International Association of Microbiological Societies
Permanent Section for Microbiological Standardization

Proceedings

OF THE

7TH INTERNATIONAL CONGRESS FOR MICROBIOLOGICAL STANDARDIZATION

LONDON 1961

E. & S. LIVINGSTONE LTD.,
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ADDRESS BY THE PRESIDENT OF THE CONGRESS

THE RT. HON. LORD COHEN OF BIRKENHEAD

MY first Presidential words must be of warm welcome both to the members of, and the contributors to, this Seventh International Congress of the Permanent Section on Biological Standardization, and also to those distinguished guests who have honoured us by attending our inaugural session.

It is to these and not to the experts that my introductory words are addressed in order to explain what our functions and purpose are.

Most of the therapeutic agents which are now used in both human and veterinary medicine are of well-established chemical and physical constitution, for example, iron salts, alkalies, the sulpha drugs and corticosteroids, and their purity can be accurately attested. But there are others of great value in the recognition, prevention and treatment of disease which remain of undefined structure, whose potency is assayed by biological methods, that is by testing their effects on living structures and systems—the whole animal or isolated tissues or organs, bacteria, red blood-cells and the like. Many of these drugs are of ancient lineage, for example digitalis, which has been used in medicine for over two centuries. It was given in the form of dried leaves, infusion or tincture, so that the amount of its active principle administered to a patient varied between very wide limits. Thus the giving of an unknown quantity of a drug to a patient of varying response, since in the biological sense men are certainly not born equal, explains much past therapeutic disputation and doubt which is not unknown even today. In the past seventy years there has been introduced to medicine a large number of new biological preparations including antisera, vaccines, antibiotics, hormones, and vitamins, which have revolutionized the control of disease. Some of these have later had their chemical constitution unmasked, *e.g.* most of the vitamins, and a few antibiotics, *e.g.* chloramphenicol. Thus an exact dose can be administered to a patient in pure form, and the only variable is the patient's response. But many of these biological products still defy the chemist's attack; they remain unassayable by chemical methods. Yet if they are to be given in a dosage which combines safety and efficiency, thus shielding the patient from the hazards, on the one hand, of underdosage with an inadequate therapeutic effect, or on the other hand, of overdosage with ensuing toxic effects, it is clearly necessary to establish and define biological standards and units, and to try and ensure that these standards are made freely and generally available, so that they will be adopted and effectively applied by research workers, by manufacturers and by clinicians in the care of their patients.

The grave consequences of lack of standardization are demonstrated by an experience of Sir Henry Dale, whose pioneering genius in this, as in many other fields, has placed humanity deeply in his debt. He found, in the 1920s working with Professor Burn, that extracts of the posterior pituitary gland, obtainable in this country and used as a stimulant to uterine contraction, "varied over such a range that the strongest had eighty times the activity of the weakest. Even in our country, then, preparations all labelled 'physiologically standardized' and offered for use, mostly in emergency, with the same dosage, could be completely ineffective at one end of the scale, and strong enough to threaten rupture of the labouring uterus at the other."

It seems astonishing in retrospect that the need for biological standardization was not earlier recognized. But for past default we have made full amends, and today there is a large measure of international uniformity, and results in this field from all over the world can now be correlated and compared on the basis of a common quantitative notation.

How has this come about? The story begins, as do so many in the saga of medicine, with Paul Ehrlich, who in 1897, established a standard preparation of diphtheria antitoxin by which all others, wherever in the world they were prepared, could be standardized. Subsidiary standards in terms of Ehrlich's unit were made; as it turned out, a wise precaution since these alone remained available outside Germany during the First World War. After the War in 1921, when the League of Nations was established, its Health Committee, at the instigation of Dr Thorvald Madsen, who was Director of the State Serum Institute in Copenhagen, convened an International Conference which found that the subsidiary standard used during the War, and held at the Hygienic Laboratory, Washington, U.S.A., had not varied significantly from the original Ehrlich standard at Frankfurt, and the Conference recommended that this unit should be adopted as the international unit for diphtheria antitoxin. The following year, at a second Conference, an international unit for tetanus antitoxin was defined. Later, through the outstanding work of a group of workers led by Sir Henry Dale, and then by Sir Percival Hartley, international standards and units for substances other than antitoxins were established. These included digitalis, posterior pituitary extracts, insulin, and the organic arsenicals.

In 1924, the League of Nations set up, through its Health Organization, the Permanent Commission on Biological Standardization, and divided the responsibility for international standards between the State Serum Institute, Copenhagen, for sera, and the National Institute for Medical Research in London, for all other substances. In London was created the Department of Biological Standards under the direction of Sir Percival Hartley, and later Professor A. A. Miles and Professor D. G. Evans, who are playing so prominent a part in the work of this Congress. The health activities of the League of Nations were, after the Second World War, carried on by the World Health Organization (WHO) which gives official recognition to this Section. WHO has left the Institutes at Copenhagen and London as custodians of its international standards and reference preparations, in collaboration with many research and manufacturing laboratories throughout the world.

In 1935, there were 27 biological standards; in 1939 there were 31. Since the war the number has rapidly increased. Today, Copenhagen and London hold, on behalf of WHO, standards and reference preparations for approximately 65 immunological products (antigens and antibodies), 20 antibiotics, 10 hormones and 12 other miscellaneous preparations (*e.g.* enzymes)—that is more than 100 standards. And this increase, despite the fact which I earlier noted, that some biological standards have been discontinued because it is increasingly possible to assay these preparations by chemical and physical tests. Indeed, because of the striking advances in physicochemical methods, it may well be that biological standardization is moving towards its own future extinction. But at the present time further increases in the number of standards are bound to occur due essentially to the continued developments in the antibiotic field, to virus vaccines, to the extension of vaccines in the veterinary field, to the standardization of antivenins and such enzymes of therapeutic activity as streptokinase, streptodornase, fibrinolysin and trypsin, to helminth vaccines, and to diagnostic sera (especially for virus infections). Many of the problems to which these give rise are to be discussed at this Congress.

This vast expansion of the field of biological standardization carries with it an increasing responsibility and burden on the part of WHO and the two laboratories in Copenhagen and London. Can this Section ease their tasks and lighten their burdens? These Congresses, first held in 1954, make their contribution. They gather together workers in this field, who in their formal sessions pool knowledge by reporting their own researches and provoke discussion or throw out ideas which may well initiate a fruitful research project. Informally, the personal contacts and discussions establish the atmosphere which propitiates communication. The Section also arranges the exchange of scientific and bibliographic information; it organizes exchanges of personnel and holds regular meetings; in it are united controllers, producers and independent research workers.

But can more than this be done? Can the Section play a greater part in the actual establishment of biological standards? In the international sphere the Section has wisely placed itself in a position where it can be most effective in promoting the establishment of a new standard for which the need is felt. By its Statutes it is closely linked with the ultimate arbiter of international standardization, the WHO, and as a result of this link, interested workers, who collaborate in the sometimes vast and tedious task of paving the way for an international standard, can so co-ordinate their activities that when the researches are ripe, the time-consuming scrutiny and the independent tests, which the WHO experts rightly demand before establishing an international standard, will be reduced to a minimum. I see that such a procedure is envisaged by those working on freeze-dried BCG vaccine and reporting to this Congress and may well be followed by those working with the staphylococcus antigens.

Again during recent years, the WHO has devoted much attention to preparing international requirements for some of the more important biological products, *e.g.* vaccines against poliomyelitis, smallpox, yellow-fever, and cholera. This policy is an important contribution towards the effective control of biological products, for control must be exercised not only by tests of the final product, but by following certain minimum requirements in manufacture and testing. This is especially important in the case of live virus vaccines The WHO requirements have been drawn up so as to provide guidance to those concerned in preparing biological products, to promote a greater uniformity in the methods of manufacture and testing, and to facilitate the exchange of biological products between different countries.

In its task of drawing up these international requirements, could not WHO be assisted by this Section on Biological Standardization? For example, the present Congress will be discussing the question of extraneous viruses in vaccines prepared from living tissues, and also tests for sterility and toxicity; from these discussions important information could be transmitted to WHO. But these Congresses cannot possibly cover the wide field of problems connected with the manufacture and testing of biological products. It is, therefore, important that the various national control laboratories should draw up their own requirements, based on their own experience, for consideration by WHO. Some control laboratories are already doing this, and it is with their collaboration that WHO is able to formulate comprehensive international requirements.

There are many other problems which need consideration, such as those relating to the potency of biological products, and the need for field investigations to be undertaken in parallel with laboratory tests. But time speeds, so may I end on a brief personal note?

I am alertly conscious of and deeply grateful for the signal honour you have done me in inviting me to preside over this Congress. My presence in this Chair is not the outcome of fervent prayer, and I readily concede, without mock modesty, that my claims to so marked a distinction are not irresistable. My professional life has been devoted largely to the medicine of the bedside, and many of you will justifiably ask of your President—Que diable faites-vous dans cette galère? My two pleas in mitigation are: first, that in your field I represent the consumer and have played a minor rôle in decisions concerning immunisation procedures in this country; and secondly and most weighty, that I yield to none in my admiration and gratitude for the remarkable and enviable contribution which workers in your scientific fields have made to the welfare and happiness of mankind, which remain the surest foundations for peace in a troubled world.

ADDRESS BY THE CHAIRMAN OF THE CONGRESS

PROFESSOR A. A. MILES

STANDARDIZATION AND SPECIFICATION

MR PRESIDENT, LADIES AND GENTLEMEN,

I am grateful for the honour of your asking me to be Chairman of this Congress; but I am in one respect going to be ungrateful and bite the hand that honoured me by taking advantage of my chairmanship to make some observations and to offer some advice on the subject of biological standardization. If you don't like my advice, you may discount it on the ground that, as far as standardization is concerned, it is in these days advice from the armchair and not from the bench.

Some years ago Professor J. H. Burn, in his classical book on biological assay, told the story of how the English foot became a standard measure of length. In mediaeval time the foot was indeed the length of a human foot, the King's foot being the standard. Unfortunately for the standard, the successive English monarchs, like all biological material, were subject to variation. When the King's foot was small, those who sold merchandise by length no doubt flourished, and when the King's foot was large, the buyers were greatly benefited; but also without doubt there was much confusion, until the foot length was established as a fixed distance between two marks on a piece of rigid material. At this moment the foot was, in fact, standardized. The analogous moment in the biological field is when a preparation of an active material is designated as a standard. I make this point, because although the word "standardization" is mentioned in the titles of thirteen of the communications listed in the programme, in only one of them is it used in the correct sense of establishing a standard. In three, the usage is ambiguous, and in the remaining nine the meaning is extended to cover a variety of quantitative researches that have no immediate connection with the act of standardization.

This usage of the word in its wider meaning is universal and accepted, but even so I would plead for restricting the meaning of "standardization" to the kind of thing mediaeval statesmen did with the King's foot. The foot was standardized, and working standards—the footrule—were made for general use; ever after that ships, spears and lengths of cloth for sale are—not standardized—but *specified* in terms of the standard foot. By analogy, it is proper to say that when diphtheria antitoxin was standardized, a standard preparation of defined unitage was established and all subsequent assays were directed to the *specification* of diphtheria antitoxins in terms of the standard preparation. There are many reasons for maintaining this distinction between standardization and specification. For example, it avoids the confusion that arises if somebody claims to have standardized a vaccine, when there is, in fact, no established standard, and nothing more has been done with that vaccine than in some way or another to measure one of its biological properties. The distinction is very relevant to our discussion this week and indeed to any discussion about standardization. We standardize a substance as a unique act, in order to make specification of like substances easy and accurate. As we move toward standardization there are three questions I suggest we should ask and answer. What do we want to specify? Why do we want to specify it? And, thirdly, at what stage in our investigation should we devise a standard for the purpose of specification?

What are we trying to specify? Our President made it clear that our field is active substances that cannot be specified in terms of chemistry and physics. And this in practice, as we all well know, means that our preparations are often mixtures. The biologically active substance we are interested in is usually contaminated either with inert substances or with other active substances. When the substance we are interested in is large-molecular, the odds are against Nature's having endowed a unique molecular configuration with the particular biological activity; she is more likely, as she did, for example, with the D vitamins and the penicillins, to produce *families* of related subtsances with similar activities but significantly different properties.

The standard doesn't necessarily solve the problems created by inert impurities, families of related active molecules or contamination with unrelated active material; but it is quite essential if we are to begin to solve them. When we try to specify material of this kind in terms of a standard, we shall probably find that the numerical results differ according to the type of preparation we test; according to the biological system we use for the test; and according to variations in our handling of the test system. We find that we get consistent answers when the material is prepared in a given way, and rigid conditions of assay are observed. We are then gravely tempted to fix this assay as the one true method. If we are very lucky indeed, it may prove to be not only consistent, but truly to measure the activity of the material. But we shall never know that it is true by sticking to the one type of assay, because in circumscribing the test system we have of necessity ensured that we shall get only circumscribed answers.

Tetanus antitoxin from the horse can be assayed with great precision if we always use the same breed of test animal and the same preparation of tetanus toxin. But if this course had been followed exclusively in the last fifty years, we should never have discovered the heterogeneity of tetanus toxin, and would still be turning out tetanus antitoxins whose potency in the poor patient is properly indicated by the potency on the label only when he happened to be attacked by a tetanus bacillus that was turning out exactly the same kind of toxin as that used in the assay. In much the same way, substitution of the chick in place of the orthodox test rat in the assay of vitamin D, led to the discovery of the multiplicity of D vitamins; and when the test microbe in the assay of penicillin was changed from *Staphylococcus aureus* to *Bacillus subtilis*, the multiplicity of penicillins became apparent.

These discoveries of heterogeneity, please note, arise from *quantitative* anomalies in the assay of what proves to be variable mixtures of active substances. Specification in terms of a standard has the declared aim of giving a numerical value to a biological property. But in practice, especially in the early stages of investigation, it makes possible the uncovering of anomalies—including heterogeneity of the standard itself—and all the fruitful discoveries that arise from resolving those anomalies. We start by trying to specify an activity we hope is the property of a single substance; but if past experience is any guide, we are likely to finish by defining a family of like substances. It follows that as the active substances become more precisely defined a heterogenous standard becomes out-of-date, and must be replaced by a better one. We must indeed be prepared, if necessary to make a series of standards before bio-assays are reliable enough for specifying a substance intended for human or animal use.

In discussing *what* we are trying to specify, I have partly answered the second question, of why we want to specify a biological substance. We need to do so for research; and most urgently need to do so in the most precise way that is practicable

when the substance is exploited as a drug or a diagnostic reagent. It is in the research period that a standard's main function is to uncover anomalies, and in the exploitation period that its greater importance is in specification of potency, though it must never be lost sight of that even in this second period the uncovering of anomalies is of fundamental importance.

The answer to the third question, at what stage in our investigation should we devise a standard, is simple; *at the beginning*. Even a single investigator does best when he establishes his own private standard; and collaboration, between even two investigators, demands a common standard preparation; and the demand grows as more and more investigators become interested in the numerical aspects of a biological activity. But these standards haven't and should not have the status of an international standard or even an international reference preparation. International and national standards must be both useful and highly respectable. Respectability of the standard is essential when research is on the threshold of exploitation in medicine; and that threshold is reached when we have really proved the clinical or diagnostic significance of the substance we are pursuing. As scientific investigators we can afford not to have respectable standards, provided they are useful, and until we *are* on the threshold of exploitation we must not be shy of establishing what we might call exploratory standards.

We are perhaps far too reluctant to establish exploratory standards. I think sometimes our very proper reverence for the internationally established standards has infected us with an inhibitory reverence for *any* standard. Let us be irreverent and flexible-minded. For after all, standards are really very simple. Once we can say of a preparation that it is stable, and can divide that preparation into a number of ampoules for use with the certainty that each ampoule will have the same potency, there is our standard. For the purposes of our investigation, it doesn't matter that the stuff is impure or has other imperfections. We may hope ultimately to use substances of the kind we are trying to standardize for parenteral injection in man but if at the moment we are confining ourselves to tests in, say, the rabbit, provided that the standard is non-toxic, it doesn't matter one bit that it is soluble with difficulty or isn't a pretty white powder that would satisfy those who are in love with "ethical" products. There is no need to examine it for suitability in all possible contingencies. All it need do is work reasonably well in the kind of assays we have in mind at the moment. It is much better to have an imperfect standard for a group of research workers or a group of laboratories and use it to uncover the fruitful anomalies, than to delay setting up a standard until meticulous tests have satisfied a large number of people that no gross anomalies are likely to arise.

The criteria for the ultimate perfection of an established international standard are not for us at this stage. We are not committed to a process of scrutinizing a proposed standard preparation as though we were deciding whether to canonize a holy man. The WHO many years ago recognized a hierachy of standards starting with what they call the "author's preparation" of a substance, which might later move up to the status of an "international reference preparation", and ultimately be admitted to the highest circles of heaven as a recognized international standard. And it is in expanding the implications and use of "author's preparation" and in laying foundations for the ultimate establishment of international standard that our Section can make the greatest contributions to this important field. The exploratory standard or even a succession of exploratory standards need in no way prejudice the final standards. Those who feel that all standards should have their share of sanctity may take comfort that the intermediate stage of reference preparations

stands between the exploratory standard and the final international preparation. This is indeed a bar to the impulse to canonize a standard too early. And those who think that in adopting this rather carefree attitude to standards as tools for uncovering anomalies, we may delay the much-to-be-desired stage of ultimate canonization, may take comfort from the realization that the circumstances which hasten the establishment of an international standard are exactly those incumbent upon us to furnish as investigators of the substance; namely, to prove *unequivocally* its prophylactic, therapeutic or diagnostic significance. Only then can we justifiably demand to specify in terms of an international standard.

ADDRESS BY THE PRESIDENT OF THE PERMANENT SECTION

PROFESSOR G. PENSO

MY LORD PRESIDENT, DEAR COLLEAGUES, LADIES AND GENTLEMEN

I am very much honoured and very touched indeed to have to bring to all scientists assembled here for the seventh successive time my welcome and the welcome of the whole permanent section of microbiological standardization.

Our international meetings follow each other with a constant rhythm and with always greater success. Seven meetings have been held from our small and timid first "recontre" in Lyon up to the present one. In all these meetings problems of current interest have been discussed and the conclusions reached in these discussions have formed the foundation of important decisions which have been taken up by international organizations. These organizations have never been insensitive to our initiative, to our discussions, to our resolutions. We have been in effect the pioneers in proposing and discussing the control methods to be used for inactivated poliovaccine, live poliovaccine, combined vaccines and other products.

Today, in London, two of the subjects in discussion will be, among others, the important problem of staphylococcal infections and the problem of biological control of new chemotherapeutic substances possessing antiviral action. These chemotherapeutic substances are in effect beginning to have medical applications: therefore, now is the time to propose control methods for their activity and their innocuity. There is indeed no lack of problems for discussion: on the contrary it is necessary to limit the subjects to be discussed, and choose instead those that are worthwhile to discuss in order not to burden too much the programme of the meeting and not to make abstract proposals. We want to be free to propose and discuss problems of present interest in microbiological standardization: to others is left the task of considering our conclusions and to rule out what we have proposed and recommended.

The permanent section of microbiological standardization is one of the many sections of the International Association of Microbiological Sciences. This is a section which I have wanted and which I have created together with some colleagues of good will: it is a section which all of us have made efficient, hard-working and successful. The difficulties which we have met, and the unavoidable errors which we have made, have not stopped our increasing success. This means that the problem of standardization was felt by us; it was welcome and appreciated. I believe that by now the existence of this section has justified itself; if it is true, let me permit to express all my satisfaction because it is easy to have new ideas but more difficult to have good, realizable and constructive ideas. Your keen and active participation at our meetings, the best relations we have not only with our Association, but also with the WHO, FAO, OIE and UNESCO are the best guarantees of our vitality and serious intentions.

To all the representatives of these Organizations, branches of the United Nations, we send today our welcome and we express our gratitude for the support they have always given us and for the consideration they have shown us. We are really very happy to see them here; and very happy indeed we are to have among us the

esteemed President of the IAMS, Professor Stuart Mudd. We owe to him, to his constant advice, to his valuable support the strengthening of this section and the definite establishment of our existence.

Now I have the most pleasant task, to thank our hosts, our British friends who have taken the burden of the organization of this seventh meeting, and who have done it so well and so thoroughly. Thanks to you my Lord Cohen, thanks to you Professor Miles, and thanks to you Dr Ungar, Dr Hulse, and thanks to all the colleagues of the organizing committee. Thanks to the British authorities who have made easy your work; and thanks to all the distinguished personalities who by honouring us with their presence, ennoble this meeting.

Finally on behalf of all the foreign members and guests of the Congress, may I be permitted to pay our homage to, and express our admiration for, your gracious Queen.

THE PREPARATION, ESTABLISHMENT AND DISTRIBUTION OF BIOLOGICAL STANDARDS AND REFERENCE PREPARATIONS (INTERNATIONAL, NATIONAL AND LABORATORY)

INTERNATIONAL BIOLOGICAL STANDARDIZATION

N. K. JERNE

World Health Organization, Geneva

SUMMARY

Classical biological standardization has led to the establishment of close to 100 international standard preparations representing forty years of world-wide collaborative work. These preparations are made available by the World Health Organization (WHO) to national laboratories in all countries. For complex substances such as vaccines this work is being complemented by the formulation of international requirements. In order to meet the demand for a large number of certified diagnostic sera and prototype strains of cells and viruses, the WHO has established international reference preparations and has appointed international reference centres serving networks of national laboratories in specialized fields. In these tasks the WHO welcomes the assistance offered in the Statutes of the Permanent Section on Biological Standardization of the International Association of Microbiological Societies.

IN introducing the discussions of this Seventh Congress of the Permanent Section on Biological Standardization of the International Association of Microbiological Societies, I shall divide my subject into three parts. First, to invoke the golden past of classical biological standardization; secondly, to briefly mention the difficulties of the present time and the expansion of our field, that the WHO is now trying to cope with; and thirdly, to express the hope that in spite of all this, our combined efforts will lead us to a better future.

Classical biological standardization was restricted to the measurement of the potencies of biological substances which, by definition, cannot be measured by physical or chemical means. Instead of concluding that, therefore, such substances cannot be measured at all, the classicist showed that the potency of a preparation relative to a standard preparation of the same substance can be determined by noting the effect of the two preparations in animals, or in some other biological system. The classical biological assay is symbolized by this figure in which curves