BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 3 1942

WARTIME ARRANGEMENTS FOR INTERNATIONAL BIOLOGICAL STANDARDS AND THE NEW STANDARD FOR PITUITARY (POSTERIOR LOBE) PREPARATIONS

BY

Sir HENRY H. DALE, C.B.E., M.D., P.R.S.

Director of the National Institute for Medical Research, Hampstead

The International Standards for remedies of which the strength has to be measured by biological methods came into being between the two world wars, through action by the Health Organization of the League of Nations. The work was by no means limited, however, to the nations that had joined the League. Important shares in the enterprise, which began in 1921 on Dr. Madsen's initiative, were taken at the outset by experts from the countries that had not yet been admitted, and during the whole period by those from the United States of America. Even when Germany and her associates later withdrew from the League the international standards remained in use among their men of science, though without official recognition, and German industry continued to make important contributions to the materials required for the preparation of new standards.

There could be no doubt that these international standards were worth preserving for their value to research as well as to practical therapeutics, and it was clearly imperative that war should not endanger the existence of materials which embodied so much work of investigation, freely given to this common enterprise by leading experts of many nationalities. Steps were, in fact, very promptly taken by Dr. Hartley, Director of the Department of Biological Standards at the National Institute for Medical Research, to see that adequate quantities of the international standards held in that department were sent to the control centres of other countries, to meet any probable interruption of normal distribution; while the main stocks were so distributed as to minimize the risk of their destruction. When Denmark was invaded, and the further supply of the international serum standards from Copenhagen to many other countries became doubtful, the identical or exactly equivalent British serum standards were made available during the emergency, at the request of the League of Nations, wherever the need should arise. In addition to making arrangements thus to keep alive the mechanism of distribution in most parts of the world, even during the war, Dr. Hartley also deposited several complete sets of all the international standards (or, in a few cases, their exact equivalents) with institutions having the requisite facilities for ideal storage, in countries widely distant from one another. With these are deposited full technical records of the methods of preparing and using them. It can be regarded as practically certain, therefore, that when the war comes to an end these standards will be available from some source to any organization which can suitably assume responsibility for the matter in the interest of the whole world.

Meanwhile, apart from the special arrangements thus made for the standards which already existed, it was necessary to provide for the completion of work on the materials for certain new ones which were under investigation in Sept., 1939. In one such case the material was on international trial for its suitability to create an entirely new standard for vitamin E; and in due course, after agreement among as many of those concerned with the matter as could still be reached by correspondence, the international standard for vitamin E was adopted and made available (*British Medical Journal*, 1941, 2, 553). In another case the trials, and the subsequent agreement by correspondence, were concerned with the replacement of an existing standard, the supply of which was nearing exhaustion. This was the international standard for preparations of the posterior lobe of the pituitary gland.

International Standard for Preparations of Pituitary Posterior Lobe

The history of this standard goes back to the first International Conference, held in Edinburgh in 1923, to consider the possibility of international agreement on the biological standardization of certain hormones and drugs. Among the pharmacological experts from different countries who were its members, there were few who then recognized the general importance of the principles on which Paul Ehrlich had firmly based the standardization of the antitoxic sera some thirty years earlier—the creation of a dry stable standard of reference, the definition of the unit as the activity of an exact weight of this material, and the use of samples of this central standard as a currency, enabling the weight representing the unit to be determined by comparison for any other subsidiary standard made for national or local use. In the case of the pituitary posterior lobe, indeed, Professor Carl Voegtlin, of the Public Health Laboratory in Washington, put forward in 1923, at the Edinburgh Conference, a sample of the dried acetone-extracted gland substance for consideration and co-operative trial, with a view to its adoption as a standard of a kind. The trials were successfully carried out, and the second Conference summoned to deal with such matters, which met in Geneva in 1925, readily agreed to adopt Professor Voegtlin's proposal. At a relatively late stage of its discussion this Conference also made the important decision to define a unit of activity for post-pituitary preparations as the activity of 0.5 mg. of the dried material, which it had already agreed to recognize as a standard. The full record of its recommendations, however, makes it clear that it did not regard this definition as applicable only to a particular quantity of a standard material, kept at and distributed from a recognized centre. On the contrary, the evidence presented led its members to assume that any sample of this

material, if prepared in accordance with the prescribed method from the perfectly fresh substance of ox pituitary glands, would have exactly the same activity; so that 0.5 mg. of any such material, wherever it was prepared, would represent 1 unit of activity. And although Professor Voegtlin was asked by the Conference to make available a small quantity of the material prepared in his own laboratory, this was to be furnished "to any authority which may require it for confirmation of its own national standard." In other words, the object was clearly to enable any national centre which had not confidence in the freshness of its raw material, or in the perfection with which it had followed the prescribed routine of preparation, to assure itself that it had in fact produced a standard of the expected optimal value. There was no suggestion that it would be proper to define the unit as corresponding to some greater or smaller weight than 0.5 mg. of such a secondary national standard if it should be found in a comparative test to be weaker or stronger than the sample provided by Professor Voegtlin's laboratory.

Experience in the administration of the international standards soon showed, however, that the basis which the Geneva Conference of 1925 had regarded as suitable for this particular case was not in fact practically applicable. A British national standard for the pituitary posterior lobe had been prepared in 1926, and had been found, indeed, by tests carried out both in London and in Washington, to be indistinguishable in activity from Professor Voegtlin's original standard preparation. Fortunately this British standard, through the co-operation given by the Armour Laboratories of Chicago in providing the material for it, had been prepared on a scale large enough to enable it to be used for a wider need than that of the national control. Applications received from all over the world soon showed that, whatever had been the intentions of the Geneva Conference, the demand for samples of an international standard for this endocrine preparation would be comparable to that for others, such as the insulin standard, for which a general distribution had been arranged. The material furnished by Professor Voegtlin being in these circumstances rapidly exhausted, it was possible to meet the situation by supplying samples of the British national standard, with a suitable guarantee of its strict equivalence to Professor Voegtlin's original sample. During this intermediate period national standard preparations were made in a number of countries and compared with the British standard. None of these other preparations was found to be more active than this, but there were a few of which the activity was perceptibly lower.

Another point of some importance has come clearly into view since the decisions made in 1925. At that date the question whether the extract of the pituitary posterior lobe owed its activities to one or to several active principles was still a matter of controversy; and the Geneva Conference had accordingly been able only to decide that the unit of activity should be regarded as contained in 0.5 mg. of the standard preparation, whether the test used for its comparative determination was based on the pressor or on the oxytocic action. By the time another Conference met in Frankfurt, in 1928, the question had been settled, as regards the artificial extract in common use, by the demonstrated possibility of making separate pressor and oxytocic extracts, each practically free from the other type of activity. It was possible, therefore, to amend the original definition in detail, and to recommend that "the activity of every such preparation should be expressed in relation to the international standard in units of pressor or oxytocic activity, and that in each case *the unit of such activity* shall be that of 0.5 mg. of the standard powder." There was no further step, however, towards the recognition of a particular preparation as the standard in question; it was still possible, by implication, to make the standard anywhere by following the prescribed routine.

By Oct., 1935, the experience which had accrued enabled the League's Permanent Commission on Biological Standardization to take the further and important step of formally accepting as the international standard preparation the British standard for the pituitary posterior lobe, which had by then been semi-officially current for international purposes for some 9 years. Since the decisions made in 1928 there had been more discussion of the possibility that the third important therapeutic application of this extract, for its antidiuretic action, might

be due to yet another separable principle. This could not then (and cannot even now) be regarded as finally settled; so that the Commission at its meeting in 1935 could only provide for any possible development by authorizing the definition of a unit of antidiuretic activity, again as the activity of that type in 0.5 mg. of what was now the international standard. Unfortunately all these decisions adopting and defining the international standard and units were made, not by a conference of specialist experts, but by a Permanent Commission consisting of national representatives who left standing the implication of the earlier recommendations, according to which the standard would still serve to indicate an optimal activity rather than to fix the value of the unit and make it unalterable in either direction.

New (1940) Standard for Pituitary Posterior Lobe

It might well be suggested that such differences of conception as to the meaning and use of a standard would in this case be of theoretical rather than practical interest, since other standards had been made which were equal in potency to that being then adopted for international use, some which were weaker, but none thus far which were perceptibly stronger. And the question did not acquire practical importance until, in 1939, it became necessary to prepare a new supply of international standard material to replace in due course the one which had served for so many years in effect, and since 1935 with official recognition. With the renewed and effective co-operation of the Armour Laboratories a further quantity of the acetone-extracted and dried powder of the fresh posterior lobe substance was obtained, and was further and finally desiccated under the conditions which have now become a standard routine in the Hampstead Institute. Samples of this were distributed to expert collaborators in a number of different countries, for assay with the greatest practicable precision in comparison with the still current standard. Reports were eventually obtained from 13 different laboratories, of which 5 were in the U.S.A., 1 each in France, Holland, and Hungary, and 5 in Britain. In all, 90 determinations of the oxytocic activity of the new standard were reported from 12 of these laboratories and 38 determinations of the pressor activity from 9 laboratories; while 7 laboratories reported determinations of the antidiuretic activity involving the use of a total of more than 600 rats.

It was then at once obvious that, with very few exceptions, the results indicated a small but significantly greater activity for the new than for the current standard with respect to all the three types of activity for which the two had been compared. Two of the co-operating laboratories failed, indeed, to detect any difference in the oxytocic activities, and reported that the two standards appeared to be equivalent. All the others, however, found the new standard stronger in this respect, the highest oxytocic activity reported for it being 125% of that of the current standard. The weighted mean of the whole series of assays for oxytocic activity gave it a value of 111%. The values reported for its pressor activity varied, again, from 100 to 130%, and the weighted mean of all the determinations gave it a pressor activity of 113%. Of the 7 reports on its antidiuretic activity, 6 assigned it values between 107.5 and 122%, with a weighted mean of 116%. The seventh was quite aberrant from the others, giving a value of only 73%. It may be noted, however, that the inclusion of this aberrant finding in the calculation of the average would reduce the weighted mean of the antidiuretic values obtained for the new standard to only 112%, bringing it, in fact, into even closer correspondence with those obtained for the new standard's oxytocic and pressor activities.

By the time these results had been assembled and made available for analysis the outbreak of war had rendered it necessary to postpone, for an indefinite period, any project of an International Conference for personal discussion of their significance and of the appropriate action to be taken on them. The replacement of the current standard, however, before it had been exhausted in distribution, was becoming a matter of urgency, and it was accordingly necessary to arrive at an international agreement by correspondence. There could be no doubt that the new material was suitable for adoption as a standard, and it was further clear that the three important types of activity were represented in it in practically the same

proportions to one another as in the current standard; so that the units of oxytocic, pressor, and antidiuretic activity could still be defined as the activities of one weight of the new standard. The only question to be discussed and decided was the weight of the new standard to be recognized as representing the unit in each case: should it remain at 0.5 mg., or should it be slightly diminished, in inverse proportion to the greater activity of the new standard? The following points required consideration in arriving at an agreed decision.

Points requiring Consideration

1. The decision to regard the standard material as giving to the unit an optimal rather than a fixed and arbitrary value must be considered still effective unless a new international agreement was made, for sufficient reason, to change it.

2. There were several features in the definition of the standard material which might be expected to give scope for variation in its activity. The limited experience available at the time of its adoption had not properly justified the assumption that every sample of material so defined would have precisely the same activity, wherever prepared. The material was to be the "fresh" substance of the "posterior lobes" of oxen, extracted with acetone and dried. Even if we allow that the last two procedures have end-points in complete extraction and desiccation, the interpretation given by different workers to the expressions "fresh" and "posterior lobe" could not safely be assumed to be precisely uniform. The activity of the material has, in fact, been observed to fall rapidly with increase of the interval between slaughter and the final stabilization of the dissected substance by acetone or rapid drying. Freshness in this sense, and consequently activity, may obviously vary with the efficiency of organization at the source. This might well improve during the 13 years between the preparation of the two standard materials, and any such improvement might further be accentuated by an increased use of refrigerants even during a quick transfer from abattoir to laboratory. Improved standards of dissection, again, might well give a more severe discrimination between the material to be retained as unquestionably "posterior lobe" substance and that to be rejected as more properly belonging to the stalk; while even small tags of completely inactive dura mater might easily have been included in some preparations of the material by hasty or imperfectly trained dissectors. In these different directions greater experience and perfection of procedure might easily account for the (average) additional 12% of activity shown by the new standard material.

3. The whole basis of biological standardization in units defined by fixed weights of standard preparations, since Ehrlich introduced this method for diphtheria antitoxin, has depended on the assumption that the standard itself has a practically indefinite stability. In the case of an antitoxic serum this is inevitable, since there is no independent basis of comparison and no natural upper limit to the activity of such a standard. All experience, moreover, has shown that such sera, when dried, have a high stability even in air at ordinary temperatures; so that there is no reason to suspect that a serum standard, when kept in perfectly dry nitrogen below 0° C., will suffer any appreciable loss of activity before it is due to be replaced by a fresh one.

In the case of the standard for pituitary posterior lobe the conditions are not identical with those for the sera. Even when the drying of the gland substance has been completed a measurable loss of activity occurs, sometimes in less than a year, if the dry product is kept without special precautions in air at ordinary room temperature. This would not justify an assumption that when sealed in perfectly dry nitrogen and kept at a temperature below 0° C. it would be subject to a significant loss of activity, even in many years; and the fact that none of the subsidiary standards made during its currency had proved to be stronger, while more than one had been somewhat weaker, was against the supposition that the current standard had suffered an important loss of activity during the 13 years since its preparation. Nevertheless it had to be recognized that a loss of activity of the order of 10% would be extremely difficult to detect in the absence of an independent and certainly stable basis of comparison, and that the presumption of perfect stability for this standard substance was not so overwhelmingly strong as in the case of the serum standards.

4. From the scientific point of view the maintenance of any unit of measurement without change has great importance. From the point of view of practical application, the avoidance of any possibility of a decline in the value of the unit is more important for remedies such as the pituitary posterior lobe preparations than for an antitoxic serum. With diphtheria antitoxin, for example, the effect of a small decline in the

value of the unit over a decade or so—there is no reason whatever for suspecting its occurrence—would have been completely swamped in practice by the great increase, probably tenfold, in the customary dosage in units made possible in the same period by improvements and refinements of manufacture. Pituitary posterior lobe extract, on the other hand, cannot be given with a large margin of excess units to cover any possibility of a decline in unit value, since over-dosage may be as dangerous from excess of action as under-dosage from lack of effect in an emergency. In that respect its position is comparable to that of insulin.

5. Adoption of the same weight of the new standard as of the old—0.5 mg.—to represent the unit would involve an increase of the unit by not more than 15% of its *present* value. If the activity of the old standard had been constant for 13 years there would be this increase in the value of the unit as originally accepted, but it would be inappreciable in practice. It is to be noted, indeed, that several of the participants failed to detect it even in the laboratory assays made with the special care appropriate to the fixation of the unit value of a new standard. There was no risk, in any case, that such a fractional increase in value would be repeated indefinitely at successive replacements of the standard, since the complete preservation of the activity present in the perfectly fresh gland substance, as contemplated in the original definition, sets a natural upper limit; it is probable, indeed, that the slightly greater activity of the new standard represents a close approach to that limit, so that a further increase of value at later replacements is improbable. On the other hand, the possibility could not be excluded that the increase in the unit on its present value might represent a very slow deterioration of the old standard, with an average loss of about 1% of its activity in each year after its preparation; and if that were the case, a decision to fix the weight of the unit of the new standard at about 0.45 mg., to make it equivalent to 0.5 mg. of the old standard at the replacement date, and a continuation of this policy at successive replacements, would obviously involve the risk of a cumulative depreciation of the unit value which would eventually become of serious practical importance. It was necessary, therefore, to balance the disadvantage of a probable single increase of the value of the unit by not more than about 15% against that of a possible *succession* of depreciations of its value in the same proportion.

With all these considerations before them the participants in the preparatory investigation unanimously agreed that the weight of the new standard for the pituitary posterior lobe, representing 1 unit of activity, should be 0.5 mg., although this would entail an increase of the value of the unit of the order indicated at the time of adoption of the new standard.

Conclusion

Though such an increase in the value of the unit is not likely to be significant, or even appreciable, in practice, it will have a greater importance for the manufacturer, concerned to ensure that his product will not be found deficient if independently tested in comparison with the new standard preparation. The *British Pharmacopoeia*, 1932, in describing the assay of an extract for its oxytocic activity in comparison with the standard preparation, indicates the limit of error of the test as $\pm 20\%$. It will be obvious that a product which had been passed for issue on a test made in comparison with the old standard, and which through testing error had a true activity nearly 20% below the indicated unitage, would incur a greatly increased chance of failure when compared with a standard some 15% stronger. While the existing conditions made it unusually important to avoid wasting material which, from the practical point of view, was not significantly weaker than its stated activity in units, it was also important that the small change involved in adopting the new standard should be made with the greatest practicable speed and unanimity.

Accordingly, the Hampstead Institute has issued a new Memorandum relating to the Second International Standard for Pituitary Posterior Lobe Preparations, in which the grounds for its adoption and for the redefinition of the unit, as above described, are discussed in detail. The new Standard and the Memorandum have been supplied to those countries at present accessible and to the Health Organization of the League of Nations at Geneva; and, in this country, the licensed manufacturers of pituitary (posterior lobe) extract have been notified of the changes indicated above and have been supplied with samples of the new standard preparation and the relevant Memorandum.