Bull. Org. mond. Santé | 1956, **14**, 543-555
Bull. Wld Hlth Org. |

THE SECOND INTERNATIONAL STANDARD FOR CORTICOTROPHIN

M. V. MUSSETT, B.Sc.
Department of Biological Standards, National Institute for Medical Research, London
W. L. M. PERRY, M.D.
Director, Department of Biological Standards, National Institute for Medical Research, London

Manuscript received in November 1955

SYNOPSIS

The authors describe the steps taken to establish the Second International Standard for Corticotrophin and discuss the results of the collaborative assay in which it was compared with the First International Standard. Sixty-one assays of the blended material from two batches of crude corticotrophin prepared by the Astwood procedure, but not subjected to oxycellulose purification, were carried out by 12 laboratories in 5 countries. Almost exclusively, the assay methods used were the thymus involution method and the subcutaneous and intravenous Sayers methods. It was estimated from the statistical analysis of the results that the new standard is 1.14 times as potent as the existing standard, and the International Unit has therefore been re-defined as 0.88 mg of the new standard.

Preparation of Proposed New Standard

The First International Standard for Adrenocorticotrophic Hormone (ACTH: Corticotrophin) was established by the WHO Expert Committee on Biological Standardization at its fourth session, held in Geneva in 1950.[10] The material used for this standard was known as " La-I-A " and had been widely used by laboratories throughout the world, although it was in fact the working standard of one particular manufacturing firm. The International Unit was defined as the activity contained in 1 mg of the International Standard and this unit was the same as the United States Pharmacopeia (USP) unit.

Since the establishment of the International Standard in 1950, a great deal of knowledge has been gained about the chemistry of corticotrophin; even in 1950 the WHO Expert Committee on Biological Standardization realized that a new standard would soon be required and authorized an immediate start on the search for suitable preparations. Four contributions were collected for this purpose—two from the United Kingdom of Great Britain and Northern Ireland, one from the Netherlands, and one from the

USA—and in 1951, at its fifth session,[11] the WHO Expert Committee on Biological Standardization decided to blend these four batches to form the new standard. At this stage, however, the possibility arose of using an oxycellulose-purified preparation as the new standard, and, since early reports suggested that material of a potency of up to 80 units per mg could be obtained by such purification, this idea had much to commend it.

The Committee decided, however, at its sixth session,[12] in 1952, that the new standard should be a preparation similar to the First International Standard and confirmed its earlier decision to blend the four contributions without oxycellulose purification for this purpose; it was further agreed, however, that a part of this blend should be oxycellulose-purified and used experimentally for comparison with the crude material.

It became apparent, however, during 1952 and 1953 that there were two difficulties in this course; first, that only material extracted by the Astwood [1] process could be satisfactorily purified in this way, and, secondly, that clinically the purified material gave different results from the crude preparations, unit for unit.[9] Nevertheless, the blends were made and, despite the fact that some of the four batches of material had not been extracted by the Astwood process, the oxycellulose purification was undertaken and a final purified blend containing some 20 units per mg was obtained.[a]

The discrepancy between the unitage of purified samples assayed by the intravenous Sayers technique and their clinical effect led, however, to doubts about the nature of the crude samples themselves; and new knowledge of the chemistry of corticotrophin[8] suggested that the nature of the crude material prepared by the Astwood method was different from that of crude extracts subjected to peptic hydrolysis. It was considered undesirable to mix materials of both types and, consequently, the plan to use the blend of the four original samples was reluctantly abandoned.

Two new contributions of crude material prepared by the Astwood method were offered by manufacturing firms in the USA, and these offers were gratefully accepted. The advantages of following this course were:

(*a*) that the new standard would consist solely of one chemical type of corticotrophin;

(*b*) that since no oxycellulose purification was to occur, no " enhancement " of clinical effectiveness was envisaged;

(*c*) that the new standard would have approximately the same potency as the existing standard, so that comparison by international collaborative assay might be expected to be relatively simple and to yield valid results by all methods of assay;

(*d*) that the material to be used for the new standard could be easily and quickly dispensed—a factor of considerable importance in view of the shortage of stocks of the existing standard.

[a] The oxycellulose purification was kindly undertaken by Dr E. B. Astwood.

The two batches were separately tested for their suitability as contributions to the new standard and were then mixed in a ball-mill. The bulk mixture was dispensed in ampoules, each containing approximately 28 mg; the material was filled at constant temperature and humidity and there was no desiccation of the contents of the ampoules. The moisture content has not been estimated; the material is not hygroscopic. A total of rather more than 8000 ampoules was obtained. Of these, 5500 have been returned to the United States Pharmacopeia, New York, to serve as the USP Standard, and the remainder is stored at the National Institute for Medical Research, London, at -10^{o}C.

Supplies of the proposed new standard and of the existing standard were sent to 12 laboratories in 5 different countries. The supplies of material were accompanied by a memorandum describing them and making suggestions as to the type of assay that should be carried out. A copy of this memorandum is given as Annex 1 to this report. The list of participants is given as Annex 2 to this report, throughout which participating laboratories are referred to by a number only.

Results of Collaborative Assay

Results were received from all 12 participating laboratories. Table I lists the numbers and types of assays carried out by the different laboratories.

TABLE I. NUMBERS AND TYPES OF ASSAYS FROM EACH LABORATORY

Laboratory No.	Thymus	Sayers (subcutaneous)	Sayers (intravenous)
1	0	3	2
2	5	0	0
3	1	1	2
4	0	2	2
5	0	2	2
6	6	0	1
7	0	2	2
8	0	2	4
9	1	0	1
10	2	0	0
11	0	4	4
12	0	3	7
Total	15	19	27

A total of 61 assays were done. It will be seen that each of the three suggested methods was used by a number of laboratories. The only additional method employed was one based on melanophore stimulation, carried out by Laboratory 9. Two such assays were performed and the potency ratio of the proposed standard in terms of the existing standard was stated to be about 8.0. This was a value so discrepant from the results obtained by other methods that no data were sent and no analysis was carried out.

Of the assays listed in Table I, one thymus test, and one subcutaneous and two intravenous Sayers tests were invalid owing to there being such a degree of non-linearity of the dosage-response lines that no analysis, even of a modified kind, was possible. The results of the various types of assay are given in Tables II, III, and IV.

Thymus involution assays

Table II lists the assays done by the thymus involution method. Laboratories 2 and 6 both obtained a series of estimates of potency which were

TABLE II. THYMUS INVOLUTION METHOD

Laboratory No.	Number of assays	Potency	Weight	Dose levels	Animals	Validity	Homogeneity	
							χ^2	P
2	5	1.11	2 324	3+3	nestling rats litter mates	3 valid; 2 curvature reduced to (2+2)	20.43	<0.001
3	1	0.52	152	3+3	weanling rats	valid	—	—
6	1	1.31	2 214	2+2	nestling rats litter mates	valid	13.17	0.02
	5			3+3	4 nestling; 1 weanling litter mates	2 valid; 3 curvature reduced to (2+2)		
9	1	—	—	3+3	nestling rats	curvature and departure from parallelism	—	—
10	2	0.91	83	3+3	male albino mice	1 valid; 1 with departure from parallelism reduced to (2+2)	2.46	>0.10

The results obtained by 4 laboratories are heterogeneous : $\chi^2 = 26.86$ P<0.001
***Omitting Laboratory* 3: $\chi^2 = 7.21$ P = 0.02-0.05**
Weighted mean potency 1.201 Total weight 4621
5% limits 1.12-1.28, i.e., 93.5%-107.0% of potency
The estimate of potency (and limits) is based on 11 assays.

heterogeneous; in both cases one aberrant result was the cause of this heterogeneity and the aberrant result was obtained in an assay which, when analysed as a full (3 + 3) dose test, showed significant curvature of the dosage-response lines, and which was consequently analysed only as a (2 + 2) dose test. The omission of the single aberrant result in each case eliminated the heterogeneity. The omissions were made before the calculation of the potencies and weights shown in Table II. The one assay carried out by Laboratory 9 was invalid owing to curvature and non-parallelism of the dosage-response lines.

The weighted mean values for potency obtained in the remaining laboratories showed significant heterogeneity, which was almost wholly attributable to the single assay carried out by Laboratory 3. The 11 estimates of potency from the remaining assays, carried out in 3 laboratories, were tested for heterogeneity. The value of χ^2 was not significant at the 1% level and was just significant at the 5% level. The weighted mean potency from these 11 assays was 1.201.

TABLE III. SAYERS SUBCUTANEOUS METHOD

Laboratory No.	Number of assays	Potency	Weight	Dose levels	Validity	Homogeneity	
						χ^2	P
1	3	1.27	604	3+3	all valid	3.66	>0.10
3	1	0.80	168	2+3	valid	—	—
4	2	1.34	157	3+3	1 valid; 1 with significant curvature reduced to (2+2)	7.15	<0.01
5	2	1.93	38	3+3	both valid	0.11	>0.70
7	2	1.46	355	3+3	1 valid; 1 with curvature reduced to (2+2)	1.03	>0.30
8	2	0.94	59	3+3	both valid	0.004	>0.95
11	4	1.57	147	3+3	3 assays reduced to (2+2) since no difference between response to 2 doses	9.04	<0.05
12	3	2.94	79	4+4	2 valid; 1 with significant curvature omitted	3.53	>0.05

The estimates of potency from 8 laboratories are heterogeneous : $\chi^2 = 21.66$ $P<0.01$
The heterogeneity is caused by the very low value obtained by Laboratory 3 and the high value by Laboratory 12.
Omitting Laboratories 3 and 12: **$\chi^2 = 3.80$ $P = 0.5-0.7$**
Weighted mean potency 1.356 Total weight 1360
5% limits 1.20-1.54, i.e., 88.2%-113.3% of potency
The final estimate of potency is based on 14 assays.

Subcutaneous Sayers assays

Table III lists the assays done by the subcutaneous Sayers method. One assay of the two carried out by Laboratory 4 showed significant curvature of the dosage-response lines; furthermore, the two estimates were very heterogeneous and consequently it was decided to omit, from the weighted mean potency, the assay showing curvilinear dosage-response lines. One assay from Laboratory 12 was also omitted owing to curvature. The four assays carried out by Laboratory 11 were heterogeneous, and since this was not due to any one particular assay, the weight attaching to each was reduced sufficiently to make χ^2 insignificant. This procedure was used previously in the collaborative assay of the International Standard for Thyrotrophin;[6] an alternative method is to use semi-weights, as described by Bliss.[2]

The weighted mean estimates of potency obtained in the 8 laboratories were heterogeneous and this is attributable to the very low value obtained in the assay done by Laboratory 3, and to the very high value obtained in the two assays by Laboratory 12. Omission of these results gave a weighted mean potency, based on 14 assays, of 1.356.

Intravenous Sayers assays

Table IV lists the assays done by the intravenous Sayers method. Two assays from Laboratory 12 were invalid owing to non-linearity of the dosage-response lines and were omitted. One assay by Laboratory 7 showed significant curvature; it was then calculated as a (2 + 2) dose assay, but was finally omitted from the weighted mean potency since the estimate of potency was significantly different from the other estimate obtained by the same laboratory. In no other laboratory was there significant heterogeneity between the estimates of potency obtained in different assays. Furthermore, the mean estimates of potency, obtained in 10 laboratories, when tested for heterogeneity, gave a value of χ^2 which was only just significant at the 5% level and was not significant at the 1% level. The weighted mean potency, based on 24 assays, was 1.009.

Combination of results

The final estimates of the potency of the proposed new standard in terms of the existing standard have been made in three different ways.

1. *Weighted mean potency.* Rejecting the heterogeneous estimates referred to earlier, the weighted mean potencies for each method of assay and for all assays are shown, with limits of error, in Table V. The weighted mean potency is calculated from the equation:

$$\bar{M} = \frac{\Sigma W M}{\Sigma W}, \text{ where } W = \frac{1}{V_M}$$

TABLE IV. SAYERS INTRAVENOUS METHOD

Laboratory No.	Number of assays	Potency	Weight	Dose levels	Validity	Homogeneity	
						χ^2	P
1	2	1.23	243	3+3	both valid	0.02	>0.80
3	2	0.98	273	3+2	both valid	0.001	>0.95
4	2	1.10	462	3+3	1 valid; 1 with significant curvature reduced to (2+2)	1.77	>0.10
5	2	0.71	114	3+3	1 assay reduced to (2+2) since no difference between response to 2 doses	0.24	>0.50
6	1	1.04	89	2+2	valid	—	—
7	2	1.74	96	3+3	1 valid; 1 with significant curvature reduced to (2+2)	9.30	<0.01
8	4	1.18	233	3+3	1 assay reduced to (2+2) since no difference between response to 2 doses	1.35	>0.70
9	1	0.76	118	4+4	valid	—	—
11	4	1.03	533	3+3	all valid	3.90	>0.20
12	7	0.77	389	4+4	3 with significant curvature: 2 of these omitted, 1 reduced to (2+2)	4.76	>0.30

Estimates of potency from 10 laboratories are only slightly heterogeneous : χ^2=18.98 P<0.05
Weighted mean potency 1.009 Total weight 2550
5% limits 0.92-1.11, i.e., 91.3%-109.6% of potency
The final estimate of potency is based on 24 assays.

TABLE V. WEIGHTED MEAN POTENCY

Method	Number of estimates	Potency	Weight	Limits	Percentage limits
Thymus	11	1.201	4 621	1.12-1.28	93.5%-107.0%
Sayers (subcutaneous)	14	1.356	1 360	1.20-1.54	88.2%-113.3%
Sayers (intravenous)	24	1.009	2 550	0.92-1.11	91.3%-109.6%
Total	49	1.162	8 531	1.11-1.22	95.2%-105.0%

χ^2 = 16.66 degrees of freedom = 2 P < 0.001

There is a highly significant heterogeneity between the mean potencies estimated by the three different methods of assay (χ^2 is 16.66 with 2 degrees of freedom). The final figure of 1.162 has limits of error (95.2%-105.0%) which are almost certainly underestimated.

2. *Weighted mean potency (disregarding all heterogeneity).* If it is decided to ignore all heterogeneity and to include 57 instead of 49 assays, the results shown in Table VI, and a final estimate of potency of 1.103, are obtained.

TABLE VI. WEIGHTED MEAN POTENCY DISREGARDING HETEROGENEITY

Method	Number of estimates	Potency	Weight	Limits	Percentage limits
Thymus	14	1.132	5 522	1.06-1.20	94.0%-106.4%
Sayers (subcutaneous)	18	1.226	1 895	1.10-1.36	90.0%-111.2%
Sayers (intravenous)	25	0.978	2 882	0.90-1.07	91.8%-109.0%
Total	57	1.103	10 299	1.05-1.15	95.7%-104.6%

3. *Unweighted mean potency.* If one simply takes the arithmetic mean of all the 57 logarithms of the potency ratio (geometric mean), a mean potency ratio of 1.141 (Table VII) is obtained. The only estimate of error which can be calculated in respect of this value is that obtained directly by calculating the standard error of the distribution of individual log potencies. This discounts entirely the internal evidence of error of the assays.

Estimates of error by direct means and by estimation of fiducial limits (based on internal evidence) should be the same. The fact that the limits

TABLE VII. GEOMETRIC MEAN POTENCY AND DIRECT ESTIMATE OF ERROR

Method	Number of estimates	Potency	Limits	Percentage limits
Thymus	14	1.063	0.86-1.31	81.1%-123.4%
Sayers (subcutaneous)	18	1.503	1.16-1.94	77.3%-129.4%
Sayers (intravenous)	25	0.973	0.86-1.10	88.5%-113.0%
Total	57	1.141	1.01-1.29	88.5%-113.0%

are wider by the direct method probably reflects the heterogeneity of the estimates obtained by the different methods of assay and by the different laboratories doing the assays.

Relative accuracy of methods of assay

The average weight contributed by one animal in each of the types of assay is given in Table VIII. It will be seen that the thymus test is some four times as efficient as the Sayers test when judged by this criterion.[4] On the other hand, if the mean weight per assay is used as the criterion of efficiency, one finds that for a thymus test it is 453 and for a Sayers test it is 111.

TABLE VIII. WEIGHT PER ANIMAL

Method	Number of animals	Total weight	Weight per animal
Thymus (rats only)	522	5 438	10.42
Sayers (subcutaneous)	736	1 895	2.57
Sayers (intravenous)	1 101	2 882	2.62

Conclusions

The estimated potency of the proposed new International Standard for Corticotrophin is:

Weighted mean potency 1.162
Weighted mean potency (ignoring heterogeneity) 1.103
Unweighted mean potency 1.141

These various estimates are all very similar. It is proposed that a figure of 1.14 be accepted as the final value. This implies that the new standard is 1.14 times as potent as the existing standard. Since one unit is at present defined as 1 mg of the International Standard, the unit will become 1/1.14 mg of the new standard; and rounding off the last place of decimals this gives a value of 0.88 mg for one International Unit.

The material proposed for the new International Standard for Corticotrophin, as subjected to the collaborative assay reported here, is therefore established as the International Standard for Corticotrophin and the International Unit is re-defined as 0.88 mg of the standard preparation.

RÉSUMÉ

Les auteurs exposent les diverses étapes du travail entrepris pour établir le deuxième étalon international de corticotrophine et l'unité internationale correspondante.

Un mélange de deux échantillons de la substance brute, du même type chimique de corticotrophine, non soumis à la purification par l'oxycellulose, préparé par la méthode d'extraction d'Astwood et provenant du même pays, a été choisi comme matériel constituant le deuxième étalon de corticotrophine. Un essai antérieur, dâns lequel on avait utilisé quatre échantillons provenant de trois pays, n'ayant pas été préparés par la même méthode d'extraction, avait dû être abandonné, en raison des nombreuses différences entre les échantillons constituant le mélange.

L'échantillon choisi comme deuxième étalon de corticotrophine a été soumis à un titrage d'activité dans 12 laboratoires de 5 pays. Les tests d'involution du thymus et les tests sous-cutané et intraveineux de Sayers ont été adoptés par les laboratoires participant au titrage. L'analyse statistique des résultats de l'essai d'activité de cet échantillon indique que le deuxième étalon de corticotrophine est 1,14 fois plus actif que le premier étalon. L'unité internationale de corticotrophine correspond actuellement à 0,88 mg de l'étalon international.

REFERENCES

1. Astwood, E. B. et al. (1951) *J. Amer. chem. Soc.* **73**, 2969
2. Bliss, C. I. (1952) *Statistics of bioassay with special reference to the vitamins*, New York, p. 580
3. Bruce, H. M., Parkes, A. S. & Perry, W. L. M. (1952) *Lancet*, **1**, 790
4. Miles, A. A. & Perry, W. L. M. (1950) *Bull. Wld Hlth Org.* **2**, 655
5. Munson, P. L., Barry, A. G. & Koch, F. C. (1948) *J. clin. Endocr.* **8**, 586
6. Mussett, M. V. & Perry, W. L. M. (1955) *Bull. Wld Hlth Org.* **13**, 917
7. Sayers, M. A., Sayers, G. & Woodbury, L. A. (1948) *Endocrinology*, **42**, 379
8. Thompson, R. E. & Fisher, J. D. (1953) *Endocrinology*, **52**, 496
9. Wolfson, W. Q. & Robinson, W. D. (1952) *Fed. Proc.* **11**, 623
10. World Health Organization, Expert Committee on Biological Standardization (1951) *Wld Hlth Org. techn. Rep. Ser.* **36**, 7
11. World Health Organization, Expert Committee on Biological Standardization (1952) *Wld Hlth Org. techn. Rep. Ser.* **56**, 9
12. World Health Organization, Expert Committee on Biological Standardization (1953) *Wld Hlth Org. techn. Rep. Ser.* **68**, 13

Annex 1

MEMORANDUM TO PARTICIPANTS IN THE COLLABORATIVE ASSAY OF THE PROPOSED SECOND INTERNATIONAL STANDARD FOR CORTICOTROPHIN

At its fifth session, held in Geneva in 1951,[11] the WHO Expert Committee on Biological Standardization noted that the stock of the current (First) International Standard for Adrenocorticotrophic Hormone was running low, and authorized the Department of Biological Standards, National Institute for Medical Research, London, in consultation with the United States Pharmacopeia, to proceed with the establishment of the Second International Standard for this substance.

After consultation, it was decided that, in the light of recent knowledge, the new standard should be made of crude corticotrophin prepared by the Astwood procedure, but not subjected to oxycellulose purification. This type of standard has obvious advantages whilst controversy as to the chemistry of corticotrophin or the corticotrophins still continues. Thus:

(1) the proposed new standard consists solely of one " type " of corticotrophin;

(2) the new standard when assayed against the existing standard should yield the same potency ratio whatever method of assay is chosen—i.e., whether assayed subcutaneously or intravenously. Thus, the transfer of the International Unit from the old to the new standard should be simple and should not depend on selecting arbitrarily which route of administration in the assays is the correct one by which to determine the " true " potency ratio;

(3) the new standard should be readily adopted for use in control regulations in Member States of WHO, since all commercial " crude " corticotrophin may be assayed against it by *any* method of assay, and purified corticotrophins may be controlled by insisting on subcutaneous assays and by appropriate labelling;

(4) the new standard, being relatively crude, was easily obtainable and easily and quickly dispensed.

The proposed Second International Standard

Through the generosity of two United States manufacturing firms, two contributions, each consisting of approximately 50 g of crude corticotrophin, prepared by the Astwood method, were obtained. These two contributions were pooled and mixed in a ball-mill in the USA and sent in bulk, on 15 May 1954, to the Department of Biological Standards, where the material was distributed into ampoules, each containing approximately 28 mg. The material was filled at constant temperature and humidity and there was no desiccation of the contents of the ampoules. The moisture content has not been estimated; the material is not hygroscopic.

The existing (First) International Standard

The existing International Standard for Corticotrophin consists of the original Armour laboratory working standard, formerly known as " La-I-A ". It is a crude preparation and by definition contains one International Unit per milligram. Two sizes of vial are available, one containing approximately 1.3 mg and the other approximately 4.8 mg.

The collaborative assay

It is suggested that participants should carry out assays by at least two methods—one a method employing intravenous dosage, and the other a method employing intramuscular or subcutaneous dosage. Any recognized method is suitable provided that it permits the calculation of fiducial limits of error from the internal evidence of the test. The following three methods are in common use, but any other method in routine use in your laboratory would be equally acceptable.

(*a*) *Intravenous dosage.* The adrenal ascorbic acid depletion method of Sayers, Sayers & Woodbury [7] with or without the Munson modification.[5]

(*b*) *Subcutaneous or intramuscular dosage.* The Munson modification of the method of Sayers, Sayers & Woodbury.

(*c*) *Subcutaneous dosage.* The thymus involution test, as described by Bruce, Parkes & Perry [3] or modified for use with weanling rats.

Samples being dispatched

Participants are being sent, under separate cover:

(*a*) 10 vials of the proposed Second International Standard, labelled " ACTH 2 ", each containing approximately 28 mg of material;

(*b*) 5 vials of the existing (First) International Standard, labelled " International Standard for Adrenocorticotrophic Hormone ", each containing approximately 1.3 mg;

(*c*) either 15 or 30 * vials of the First International Standard, labelled " International Standard for Adrenocorticotrophic Hormone ", each containing approximately 4.8 mg.

Reporting of results

All results should be sent direct in their original form to the Department of Biological Standards, National Institute for Medical Research, London, where the over-all statistical analysis will be carried out. In view of the urgency of the matter, participants are asked to send on their results as soon as possible, preferably by 2 October 1954.

* 30 vials are being sent to those participants known to carry out routinely assays on non-hypophysectomized animals.

Annex 2

PARTICIPANTS IN THE COLLABORATIVE ASSAY OF THE PROPOSED SECOND INTERNATIONAL STANDARD FOR CORTICOTROPHIN

AUSTRALIA	Dr K. C. Porter and Mr A. H. Mengoni Commonwealth Serum Laboratories Parkville, N.2 Victoria
DENMARK	Dr C. Hamburger and Dr Eric Thing Statens Seruminstitut Amager Boulevard 80 Copenhagen
NETHERLANDS	Dr J. Lens N. V. Organon Oss
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	Mr R. N. Chapman and Dr J. B. Dekanski The Organon Laboratories Newhouse-by-Motherwell Lanarkshire
	Dr J. I. M. Jones and Mr J. A. Lock The Crookes Laboratories Park Royal London, N.W.10
	Dr D. M. V. Parrott National Institute for Medical Research Mill Hill, London, N.W.7
UNITED STATES OF AMERICA	Dr G. Bond The Upjohn Company Kalamazoo, Mich.
	Dr R. A. Brown and Dr M. Ruth Pascoe Parke, Davis & Company Detroit, Mich.
	Dr J. Curtis Food and Drug Administration Washington 25, D.C.
	Dr S. W. Hier The Wilson Laboratories Chicago 9, Ill.
	Mr P. J. McCall The Armour Laboratories Chicago 9, Ill.
	Dr R. Varney E. R. Squibb & Sons New Brunswick, N.J.