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INTERNATIONAL STANDARD FOR PHENOXYMETHYLPENICILLIN

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SYNOPSIS

A batch of highly purified phenoxymethylpenicillin has been examined by eight laboratories in seven different countries, and has been assayed against the phenoxymethylpenicillin standard of the Food and Drug Administration of the US Department of Health, Education, and Welfare. The material examined has been established as the International Standard for Phenoxymethylpenicillin, and the International Unit of Phenoxymethylpenicillin is defined as the activity contained in 0.000590 mg of the International Standard.

At a session of the Expert Committee on Biological Standardization of the World Health Organization, held in Geneva in October 1956 (World Health Organization, 1957), the Department of Biological Standards at the National Institute for Medical Research, London, was authorized to obtain a preparation of phenoxymethylpenicillin suitable for use as an International Standard and to proceed with its characterization and assay.

The Proposed International Standard consists of 60 g of a single batch of highly purified phenoxymethylpenicillin (free acid), which was obtained through the generosity of the Distillers Company (Biochemicals) Ltd., England.[1] Some of the properties of this material have already been described in a series of papers where it is referred to as sample E (Goodey et al., 1955; Parker et al., 1955; Stephens & Grainger, 1955). It was found to be homogeneous when examined by solubility analysis and by chromatography.

The material was received at the National Institute for Medical Research, London, in a single sealed container, in which it was stored for six months at 4°C until it was distributed into ampoules, each containing approximately 75 mg. The ampoules were stored over P_2O_5 *in vacuo* for 14 days, after which they were constricted, stored for a further six days *in vacuo* over P_2O_5, and then filled with pure dry nitrogen and sealed. After sealing,

[1] The sample was prepared by Dr G. Parker, Mr R. J. Cox and Miss Dorothy Richards, to whom acknowledgement is made.

the ampoules were tested for pinholes and leaks, and stored at −10°C. The moisture content of the material in the ampoules was measured by heating at 56°C over P_2O_5 at a pressure of 0.03 mm Hg for 10 hours, and was found to be 0.2% w/w.

The Reference Preparation

The Reference Preparation consisted of 2 g of a preparation of phenoxymethylpenicillin (free acid), which was the current Standard Preparation of the Food and Drug Administration (FDA), United States Department of Health, Education and Welfare, and of the United States Pharmacopeia. The material was received at the National Institute for Medical Research, London, in October 1956, and was stored in its sealed container at 4°C for seven weeks. It was then distributed in 35-mg amounts into glass ampoules, which were constricted, stored *in vacuo* over P_2O_5 for three weeks, filled with pure dry nitrogen and sealed. The moisture content of the material in the ampoules, measured under the conditions described for the Proposed International Standard, was found to be 0.26% w/w.

The International Collaborative Assay

Samples of the two preparations were sent to the eight laboratories in seven countries listed in the Annex. The participating laboratories, which are referred to by arbitrary numbers in this report, carried out 189 assays, of which 154 were biological. The number of assays received from each laboratory are listed in Table 1, together with the methods and test organisms that were used.

Statistical Analysis and Results

The assays were analysed by the method described in the report on the International Standard for Erythromycin (Humphrey et al., 1957). Only biological assays have been used in the calculation of an over-all potency, and all estimates of potency of the Proposed International Standard are based on the assumption that the potency of the Reference Standard is unity.

An estimate of potency was obtained for each of the 154 biological assays, and the distribution of the logarithms of these potencies is shown in the figure. The mean of this distribution gives an over-all potency of 1.042 with 5% limits of 1.032–1.053.

Separate estimates of potency have also been made for each laboratory by considering the distribution of individual log potencies. These estimates are given in Table 2 with their associated weights, which have been calculated as the reciprocal of the variance of the mean log potencies.

TABLE 1. NUMBER AND TYPE OF ASSAYS RECEIVED FROM DIFFERENT LABORATORIES

Laboratory No.	Type of assay	Test organism	Number of assays
1	Plate	*Micrococcus pyogenes* var. *aureus* 209P	6
	Iodometric	—	3
2	Plate	*Staphylococcus aureus* 209P	7
3	Plate	*Sarcina lutea*	8
	Plate	*Bacillus subtilis*	8
4	Plate	*Staphylococcus aureus*	6
	Spectrophotometric	—	2
5	Plate	*Staphylococcus aureus* 209P	12
	Plate	*Staphylococcus aureus* Oxford H	12
	Spectrophotometric	—	2
	Iodometric	—	1
6	Plate	*Bacillus subtilis*	75
	Iodometric	—	5
7	Plate	*Micrococcus pyogenes* var. *aureus*	6
	Iodometric	—	8
8	Plate	*Staphylococcus aureus* S.G. 511	14
	Iodometric	—	14
Total			189

A test of homogeneity between the values in Table 2 gives a highly significant value of χ^2 of 102.3. The weighted mean potency is 1.022. Omission of the extremely high values obtained by Laboratory 5 considerably reduces χ^2 to 22.2, but makes little difference to the mean potency, which becomes 1.020 with 5% limits of 1.015–1.024.

Table 3 shows the result of assays made by physical and chemical methods. The unweighted mean potency obtained by these methods is 1.018.

It is evident from Tables 2 and 3 that, with the exception of Laboratory 5, there is good agreement that the activity of the Proposed International Standard is approximately 1%–3% greater than that of the Reference Preparation. Since Laboratory 5 used the same solutions for all its 27 assays, the possibility cannot be excluded that there may have been some unsuspected error—possibly in weighing or dilution.

As may be seen from the figure, the skewed nature of the distribution curve of the log potencies is due to the contributions from this laboratory,

FREQUENCY DISTRIBUTION OF LOG POTENCIES DERIVED FROM 154 BIOLOGICAL ASSAYS

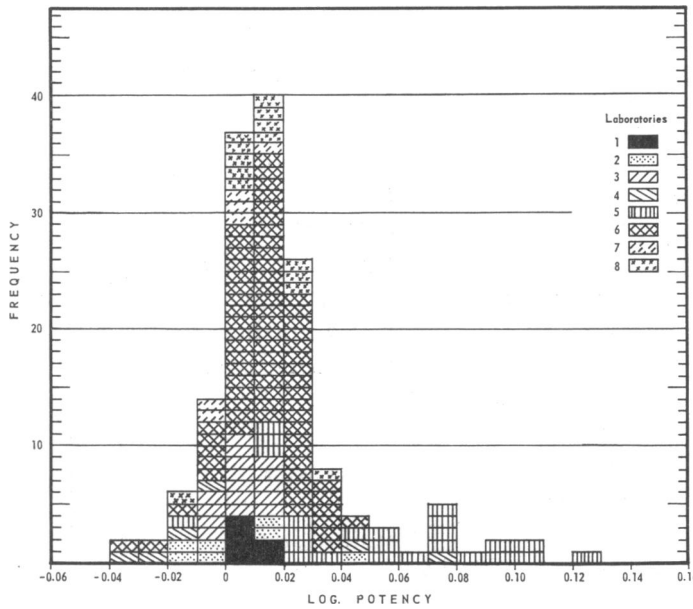

TABLE 2. MEAN POTENCIES OF THE PROPOSED INTERNATIONAL STANDARD, IN TERMS OF THE REFERENCE PREPARATION, OBTAINED IN DIFFERENT LABORATORIES BY BIOLOGICAL METHODS

Laboratory No.	Number of assays	Potency	Weight
1	6	1.016	225 253
2	7	1.009	14 479
3	8	1.011	227 213
	8	1.016	204 902
4	6	1.018	3 436
5	12	1.076	14 880
	12	1.201	14 446
6	75	1.033	379 993
7	6	1.004	121 840
8	14	1.028	72 559
Total or mean	154	1.022	1 279 001

TABLE 3. POTENCIES OBTAINED BY PHYSICAL AND CHEMICAL METHODS

Laboratory No.	Method	Number of assays	Potency
1	Iodometric	3	1.015
4	Spectrophotometric	2	1.032
5	Spectrophotometric	2	1.060
	Iodometric	1	1.138
6	Iodometric	5	1.015
7	Iodometric	8	0.995
8	Iodometric	14	1.016
Total or mean		35	1.018

and the over-all potency of 104% obtained from the mean of the distribution is almost certainly an over-estimate of the true value. This value is reduced to 102.6% if Laboratory 5 is excluded. It is proposed, therefore, that the results of Laboratory 5 be disregarded for the purposes of this assay. If this is done, the Proposed Standard is found to be 2% more potent than the Reference Preparation, a value based on 130 bio-assays from seven laboratories.

The International Unit

The Reference Preparation had already been assigned a unitage of 1695 units/mg by the United States Food and Drug Administration. This value was based on the assumption that the unit of phenoxymethylpenicillin should, and could, be linked with that of benzylpenicillin. Since the activity of the International Standard for Penicillin (which is practically pure sodium benzylpenicillin, M.W. 356.4) is 1670 IU/mg (Humphrey et al., 1953), that of pure phenoxymethylpenicillin (free acid, M.W. 350.4) would be 1699 IU/mg. The value assigned to the United States Reference Preparation, i.e., 1695 u/mg, was, however, based on pure sodium benzylpenicillin having a potency of 1667 IU/mg, which was the potency of the first International Standard for Penicillin.

The argument on which this unit for phenoxymethylpenicillin is based presupposes that, for practical purposes, the activities of phenoxymethylpenicillin and of benzylpenicillin will be equal molecule for molecule. Although this may be true for some micro-organisms, it is not always so, and the very fact that separate standards have been found necessary (Goodey et al., 1955) underlines the difference between the two. It seemed to the authors to be most logical to define a unit for phenoxymethylpenicillin

which was quite distinct, and not liable to confusion with that for benzylpenicillin. Unlike benzylpenicillin, whose unit was defined before the substance was available in practically pure form, phenoxymethylpenicillin was available in a very pure form and its constitution known *ab initio*. It was proposed, therefore, that the International Unit should be defined along the lines followed recently with other highly purified antibiotics, so that 1 unit of activity would be contained in 1 μg of "pure" phenoxymethylpenicillin.

This proposal was considered by the Expert Committee on Biological Standardization in 1957 (World Health Organization, 1958), which, however, decided that the basis of the existing FDA unit should be adhered to. It is customary in setting up an International Standard to give precedence to to any existing unit if one has been established. The US Food and Drug Administration favoured the retention of the basis of their existing unit so that the two forms of penicillin could continue to be prescribed clinically on the same level, as has been the practice in the USA.

In terms of the Reference Standard used in the collaborative assay the International Standard would have a potency of 1729 IU/mg. Since, however, the basis of the FDA unit was that pure phenoxymethylpenicillin (free acid) should have a potency of 1695 u/mg, the collaborative assay was interpreted as showing the FDA standard to be only 98% pure. The US standard was therefore devalued 2% by the Food and Drug Administration and the International Standard was established by the Expert Committee on Biological Standardization with a potency of 1695 IU mg (World Health Organization, 1958). The International Unit for phenoxymethylpenicillin is therefore defined as the activity contained in 0.000590 mg of the International Standard.

Annex

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POLAND	Professor Dr T. Korzybski and Dr Mgr I. Dzierzynska Department of Antibiotic Control State Institute of Hygiene Warsaw
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	Mr R. Goodey and Mr K. Galloway Distillers Co. (Biochemicals) Ltd. Bromborough Research Station Great Burgh, Epsom, Surrey
	Mr J. G. Chattwood and Miss Dorothy Baron Distillers Co. (Biochemicals) Ltd. Speke, Liverpool
	Dr J. H. Humphrey and Mr J. W. Lightbown National Institute for Medical Research London
UNITED STATES OF AMERICA	Dr D. C. Grove and Dr William W. Wright Division of Antibiotics Food and Drug Administration Washington, D. C.

RÉSUMÉ

Un lot de phénoxyméthylpénicilline hautement purifiée a été examiné par huit laboratoires de sept pays, et comparé avec l'étalon de phénoxyméthylpénicilline du Food and Drug Administration (FDA) (Etats-Unis) en vue d'établir un Etalon international de ce produit. Les laboratoires sollicités effectuèrent 189 essais dont 154 biologiques. Il apparut que l'étalon proposé était de 2% plus pur que la Préparation de référence. On avait assigné à celle-ci une valeur unitaire qui correspondait à 1695 unités par mg, ceci en supposant que l'unité de phénoxyméthylpénicilline pouvait être assimilée à celle de la benzylpénicilline, mais il apparut qu'il serait plus logique de définir une unité pour chacune des pénicillines car leur activité n'est pas forcément égale molécule pour molécule. La phénoxyméthylpénicilline pouvant être obtenue à l'état très pur, il a été proposé que, comme pour les autres antibiotiques hautement purifiés, une unité corresponde à l'activité d'un μg de phénoxyméthylpénicilline pure. Mais la Food and Drug Administration ayant préféré garder la valeur unitaire déjà définie, on a dévalué l'étalon FDA de 2% et maintenu la valeur unitaire de l'Etalon international à 1695 UI par mg. L'Unité internationale de Phénoxyméthylpénicilline correspond donc à l'activité de 0,000590 mg de l'Etalon international.

REFERENCES

Goodey, R., Reed, K. N. & Stephens, J. (1955) *J. Pharm. (Lond.)*, **7**, 692

Humphrey, J. H., Mussett, M. V. & Perry, W. L. M. (1953) *Bull. Wld Hlth Org.*, **9**, 15

Humphrey, J. H., Lightbown, J. W. & Mussett, M. V. (1957) *Bull. Wld Hlth Org.*, **17**, 527

Parker, G., Cox, R. J. & Richards, D. (1955) *J. Pharm. (Lond.)*, **7**, 683

Stephens, J. & Grainger, A. (1955) *J. Pharm. (Lond.)*, **7**, 702

World Health Organization, Expert Committee on Biological Standardization (1957) *Wld Hlth Org. techn. Rep. Ser.*, **127**, 14

World Health Organization, Expert Committee on Biological Standardization (1958) *Wld Hlth Org. techn. Rep. Ser.*, **147**, 7