CHARACTERISTICS OF THE LIPOLYTIC ACTIVITY OF FATTY TISSUE

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Processes taking place in fatty tissue include hydrogenation and dehydrogenation of fatty acids, the decomposition and synthesis of triglycerides, the conversion of carbohydrates into lipids [1, 9], and the synthesis of fatty acids from amino acids [4]. The lipolytic activity of fatty tissue permits the entry of free (nonesterified) fatty acids (NEFA) into the blood stream, to be used subsequently as energy-producing material [5]. Experiments in vivo and in vitro have shown that one of the factors influencing the production and release of NEFA from fatty tissue is the glucose level in the blood and also in the fluid bathing the fatty tissue [1, 5, 6, 7].

The object of this research was to study the relationship between the lipolytic activity of the fatty tissue and the glucose concentration in the medium and its degree of absorption by the fatty tissue.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-200 g, which were fasted for 18 h. The lypolytic activity of the fatty tissue was determined by the method of Gordon and Cherkes [5] with slight modifications. The epididymal fatty tissue was extracted and washed in cold physiological saline, after which it was quickly chopped with scissors, and a sample weighing 100 mg was placed in a test tube containing 3 ml of incubation medium (5% of human serum albumin in Krebs-Ringer-phosphate buffer, the pH of which was adjusted to 7.3-7.4 by the addition of 1 N NaOH).

The NEFA content of 1 ml of the fluid was determined before incubation, after which the test tube was covered with tinfoil and placed in the water bath of a Warburg's apparatus at 37° for 150 min, with continuous agitation. At the end of incubation the NEFA content of 1 ml of the incubation mixture was again determined.

The lypolytic activity was given by the difference between the NEFA contents before and after incubation, and expressed in meq/ml NEFA per gram of tissue.

Dole's method of estimating NEFA [3]: to 1 ml of fluid is added 5 ml of extracting mixture (isopropyl alcohol, heptane, 1 N sulfuric acid in proportions of 40 : 10 : 10); agitation for 2 min, 3 ml heptane and 2 ml bidistilled water are added, agitation for 3 min, the mixture is allowed to stand for 10 min; 2 ml of the upper (heptane) layer is transferred to a centrifuge tube, 1 ml of 0.01% thymol blue is added (as indicator) and titration is carried out in a stream of nitrogen with a 0.018 N solution of NaOH from a 0.2 ml microburet with divisions of 0.002 ml.

A parallel series of reagent control tests was carried out (2 ml heptane and 1 ml of 0.01% thymol blue solution). A calibration curve was plotted for a palmitic acid solution (15.4 mg palmitic acid in 100 ml heptane; 1 ml of this solution contains 0.6 meq/liter or 0.6 μeq/ml).

In the experiments to study the effect of glucose on lipolysis, the glucose was added in a sufficient quantity to give a concentration of 50 and 300 mg% in the incubation medium. Glucose was determined by the Hagedorn-Jensen method.

The control determinations showed that after preliminary heating to 60° for 45 min, the lipolytic activity of the incubation mixture was unchanged, thereby demonstrating the enzymic nature of lipolysis.

EXPERIMENTAL RESULTS

Experiments conducted on 66 rats confirmed the presence of lipolytic activity in the fatty tissue. Its average magnitude was 4.3 ± 0.154 μeq/mg per gram of tissue (maximum 6.5, minimum 2.2 μeq/mg per gram of tissue).

When the incubation medium contained 50 mg% of glucose, the lipolytic activity of the fatty tissue (difference between the NEFA contents before and after incubation for 150 min) fell on the average from 4.85 (±0.595) to 1.58 (±0.522) μeq/ml per gram of tissue (67.4%). If the glucose concentration was 300 mg%, the lypolytic activity fell to 0.261 (±0.371) μeq/ml per gram of tissue (94.6%). In each case the difference was significant ($P < 0.001$; No. of experiments 11). If the incubation mixture contained 50 mg% of glucose, the fatty tissue utilized 10.3 (±2.01) mg% of glucose, and if 300 mg% of glucose – 24 (±5.72) mg% (No. of experiments 10).

Hence, the addition of glucose directly to fatty tissue inhibits its lipolytic activity, and this inhibition bears a definite relationship to the degree of absorption of glucose by the fatty tissue. The inhibition of the lipolytic activity by glucose may be the result of stimulation of the synthesis of triglycerides or of inhibition of their hydrolysis.

Investigations using palmitate-1-C^{14} [2] have shown that if up to 5 mM of glucose is added to fatty tissue, the incorporation of palmitate-1-C^{14} into triglycerides is increased and its oxidation depressed. Since the synthesis of triglycerides in fatty tissue is effected through α-glycerophosphoric acid, which is formed in the course of glucose metabolism, the workers cited concluded that the addition of glucose stimulates the resynthesis of triglycerides by fatty tissue, for under these circumstances α-glycerophosphoric acid is formed.

Experiments on isolated fatty tissue [8] have shown that if 0.5 mg/ml of glucose is added to the medium (50 mg%), the incorporation of palmitate-1-C^{14} into the triglycerides of the fatty tissue is doubled; the concentration of NEFA in the medium falls. These workers asserted on this basis that the addition of glucose to fatty tissue acts principally by stimulating the resynthesis of triglycerides in the fatty tissue and not by inhibiting their hydrolysis.

It may be concluded from these facts and considerations that the addition of glucose to fatty tissue in our experiments stimulated (through the formation of α-glycerophosphoric acid) the processes of resynthesis of triglycerides, so that the concentration of NEFA in the medium was decreased.

SUMMARY

It has been confirmed that in definite incubation conditions fatty tissue possessed a lipolytic activity manifested in the production of free (nonesterified) fatty acids. Addition of glucose depresses the lipolytic activity of the fatty tissue, the extent of depression depending on the glucose concentration in the medium. The glucose uptake by the fatty tissue increases with the rise of its concentration.

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