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THE STRENGTH OF INSULIN PREPARATIONS:

A COMPARISON BETWEEN LABORATORY AND CLINICAL MEASUREMENTS.

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I.—INTRODUCTORY.

THE decision of the discoverers of insulin and their colleagues in Toronto to indicate the strength of the preparation in "units" of activity was undoubtedly sound, and has led to a highly satisfactory precision in its dosage. So far as this country is concerned, experience may be divided roughly into three periods.

In the first no final definition of the unit had been given, and only rough methods were available for its measurement in the laboratory. On the other hand, physicians had yet to learn what regularity of effect could be obtained with a really constant preparation, so that comparatively few complaints were heard of the differences in strength between similarly labelled batches of the solution, though it can hardly be doubted that such occurred.

In the second period, though the unit had received its final physiological definition, and a representative batch of solution had been received in this country to serve as a standard of comparison, a trustworthy method for its measurement on normal animals was still being sought. Physicians, on the other hand, with time and opportunity, had found that a patient whose diet and activities were under complete control was a very constant reagent for insulin. The effect of each daily dose on such a patient, if followed with sufficient care, provided a reasonably accurate measure of the activity of the preparation. Clinical observation, for the time being, had attained a greater accuracy than laboratory methods of assay, and it was to be expected that discrepancies would be observed, such as those collected below by Drs. Harrison and Lawrence in Table III. The suggestion naturally arose that a clinical assay of each batch of insulin should supplement that made in the laboratory before the final adjustment of its strength for issue. The Insulin Committee of the Medical Research Council decided first to organize a careful and deliberate comparison between the results of laboratory and clinical assays, on a selected series of samples.

By the time this decision was taken, however, the third period had been reached. There was reason now for hope that the laboratory test had again overtaken clinical observation in accuracy. The method devised and described

below by Mr. Marks had aimed at eliminating the effect of the wide individual differences of response shown by different animals, as thoroughly as such differences were eliminated in the clinical tests on patients of known reaction. It had endeavoured to go further, and to neutralize even the day-to-day variations of sensitiveness to insulin shown by the same animals. The comparative trials now undertaken had, moreover, the advantage that clinical and laboratory measurements were to be made at the same time; whereas many of the discrepancies earlier apparent had resulted from clinical trials made many months after the laboratory assay, at a time when the preparations of insulin were less stable than the highly purified products now available. The results of this organized comparison, as shown in Table IV of the report of Drs. Harrison and Lawrence, fully realized the expectation that the laboratory method of assay, now for some time in use at the National Institute, would be able to hold its own with the most careful clinical measurements. The correspondence is so good, indeed, as to justify the conclusion that the assay on rabbits here described by Mr. Marks, if conscientiously carried out, can fix the activity of a preparation with an accuracy that no clinical test is likely to improve.

It should be added that experience in this department with preparations standardized by other methods suggests that these also are capable of an accuracy well within the range of clinical needs, when carefully carried out in comparison with the stable standard now adopted for international use.

H. H. D.

II.—THE BIOLOGICAL ASSAY OF INSULIN PREPARATIONS IN COMPARISON WITH A STABLE STANDARD.

BY

H. P. MARKS.

The original physiological unit of insulin was defined as the smallest dose which would lower the blood sugar of a 2 kilogram rabbit, starved for twenty-four hours previously, to the convulsant level of 0.045 per cent., and this unit, with the clinical unit later based on it (1 rabbit unit = 3 clinical units), has been generally accepted. The word "unit" is here used throughout to mean such a "clinical" unit, the original or "physiological" unit being now only of historical interest.

All the earlier methods of assay based on this definition, whether they involved the observation of actual convulsions or of the fall in blood sugar produced by a subconvulsive dose, aimed at determining the potency of a sample of insulin absolutely in terms of an animal reaction.

THE STANDARD OF REFERENCE.

As it became evident that such a reaction could not furnish an invariable standard, an attempt was made in this laboratory to standardize rabbits by determining their response to a given dose of a stable reference sample whose potency had been verified at Toronto. The response of these rabbits to the sample under test was then compared with their response to the standard sample, so that, in effect, the potency of the unknown sample was expressed in terms of that of the standard preparation. Such was the state of affairs at the time when the laboratory tests were performed on the batches in Table III of the clinical report. Effort was directed to improvement of the laboratory test, so as to take full advantage of direct comparison with a standard sample of constant potency. Meanwhile we had replaced the original reference sample of insulin solution by a purified, dry preparation which could be relied upon to retain its potency unchanged. A similar preparation, made at the request of the International Conference on Biological Standards, has now been accepted for international use, and the unit of insulin has been defined by the Insulin Committee of Toronto in terms of that standard preparation.

THE METHOD OF COMPARISON.

In the experience of this laboratory, as also in that of Macleod and Orr, it has been found repeatedly that, for either of two reasons, convulsions may fail to occur in a

rabbit injected with a dose which produces them in others. The rabbit may be abnormally insensitive to the action of insulin on the blood sugar, so that this only falls to a trivial extent; or, on the other hand, the rabbit may be quite normally sensitive, according to the indication of the effect on its blood sugar, but show practically no symptoms.

We might distinguish these two types of insensitiveness to the convulsant action of insulin as "metabolic insensitiveness" and "nervous insensitiveness." The latter condition, in which the rabbit may exhibit no overt abnormality, though its blood sugar fall as low as 0.03 per cent., can apparently be developed to some extent by habituation. It is certainly much commoner in rabbits which have been used for many tests with these larger doses than in those which have been so treated a few times only.

In view of these facts, it is obvious that no reasonably accurate estimate of the activity of a sample can be made from the proportion of convulsions observed on a small number of rabbits; and, indeed, we are informed that those who have used this method with success have found it necessary to use relatively enormous numbers in order to obtain consistent results. Attention has therefore been directed to determining as accurately as possible the hypoglycaemic response to a subconvulsive dose, as involving fewer factors of possible variation than those which must complicate the tests based on observation of convulsions or death. This direct determination of hypoglycaemic effect is the basis of Macleod and Orr's method, as now used by the Insulin Committee of Toronto. But, in that form of the test, individual differences and periodic fluctuations of metabolic sensitiveness remain, and have to be eliminated as far as possible by many repetitions. Our aim has been to make the test based on blood sugar determinations strictly comparative, and to eliminate individual variations altogether, by using the same animals for the test on the unknown sample and on the standard.

The matter would be simple if the animals, though differing widely among themselves, exhibited an individual constancy of reaction. It would only be necessary to standardize each rabbit by determining its reaction to a given dose of the standard, with which the reaction to a dose of the sample could then be compared. For some time we experimented with this method of comparison; but control experiments, in which the same group of rabbits received the same dose of the same preparation once a week, brought to light the fact that each rabbit showed, in varying degree, fluctuations of response on either side of its characteristic average. It might be supposed that, if a sufficient number of animals were used, these fluctuations would tend to cancel one another; but we found pronounced tendency to a simultaneous rise or fall from the characteristic averages affecting all the rabbits together.

No increase in the number of animals used could eliminate a variation affecting the whole stock. On the other hand, if the variations were really simultaneous and equal, we could eliminate their effect entirely by dividing the rabbits used for the test into two equal groups—one receiving the sample under test and the other the standard preparation—and then repeating the test a few days later with reversal of the groups. This method has been used here for some time, and was employed in that later part of the present comparison which was officially organized.

It is not suggested that the periodic fluctuations really show the strict parallelism required to make the method perfect. Different animals would appear to vary in their tendency to these fluctuations. All that is claimed is that the method, by ensuring that each rabbit receives both the sample under test and the standard, and that the effect of day-to-day variations is spread as evenly as possible over the whole series, eliminates the effect of permanent individual differences altogether, and that of periodic variations as far as is practicable.

DETAILS OF METHOD.

The details of the method employed are as follows.

1. Choice and Care of Stock.

There is no restriction on the breed of rabbits used, but greater uniformity in response is aimed at by eliminating from the stock all rabbits in which a dose of 1 unit per kilogram produces convulsions (usually about 15 per cent. of the rabbits), and all those

which prove to be abnormally insensitive. Their weight should be between 2 and 3 kilograms.

The temperature of the animal room, and of the room in which the tests are carried out, is maintained as nearly as possible at 65° F. throughout the year. The diet consists mainly of oats (about 100 grams a day for a rabbit of 2 kilos), with cabbage leaves, and fresh grass when obtainable. A diet containing swede turnips was discontinued, owing to the rapid decrease in sensitiveness which followed.

Twenty-four hours before a test all food and litter is removed from the cages of the rabbits to be used, but water is left till just before the commencement of the test. Rabbits should not be used more than twice a week, and the stock should be large enough to enable each rabbit to have a frequent rest.

2. General Procedure.

A batch of not less than six rabbits having been prepared for test by withholding food as above, samples of blood for the estimation of blood sugar are obtained by puncture from the lateral ear-vein, a vigorous circulation being promoted by warming the ear over a carbon-filament lamp, and by friction if necessary. Slightly more than 1 c.cm. of blood is collected in a small glass vessel containing a trace of powdered potassium oxalate, and the estimation of blood sugar carried out according to the method of Shaffer and Hartmann (1920). This method lends itself well to a large number of estimations, but any other suitable method may be used.

A sample of blood for the estimation of the normal fasting blood sugar is taken immediately before the injection, and further samples at every hour subsequently over a period of five hours. It is felt that by this means a more accurate picture of the hypoglycaemia following the injection is obtained than by determining the blood sugar level at only three intervals after injection, as in Macleod and Orr's method. The period of five hours, as used by Macleod and Orr for the duration of the experiment, is adhered to, as in our experience the blood sugar usually returns to about the normal level at the end of five hours.

The injection is given subcutaneously. To obtain a suitable volume for injection, commercial samples of the usual strength—namely, 20 units per cubic centimetre—should be diluted ten times with distilled water, and the appropriate volume injected with a syringe graduated to 0.01 c.cm. ("tuberculin syringe").

Half the rabbits are injected with a dose of the standard preparation (diluted as above said) adjusted to the weight of the rabbit, and representing 1 unit per 2 kilograms, while the other half are given a theoretically corresponding dose of the sample under test (see 3 below). After an interval of at least three days the test is repeated on the same batch of rabbits, with the difference that the rabbits which previously received the standard are now injected with the unknown sample, and vice versa.

3. Choice of Dose.

Consideration must be given to the fact that the effect on blood sugar does not increase in direct proportion to the dose, but reaches a limit as the convulsant dose is approached. Equal hypoglycaemic effect does not necessarily imply equality of dosage, when the doses employed fall near the region of the convulsant dose. It is desirable, therefore, to choose a dose as far below the convulsant limit as is consistent with obtaining a reaction of sufficient magnitude to be reasonably free from experimental error. A dose of 1 unit (one-third of the generally convulsant dose) per 2 kilogram rabbit is found to fulfil these requirements, especially when abnormally sensitive and insensitive rabbits from the stock are eliminated as recommended above. Where the general level of sensitiveness of the rabbits is high, a smaller dose should be employed—for example, 0.8 unit per 2 kilograms.

It has been our custom to adjust the dose to the weight of the rabbit, but there seems to be no reason why body weight should not be regarded as an individual characteristic for the short period occupied by the test, and the simpler plan adopted of giving all the rabbits the same amount of injection, regardless of their weight. In this case, however, it would seem desirable to set narrower limits on the weights of the animals used.

Although, by reference to Table B, it may be possible to compare unequal doses with a fair degree of accuracy, the most reliable results are obtained when the dosage of the unknown preparation is adjusted so as to be reasonably near the dose of standard used—namely, 1 unit per 2 kilogram rabbit.

4. Calculation of Hypoglycaemic Effect.

If the test is used in this way, as it is primarily intended to be used—namely, to compare the effects of two approximately equal doses—the exact relation between hypoglycaemic effect and dose is of little moment, and, so long as we can arrive at a figure truly representing the hypoglycaemic effect, it is unnecessary to bring it into relation with any absolute unit of reaction, such as the convulsant dose. For this reason we were led to abandon the Macleod and Orr formula, and to adopt the more convenient percentage blood sugar reduction.

This figure—namely, the average reduction in the blood sugar over a period of five hours following the injection, expressed as a percentage of the initial blood sugar—is arrived at by subtracting the mean of the five-hourly blood sugar estimates following the injection, from the initial estimate, and expressing this difference as a percentage of the initial blood sugar. The following example will make this clear: Initial blood sugar 106 mg. per 100 c.cm. Blood sugar at 1, 2, 3, 4, and 5 hours after the injection=65, 64, 83, 88, and 99 mg. per 100 c.cm. respectively. The mean of the figures for the five hours following the injection is 80, so that the figure for the hypoglycaemic effect is

$$\frac{106-80}{106} \times 100 = 24.5 \text{ per cent.}$$

Having calculated the percentage blood sugar reduction in this way for each injection, we now find the sum of all the figures relating to injections of the sample, and of all those relating to injections of the standard. A comparison of these two totals will then indicate whether the particular dose of the sample under test is more or less active than that of the standard preparation, and approximately the extent of the difference.

TRIAL OF THE METHOD.

The essential requirements for the validity of the method, applied in this manner, are that equally active doses should give equality of effect, and that differences in dosage should be made manifest by roughly proportional differences in effect. To what extent these requirements are satisfied may be gathered from a consideration of the following results of experiments, in which (1) the standard preparation was simply substituted for the unknown sample, so that, in effect, the activity of the standard preparation was compared against itself; and (2) the effects of various proportional doses of the standard were compared with that of the standard dose of 1 unit per 2 kilograms.

1. Comparison of Equal Doses of the Standard Preparation.

The results of a typical experiment are set out in the following table.

TABLE A.

Rabbits receiving Standard Preparation (1 unit per 2 kilos).	Percentage Blood Sugar Reduction.	Rabbits receiving Sample (in this Case Standard Preparation—1 unit per 2 kilos).	Percentage Blood Sugar Reduction.
First day—			
No. 7	52.2	No. 43	37.0
No. 8	29.3	No. 44	40.6
No. 9	35.8	No. 45	41.7
	117.3		119.3
Second day—			
No. 43	49.6	No. 7	39.1
No. 44	44.6	No. 8	53.1
No. 45	41.8	No. 9	48.6
	136.0		140.8
Total for both days	253.3		260.1

It will be seen that, although the rabbits as a whole gave a greater response on the second day than on the first, the totals for the whole test differed by only 2.7 per cent. Theoretically they should be identical.

This is the type of variation which the cross-over test particularly aims at eliminating. In addition to this, however, erratic responses in single rabbits are occasionally encountered. In order to minimize the effect of these, it seems desirable to employ more than six rabbits for a test.

The percentage differences between totals in ten such tests were 1.4, 0.2, 2.7, 13.4, 15.8, 3.9, 1.9, 5.0, 2.5, 1.2, and indicate the degree of accuracy to be expected. The standard error for the series is 7.0. Two of the above differences are unusually great, and, if full data had been available concerning the normal response of the rabbits concerned, might have been eliminated. They have been included to show the method at its worst, as well as at its best. If these are eliminated, the standard error is only 2.5.

2. Comparison of Unequal Doses of the Standard Preparation.

As already pointed out, it is not sufficient merely that equal doses should produce equal effects; a significant difference in dosage must, further, give rise to an unmistakable difference in effect. In order to determine what difference in effect was produced by a difference of 10 per cent. in the dose, further tests were carried out, on the lines of those already described, with the difference that a dose of nine-tenths of a unit was compared with the standard dose of 1 unit per 2 kilogram rabbit. The effects of nine-tenths of a unit, expressed as a percentage of the effect of 1 unit, were 92.3, 93.6, 91.4, 96.0, 89.0, 97.9, 97.7, 92.3, 98.5, 91.5. The mean of all the tests gives a figure of 94 per cent. as the percentage effect produced by a dose of 90 per cent. of the standard dose, while the variation from the mean is 3.1.

Similar series of tests were carried out on other proportional doses. The results of all the tests are summarized in the following table, from which the potency of a sample, giving a total hypoglycaemic effect different from that given by the "standard," may be roughly estimated.

TABLE B.—Effect of Unequal Doses.

No. of Tests.	Dose, as Percentage of "Standard" Dose.	Effect as Percentage of Effect of "Standard" Dose.	Standard Error.
7	110	106	3.7
10	100	100	7.0 (2.5)
10	90	94	3.1
7	80	83	6.3
7	75	78	10.7

A Simplified Procedure.

The desirability of using more than six rabbits for a test has already been indicated. With a view to enabling this to be done, by reducing to a minimum the labour involved in taking samples of blood and estimating the blood sugars, trials have recently been made in which the mean value of the blood sugars for the five hours following the injection has been determined directly on a pooled sample. It is then only necessary to collect 0.2 c.cm. of blood at each hour, the five-hourly samples for a particular rabbit being delivered into the same tube containing 8 c.cm. of N/12 sulphuric acid. It is better, however, to take all samples in duplicate, so that 0.4 c.cm. of blood is taken at each bleeding. At the end of the experiment the whole is defaecated by the addition of 1 c.cm. of 10 per cent. sodium tungstate. This simplified procedure is found to give results agreeing closely with those based on separate 1 c.cm. samples, taken at the same times, estimated separately, and arithmetically averaged.

Using this method, it is quite practicable to conduct a test on ten or twenty rabbits, and so minimize the error introduced by occasional erratic responses.

III.—CLINICAL ASSAYS OF INSULIN PREPARATIONS.

BY

G. A. HARRISON AND R. D. LAWRENCE.

Commercially manufactured insulin became generally available in Great Britain in 1923. In those early days it was obvious clinically that batches from different sources varied widely in strength (Table I). It was necessary, however, in order to save the lives and relieve the sufferings of diabetics, to place insulin on the market as quickly as possible.

TABLE I.—Comparison between Two Batches of Insulin under Identical Conditions on Consecutive Days. (G. A. H.)

Period after Insulin.	Blood Sugar: Mg. per 100 c.cm.	
	Batch A (10 Units).	Batch B (20 Units).
Immediately before	205	220
One and a half hours	164	223
Two and a half hours	118	174
Four hours	76	110

It will be seen that 20 units of Batch B was not so efficacious as 10 units of Batch A. This comparison was made in early days (1923) before the methods of standardization were satisfactorily established. Batch A was made by a different firm from Batch B. Such gross differences are probably never to be met with nowadays. Certainly two batches made by the same firm would never exhibit such wide differences now.

Complaints from clinical workers as to variations in the potency of different batches were very few and then sometimes contradictory. This, no doubt, was mainly due to two reasons. First, very few physicians maintained the blood sugars of their patients for long periods close to the level at which hypoglycaemic symptoms occur, and most therefore failed to observe variations in the strength of different batches. Secondly, all physicians were intent on learning how to use the drug to the best effect, and were trying different doses and methods of treatment, and therefore

TABLE II.—*Clinical Comparison of Different Batches of Insulin which had been passed as of Uniform Strength as a Result of Rabbit Tests. (R. D. L.)*

Hours after First Dose of Insulin.	April 15th, 1924.		April 16th.		April 17th.		April 18th.		April 19th.		April 20th.	April 21st.	
	Batch C, 18 and 14 units.		Batch D, 18 and 8 units.		Batch E, 18 and 14 units.		Batch F, 18 and 12 units.		Batch E, 18 and 12 units.		Batch D 14 units and Batch F 12 units.	Batch G 18 units and Batch F 12 units.	
	Glycosuria.	Glycaemia.	Glycosuria.	Glycaemia.	Glycosuria.	Glycaemia.	Glycosuria.	Glycaemia.	Glycosuria.	Glycaemia.	Glycosuria.	Glycosuria.	Glycaemia.
0	0	150	0	166	v. sl. tr.	182	0	165	0	167	0	0	157
1	+	—	0	—	++	—	0	—	v. sl. tr.	—	—	0	—
2	+++	—	0	92*	+++	—	sl. tr.	—	+	—	0	trace	—
3	++	—	0	—	—	—	0	—	trace	—	—	+	—
4	trace	160	0	105	trace	157	0	92	0	146	0	sl. tr.	171
5	v. sl. tr.	—	—	—	0	—	—	81*	—	—	—	0	—
6	—	—	0	—	0	—	0	—	—	—	—	0	—
7	sl. tr.	165	—	—	—	—	0	145	—	—	—	v. sl. tr.	132
8	—	—	0	—	—	—	0	—	—	—	—	—	—
9	v. sl. tr.	—	—	—	—	177	—	—	v. sl. tr.	—	trace	0	—

Note.—Breakfast half an hour and lunch four and a half hours after insulin. Second dose of insulin given eleven hours after first. Glycaemia as mg. per 100 c.cm. blood.

* Hypoglycaemic symptoms; 3 grams of sugar given April 16th, but no sugar on April 18th.

ascribed observed changes to factors other than insulin. It was only later that constant methods and refinements in handling patients led to the detection of small variations in the strength of different batches.

Early in 1924 a few workers, whose plan was to keep the blood sugars of their patients fairly normal (between 180 and 80 mg. per cent.), and who were employing large doses of insulin to do so in severe cases, reported that the variation in the potency of insulin was sometimes very noticeable. A typical experiment by one of us (R. D. L.) is given in Table II, and the conclusions in Table III are based on such experiments. It was further noted that the results of assay on rabbits and of clinical tests on human patients occasionally showed marked discrepancies.

Early in 1925 we began the clinical assay of the specially selected series of samples, under the conditions mentioned above by Dr. Dale. We always worked independently of each other, and on different patients. At the end of the inquiry the results from the three sources—the National Institute, the Hospital for Sick Children, and King's College Hospital—were compared. A summary is given in Table IV. It will be seen that, considering the many incompletely controllable factors (such as a variable glycogen store, etc.) inherent in such experiments, the three sets of results are exceedingly close to one another.

TABLE III.—*Comparison between Rabbit Tests and Clinical Tests in 1924.*

Results Expressed as Percentages of a Common Standard.

Batch No.	Rabbit Tests. (H. P. M.)	Clinical Tests. (R. D. L.)	Batch No.	Rabbit Tests. (H. P. M.)	Clinical Tests. (R. D. L.)
D	67	100	I	100	100
H	100	100	N	95	70
C	83	63	J	100	100
G	120	70	K	98	145
E	95	63	L	87	70
F	120	90	M	100	100

It will be noted that Batch I is 100 per cent. in Table III but 80 per cent. in Table IV. In the summer of 1924 it was 100 per cent. according to both rabbit and clinical tests. Six months later it was 80 per cent. according to clinical tests. As a result of repeated clinical tests its potency appeared constant at 80 per cent. for the next five months. At the end of eleven months the total rabbit tests resulted in a figure of 80 per cent. of the standard. It would appear,

TABLE IV.—*Comparisons between Rabbit Tests (Marks's Method) and Clinical Tests in 1925.*

Results Expressed as Percentages of a Common Standard.

Batch No.	Rabbit Tests. (H. P. M.)	Clinical Tests.		Batch No.	Rabbit Tests. (H. P. M.)	Clinical Tests.	
		R. D. L.	G. A. H.			R. D. L.	G. A. H.
I	80	71	80	S	112	104	112
O	75	80	80	T	106	100	103
P	124	127	119	H	100	100	94
Q	100	100	100	U	120	125	133
R	95	90	80				

therefore, that some solutions of insulin may deteriorate with long keeping.

We have been led by this comparative experiment to the following conclusions.

1. Rabbit tests by Mr. Marks's method are quite satisfactory for the control of the potency of different batches of insulin for clinical use.

2. Clinical tests, if carefully carried out, are quite as delicate as the best of rabbit tests. Owing to the practical difficulties of clinical testing, it is fortunate that they are not essential for the final adjustment of the strength of batches of insulin for clinical use.

3. There would appear to be no evidence of any fundamental difference in the results of tests performed on *normal* rabbits and on *diabetic* patients.

4. To exclude variations in potency which might be inconvenient in the clinical use of insulin, it would appear sufficient to recommend that only variations exceeding 10 per cent. from the standard need be corrected before issue. The method of assay described by Mr. Marks appears to be adequate to secure this.

5. Some solutions of insulin may deteriorate when kept for several months.

METHOD OF PERFORMING CLINICAL TESTS.

The five selected patients had been on a fixed daily intake of carbohydrate, protein, fat, and calories for many weeks or months before the period of the tests. The insulin (two doses daily) had been carefully adjusted so that there was no glycosuria (or at most occasional traces) at any time. The blood sugars usually varied between 60 and 200 mg. in each twenty-four hours. In the intervals between tests the patients were continuously on the same batch of insulin (Batch I at first, and Batch Q later in the period under review). They were all out-patients and arranged and weighed their own diet.

On the day before each test and on each of the test days the diet was identical, not only in its total food values, but also as regards the articles of food taken. Likewise every detail of the patients' daily routine (the amount of exercise, etc.) was made as exactly alike as possible on each of these days. Charts 1 and 2 show how almost identical curves can be obtained on different days under standard conditions with the same dose of the same batch of insulin.

CHART 1.—Blood sugar curves, with an interval of six days, after the same dose of the same batch of insulin, and on the same diet.

Under these conditions it is fair to conclude that differences in the blood sugar curves are due to variations in insulin alone.

On the first test day the usual dose of the standard batch (I or Q) was administered half an hour before breakfast. The sugar content of the blood was estimated before and at hourly intervals after the insulin for five or six hours. The blood sugar results were then plotted. On the second

CHART 2.—Blood sugar curves, with an interval of ten days, after the same dose of the same batch of insulin, and on the same diet.

day what was judged to be the equivalent dose of the second batch of insulin (usually the same dose, unless we had obtained previous evidence to the contrary) was injected instead of the standard batch. The second curve so obtained was plotted beside the first. When the curves practically coincided, the doses of the two batches were regarded as equivalent. If the curves did not coincide the test was repeated, making a suitable alteration in the dose of the second batch of insulin. Sometimes the standard was given on the second and the unknown on the first test day. The tests were confined to the five selected patients, who had

much experience in tests of this kind, involving accurate quantitative dieting. Three of the cases were severe and

CHART 3.—Comparison of Batch Q and batch I (first test). B=Breakfast (half an hour after insulin) of carbohydrate 15 grams, protein 19½ grams, fat 43½ grams. L=Lunch (three and a half hours after insulin) of carbohydrate 15 grams, protein 20 grams, fat 35½ grams. Batch Q is obviously stronger than Batch I. It was estimated from the curves in Chart 1 that about 16 units of Batch Q would be the equivalent of 20 units of Batch I. The test was therefore repeated a week later. (See Chart 4.)

CHART 4.—Comparison of Batch Q and Batch I (second test). B=Breakfast (half an hour after insulin) of carbohydrate 15 grams, protein 20½ grams, fat 45 grams. L=Lunch (three and a half hours after insulin) of carbohydrate 15 grams, protein 20½ grams, fat 35½ grams. Sixteen units of Batch Q are equivalent to 20 units of Batch I; or, taking Batch Q as 100 per cent., Batch I is 80 per cent.

two moderately severe, thus differing in their power to produce endogenous insulin. But in spite of this the results

obtained in these five individuals were remarkably constant, and in some fifty tests rarely differed more than 10 per cent. from the average results given in Table IV. The results are expressed as percentages of the standard Batch Q. Charts 3 and 4 illustrate the manner in which the tests were performed.

It was soon found that certain conditions, apart from the standard conditions mentioned above, had to be observed if the tests were to be trustworthy. Hypoglycaemic symptoms had to be avoided to obtain comparable curves. When an excess of insulin was given, the second half of the curve tended to be flattened out at a level of about 70 to 90 mg. per cent., the blood sugar falling but slightly although the action of insulin was still potent (Chart 5). The next dose—that is, the evening dose—of insulin also appeared unusually efficient. Presumably the failure of the curve to continue its fall was due to the extra call to mobilize glycogen as a response to hypoglycaemia, and the unusually good response to the next dose of insulin was due to the fact that there was less glycogen upon which to draw. The best comparisons were obtained when the fasting levels on the two days were approximately the same. Comparisons between standards and unknowns were invariably made on consecutive days. It was of little value to make comparisons when marked hyperglycaemia and glycosuria were present. This would be expected owing to the difficulty of ensuring that the same quantity of dextrose “escaped” the action of insulin in tests lasting for relatively short periods.

All determinations of blood sugar were made in duplicate

and the mean taken. Maclean's method of estimation was used throughout. The dose of insulin was measured in a 1 c.cm. syringe graduated in hundredths. Subcutaneous injections were given by the same person in one limb one day and in the corresponding site in the opposite limb on the next day.

An attempt was made to work out an arithmetical method

of comparing different batches. The one which gave the most uniform results was a comparison of the totals of the blood sugar figures obtained on the different test days. For example, if with the same dose of different batches the totals were 500 and 750 mg. the second batch was taken as 50 per cent. weaker than the first, the standard batch. This gave results closely similar to the plotted curves. When the initial fasting levels were different by 10 or 20 mg., an adjustment was made by subtracting 10 or 20 mg. from all the figures, and in most cases this gave very satisfactory results. Thus, if one fasting level was 10 mg. higher than the other, and six blood sugar estimations were made, before making the arithmetical comparison 60 mg. were subtracted from the higher fasting level total. Sometimes these correc-

tions gave results which were manifestly inaccurate, especially when the fasting levels were 30 or more milligrams apart. Other arithmetical calculations based on the fall of the blood sugar—either total or over various parts of the curves—were still more inaccurate, and the comparison of the general appearance and range of the plotted curves was found to be far the most consistent mode of comparison.

CHART 5.—Effect of hypoglycaemia on the shape of the curve. Curve (a): Insulin was not given in doses sufficient to produce hypoglycaemia. It will be noted that the blood sugar was falling rapidly between the tests at two and a half and at four and a half hours. Curve (b): A larger dose of insulin was given; hypoglycaemic symptoms started at the end of three and a half hours, and continued until lunch (four hours forty minutes). It will be noted that Curve (b) is flattened out between two and a half and four and a half hours, whereas Curve (a) is falling steeply at this period. B=Breakfast. L=Lunch.

RIGHT- AND LEFT-HANDEDNESS IN PRIMITIVE MEN.*

BY

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THE statement that the fossilized skull recently found in London was that of a left-handed woman has given rise to widespread incredulity, which in some cases has found expression even in derision. This has not been entirely allayed by the statement published in the *BRITISH MEDICAL JOURNAL* of November 7th, in which I called attention to the very definite evidence provided by the fossilized skull of the reversal of the normal asymmetry of the brain case, and the reasons for associating this reversal with the condition of left-handedness. In that communication (as well as in the *BRITISH MEDICAL JOURNAL* of August 29th, 1908, p. 596) I called attention to the fact that in the winter of 1907-8 Professor Wood Jones and I investigated the significance of this reversal of the asymmetry of the brain case, accepting as the criterion of right- or left-handedness respectively the observation whether the right or the left humerus was the longer and stronger. In the ranium I found that the asymmetrical impressions upon the occipital bone were reversed in those cases where the left humerus was longer and more robust than the right.

* Address to the Westminster and Holborn Division at the Social Evening on December 2nd.

The question has been raised by several critics as to the validity of these inferences from the size of the humeri as indications of right- or left-handedness. Since 1845, when Arnold raised this problem for consideration, an extensive literature has accumulated from the repeated discussions of the asymmetry of the human body and the difference in the length of the right and left arms. An admirable summary of these discussions is given by the late Professor Gaupp in two small books published at Jena in 1909.† On page 7 of the former he summarizes the researches of Arnold, Rollet, Matiegka, Hasse, Dehner, and Guldberg on the question of the excess in length of the left or right arm, and shows that these differences are usually associated with left- and right-handedness respectively. However, he calls attention to the fact that at the time of birth the length of the bones in the two arms is identical. In other words, the asymmetry manifests itself in the course of post-embryonic life. Gaupp further points out that occasionally it happens (in people whose occupation compels them to exercise the left arm more than the right) that a person with a congenital tendency to right-handedness may have longer and stronger bones in the left arm. This state of affairs, however, is altogether exceptional, and should not be allowed to discredit the clear inference from a large mass of evidence that the length of the arm-bone in the great majority of cases is a safe indication of right- or left-handedness.

† *Über die Rechtshändigkeit des Menschen, and Die normalen Asymmetrien des menschlichen Körpers.*