This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

No. 530

WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-fifth Report

WORLD HEALTH ORGANIZATION

GENEVA

1973

PRINTED IN SWITZERLAND

CONTENTS

PHARMACOLOGICAL SUBSTANCES

Antibiotics Page

1. Doxycycline 5
2. Minocycline 6
3. Neomycin 6
4. Gramicidin S 6

Hormones, vitamins, enzymes, and miscellaneous substances

5. Glucagon 6
6. Heparin 7
7. Vitamin D 7
8. Sulfarsphenamine, neoarsphenamine, oxophenarsine 8
9. Mel B (melarsoprol), dimercaprol, MSb 8
10. Thrombin 8
11. Opacity Reference Preparation 9

IMMUNOLOGICAL SUBSTANCES

Antigens

12. Diphtheria toxoid, plain 9
13. Yellow fever vaccine 10
14. Cholera vaccine 10

Antibodies

15. Human immunoglobulin IgE 10
16. Diphtheria antitoxin for flocculation test 11
17. Gas-gangrene antitoxins 11
18. Gas-gangrene antitoxin (*Clostridium histolyticum*) 12
19. Anti-*Salmonella pullorum* sera 12

REQUIREMENTS FOR BIOLOGICAL SUBSTANCES

20. Requirements for inactivated influenza vaccine 12
21. Requirements for cholera vaccine 13
22. Requirements for rabies vaccine for human use 13
23. General requirements for the sterility of biological substances 13

ANNEXES

Annex 1. Requirements for inactivated influenza vaccine (Addendum 1973) . . . 15
Annex 2. Requirements for cholera vaccine (Revised 1968) (Addendum 1973) . . 18
Annex 3. Requirements for rabies vaccine for human use 22
Annex 4. General requirements for the sterility of biological substances (Revised 1973) 40
Annex 5. Requirements for biological substances and other sets of recommendations 62
Annex 6. International standards and international reference preparations 64

INDEX 66

WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 24–30 April 1973

Members :

Dr H. Cohen, Director, National Institute of Public Health, Bilthoven, Netherlands

Dr J. Desbordes, Director, Microbiology Section, National Public Health Laboratory, Paris, France (*Vice-Chairman*)

Professor S. G. Dzagurov, Director, Tarasevič State Institute for the Standardization and Control of Biological Preparations, Moscow, USSR

Professor D. G. Evans, Director, National Institute for Biological Standards and Control, London, England (*Chairman*)

Dr Sutas Guptarak, Chief, Biological Division, The Government Pharmaceutical Organization, Bangkok, Thailand

Dr P. Krag, Director, Department of Biological Standardization, Statens Serum-institut, Copenhagen, Denmark

Dr H. Mirchamsy, Associate Director, Razi State Institute for Serum and Vaccine Production, Teheran, Iran

Dr R. Murata, Director, The Second Department of Bacteriology, National Institute of Health, Tokyo, Japan

Dr E. B. Seligmann, Jr, Director, Division of Control Activities, Bureau of Biologics, Food and Drug Administration, Rockville, Md., USA

Dr M. Yu, Senior Pathologist, Department of Pathology, Singapore (*Rapporteur*)

Secretariat :

Dr A. S. Outschoorn, Chief Medical Officer, Biological Standardization, WHO, Geneva, Switzerland (*Secretary*)

WHO EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Twenty-fifth Report

The WHO Expert Committee on Biological Standardization met in Geneva from 24 to 30 April 1973. Dr T. A. Lambo, Assistant Director-General, opened the Meeting on behalf of the Director-General. He welcomed the Members of the Committee and thanked them for coming to Geneva to participate in this Meeting. He drew attention to the long history of biological standardization, an activity inherited by WHO from the League of Nations Health Organization and the Interim Commission. The WHO Expert Committees on Biological Standardization continue the work carried out previously by the Permanent Commission on Biological Standardization of the League and it was interesting to note that in the year of WHO's 25th anniversary there was also convened the 25th Expert Committee on Biological Standardization. He was sure that the discussions of this Committee would be in keeping with the high standards and traditions maintained over the years.

PHARMACOLOGICAL SUBSTANCES

ANTIBIOTICS

1. Doxycycline

In regard to the authorization in the twenty-fourth report[1] for the establishment of the preparation studied as the international reference preparation of doxycycline the Committee was informed that it had not yet been possible to establish this preparation. The Committee therefore endorsed the previous authorization for the National Institute for Biological Standards and Control, London, to establish the material as the International Reference Preparation of Doxycycline on the basis of the results of the collaborative assay and to define the international unit with the agreement of the participants.

---

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 9.

2. Minocycline

The Committee was informed that the limited collaborative assay of the preparation of minocycline referred to in the twenty-fourth report [1] with a view to the establishment of an international reference preparation was in progress.

3. Neomycin

The Committee noted [2] that, in accordance with the request in the twenty-third report,[3] the National Institute for Biological Standards and Control, London, had obtained a preparation of neomycin that was more representative of preparations of neomycin in use throughout the world than the existing reference preparation. A collaborative assay had been carried out and the results were being analysed.

The Committee authorized the National Institute for Biological Standards and Control to establish the material studied as the second International Reference Preparation of Neomycin and to define the international unit on the basis of the results of the collaborative assay with the agreement of the participants.

4. Gramicidin S

Since replacement of the International Reference Preparation of Gramicidin S was no longer necessary because this antibiotic could be adequately characterized by chemical and physical means, the Committee discontinued this international reference preparation.

The Committee was informed that, in accordance with the request in the twenty-fourth report,[4] the WHO Secretariat was investigating the possibility of providing this antibiotic in the form of a suitable chemical reference substance.

HORMONES, VITAMINS, ENZYMES, AND MISCELLANEOUS SUBSTANCES

5. Glucagon

The Committee noted [5] that the collaborative assay of the proposed international standard for glucagon, referred to in the twenty-fourth

---

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 10.

[2] Unpublished working document WHO/BS/73.1063.

[3] *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 463, p. 11.

[4] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 9.

[5] Unpublished working document WHO/BS/73.1064.

report,[1] had been completed. The result had shown that the material was suitable to serve as the international standard and the Committee therefore established this material as the International Standard for Glucagon, Porcine, for Bioassay. The Committee also noted [2] a proposal for the definition of the international unit, which was equivalent to the only known existing national unit and which was acceptable to the participants. The Committee agreed with this proposal and defined the International Unit for Glucagon, Porcine, for Bioassay as the activity contained in 4.5302 mg of the International Standard for Glucagon, Porcine, for Bioassay.

6. Heparin

The Committee noted [3] that stocks of the second International Standard for Heparin were nearly exhausted and agreed that it should be replaced. A preparation of heparin of porcine mucosal origin had been examined in a collaborative study of heparins from different sources [4] and found suitable to replace the current International Standard when this became necessary. The Committee agreed that this preparation could serve as the replacement without the necessity of a further collaborative assay. The Committee therefore established the material as the third International Standard for Heparin in replacement of the second international standard. The Committee also noted [3] a proposal on which to base the unitage assigned to the preparation from the results of the collaborative study. The Committee was informed that this was acceptable to the participants and therefore defined the International Unit for Heparin as the activity contained in 0.005766 mg of the third International Standard for Heparin.

7. Vitamin D

The Committee considered further the question of the discontinuation of the International Standard for Vitamin D discussed in the twenty-fourth report.[1] In response to the request in that report,[5] the WHO Secretariat had investigated the possibility of making vitamin D available as a chemical reference substance and action was being taken with a view to providing such a reference substance. As it would be advisable also to have available for international use a well characterized, stable preparation of vitamin D that could be used for biological assays, the Committee agreed that the current International Standard should not be discontinued for the present.

---

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 11.

[2] Unpublished working document WHO/BS/73.1064.

[3] Unpublished working document WHO/BS/73.1065.

[4] *Bull. Wld Hlth Org.*, 1970, **42**, 129.

[5] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 12.

8. Sulfarsphenamine, Neoarsphenamine, Oxophenarsine

The Committee was informed that, in response to the request in the twenty-fourth report,[1] the WHO Secretariat had ascertained that there was little or no use of the substances sulfarsphenamine, neoarsphenamine and oxophenarsine in clinical medicine and hence no need for reference materials. The Committee therefore discontinued the international reference preparations of these substances.

9. Mel B (Melarsoprol), Dimercaprol, MSb

The Committee noted[2] that, in response to the request in the twenty-fourth report,[1] the WHO Secretariat had ascertained that there was a need for reference material of Mel B (Melarsoprol) and of Dimercaprol for use in toxicity tests. The Committee agreed therefore that it was advisable to retain the International Reference Preparations of these two substances.

The WHO Secretariat had also ascertained that there was no interest at present in the use of MSb. The Committee therefore discontinued this international reference preparation.

In view, however, of current interest in research on trypanocidal drugs as well as the possibility that there may be further work on the use of antimonial compounds in filariasis, the Committee agreed that remaining stocks of the discontinued preparation should be kept for possible use in such studies.

10. Thrombin

The Committee noted[3] that studies of certain components of the blood coagulation and fibrinolytic systems, referred to in the eighteenth[4] and nineteenth[5] reports, had been continued and that a collaborative study of a preparation of human thrombin had been made with a view to assessing its suitability for the assay of human and bovine thrombin preparations. In this study 5 preparations of thrombin, and a common substrate of freeze-dried fibrinogen were provided. Various kinds of local substrates were also included. The results showed that the preparation studied was suitable for the assay of both human and bovine thrombin preparations. There was, however, some indication that there were significant differences in the potency estimates obtained with different species of the substrate fibrinogen.

---

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 486, p. 12.
[2] Unpublished working document WHO/BS/73.1068.
[3] Unpublished working document WHO/BS/73.1069.
[4] *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 329, p. 11.
[5] *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 361, p. 13.