Special Article

International Insulin Standards

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In a previous paper,[1] the International Insulin Standards have been dealt with in some detail in tracing the definition of the unit of insulin since the discovery of the hormone in 1921 to the present time. Four International Insulin Standards have been established during this period, each being official at different times, and in terms of these the unit of insulin has been defined. The First Standard was assigned a potency of 8 U. per milligram after international collaborative biological assays, based on local experience and usage of the term "unit."[2] The other standards were assessed in terms of the standard official at the time.[3-5] Some data pertaining to the standards are shown in table 1.

In establishing successive standards in terms of the preceding standard it was assumed that each of the preceding standards had suffered no loss in activity during the years of use. From time to time, however, the stability of the standards has been a matter of consideration. Accelerated degradation tests, whereby the preparation was subjected to elevated temperature for periods of time and the change in potency ascertained, have given unsatisfactory evidence of stability since standards of insulin are required to be stored at low temperature.

If during the period of use of the standards there had been a gradual loss in potency the unit of insulin would have suffered a loss in value over the years. During use of any one standard, assuming a gradual continuous deterioration of the standard, potency values assigned to preparations of consistent manufacture in a sequence would show an upward trend. In recent years and particularly since the establishment in 1958 of the Fourth Standard, which unlike former standards also serves for routine assays, and with the customary use of zinc-insulin crystals in regular preparations it has been possible to observe such a trend in potency of successive lots of crystals. The study, based on assays of regular lots of zinc-insulin crystals in the Insulin Committee Laboratory, indicates an increase of about 0.14 U. per annum. This has posed the question as to whether this represents a true increase in potency or whether it is related to a loss in activity of the Fourth Standard.

In the Insulin Committee Laboratory a supply of each of the four standards was available. With the exception of a single available ampoule of the First Standard all had been maintained at about 6° C. throughout the years. The ampoule of the First Standard had been subjected to room temperature for many years, probably 35 or more, and in addition, being amorphous and consisting of about 65 per cent impurities, a loss in activity might be anticipated. However, sufficient of the four standards being available for adequate assays it was felt that to re-assess these preparations in terms of the unit current at the time each became official could be useful in respect to the question of stability.

In table 2 are shown the results originally reported for the four standards based on assays conducted in the Insulin Committee Laboratory using rabbits, in comparison with the results of collaborative assays and with the final established potency, the latter being officially established by rounding off the values for convenience. Although rabbits were the test animals in all Insulin Committee results shown in the table for each of the preparations the technic used was that current at the time of the tests and differed in each instance.

EXPERIMENTAL

In the present study the method of assay was the twin cross-over procedure,[6] with modifications and calculations as set forth in the United States Pharmacopeia XVI. The blood sugar determinations were made by means of an AutoAnalyzer. All solutions used for injection were made to contain 1 and 2 U. per cc. assuming the preparations to contain the officially established potencies. In each case the separate estimates of the four tests comprising an assay have yielded results which are mutually consistent and therefore have been combined by the appropriate U.S.P. procedure[7] to yield the most probable single value for the preparation under test. The results are reported in tables 3, 4, and 5.

It will be observed from table 3 that essentially iden-

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TABLE 1

International Standards for Insulin

International Insulin Standard	Physical state	Contents of ampoule (mg.)	Moisture (per cent)	Atmosphere	Year established	Official potency (U. per mg.)
First	amorphous	100	0	nitrogen	1925	8.0
Second	crystalline	20	0	nitrogen	1935	22.0
Third	crystalline	20	5.93	nitrogen	1952	24.5
Fourth	crystalline	100	5.65	air	1958	24.0

TABLE 2

Potency values assigned to the Four International Insulin Standards

International Insulin Standard	Insulin Committee rabbits (U. per mg.)	Collaborative assays (U. per mg.)		Established potency (U. per mg.)
		Rabbits	All methods	
First (1925)	8.5	8.5	8.6	8.0
Second (1935)	21.3	21.5	22.1	22.0
Third (1952)	24.6	approx. 25.0	24.46	24.5
Fourth (1958)	23.80	23.8	23.9	24.0

TABLE 3

Data from assay of Fourth Standard (24.0 U./mg.) in terms of the Third Standard (24.5 U./mg.)

Test No.	Number of rabbits	Weight of test	Fourth Standard (U. per mg.)	95 per cent limits
1	32	1,698.79	23.96	21.37-26.88
2	32	2,067.00	22.91	20.63-25.39
3	32	1,848.46	24.18	21.67-26.99
4	32	1,267.67	24.62	21.58-28.13
Combined results	128	6,881.92	23.81	22.47-25.24

TABLE 4

Data from assay of Third Standard (24.5 U./mg.) in terms of the Second Standard (22.0 U./mg.)

Test No.	Number of rabbits	Weight of test	Third Standard (U. per mg.)	95 per cent limits
1	32	2,954.93	23.05	21.11-25.11
2	32	1,477.46	25.30	22.41-28.63
3	32	1,323.66	24.84	21.83-28.28
4	32	1,565.67	25.69	22.83-28.98
Combined results	128	7,321.72	24.36	23.03-25.77

TABLE 5

Data from assay of Second Standard (22.0 U./mg.) in terms of the First Standard (8.0 U./mg.)

Test No.	Number of rabbits	Weight of test	Second Standard (U. per mg.)	95 per cent limits
1	32	877.82	23.20	19.84-27.29
2	32	1,651.97	27.26	24.40-30.78
3	32	2,397.71	26.11	23.79-28.84
4	32	1,665.08	24.82	22.19-27.95
Combined results	128	6,592.58	25.65	24.17-27.22

tical evaluation of the Fourth Standard is obtained as was originally obtained (23.80 U./mg.) in the Insulin Committee Laboratory in 1958. The relative potency therefore appears unchanged and unless the two preparations have changed proportionately the unit of insulin is unchanged since the establishment of the Third Standard in 1952, except for small changes brought about by rounding off values as was done officially. The indicated increase in potency of successive lots of zinc-insulin crystals, to which reference has been made, therefore appears to be a true increase resulting from refinements in methods of purification. Of course there is the unlikely possibility that there has been a proportionate change in both the standards.

In table 4 the indicated potency of 24.36 U. per milligram for the Third Standard in terms of the Second Standard compares with 24.6 U. per milligram obtained in the Insulin Committee Laboratory in 1952 (table 2). This represents a difference of about 1 per cent which is well within the error of the assays. It therefore

appears from the assays recorded in tables 3 and 4 that unless the Second, Third and Fourth Standards have all changed proportionately, which would seem unlikely in view of the great differences in age, the value of the unit of insulin has not changed inadvertently since the establishment of the Second Standard in 1935.

In table 5 the indicated potency of 25.65 U. per milligram for the Second Standard in terms of the First Standard compares with 21.3 U. per milligram obtained in the Insulin Committee Laboratory in 1935 (table 2). This represents a difference of about 20 per cent and is interpreted to mean that the First Standard has suffered a loss in activity of about 20 per cent. This interpretation seems the most reasonable explanation, particularly when it has been shown in tables 3 and 4 that the potency values of the Second, Third and Fourth Standard remain about the same as originally determined. The only other interpretation is that there has been an increase in potency of the Second Standard and this is unrealistic. As pointed out above a loss in activity of the First Standard was anticipated and probably the most surprising finding is that the loss is not of greater magnitude for an impure material maintained under adverse conditions of storage for such a prolonged time.

Technic of assays using rabbits has changed greatly over the years in important ways including such basic details as diet, breed of rabbits and blood sugar determination methods. Consequently the measured effects of injection of insulin to rabbits as prevailed as long ago as forty years are not strictly comparable to those observed now. It is therefore impracticable to evaluate the First Standard in the manner of its original assessment, that is, in terms of animal response and vague unit value. In these circumstances and being unaware whether there was a change in activity of the First Standard during its ten years of use between 1925 and 1935 it is not possible to state with certainty that the activity of the unit of insulin has remained unchanged since 1925 except for those minor changes involved in rounding off determined values. It is postulated that, of the moderate loss under adverse storage conditions of a low grade material at least forty-three years old, only a small part, if any, of this loss would have occurred in the preparation when stored in the cold before 1935, at which time the Second Standard was established, and that the value of the unit of insulin was not appreciably changed unmindfully on account of any loss in activity of the First Standard.

CONCLUSIONS

The four International Insulin Standards, each being official at different times since 1925, were available in the Insulin Committee Laboratory and have recently been used in comparative assays on rabbits. In assays involving the Second, Third and Fourth Standards the evidence points to unchanged relative potency among these preparations. The First Standard, a very impure material maintained at room temperature for thirty-five or more years, was found to be discordant in its potency relation to the other three. It is concluded that the activity of the First Standard has declined by about 20 per cent under these adverse conditions. Except for minor changes effected officially, it is deduced that the unit of insulin has remained unchanged in value since 1935 and that the unit has not changed appreciably, if at all, since 1925.

ACKNOWLEDGMENT

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[4] Bull. WHO: 7:445-59, 1952.

[5] Bull. WHO: 20:1209-20, 1959.

[6] Smith, Marks, Fieller and Broom. An extended crossover design and its use in insulin assay. Quart J. Pharm. Pharmacol. *17*:108-23.

[7] United States' Pharmacopeia *16*.