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The International Reference Preparation of Erythropoietin *

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The WHO Expert Committee on Biological Standardization authorized the National Institute for Medical Research to establish an International Reference Preparation of Erythropoietin on the basis of the results of an international collaborative assay and to define an international unit with the agreement of the participants.

The material investigated was a research standard (Erythropoietin Standard B) that had been in widespread use for some time. The preparation of this material and the estimation of its potency in terms of an earlier research standard (Erythropoietin Standard A) are described. By comparisons with other preparations of erythropoietin, it was found that Erythropoietin Standard B is a suitable standard for the bioassay of many preparations of erythropoietin in current use.

On the basis of the information obtained, Erythropoietin Standard B has been established as the International Reference Preparation of Erythropoietin and the International Unit for Erythropoietin has been defined as the activity contained in 1.48 mg of the International Reference Preparation. This amount has equivalent biological activity to the unit of Erythropoietin Standard A and Standard B and thus ensures the continuity of the units already in widespread use.

ERYTHROPOIETIN STANDARD A

The need for a commonly accepted and readily-available standard for the bioassay of erythropoietin was stated by Gordon (1959) and Bangham (1960). In 1961 a research standard, Erythropoietin Standard A, was made from part of a batch of sheep plasma erythropoietin.

The material consisted of an extract of plasma from anaemic sheep prepared by the Armour Pharmaceutical Company in collaboration with Dr L. Jacobson and colleagues and made available by the Hematology Study Section of the National Institutes of Health, Bethesda. The material was distributed in equal portions into 500 ampoules, each containing approximately 0.5 mg of protein with carrier lactose and buffer, and freeze-dried in the Division of Biological Standards, National Institute for Medical Research, London. A potency of 10 units per ampoule was assigned; the activity contained in this unit was roughly equivalent to the so-called " cobalt unit " with which a number of batches of a widely distributed extract of sheep plasma were labelled. This standard was examined in several laboratories by the common assay methods: stimulation of incorporation of ^{59}Fe into circulating erythrocytes in starved rats (Fried et al., 1957; Hodgson et al., 1958) or polycythaemic mice (Jacobson et al., 1960; Cotes & Bangham, 1961). Erythropoietin Standard A was used to calibrate laboratory standards, but supplies of it were almost exhausted within a year.

THE PROPOSED INTERNATIONAL REFERENCE PREPARATION

Since many investigators were interested in the quantitative estimation of erythropoietin in samples of clinical material, it was considered advisable to select material of human origin for use as a standard, in preference to material obtainable from sheep or rabbits. Material was obtained from human urine, as described below, and was designated Erythropoietin Standard B. The WHO Expert Committee on Biological Standardization (1964a) requested the National Institute for Medical Research to determine the suitability of this material as an international reference preparation. It was later noted

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(WHO Expert Committee on Biological Standardization, 1964b) that the material was considered suitable as such a preparation and the National Institute for Medical Research was authorized to establish the material as the International Reference Preparation of Erythropoietin on the basis of the results of the collaborative assay and to define an international unit with the agreement of the participants. This paper presents evidence that the material is suitable for the estimation of potency of different preparations of erythropoietin by comparative bioassay.

The bulk material

The starting material consisted of four batches of erythropoietin prepared from urine from severely anaemic patients of different sexes, ages and pathology.

Batch 1 was kindly supplied by Dr G. Keighley and Dr P. H. Lowy (California Institute of Technology) and by Dr D. Hammond (Children's Hospital of Los Angeles). This material was precipitated from urine by the addition of 3.5 volumes of ethanol in the presence of 0.1% phenol (Lowy & Keighley, 1961) and subsequently reprecipitated from an isotonic saline solution.

Batch 2 was kindly supplied by Dr D. C. Van Dyke (Donner Laboratory, University of California). This material was concentrated by adsorption on collodion membrane (Van Dyke, 1960).

Batches 3 and 4 were prepared in the Division of Biological Standards at the National Institute for Medical Research. The starting material was urine from patients with paroxysmal nocturnal haemoglobinuria, which contained some haemoglobin. *Batch 3* was prepared from material collected in 1960 and 1961 by precipitation from urine by addition of ammonium sulfate (550 g/litre). In 1962 the precipitate was dialysed to remove ammonium sulfate, extracted with saline and then fractionated with alcohol. This gave a crude preparation of erythropoietin, brown and of low specific activity. *Batch 4* was prepared by precipitation from urine with ethanol by the method of Lowy & Keighley (1961) (and Keighley, personal communication, 1962).

In May 1963 the four batches of erythropoietin were pooled and dissolved in 4 litres of glass-distilled water; 20.6 g of lactose were added. This solution was filtered through a bacterial sterilizing membrane filter (Millipore, average pore diameter 0.45 μ) and then distributed into some 3500 ampoules in equal (approximately 1.0-ml) amounts. The maximum difference in weight of contents of 47 ampoules taken at random during filling was 2%. The filled ampoules were freeze-dried as one batch in a shelf freeze-dryer, stored over fresh phosphorus pentoxide in evacuated dessicators for two weeks, filled three times with pure dry nitrogen and sealed. The ampoules were subsequently checked for cracks and pinholes and stored at −10°C in the dark. Although processing of the material included filtration through a sterilizing membrane, all other steps were carried out in a routine clean laboratory manner and it cannot be assumed that the ampoule contents are sterile.

For practical purposes it may be assumed that all ampoules of the International Reference Preparation contain the same amount of erythropoietin and about 5 mg lactose. It is not known whether the erythropoietin is evenly distributed within the freeze-dried plug and therefore portions of the powder should not be extracted and weighed. It is intended that the entire contents of each ampoule be dissolved in a known amount of solvent so that the resulting solution contains 10 International Units.

ACCELERATED DEGRADATION TESTS ON THE PROPOSED INTERNATIONAL REFERENCE PREPARATION

In order to assess the stability of the proposed international reference preparation, some ampoules of it were stored at elevated temperatures for various periods. The activity of the material in these treated ampoules was compared with that of the proposed international reference preparation, which had been stored at −10°C with the main stock of ampoules. Assays were carried out in polycythaemic mice (method IA; Table 1) and each preparation was tested at two or more dose levels. The results of these studies are shown in Table 2; there was no evidence of loss of activity after storage at 37°C for 10 months, although it is probable that after 17 or 21 months there was some loss of activity. After storage at 20°C for two years there was no detectable loss of activity.

ASSAY OF ERYTHROPOIETIN STANDARD B IN TERMS OF ERYTHROPOIETIN STANDARD A

There were not enough ampoules of Erythropoietin Standard A remaining to permit the calibration of the proposed international reference preparation (Erythropoietin Standard B) to be carried out by an extensive collaborative assay. Instead, three laboratories made 10 assays in mice (of different strains,

TABLE 1
METHODS USED FOR BIOASSAY OF ERYTHROPOIETIN

Method	Laboratory	Test animal			Treatment with erythropoietin		Metameter of erythropoietin action	References
		Species	Sex	Preparation	Route [a]	No. of injections		
IA	1, 3, 6, 8, 11, 12, 17, 18	Mouse	Female (male in lab. 1)	Polycythaemic (exposure to air at reduced pressure)	ip *or* sc	1 or 2	Incorporation of ^{59}Fe into erythrocytes 68 h-124 h after (first) injection of erythropoietin and 20 h-72 h after ^{59}Fe injection.	Cotes & Bangham (1961) DeGowin et al. (1962) Gordon & Weintraub (1962)
IB IC	9	Mouse	?	Polycythaemic (exposure to air at reduced pressure)	ip	1	Incorporation of ^{59}Fe into erythrocytes (IB) and reticulocyte count (IC) 29 h after injection of erythropoietin and 24 h after ^{59}Fe injection.	Korst, personal communication, 1964
II	4, 8, 10, 14, 18	Mouse	Female	Polycythaemic (transfusion)	ip *or* sc	1 or 2	Incorporation of ^{59}Fe into erythrocytes 92 h-120 h after (first) injection of erythropoietin and 20 h-72 h after ^{59}Fe injection.	Jacobson et al. (1960)
III	18	Mouse	Female	Polycythaemic (exposure to air at a reduced pressure with subsequent transfusion)	ip	2	Incorporation of ^{59}Fe into erythrocytes 92 h after first injection of erythropoietin and 20 h after ^{59}Fe injection.	
IV	5, 7, 13, 15, 16	Rat	Male	Starved	sc	1 or 2	Incorporation of ^{59}Fe into erythrocytes 64 h-72 h after (first) injection of erythropoietin and 20 h-24 h after ^{59}Fe injection	Fried et al. (1957) Hodgson et al. (1958) Hansen (1963)

[a] ip = intraperitoneal; sc = subcutaneous.

TABLE 2

ACCELERATED DEGRADATION TESTS ON PROPOSED INTERNATIONAL REFERENCE PREPARATION OF ERYTHROPOIETIN (ERYTHROPOIETIN STANDARD B)

Storage temp. (°C)	Storage period (days)	Potency ratio (Treated material: proposed reference material)	Statistical weight	Confidence limits (P = 0.95)	Weighted mean potency and confidence limits (P = 0.95)
37	311	0.997	32	0.649-3.944	
37	503	0.589	90		0.630
37	517	0.723	44		0.422-0.941
37	646	0.509	24	0.170-0.909	
20	724	1.021	471	0.822-1.270	

made polycythaemic by anoxia and/or transfusion) and in starved rats (Table 3, assays 26-35).

In all assays there was a tendency for the variance of response to increase with increasing dose and so the log response was related to the log dose. One assay was rejected as there was no significant slope for Erythropoietin Standard A. The nine valid assays gave log potencies that were homogeneous ($\chi^2 = 3.92$; $0.80 > P > 0.70$) and were thus combined to give a potency estimate of 10.1 units per ampoule of Erythropoietin Standard B, with confidence limits ($P = 0.95$) of 8.8 - 11.4 units per ampoule (Table 4).

The slope of the log-dose - log-response line of Erythropoietin Standard A in all nine assays was slightly steeper than that of Erythropoietin Standard B, although the non-parallelism term was not significant in any one assay.

Allocation of unitage

It was thus decided to assign a unitage of 10.0 units per ampoule to Erythropoietin Standard B.

THE COLLABORATIVE STUDY

After Erythropoietin Standard B had been in use for about one year, a request for information on the usefulness of the material as a bioassay standard was sent to 14 laboratories concerned with research on erythropoietin. Data were obtained from 12 of these and from two additional laboratories. The information collected is listed in Table 3, together with details of the assay of the proposed international reference preparation (Erythropoietin Standard B) in terms of Erythropoietin Standard A. Those who contributed assay data are listed in the Annex; throughout this paper each contributing laboratory is referred to by a number, which is not related to the order in which the laboratories are listed in the Annex.

In all, 32 assays were reported on by 17 different laboratories. In 25 of these assays Erythropoietin Standard B was compared with another preparation of erythropoietin; seven assays gave data on the dose-response relationship obtained with Erythropoietin Standard B. In three assays (No. 7, 8 and 24) other preparations of erythropoietin were studied.

Since it was found that there was a tendency for the variance of response to increase with increasing dose, assays were analysed by relating log response to log dose. From the analyses of variance, seven assays were considered to be invalid because (1) regression of the log-dose-log-response lines was not significant ($P > 0.05$) (four assays) or (2) the slopes of the log-dose-log-response lines differed significantly ($P < 0.01$) (three assays). Deviation from linearity was observed in three assays (No. 28, 31 and 32), but was not considered to make the assays invalid. Summarized data tabulated in Table 3 include the slope of the log-dose-log-response lines given by the preparations being considered, the estimated potency or relative potency (with fiducial limits, $P = 0.95$) of the test preparation and the estimate of precision of the assay (λ).

From this analysis it may be seen that:

(1) The proposed international reference preparation (Erythropoietin Standard B) gave a satisfactory linear log-dose-log-response relationship in 29 out of 32 assays. In three assays (No. 1, 2 and 5), regression of the log-dose-log-response relationship was not significant. The invalidity of

assay 33 was attributable to the apparent inactivity of the other preparation tested. These studies were made with test animals prepared in three different ways (I, II and IV: Table 1) and with various strains of mice and rats. In all studies, stimulation of the incorporation of ^{59}Fe into erythrocytes was used as a metameter of erythropoietin action. In one test (No. 19), induction of reticulocytosis was also observed and this also gave a satisfactory linear log-dose-log-response relationship.

(2) When other preparations of erythropoietin were compared with Erythropoietin Standard B and the regression of the log-dose–log-response lines was significant, there were no significant departures from parallelism in 21 out of 24 assays. These assays comprised comparisons with erythropoietin from

(*a*) sheep plasma (satisfactory parallelism in 14 out of 16 assays),

(*b*) human urine (satisfactory parallelism in 3 out of 4 assays),

(*c*) rabbit plasma (satisfactory parallelism in 4 out of 4 assays).

Nevertheless, it may be noted that in all assays of Erythropoietin Standard B against one preparation of sheep plasma erythropoietin (Erythropoietin Standard A), the slope of the log-dosel–og-response regression line of Standard B was less steep than that of Standard A (see original comparison of Standard B with Standard A on pp. 752-754).

(3) In one assay (No. 19), simultaneous measurements of stimulation of reticulocytosis and incorporation of ^{59}Fe into erythrocytes were observed. The activity of Erythropoietin Standard B was compared with that of a preparation of sheep plasma erythropoietin (Table 3, preparation 8); it was found that the two activities were present in different proportions in the two preparations. The significance of these findings is being investigated, but they suggest that there may be at least two factors, which influence reticulocyte release and maturation of erythrocyte precursors to different extents, and which may be present in different proportions in various preparations of erythropoietin.

DISCUSSION

From these studies it was concluded that Erythropoietin Standard B is suitable for use as a standard for the bioassay of several different types of preparation of erythropoietin in various widely used assay systems dependent upon the stimulation of incorporation of ^{59}Fe into erythrocytes.

The specific activity of the material is relatively low. This is because a considerable proportion was derived from the urine of patients with paroxysmal nocturnal haemoglobinuria, and the extract contained quantities of inactive pigment and protein. A low specific activity need be of no consequence provided that the slope of the log-dose–log-response (or log-dose–response) line is not significantly different from that for preparations against which the material is (most frequently) assayed. Differences in slope may also be due to other factors besides purity, such as species or source (blood or urine) of the extract. Indeed, it has been found that slope differences occur between preparations of erythropoietin from urine from different human subjects (see also Weintraub et al., 1963). Three preparations of human urinary erythropoietin (preparations 4, 9 and 10 in Table 3) could be satisfactorily assayed against the proposed international reference preparation (Erythropoietin Standard B), which is also derived from human urine, whereas another preparation (No. 3) did not give a valid assay against Standard B but could be assayed against two preparations of sheep plasma erythropoietin (Erythropoietin Standard A and preparation No. 2).

From these observations it may be concluded that, for use as laboratory standards, preparations of erythropoietin derived from human urine or from sheep or rabbit plasma may be satisfactory. Indeed, the ease of preparation of highly active plasma (cf. preparations 6 and 7) in rabbits made anaemic with phenylhydrazine is likely to favour this source of material, except when urine from suitable anaemic human subjects is easily available.

Assays using various species, strains and preparations of test animal differed in their sensitivity and precision. Valid assays with a low index of precision ($\lambda < 0.2$) were obtained in 10 assays: in eight of these (carried out in various laboratories) starved rats were used as test animals (method IV, Table 1) and in two others mice made polycythaemic by exposure to hypoxia (methods IA and IC, Table 1) were used. Despite the sensitivity and specificity of response in the hypoxic-polycythaemic mouse treated with small doses of erythropoietin, the present study demonstrated that to estimate the potency of a preparation of erythropoietin the same amount of material may be needed for an assay in starved rats of a suitable strain as for one of comparable precision in polycythaemic mice. The doses used in various laboratories

TAE

RESULTS OF BIOASSAY EXPERIMENTS WITH ERYTHROPOIETIN STANDA

Labora-tory	Assay method (see Table 2)	Standard (S) [a]	Test preparation (T)					Assa no.
			No.	Species	Source	Preparation	Type/Batch no.	
1	IA	IRP	1	Sheep	Plasma	Purified–Armour/NIH	Lot K-147192A	1
	IA	IRP	1	Sheep	Plasma	Purified–Armour/NIH	Lot K-147192A	2
3	IA		2					3
	IA	IRP	2	Sheep	Plasma	Purified "step 4" Armour ESL	Lot K-103124	4
	IA		2					5
	IA		2					6
	IA	Preparation (2) purified from sheep plasma and stated to have 23 units/mg.						7
	IA	Preparation (2) purified from sheep plasma and stated to have 23 units/mg.	3	Human	Urine	?		8
4	II	IRP	4	Human	Urine	Partially purified		9
5	IV	IRP	5	Sheep	Plasma	Discard precipitate with polyphosphoric acid at pH 4, then concentrate with DEAE sephadex		10
6	IA	IRP						11
	IA							12
7	IV		6				Batch 1	13
	IV	IRP	6	Rabbit	Plasma	Not purified	Batch 1	14
	IV		6				Batch 1	15
	IV		7				Batch 2	16
8	IA	IRP						17
	II	IRP						18
9	IB	IRP	8	Sheep	Plasma	Purified Armour	ALO336	19
	IC	IRP	8	Sheep	Plasma	Purified Armour	Lot K 103-214A	19
10	II	IRP	9	Human	Urine	Crude DEAE cellulose concentrate		20
12	IA	IRP	1	Sheep	Plasma	Purified Armour	ALO566 Lot K147192A	21
13	IV	IRP		Sheep	Plasma	See notes on preparation of Standard A	Standard A	22
	IV	IRP	3	Human	Urine	?		23
	IV	Standard A	3	Human	Urine	?		24
14	II	IRP	10	Human	Urine	Collodion adsorption		25
11	I	IRP						
15	IV	IRP						
16	IV	Standard A		Sheep	Plasma		IRP	26
	IV	Standard A		Sheep	Plasma		IRP	27
17	IA	Standard A		Sheep	Plasma		IRP	28
18	IA							29
	IA							30
	III							31
	II	Standard A		Sheep	Plasma		IRP	32
	IA							33
	IA							34
	IA							35

[a] IRP = proposed international reference preparation (Erythropoietin Standard B).

[b] Detailed figures not available.

(PROPOSED INTERNATIONAL REFERENCE PREPARATION; IRP)

Slope of log dose–log response regression lines S	T	Potency of T in units of IRP (Standard B) (except as otherwise stated)	Fiducial limits (P = 0.95)	λ	Validity and comments
0.454	[b]			0.711	invalid regression (P > 0.05)
0.549				0.646	Invalid regression (P > 0.05)
0.244	0.529	(17.4 units/mg)		0.103	Invalid (departure from parallelism [c])
0.157	0.204	33.3 units/mg	13.5-98.5	0.470	Valid
0.041				0.624	Invalid regression (P > 0.20)
0.229	0.351	15.8 units/mg	7.2-32.1	0.403	Valid
0.454				0.248	Valid
0.458	0.564	48.19 units/ml [d]	25.67-82.80	0.314	Valid
0.195 [e]	0.259	(Potency ratio 1.596)	0.996-2.602	0.325	Valid
0.734	0.769	53.7 units/ampoule	44.5-63.3	0.102	Valid (all data combined despite not being obtained on same day)
0.460				0.243	Valid
0.466				0.267	Valid
0.678	0.357	10.34 units/ml	6.29-24.32	0.137	Valid ⎫ Weighted mean potency 10.51 units/ml; confidence limits 8.80-12.56
0.828	0.762	11.65 units/ml	9.23-14.93	0.092	Valid ⎭
0.766	0.455	7.76 units/ml	5.29-11.82	0.195	Valid
0.488	0.751	7.37 units/ml	4.42-9.94	0.134	Valid
0.350				0.217	Valid
0.299				0.283	Valid
0.580	0.806	29.95 units/mg	17.807-52.334	0.261	Valid ⎫ Simultaneous estimate using different parameters of erythropoietin activity
1.331	1.831	9.702 units/mg	6.435-14.533	0.189	Valid ⎭
1.042	1.085	27.78 units/ml	20.98-37.82	0.222	Valid (data from two assays combined)
0.524	0.557	(Potency ratio 2.509)	1.692-4.267	0.248	Valid
0.383	0.639	(Potency ratio 0.709)		0.195	Invalid (departure from parallelism) [c] ⎫ Multiple assay but data analysed as
0.383	0.611	72.2 units/ml		0.204	Invalid (departure from parallelism [c] ⎬ though separate assays
0.639	0.611	101.56 units/ml	84.33-122.55	0.138	Valid ⎭
0.594	0.508	(Potency ratio 1.070)	0.741-1.548	0.337	Valid (all data combined for analysis)
[b]					
[b]					
0.70	0.63	10.2 units Std A/ampoule	7.35-13.86	0.176	Valid ⎫ Weighted mean potency 10.1
0.78	0.66	10.1 units Std A/ampoule	7.25-14.05	0.190	Valid ⎭ (Confidence limits (P = 0.95) 8.1 — 12.6)
0.51	0.46	10.1 units Std A/ampoule	8.07-12.6	0.167	Curvature Standard A ⎫ (0.05 > P > 0.01) Curvature Standard B ⎭
0.45	0.37	14.4 units Std A/ampoule		0.518	Valid ⎫ Weighted mean potency 9.9 units Std A/ampoule (Confidence limits (P = 0.95) 7.73-12.6)
0.41	0.30	9.2 units Std A/ampoule		0.511	Valid
0.58	0.44	10.7 units Std A/ampoule		0.315	Curvature Standard A [c]
0.71	0.63	7.2 units Std A/ampoule		0.351	Curvature IRP [c]
0.05	0.24	Invalid		1.883	No slope for Standard A
0.48	0.38	9.8 units Std A/ampoule		0.433	Valid
0.45	0.31	14.9 units Std A/ampoule		0.523	Valid

[c] 0.01 > P > 0.001. [d] Based on stated potency of preparation No. 2.
[e] Slope = 0.239 if top dose omitted.

TABLE 4

ASSAYS OF PROPOSED INTERNATIONAL REFERENCE PREPARATION (ERYTHROPOIETIN STANDARD B) IN TERMS OF ERYTHROPOIETIN STANDARD A

Laboratory	No. of assays	Test animal	Activity (units/ampoule) and fiducial limits (%) (P = 0.95)	
18	6	Polycythaemic mice (methods IA, II and III)	9.9 (78.2-127.7)	10.1 (87.9-113.7)
16	2	Starved rats (method IV)	10.1 (80.2-124.7)	
17	1	Polycythaemic mice (method IA)	10.1 (79.9-125.1)	

suggest that there is a great difference in the sensitivity of preparations of test animals. For the economical use of erythropoietin, as well as for the achievement of precise assays, the selection of a suitable strain and preparation of test animal has been found to be of great importance.

One of the reasons why a common standard for erythropoietin was not in use among laboratories before 1961 was that material with suitable activity was difficult and costly to prepare and sometimes proved unstable. Laboratories were therefore reluctant to allocate material for use as a standard. Several workers have now demonstrated that adequate amounts of erythropoietin for use as a working standard can be made from extracts of anaemic rabbit or sheep plasma or of suitable human urine. A large quantity of these materials can be divided among several small containers and stored sealed in a deep-freeze (with or without freeze-drying). The evidence collected shows that the proposed international reference preparation is suitable for the calibration of such laboratory standards so that their potencies can be expressed in International Units of Erythropoietin.

THE INTERNATIONAL REFERENCE PREPARATION FOR ERYTHROPOIETIN

In accordance with the authorization of the WHO Expert Committee on Biological Standardization (1964b) and on the basis of the results of the collaborative assay, Erythropoietin Standard B has been established as the International Reference Preparation of Erythropoietin. The International Unit for Erythropoietin is defined as the activity contained in 1.48 mg of the International Reference Preparation of Erythropoietin.

The biological activity in the International Unit is thus identical with those of the preceding research standards, Erythropoietin Standards A and B; the historical continuity of these units is thus ensured.

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Annex

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RÉSUMÉ

Le Comité OMS d'experts de la standardisation biologique a autorisé le National Institute for Medical Research de Londres à établir une préparation internationale de référence d'érythropoïétine d'après les résultats d'un titrage international collectif et à définir une unité internationale après accord des participants.

Ce n'est que depuis 1961 que plusieurs chercheurs ont montré qu'il était possible d'extraire des quantités d'érythropoïétine utilisables comme étalon de travail à partir du plasma de lapins ou de moutons anémiques ou de l'urine de certains malades humains. Il devenait indispensable de pouvoir titrer ces étalons de laboratoire grâce à l'établissement d'une unité internationale d'érythropoïétine.

Le présent travail décrit la préparation d'un étalon B d'érythropoïétine et l'estimation de son activité par rapport à un étalon antérieur, l'étalon A d'érythropoïétine. Par comparaisons avec d'autres préparations, il a été conclu que l'étalon B d'érythropoïétine convenait aux titrages biologiques de nombreuses préparations d'érythropoïétine d'usage courant. A la suite de cette étude l'étalon B d'érythropoïétine a été constitué en préparation internationale de référence et l'unité internationale d'érythropoïétine a été définie comme l'activité de 1,48 mg de la préparation internationale de référence. L'activité de cette unité correspond donc à celle des unités de l'étalon B et de l'étalon A et permet la continuité d'utilisation de ces unités déjà très répandues.

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