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Twenty-sixth Report

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Geneva, 26 November – 2 December 1974

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WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-sixth Report

The WHO Expert Committee on Biological Standardization met in Geneva from 26 November to 2 December 1974. Dr A. S. Pavlov, Assistant Director-General, opened the Meeting on behalf of the Director-General. He welcomed the participants, including the Representative of the International Atomic Energy Agency, and thanked them for coming to Geneva for this Meeting. He drew attention to the long history of biological standardization and pointed out that this meeting was concerned exclusively with hormones. He was sure the report of the Committee would be useful in the standardization and control of hormones of importance in human health and in the improvement of hormone assays.

GENERAL

The Committee discussed policies concerning criteria for including or excluding apparently aberrant data (outlying results or outliers) obtained from collaborative assays, in the calculation of combined potency estimates on the basis of which an international unit was defined for international standards for various hormones. It was pointed out that such outliers among assay results were discussed with the participant responsible and excluded only when there were clear technical grounds for doing so. If there was no reasonable explanation, however, weighted and unweighted mean potencies were calculated in the same way for all estimates and recorded in the report of the collaborative assay. Thus heterogeneity of variance has not necessarily been a basis for excluding assay results. Furthermore in the overall combination of results, analysis of variance is made on both weighted and unweighted potency estimates.

The Committee agreed that it is desirable to include tests for homogeneity of variance in statistical analyses of bioassay results and accepted the current practice of not excluding results on the basis of heterogeneity of variance. An exception is made only for results of those methods that clearly did not measure the same property of the hormone, e.g., biological and immunological activities. Such distinctions are clear from the name of the international standard or international reference preparation when it is established (i.e., "for immunoassay" or "for bioassay").

The Committee was of the opinion that inclusion of all such assay results has introduced only minimal differences in potencies assigned to international standards or international reference preparations in collaborative studies in which a large number of assays by different methods are generally performed; as a result, the 95% fiducial limits of the combined potency estimates are narrow.

Since both weighted and unweighted means derived from all data are recorded in the reports of collaborative studies, users of international standards and reference preparations should take these facts into consideration in the calibration of national and other standards by their own assay methods.

PART I. INTERNATIONAL STANDARDS AND INTERNATIONAL REFERENCE PREPARATIONS

1. Human Urinary Gonadotrophins

The Committee noted [1] the results of the collaborative assay referred to in its twenty-third report [2] of the material intended for the replacement of the second International Reference Preparation of Human Menopausal Gonadotrophins (FSH and ICSH), Urinary, for Bioassay (2nd IRP).

Since preparations of human urinary menopausal gonadotrophins are administered to man in many countries, it was desirable to have an international standard for the control of potency of such preparations. The collaborative assay showed that the preparation studied was suitable for the bioassay of both urinary FSH and urinary LH. The Committee therefore established this preparation as the International Standard for Human Urinary FSH and for Human Urinary LH(ICSH), for Bioassay, in replacement of the second International Reference Preparation of Human Menopausal Gonadotrophins (FSH and ICSH), Urinary, for Bioassay.

The Committee also noted [1] a proposal for defining the international units on the basis of the results from the collaborative bioassay which would maintain continuity of the units of the preparation being replaced. It was informed that this was acceptable to the participants in the collaborative assay. On this basis, the Committee defined the International Unit for Human Urinary FSH, for Bioassay, as the activity contained in 0.11388 mg and the International Unit for Human Urinary LH(ICSH), for Bioassay, as the activity contained in 0.13369 mg of the International Standard.

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[1] Unpublished working document WHO/BS/74.1080.

[2] WHO Technical Report Series, No. 463, 1971, p. 14.

For practical purposes each ampoule can be used as containing 54 IU of FSH activity and 46 IU of LH(ICSH) activity. It is to be noted that the preparation it replaced (2nd IRP) contained, by definition, 40 IU of FSH and 40 IU of LH per ampoule. The Committee stressed that, as for this new standard, future standards and preparations calibrated against it should have their separate activities (FSH and LH(ICSH)) individually assessed.

The Committee noted that among the various bioassay methods employed, some heterogeneity of results was obtained by two laboratories using the ovarian ascorbic acid depletion assay method for LH(ICSH) and using the 2nd IRP as a standard. This may have been due to certain toxic constituents in the 2nd IRP, which are known to have such an effect on intravenous injection.

The preparation established was made from material processed in three batches, each of approximately 3500 ampoules, of which one was intended to serve as the replacement of the international reference preparation while the other two were to be distributed internationally as working material. Although all three batches had been processed under identical conditions, the results of accelerated degradation studies showed certain small differences in their stability. The reasons for these differences have not been resolved and the Committee therefore considered that continued stability studies should be made.

The Committee was informed that there were still available some 500 ampoules of the 2nd IRP now replaced. It noted that immunoassays of urinary FSH and LH(ICSH) are of clinical value; and that the IRP has been widely used as a working standard for these assays. The Committee therefore recommended that the remaining stocks should continue to be made available for this purpose, labelled as working material and not as the 2nd IRP.

The Committee further requested the National Institute for Biological Standards and Control, London, to obtain more highly purified preparations of the individual urinary gonadotrophins and arrange collaborative studies of their suitability to serve as reference material for the appropriate immunoassays.

2. Insulin

The Committee noted [1] the results of stability studies on the fourth International Standard for Insulin, Bovine and Porcine, for Bioassay, which were requested in its twelfth report [2] because the material was sealed

[1] Unpublished working document WHO/BS/74.1083.

[2] WHO Technical Report Series, No. 172, 1959, p. 10.

in ampoules in air and had some 6% average moisture content. This procedure differed from that customarily followed for the preparation of international standards (drying to less than 1% moisture and sealing under dry nitrogen). These studies had been made over the last 14 years and an interim report [1] was made in 1963.

The results showed that the preparation, which consists of insulin in a crystalline form, retained 95.8% of the original biological activity after 12 years storage in the dark at 20°C and 65.7% after 14 years in the dark at 37°C. Estimates derived from these data indicate that the preparation stored at −20°C for 20 years would have retained at least 99.93% ($P = 0.95$) of its original activity. The Committee was informed of similar studies in the State Research Institute of Standardization and Control of Drugs, Moscow, the results of which were in substantial agreement with the above. The present international standard could be expected to serve as a satisfactory material for many years if the present rate of distribution is maintained.

The Committee was informed that this batch of highly stable crystalline insulin had been prepared with particular attention to the removal of contaminating proteolytic enzymes. Comparable stability should not be assumed for all crystalline insulin preparations that are intended to serve as national standards.

3. Calcitonins

The Committee noted [2] the information, collected in response to the request in its nineteenth report,[3] on the nature and usage of calcitonin preparations in man. In therapy, preparations of porcine and salmon calcitonins are used in the treatment of Paget's disease and may also be used in acute hypercalcaemia.

The two calcitonins differ in their primary chemical structure, and comparisons of their activities in various bioassays have given widely differing estimates of relative potency. This is particularly relevant to the dosages used therapeutically. An international reference preparation for each calcitonin was therefore required for the assay of the respective hormones.

The Committee also noted [2] that preparations of these calcitonins had been obtained and studied. A stable preparation of porcine calcitonin was available and the Committee agreed that this was suitable to serve as

[1] Unpublished working document WHO/BS/631.
[2] Unpublished working document WHO/BS/74.1077.
[3] WHO Technical Report Series, No. 361, 1967, p. 15.