The low boiling vitamin D, however, failed to elicit femur ash response, although it was administered at a level at which good response was expected.

In Series 2, the reference oil gave a response close to normal. 16 units of low boiling vitamin D had an efficacy ratio of about half normal, and 60 units about one-quarter normal. These are significant differences. The fact that the efficacy ratios at 16 units and 60 units were not the same merely indicates, as has been pointed out before in another connection (5), that the response to the vitamins involved is dissimilar. It is additional evidence that the low boiling distilled vitamin is different from the bulk of the vitamin in the original oil.

In the paper (3) in which the distillation of the several vitamins D from cod liver oil was described, the point was made that the lowest boiling form may differ from the familiar forms in the complete absence of the side chain on the cholane nucleus. This supposition is based only on boiling point analogies under the conditions of molecular distillation, but if it is true, the form of vitamin D investigated in the present work would be the simplest possible one, and in a sense, the prototype of all the others.

SUMMARY

The most volatile of several vitamins D which occur in cod liver oil was assayed with rats and chickens. Per rat unit, it was from one-half to one-fourth as effective for chickens as the total vitamin D of the oil. The assays substantiate the evidence, previously obtained from molecular distillations, that cod liver oil contains several vitamins D.
BIBLIOGRAPHY

A NEW VITAMIN D IN COD LIVER OIL
Charles E. Bills, O. N. Massengale, K. C. D. Hickman and E. LeB Gray

J. Biol. Chem. 1938, 126:241-244.