

Update Le point

Articles in the Update series give a concise, authoritative, and up-to-date survey of the present position in the selected fields, covering many different aspects of the biomedical sciences and public health. Most of the articles are written by acknowledged experts on the subject.

Les articles de la rubrique *Le point* fournissent un bilan concis et fiable de la situation actuelle dans les domaines considérés, couvrant de nombreux aspects des sciences biomédicales et de la santé publique. La plupart de ces articles auront donc été rédigés par les spécialistes les plus autorisés.

Evolution of activities in international biological standardization since the early days of the Health Organisation of the League of Nations*

P. SIZARET¹

The main activities in international biological standardization during the 18 years that followed the first international biological standardization meeting in London in 1921 were concerned with expressing the potencies of test preparations in comparison with reference materials. After the Second World War, however, it became clear that the testing of biological substances against international reference materials was only one among several measures for obtaining safe and potent products. The activities in international biological standardization were therefore widened so that, by the strict observance of specific manufacturing and control requirements, it was possible to gain further in safety and efficacy. At the end of 1987, 42 international requirements for biological substances were available and were being used as national requirements, sometimes after minor modification, by the majority of WHO's Member States. This is of utmost importance for the worldwide use of safe and potent biological products, including vaccines.

INTRODUCTION AND OVERVIEW

The problems inherent in international biological standardization as well as the achievements have been examined in the past by Miles in 1950 (1), Matthews in 1968 (2), and Outschoorn in 1973 (3). Following an initiative by Thorwald Madsen, Director of the Statens Seruminstitut in Copenhagen, the Health Organisation of the League of Nations in December 1921 convened an international conference in London for the standardization of immune sera and biological reagents. This date marks a milestone in the development of international activities in biological standardization; before that, however, a few inter-

national standards for biological substances had been established and distributed by the Statens Seruminstitut in Copenhagen. The London conference was attended by 18 scientists from eleven countries, 8 technical advisers from the United Kingdom, and two members of the Health Organisation Secretariat. The agenda of the conference included the diagnosis of syphilis and the titration of antisera against diphtheria and tetanus, and shigella, meningococcus and pneumococcus infections (4). The participants agreed that there was a need to coordinate the activities in biological standardization at the international level and to provide reagents with assigned units.

For the sake of comparison, the thirty-eighth meeting of the WHO Expert Committee on Biological Standardization, which today is the authority on matters relating to international biological standardization, took place in December 1987 in Geneva, and

* A French translation of this article will appear in a later issue of the *Bulletin*.

¹ Scientific Officer, Biologicals Unit, World Health Organization, 1211 Geneva 27, Switzerland.

was attended by 22 members or temporary advisers from sixteen countries as well as one observer and two members from the WHO Secretariat (20). Twelve items of general interest were discussed and progress reports on reference materials for 38 biological substances were examined. Certain sections of the WHO requirements on yellow fever vaccines, BCG vaccines, oral polio vaccines, and agar diffusion tests using antimicrobial susceptibility discs were modified; the WHO requirements on hepatitis B vaccines produced from plasma and on measles vaccine (live) were revised; and requirements for two new biological substances (i.e., Japanese encephalitis vaccines and interferons produced by recombinant DNA technology) were adopted. In 1986, more than 11 000 samples of International Reference Materials (IRM) had been distributed to WHO Member States by the four international laboratories in Denmark, Netherlands, and the United Kingdom, and additional IRMs had been distributed by the three WHO custodian laboratories in Atlanta and Bethesda (USA) and Bilthoven (Netherlands). The 1986 list of biological substances, published by WHO (6), includes over 200 International Standards or other materials with equivalent status, 18 International Reference Preparations (IRP) without assigned international units, and 102 International Reference Reagents available for worldwide distribution.

The main purpose of the London conference in 1921 was to prepare reference materials that would allow national public health authorities to ensure that the biological products used for the prophylaxis, diagnosis or treatment of diseases were potent. The two main characteristics of such materials were (and still are): that, for given products, they were unique; and that their content was expressed in arbitrary units. Reference materials have, since then, become widely used as more and more biological substances were produced; and increased safety and efficacy were gained after 1945 by the production and control of important biologicals according to the WHO requirements for biological substances.

STRUCTURE AND FUNCTIONING

The main activities in international biological standardization during the 14 years that followed the 1921 London conference have been described in detail by Gautier (7). In 1924 the Health Committee decided, in order to secure continuity of work, to set up a Permanent Commission on Biological Standardization which thereafter organized meetings, usually every two years, in various capitals; the two main "international laboratories" which were most active in international biological standardization were the Hampstead Institute (London) and the Statens Serum-

institut (Copenhagen). During the Second World War, Denmark was under occupation and the international activities of the Statens Seruminstitut were taken over temporarily by the Hampstead Institute. In 1947, the several International Standards that had been prepared and made available in the United Kingdom between 1939 and 1945 were formally established by the WHO Expert Committee, which also adopted a plan of action for preparing reference materials for various biological substances (8). The WHO Constitution was signed in 1946, and the administrative structure dealing with international biological standardization has since then been represented by the WHO Secretariat (through the Biologicals Unit) which acts in conjunction with the Expert Committee. As a rule, the Expert Committee meets once a year in Geneva and makes decisions on International Reference Materials and concerning international requirements; the IRMs are held and distributed by four international laboratories (in Amsterdam, Copenhagen, Potters Bar and, for veterinary products, Weybridge)^a and the three WHO custodian laboratories in the Netherlands and USA already mentioned. Several nongovernmental organizations, such as the International Association of Biological Standardization, the International Committee for Standardization in Haematology, and the Standardization Committee of the International Union of Immunological Societies, contribute actively to the WHO programme of international biological standardization.

Reasons why International Reference Materials are needed

The main reason why IRMs for biological substances are needed is that, because of their complexity and impurity, these substances cannot be assayed by simple physico-chemical means. In fact, whenever the structure and the chemical composition of a biological substance becomes known, or when the substance is produced in a sufficiently pure form and when a biological test is no longer necessary, the corresponding international *biological* reference materials are discontinued; if necessary, they are replaced by international chemical reference substances for pharmaceuticals which are held and distributed by the WHO Collaborating Centre for Chemical Reference Substances in Stockholm. Biological substances can only be assayed by biological or complex immunological assays, the results of which may be profoundly affected by certain parameters such as the duration and temperature of incubation of the reactions, the origin of the reagents, the strain of laboratory animals, etc. Experience has

^a Potters Bar and Weybridge are in the United Kingdom.

shown that, in order to minimize between-assay variations, it is useful to assay under similar conditions both the test preparations and the corresponding reference material which is the same from one assay to another. Any changes in assay conditions will then similarly affect both test and reference preparations, so that if the contents of test preparations are expressed by comparison with the reference, the relative potencies will not change markedly from assay to assay. The contents of IRMs are usually expressed in arbitrary units, as opposed to mass units, for two reasons:

(a) The estimates by laboratories of the amount of the active substance contained in a reference preparation may vary; on the other hand, there cannot be disagreement on the contents of International Standards because these are assigned in arbitrary units.

(b) Should there be agreement on the mass units of the "specific" substance in a reference material, this may be of little practical importance if, for example, the immunogenicity of the preparation, e.g., tetanus vaccine, is due to the combination of a "specific" substance, i.e., the toxoid, and an adjuvant on which it is adsorbed. However, it is not possible to reliably predict the immunogenicity of such vaccines from various origins by simply determining by *in vitro* tests the respective amounts of toxoids and adjuvants, especially since immunogenicity also depends on the way the toxoids are combined to the adjuvants; hence, the idea of expressing the contents of unknown toxoid vaccines by comparing them with those of reference vaccines, to which arbitrary units have been assigned.

Problems of nomenclature

In 1952, 40 International Standards and four IRPs, including cardiolipin, beef heart lecithin, egg lecithin and penicillin K established as "provisional IRP", were available for distribution (9). In 1954, the Expert Committee created an additional category of reference material (called "Authors' preparation") for substances such as bradykinin, hypertensin, kallikrein, prostaglandin and renin (10); it was agreed that if units were to be assigned to such preparations, evidence of "reasonable stability" should be supplied by the "authors". In 1958, the Expert Committee noted that there was no more need for the category of "Authors' preparations" which was therefore discontinued (11); the Committee also decided to make a clear distinction between IS and IRP. It was agreed that an IS is "a preparation to which an international unit has been assigned on the basis of an extensive international collaborative study", whereas an IRP is "a preparation to which the international unit has *not* been assigned". This decision was unfortunately reversed in 1962 (12),

when it was accepted that international units could also be assigned to IRPs; in the resulting confusion since then, some 80 IRPs with true international standard status, as recognized by the Thirty-seventh World Health Assembly in 1984 (in resolution WHA37.27), and 18 IRPs without such status were labelled similarly. Consequently, the Expert Committee which met in 1983 decided (13) that the future designation of IRMs would be restricted to preparations that did not define international units of activity—as had already been decided in 1958! In practice, however, the 80 IRPs which had become "true" International Standards remained labelled as IRP, since relabelling would have required a substantial amount of work; in order seemingly to avoid further confusion, the Expert Committee decided in 1986 (5) that newly established reference materials would thereafter fall into either the category of IS (most of the time with international units rather than mass units) or IRR (without international units).

The category of International Reference Reagents appeared first in 1965 (14) on the occasion of the establishment of anti-tickborne virus and anti-enterovirus sera. Further to the above-mentioned decision of the Expert Committee in 1986, it is clear that the IRR category may include preparations with assigned content, which implies providing evidence for stability in the submission report.

The above-mentioned changes in the nomenclature illustrate the difficulty of properly classifying substances which are complex and varied; indeed, certain biological products are useful for the diagnosis or prophylaxis or treatment of human and animal diseases, and, to a lesser extent, to biological research; they belong to several categories such as antibiotics, antibodies, antigens, immunogens, blood products, allergens, hormones and others such as pyrogens, interferons, etc. Furthermore, there is some inherent conflict between the wish to establish quickly a reference material in order, for example, to prevent groups from embarking upon using different units for similar substances, and the concern about assigning units only after proper characterization (including evidence of stability and of comparability with other preparations from various sources). In cases where International Standards need to be established before full characterization has been carried out, the simplest designation might be that of a "provisional" IS; later on, when proper characterization becomes available, the "provisional" qualification would be deleted. In practice, four criteria are considered for establishing an IS:

- (1) The importance of the products for health matters.
- (2) The extensive characterization through international collaborative studies of the Proposed Inter-

national Standard (PIS).

(3) The suitability of the PIS for the purpose for which it is intended, having regard to the nature of the substances to be evaluated; this implies that using relevant test systems, the PIS gives results that can be compared with the results of other commercial biological preparations having similar biological activities. Inasmuch as, for example, certain live attenuated vaccines (e.g., BCG vaccines) are derived from several strains having different growth characteristics and biological properties, there cannot be a single IS for all such vaccines. The conditions to be fulfilled for results of biological assays to be valid and meaningful have been extensively reviewed by Jerne & Wood (15).

(4) The strict adherence to appropriate technical requirements, especially those dealing with the precision of filling of containers and the stability of the contents.

International Standards are established through a series of steps including the provision, through the Biologicals Unit of WHO to the members of the Panel of Biological Standardization, of unpublished technical documents, followed by a submission to and a decision by the WHO Expert Committee, then the forwarding by the Director-General to the WHO Executive Board of the Expert Committee's published reports, and finally the formal adoption of resolutions by the World Health Assembly.

The line of division between preparation with and without international standard status is not always straightforward. It is an important one, however, since only International Standards are binding on WHO's Member States. Resolution 37.27 on biological substances adopted in 1984 by the World Health Assembly recommends that Member States officially recognize as International Standards a list of Proposed IS; furthermore, it recommends: (1) that those standards and units, or their equivalent, be cited in the relevant pharmacopoeias; (2) that, where applicable, those standards and units, or their equivalent, be recognized in relevant national regulations; and (3) that in those countries which do not possess a national pharmacopoeia or national standards, when it is necessary that the potency of the product should be stated on the label, such potency be expressed in international units.

The words "or their equivalent" contained in the first part of the recommendation allude to the WHO recommendation of using International Standards for calibrating national standards, the reason being that ISs are too valuable and too limited in supply to be used as routine calibrators. Providing national standards is the responsibility of national control laboratories, the establishment of which was recommended by the intergovernmental conference on biological standardization held in 1935 in Geneva (16). Today,

the implementation of this recommendation has still not been realized in many countries.

The above-mentioned resolution also authorizes the Director-General to make additions or replacements to the list of WHO International Standards, "subject in each case to the satisfactory completion of the technical procedures now established of international collaborative studies and assays and under the advice of the members of the Expert Advisory Panel on Biological Standardization or other experts designated to deal with the standardization of particular biological substances". This provision is provided because although new or replacement ISs are adopted each year by the Expert Committee, updated lists of IS are not proposed for adoption every year to the World Health Assembly.

INTERNATIONAL REGULATIONS FOR BIOLOGICAL SUBSTANCES

The initial concept upon which biological standardization was based was that the preparation of potent and safe biological products could be achieved essentially by assaying final test products against reference materials, and then expressing the concentrations of active principles in units that were accepted worldwide. However, that simple notion was progressively replaced by a more general concept which also recognized the necessity to codify all the manufacturing steps and to carry out "in-process controls". It was clear that even greater security would be achieved if such codification was to be carried out by internationally recognized experts. Setting international regulations for biological products moving in international commerce was clearly of concern to the 61 WHO Member States whose representatives signed the WHO Constitution on 22 July 1946 at the International Health Conference in New York. Article 21 of the WHO Constitution (17) states that "the Health Assembly shall have authority to adopt regulations concerning (a)...(b)...(c)...(d) standards with respect to the safety, purity and potency of biological, pharmaceutical and similar products moving in international commerce".

It is not by chance that yellow fever vaccine (18), a live viral attenuated vaccine, was the first product for which international requirements for manufacture and control were adopted. Although it was known that safe and potent yellow fever vaccines could be produced for preventing the spread of the disease by international travellers, it was felt important to agree internationally on "standards" for the manufacture and control of these vaccines. The main test capable of giving assurance that yellow fever vaccines are safe and efficacious is the intracerebral challenge test

of monkeys, but it is an expensive and cumbersome test; experience has validated the concept that the testing of yellow fever vaccines produced routinely could be considerably simplified if (a) the vaccine virus seed lots and initial batches of vaccines were tested successfully by the monkey neurovirulence test, and (b) strict adherence to protocols for the production of satisfactory vaccines was maintained. Later on, the seed lot concept was successfully extended to cell substrates such as human diploid cells and continuous cell lines used for the production of oral or inactivated poliomyelitis vaccines, inactivated rabies vaccine, and live measles vaccine, and this resulted in further simplification of the production procedures and safety of these vaccines.

BCG vaccine, which was needed for a UNICEF vaccination campaign, was the second product for which international requirements were adopted. This was in 1949 at the third meeting of the Expert Committee (19), whose report includes four pages dealing with various problems such as staff, building, animal quarters, precautions against contamination, methods for cultivating BCG strains and control tests. In the section on staff, the report even specifies that "the number of workers on the staff should be sufficient to ensure that in the case of the absence of one of its members, the duties can be carried out by one of the others".

It is only in 1958 that the international requirements for biological substances began to be examined by the Expert Committee in a systematic way. As a result, the general WHO requirements for manufacturing establishments and for control laboratories, the WHO requirements for inactivated poliomyelitis vaccines, and the WHO requirements for cholera vaccines were published in 1959 (11). The structure of the WHO requirements, which from the beginning included sections on microbiological seeds, substrates, single harvests, bulks, final bulks, and final products, has remained basically unchanged. Requirements are usually drafted by a small group of experts; the draft requirements are then circulated for comments by the WHO Expert Panel on Biological Standardization; after suitable amendments, they are proposed for adoption to the WHO Expert Committee and, if accepted, are published in their reports. These reports are finally forwarded by the Director-General to the WHO Executive Board, as well as to Ministries of Health in Member States. At the end of 1987, 42

WHO requirements for the manufacture and control of biological substances had been adopted and often updated (20).

The WHO requirements are most often incorporated by Member States into their own national requirements. Even when they are not incorporated, they exert a considerable influence on manufacturers since they contain recommendations made by the best experts. Moreover, the fact that a biological product meets the WHO requirements helps the promotion of its export; indeed, the WHO requirements form a ready-made and authoritative basis for the exchange of biological substances among countries. Furthermore, the WHO requirements are constantly referred to when vaccines have to be acquired for mass campaigns or international immunization programmes; the fact that in 1987 an organization like UNICEF bought about 23 million doses of diphtheria-tetanus vaccines, 83 million doses of tetanus vaccines, 85 million doses of measles vaccines, 107 million doses of BCG vaccines, 156 million doses of diphtheria-pertussis-tetanus vaccines, and 188 million doses of oral polio vaccines gives an idea of the magnitude of international exchanges of certain biological products.

CONCLUSIONS

The increased use of continuous cell lines as substrates and of recombinant DNA techniques are two important recent developments in the production of biological substances. Both offer interesting prospects for the manufacture of biologicals in a more economical way, but also pose new problems to the regulatory authorities. At the same time, the spread of diseases like AIDS (acquired immunodeficiency syndrome) has led to intensive efforts at devising protective vaccines, in addition to similar efforts for the development of vaccines against classical diseases, such as malaria, schistosomiasis, leprosy and tuberculosis, and even for controlling fertility in humans. The need for the early formulation of guidelines and WHO requirements and for the establishment of International Reference Materials for producing and controlling these new products indicates the direction of activities in international biological standardization over the next few years.

ACKNOWLEDGEMENTS

I am indebted to Dr. J. Lyng (Head of the WHO International Laboratory for Biological Substances, Statens Seruminstitut, Copenhagen) for his critical review of the manuscript and pertinent remarks, and to Ms J. Brunt and Ms L. St Maxens for secretarial assistance.

REFERENCES

1. MILES, A. A. Biological standards and the measurement of therapeutic activity. *British medical bulletin*, 7: 283-291 (1950).
 2. MATTHEWS, A. G. WHO's influence on the control of biologicals. *WHO Chronicle*, 23: 3-15 (1968).
 3. OUTSCHOORN, A. S. The biological standardization programme of the World Health Organization. *Journal of biological standardization*, 1: 203-213 (1973).
 4. International Conference on the Standardisation of Sera and Serological Tests, 12-14 December 1921, British Ministry of Health, London. Geneva, League of Nations, 1921, pp. 1-18.
 5. WHO Technical Report Series, No. 760, 1987 (*WHO Expert Committee on Biological Standardization*: thirty-seventh report).
 6. *Biological substances. International Standards and Reference Reagents, 1986*. Geneva, World Health Organization, 1987.
 7. GAUTIER, R. The Health Organisation and biological standardisation. *Quarterly bulletin of the Health Organisation, League of Nations*, 4: 497-554 (1935).
 8. Report of the Expert Committee on Biological Standardization. First session, Geneva, 9-13 June 1947. *Bulletin of the World Health Organization*, 1: 7-20 (1947).
 9. WHO Technical Report Series, No. 56, 1952 (*Biological standardization*: fifth report of the Expert Committee).
 10. WHO Technical Report Series, No. 96, 1955 (*Biological standardization*: eighth report of the Expert Committee).
 11. WHO Technical Report Series, No. 172, 1959 (*Biological standardization*: twelfth report of the Expert Committee).
 12. WHO Technical Report Series, No. 259, 1963 (*Biological standardization*: fifteenth report of the Expert Committee).
 13. WHO Technical Report Series, No. 700, 1984 (*WHO Expert Committee on Biological Standardization*: thirty-fourth report).
 14. WHO Technical Report Series, No. 329, 1966 (*WHO Expert Committee on Biological Standardization*: eighteenth report).
 15. JERNE, N. K. & WOOD, E. C. The validity and meaning of the results of biological assays. *Biometrics*, 5 (4): 273-299 (1949).
 16. Report of the Inter-Governmental Conference on Biological Standardisation. *Quarterly bulletin of the Health Organisation, League of Nations*, 4: 631-642 (1935).
 17. *Constitution of the World Health Organization*, Geneva, World Health Organization, 1985, pp. 1-18.
 18. Standards for the manufacture and control of yellow fever vaccine. *Epidemiological information bulletin, (UNRRA)*, 1: 365-370 (1945).
 19. Requirements for laboratories engaged in the preparation of BCG vaccine for the UNICEF vaccination campaign. In: WHO Technical Report Series, No. 2, 1950. (*Biological standardization*: report on the third session of the Expert Committee), Annex 1.
 20. WHO Technical Report Series, 1988 (*WHO Expert Committee on Biological Standardization*: thirty-eighth report) (in press).
-