U. S. P. BIOLOGICAL ASSAY OF COD LIVER OIL, PRESENT AND FUTURE.*

BY JOHN F. ANDERSON, M.D.

The Revision Committee of the United States Pharmacopœia has been in advance of those of other countries as regards the biological assay of various preparations. U. S. P. IX provided for the first time optional methods for the biological assay of certain potent preparations such as Ergot, Digitalis, Strophanthus, etc. These optional methods were extended and made obligatory in U. S. P. X. For the first time in any Phamacopœia there was included in U. S. P. X an optional method for the biological assay of one of the potent principles of Cod Liver Oil. A special committee of those interested in the biological assay of Cod Liver Oil was appointed by the Revision Committee to make a report with recommendations as to the biological assay of Cod Liver Oil for U. S. P. X.

This Committee made a report in which the biological assay of Cod Liver Oil for its vitamin A content was recommended and an assay method for this purpose was given. The report in part stated that "Cod Liver Oil may be assayed for its vitamin A potency and should then contain at least 50 units per Gm. Cod Liver Oil so assayed must be labeled 'This unit is not a measure of the antirachitic activity of Cod Liver Oil.'" While this statement appears to be perfectly plain nevertheless a portion of it has been misinterpreted by many.

It is to be noted first of all that the assay of Cod Liver Oil by U. S. P. X method is optional for the words are "may be assayed for its vitamin A potency." It is also to be noted that Cod Liver Oil when so assayed should contain at least 50 units per Gm. This is not to be interpreted as establishing 50 units per Gm. as the standard for Cod Liver Oil but only as defining the lowest value which Cod Liver Oil can have in order that it may be labeled as having been assayed by U. S. P. X method. Lastly, it will be seen that the U. S. P. X method of assay does not provide for the assay of the antirachitic or vitamin D content of the oil.

It was not long before it was found that certain modifications were necessary in the U. S. P. X method of assay for vitamin A and by unofficial agreement these necessary modifications have been made in the assay method. The most important of these modifications has to do with the necessity of including in the vitamin deficient diet an adequate amount of vitamin D. Criticisms have also been advanced against the method in that it establishes as an end-point the curing of induced symptoms of vitamin A starvation in young albino rats and a specified gain in weight during the period of the test rather than the cure of xerophthalmia. However, it is believed that the majority feel that although the cure of advanced xerophthalmia may be qualitatively more convincing it is at the same time quantitatively more indefinite and gives more irregular results as a criterion of activity than does growth recovery. Also, it is a matter of common experience that larger daily doses of Cod Liver Oil are required to cure definite symptoms of xerophthalmia than are required to induce growth recovery for a period of 35 days.

For the past several years a committee from the American Drug Manufacturers Association has been doing coöperative experimental work upon the

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biological assay of Cod Liver Oil. This committee has not yet rendered a final report and their results cannot be given. It will suffice to say, however, that it is believed that the report from this committee will support the use of the criteria of growth recovery with definite specifications regarding vitamin A depletion at the beginning of the test period rather than the cure only of advanced xerophthalmia as the distinctive end-point. Several modifications have also been necessary in regard to the vitamin A free diet, the diet for the colony and the diet for the mother of the test animals.

The general purpose of these modifications is to obtain a vigorous stock; to accomplish the vitamin A depletion of the test rats in gradual stages (avoiding drastic changes of diet); and to obtain rapid growth during the vitamin A free period.

One of the features of the present U. S. P. X method which requires correction is the possibility that other factors in addition to deficiency of vitamin A may influence weight decline during the vitamin A free period as well as weight recovery during the period of feeding Cod Liver Oil. I have already mentioned vitamin D in this connection and also have in mind the physical character of the ration, vitamin B complex, quantity and quality of protein consumed and possibly other factors which influence food consumption.

U. S. P. X contains no method for vitamin D assay. In the last few years, however, there has been general recognition of the importance of vitamin D and consequent appreciation of the equal necessity for U. S. P. methods for the assay of D as well as for A.

The Wisconsin Alumni Research Foundation which administers the Steenbock patent for the irradiation of ergosterol has established a standard method for the assay of irradiated ergosterol. This method provides, in general, for the use as a rickets-producing diet, of Steenbock's No. 2965 or McCollum's No. 3143 and a comparison with definite amounts of Cod Liver Oil of which 75 mg. fed over a period of about 6 days (depending on the food consumption of the individual rats), with examination on the tenth day, is the curative dose.

The rickets-producing effect of these diets depends upon their low phosphorous and high calcium content and it would appear that better quantitative consistency in results might be obtained in a pharmacopœial method if certain limitations were placed upon the phosphorous content of the diet and the exact ratio of calcium to phosphorus were specified. Such specifications would require an analysis of the diet and an adjustment of the addition of calcium carbonate according to the results of this analysis.

It will at once be apparent that whereas the unit for vitamin A is based upon the daily dose, the unit for vitamin D, according to the Wisconsin Alumni Research Foundation, is based upon the total dose. The result is that there is a very great apparent discrepancy in the number of vitamin A and vitamin D units in the same Cod Liver Oil. This apparent discrepancy can be very closely reconciled if the unit for vitamin A is also based upon the total dose rather than upon the daily dose. For example, an oil having 500 present U. S. P. units per Gm. would have a minimum total dose corresponding to approximately 14 units per Gm., and according to the Wisconsin Alumni Research Foundation standard would have 13.3 units per Gm. of vitamin D.

In this connection it may be pointed out that if both units are calculated from a minimum effective total dose that the very convenient value of 10 units per Gm. would be a reasonable minimum standard of activity for both vitamins in Cod Liver Oil. Such a figure for the minimum standard would have the further advantage in that assay results could be directly converted into per cent of U. S. P. minimum standard by simply multiplying the number of units per Gm. by the figure 10, for example, if a sample of Cod Liver Oil assayed 13.3 units of vitamin D and 15 units of vitamin A per Gm. it would have a vitamin D content of 133% and a vitamin A content of 150% U. S. P. minimum standard.

A very practical and significant question arises in connection with the quantitative interpretation of the assay data in both the vitamin A and vitamin D assays. That is—shall the deviation of response which is a biological occurrence common to any bio-assay be made use of in the evaluation of the results or shall it be ignored. This fact of deviation is made use of in the standard method for the assay of irradiated ergosterol in that the method specifies the unit dose in the terms of a definite percentage of positive responses—*i. e.*, 60%. The quantitative importance of also including such a specification in the vitamin A assay cannot be too strongly emphasized.

It is of the highest importance that the required methods for the assay of Cod Liver Oil both for vitamin A and vitamin D be provided in U. S. P. XI. This is of especial importance in view of the great variation in the labeled statement of many brands of Cod Liver Oil, due to the fact that the various firms distributing such oil have established different standards for vitamin potency. It would also seem desirable that the U. S. P. method require a uniform method of labeling, preferably in terms of the number of units per one Gm. of oil and should not permit a labeled statement as to the number of units per ounce, multiple of a gram or any fraction of the permitted figure. Such variations in the labeled statement result in confusion not only to the consumer, but also to the druggist and physician.

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THE RELAXANT ACTION OF BENZYL DERIVATIVES.*

BY LEWIS C. BRITT AND E. V. LYNN.

We are all familiar with the facts surrounding the introduction of benzyl benzoate as a relaxant of smooth muscle. On the basis of extensive experiments on animals and of some clinical data, Macht (1) in 1918 proposed the use of this compound and other benzyl derivatives as remedies in any condition of heightened tonus, such as uterine colic, vesical spasm, angiospastic conditions, spastic constipation, hiccup, etc. His work was apparently substantiated in practical therapeutics by a number of other observers (2), but the evidence therefor was obtained largely without controls and, hence, liable to considerable misinterpretation. The end-result, however, was an almost immediate adoption of the drugs by a considerable share of the medical profession. If many of the early written reports could be taken literally, benzyl benzoate and its relatives might well be ranked as panaceas for any disorder of smooth muscle due to spasm or increased tonicity.

* Scientific Section, A. PH. A., Baltimore meeting, 1930.