TESTING SMALL INSULIN CONCENTRATIONS BY MEANS OF ISOLATED EPIDIDYMAL RAT FAT

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 121-124, July, 1961
Original article submitted March 17, 1960

In recent years, methods have been proposed for testing small insulin concentrations by means of their action on fatty tissue. The basis of the method is that insulin increases the utilization of glucose, its oxidation to carbon dioxide, the formation of fat from glucose, etc. The effect of insulin on glucose utilization by fatty tissue has not been sufficiently studied, and the different laboratories concerned have usually used quite a small range of insulin concentrations. The results from different laboratories, and even from those using similar methods of its action on epididymal rat fat are widely divergent: in one case the minimum concentration of insulin was 1 micro-unit per ml [2], in another it was 10 micro-units [6], in a third 100 micro-units per ml [11]. The maximal effect has been reported to occur in one case at a concentration of 100 micro-units per ml [2], and in another at 10,000 micro-units per ml [11]. The differences may to some extent depend on the strain of rat used, and on their nutrition [7]. A relevant factor may also be the use in many cases of gelatine to prevent absorption of insulin on the walls of the glass vessel [5]. However, these considerations cannot explain the whole of the divergence in results, which are probably to be attributed to other differences in technique.

METHOD

The experiments were carried out on white male rats of various weights, fed on a free choice of diet. They were killed by decapitation, and the epididymal fat was quickly removed. It was important to avoid damage to the fatty tissue, and to reduce to a minimum the time for which it was cooled in the air. In our experiments, not more than 2-3 min elapsed between removing the fat and placing it in the incubator. After it had been removed, it was weighed on a torsion balance and placed in a 25 ml Erlenmeyer flask containing the incubation medium. The fatty tissue was weighed before and after incubation, and in many cases it was found to increase by 10-60%, and the amount of water taken up was not proportional to the weight of tissue. We have therefore thought it incorrect to determine the weight of the tissue after incubation, and in all our experiments the calculations were made in terms of the freshly removed fat.

After the fat had been placed in the flask, the latter was closed with a rubber cork containing tubes through which a gaseous mixture was passed for $1\frac{1}{2}$ -2 min. It was found from many experiments that a suitable composition for the gaseous mixture was 5% carbon dioxide, while the remaining 95% of the mixture could be either air or oxygen. When the air was replaced by oxygen, no change in the results was found. Similar results for the formation of carbon dioxide by fatty tissue were obtained whether the mixture contained air or oxygen [4]. On this account we used a mixture consisting of 5% carbon dioxide and 95% air. After aeration, the tubes were closed, and the flask was placed in a Warburg apparatus. Incubation at 37° was continued for 3 hours, and the flask was shaken at a rate of 120 strokes per minute.

The absorption of glucose by epididymal rat fat, and the effect on it of insulin vary greatly according to the animal used, and for this reason, in order to obtain reliable results, it is important to incubate tissue from several animals simultaneously in a single flask, or else to repeat the experiment many times. On account of the wide variations in the sensitivity of the fat of different animals to incubation, it is justifiable to incubate the fat from several animals at the same time in a single flask, only if the amount of fat contributed by each animal is the same

and this condition is not easily attained without causing trauma to the fatty tissue. It is therefore preferable to use fat from a single animal, and to repeat the determination several times. For this reason we took two portions of epididymal fat from each animal, one from each side. One was incubated in a medium with, and the other in a medium without insulin. It should be noted that different portions of the fat of a single animal may show different sensitivities [1].

The experiments were made on 48 portions of fat weighing 100-700 mg, and it was found that for an insulin concentration of 1,000 micro-units per ml the absorption of glucose was proportional to the weight of the tissue, the co-efficient of proportionality being 0.72. The absorption was greatly reduced when the weight of the sample exceeded 300 mg, and in subsequent experiments samples of such a size were not used. In many cases it was found that the sensitivity of the deeper portions of epididymal fat was less than the more peripheral parts'. Similar results were obtained by determining the formation of carbon dioxide from glucose due to epididymal fat [4]. We therefore used two weighed portions of peripheral epididymal fat from each animal.

The incubation medium was that of Krebs and Henseleit [8], which contained 400 mg% of glucose and 200 mg% of gelatine. Previous experiments have shown that when gelatine is added the effect is considerably enhanced.

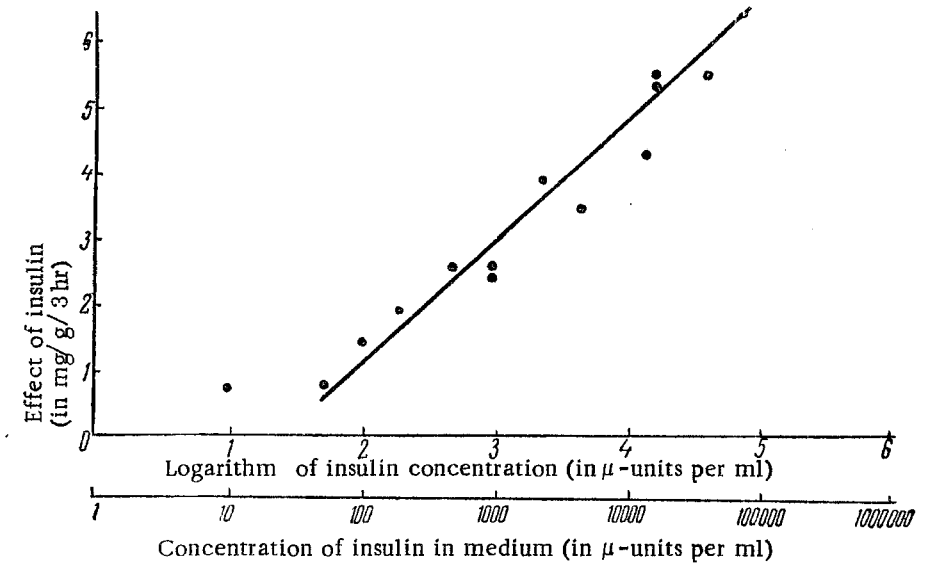
The insulin was supplied by the Leningrad Meat Plant; it was series No. 39, the activity was 24 units per mg, it was not free from glucagon, and it was recrystallized once. A stock solution of insulin was made in 0.01 N hydrochloric acid, and it had an activity of 50 units per ml; subsequently, portions were diluted to the required concentration.

The experiments were carried out simultaneously on 5 animals. The absorption of glucose by fatty tissue was calculated from the excess glucose remaining in the medium after incubation, and it was expressed as mg of glucose absorbed per g of fat per 3 hours' incubation. The effect of insulin was measured by the difference in the amount of glucose absorbed in a medium with or without insulin, and was expressed as mg of glucose per g of fat per 3 hours. The glucose concentration of the medium was determined by the method of Somodzhi and Nelson [9,10], and by the protein precipitation method of Frank and Kirberger [3].

Because the experiment was carried out on 5 animals, the correlation between insulin concentration and its effect was obtained in terms of the mean value for the group, and not from the separate determinations. In fact, by this method, the co-efficient of correlation shows how accurately it is possible to determine insulin concentration in the medium from its mean effect on 5 animals. Of course, it would be possible to obtain greater accuracy with a greater number of rats, but two Warburg apparatuses would then be required, which is inconvenient.

Effect of Various Insulin Concentrations on the Absorption of Glucose by Weighed Portions of Epididymal Rat Fat

Insulin concentration in medium (in micro-units per ml)	Log. of insulin concentration	Number of animals	Effect of insulin (in mg of glucose/g of tissue/3 hr)	
			Results of different determinations	Mean value
10	1.0	5	0; 0.71; 0.76; 0.84; 1.23	0.71
50	1.7	5	0; 0.48; 0.54; 0.55; 2.16	0.75
100	2.0	4	0.70; 2.45; 2.90; 3.73;	2.44
100	2.0	5	0.33; 0.79; 1.26; 2.26; 2.43	1.41
200	2.3	5	0.55; 1.67; 2.06; 2.45; 2.56	1.84
500	2.7	5	0.28; 1.98; 2.25; 3.03; 4.97	2.50
1 000	3.0	5	1.40; 2.15; 2.37; 2.85; 3.05	2.36
1 000	3.0	5	0.97; 2.31; 2.49; 2.95; 3.86	2.50
2 500	3.4	5	1.36; 1.83; 3.88; 5.57; 6.36	3.80
5 000	3.7	5	0.05; 3.01; 3.55; 4.71; 5.62	3.38
15 000	4.2	5	1.90; 2.50; 2.75; 6.30; 7.68	4.22
20 000	4.3	5	3.64; 4.41; 5.18; 5.92; 7.45	5.32
20 000	4.3	5	2.94; 4.94; 5.42; 5.46; 7.40	5.23
50 000	4.7	5	3.62; 3.92; 5.18; 5.90; 8.13	5.35
100 000	5.0	5	3.08; 5.32; 6.68; 7.37; 9.25	6.34
500 000	5.7	4	2.60; 5.28; 5.90; 9.78	5.89



The effect of insulin at different concentrations. The points represent mean values for 4-5 determinations. The regression line has been drawn.

In determining the correlation between insulin concentration and its effect, we used the well-known method of expressing not the actual concentration, but the logarithm to the base ten of the concentration in micro-units of insulin per ml of medium. The effect was calculated from the difference in the amount of glucose utilized in media with and without insulin.

In some cases, the glucose absorption by the fatty tissue was so great that the figures were not included in those from which the mean values were calculated. For example, in one of the experiments, the action of insulin in a concentration of 100 micro-units per ml was 11 mg/g/3 hours, while in another, at a concentration of 500,000 micro-units per ml it attained the value of 37 mg/g/3 hours. We were unable to find the reason for this very high sensitivity. Other authors have observed the same effect.

RESULTS

The table shows the separate and mean values of 78 determinations made in 16 experiments using 13 different insulin concentrations.

An increase in the insulin concentration of the medium above 100,000 micro-units per ml did not increase its effect, while reducing it to 5 micro-units per ml led to variable and uncertain results.

It can be seen from the table that over a range of concentrations of 50 to 100,000 micro-units per ml there is a linear relationship between the logarithm of the insulin concentration and its effect.

It could be shown statistically that for concentrations between 5 and 100,000 micro-units per ml, the co-efficient of correlation between the log. of the insulin concentration and the effect on 4-5 animals was + 0.889. For this number of experiments [14], the mean error of the co-efficient was 0.277, and therefore the co-efficient itself was 3.2 times greater than its mean error, which shows that the correlation was highly significant. The regression line was determined from the formula: $x=0.568\,y + 1.382$, where x is the logarithm of the concentration of insulin in the medium, and y is the effect of insulin in mg of glucose per gram of tissue per 3 hours.

For the concentration limits given above, the mean error of the logarithm of the concentration, calculated from the formula, is 0.25, which corresponds to a mean error in the determination of the insulin concentration of 1.78 (in certain experiments it ranged from 1.07 to 5.9; then, in 12 experiments out of the 14, the error did not exceed 2.7). For comparison, the mean error for the determination of insulin using an isolated rat diaphragm is usually taken to be 3 (i.e.,the value found may be three times less or three times greater than the true amount).

Therefore, by using the regression equation, the method of determining insulin concentration in terms of the utilization of glucose by epididymal rat fat enables unknown insulin concentrations of a wide range to be determined

with sufficient accuracy.

The co-efficient of correlation which we obtained is higher than that reported elsewhere. Possibly, some reasons for such a high degree of correspondence are as follows. The correlation was determined from the mean values of several experiments carried out in parallel, which is a more precise method than using results of separate experiments, which always give a fairly wide scatter. The calculation was made not from the absolute absorption of glucose from the medium by insulin, but from the effect of the action of insulin, i. e., from the extra amount of glucose absorbed from a medium containing insulin, as compared with one free from it. We did not incubate weighed portions of tissues from several different animals simultaneously in one flask, because such a method not only does not reduce the influence of individual variations, but may actually increase it. Other unknown factors may also play an important part, and they may include properties of the insulin itself, the strain of rats, their nutrition, etc.

SUMMARY

The study of glucose uptake by rat epididymal fat has demonstrated that the effect occurs at concentrations above 10μ units per ml; it then increases progressively up to a concentration of 100,000μ units per ml. Within a range of concentration from 50 to 100,000μ units per ml, there is a linear relationship between the logarithm of the insulin concentrations in the medium and its effect; the correlation co-efficient is + 0.889. A regression equation was calculated which gave a mean error for the determination of the insulin concentration of 1.78. Thus, this method is not only simple, but is also sufficiently precise for the determination of insulin concentrations in an incubation medium.

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