of erythrocyte catalase represent different conformations (conformers) of the same molecule[10].

By applying the standard separation procedure, it has been demonstrated that reticulocytes show a higher A/C ratio than old red cells[4,11]. In view of the results presented here, it seems probable that the considerably higher metabolic activity of the reticulocyte (e.g. regeneration of GSH) may cause a higher degree of reductive protection during the fractionation procedure. Secondary alterations of similar nature have been observed with other red cell enzymes such as G-6-PD and GOT[12], stressing the importance of a reducing environment for maintenance of the structure of red cell enzymes[14,15].

Zusammenfassung. Katalase aus Erythrocyten vom Menschen (und Pferd) lässt sich säulenchromatographisch und elektrophoretisch in drei Fraktionen A, B und C auftrennen, wobei die Fraktionen A und B die Tendenz haben, in die Fraktion C überzugehen. Durch Chromatographie unter Ausschluss von Luftsauerstoff konnte gezeigt werden, dass die Katalase in den Erythrozyten in der Form A vorliegt. Setzt man das Hämolysat dagegen einige Zeit Luftsauerstoff aus, wird die Katalase bei der Chromatographie in Form C eluiert. SH-blockierende Reagentien verhindern die Umwandlung von A in C, während C mit Mercaptoäthanol zu A reduziert werden kann. Es wird angenommen, dass dem Übergang von Fraktion A in B und C eine Bildung von Disulfidbrücken zugrunde liegt und dass es sich bei den beobachteten alternativen Formen möglicherweise um Katalase-Konformere handelt.

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Prompt Effect of Insulin in vitro on the Esterolytic Activity of Tissue Homogenates not Modified by Actinomycin D

In the course of our study of the mechanism of insulin action in the lipolytic system, we centred our attention on the role of group-specific esterases. It is possible that these enzymes might, besides lipases, take part in lipolysis. Esterases, in contradistinction to lipases[1,2], act predominantly on aqueous solutions of the substrates. In spite of the fact that most tissues contain complex mixtures of esterases with high total activities[3,4], their physiological function has so far remained unexplained[5].

In the experiments which were partly reported elsewhere[6,7], we have found that the esterolytic system of adipose tissue from starved rats is sensitive in vitro to insulin. These results were obtained after preincubation of samples of the whole tissue with insulin in the presence of NaCl. In this paper we have studied the influence of insulin in vitro on the esterolytic activity of tissue homogenates taken from fed as well as from starved rats in the presence of KCl or NaCl in the medium. Moreover, we have examined whether the tissue esterolytic activity changes during fasting and whether insulin also acts in vivo as an inducer or suppressor of the synthesis of esterases.

Materials and methods. Albino Wistar rats (inbred strain) weighing 150–200 g were used. They were fed on the usual Larsen diet[8] and, before the experiment, were fed or fasted for 48 and 96 h respectively.

One group of rats was made diabetic by using alloxan-monohydrate[9]. Diabetes produced by alloxan was stable as indicated by persistant glycosuria and hyperglycemia. Glucose was estimated by glucose oxidase test[10]. Insulin (Insulin Spofa pro injectione) and actinomycin D were applied to alloxan-diabetic rats during 24 h[9].

Epididymal fat pad or myocardium, femoral muscles, lungs, liver, and kidney were weighed immediately after killing the animals and homogenated in distilled water by using a glass homogenizer of Potter-Elvehjem type as well as sea sand (under standard conditions). For estimation were used samples of 0.1 ml of supernatant fluid obtained after centrifugation at *2000 g*. Each sample represented 2 mg of tissue.

For the determination of esterolytic activity was used 2-naphtolacetate dissolved in 0.2 *M* veronal buffer pH 7 (adipose tissue and skeletal muscle) or 7.5 (myocardium and lungs). The liberated 2-naphtol was determined colorimetrically[11] or fluorimetrically[12]. In the samples of tissue homogenates proteins were determined similarly by

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using LOWRY's method[13]. Esterolytic activity was expressed in μmol of 2-naphtol liberated/mg of tissue protein/h.

In the experiments where the effect of insulin on tissue esterases was tested in vitro, the samples of tissue homogenates were preincubated for 20 min at 25 °C in a medium containing indicated veronal buffer and NaCl or KCl in the final concentration of 40 mmol/l with or without insulin (Insulin Spofa pro injectione in the final concentration of 0.01 U/ml, unless otherwise indicated). To prevent absorption of insulin on glass walls, we added gelatine to the medium[14]. To obtain the insulin effect, all respective conditions had to be strictly adhered to, especially pH[15] and the ion composition of the medium. Guinea-pig anti-insulin serum had the binding capacity of 90 U/l. The human insulin antibodies (binding capacity 270 U/l of the serum) were taken from insulin-resistant patient. The binding capacity of the added insulin antibodies exceeded approximately 5 times the quantity of insulin in the medium. After preincubation the determination of esterolytic activity was carried out in the above-mentioned way.

The results were statistically evaluated by means of t test.

Results and discussion. In the presence of NaCl in the medium insulin significantly decreased the esterolytic activity of adipose tissue homogenate taken from starved rats (Table I). When samples from fed rats were taken, no insulin effect occurred under these conditions. When NaCl in the preincubation medium was replaced by KCl in equimolar concentration, the insulin effect on the esterolytic activity of adipose tissue homogenate was stimulatory and appeared in the tissue of starved as well as fed rats (Figure 1). The results illustrated in Figure 2 showed the sensitivity of tissue esterases even to the minute doses of insulin (50 μU/ml).

In this paper only the results with adipose tissue are presented in details. We have ascertained, however, the group-specific esterases sensitive in vitro to insulin also in myocardium, skeletal muscle and lung tissue. In all tissues indicated, there was stimulatory effect of insulin in the presence of KCl, and different effects in the presence of NaCl in the medium. The optimal pH for these effects differed in individual tissues from 7–7.5. On the contrary, we were unable to prove insulin-sensitive esterase in liver and kidney at any pH from 6.5–9.

As insulin influenced esterolytic activity after preincubation with homogenated tissue samples, intactness of the cells was not an indispensable condition for this effect. The insulin effect demonstrated after 20 min preincubation occurred already after 3–5 min preincubation of tissue homogenate with insulin. Insulin apparently participates in the reaction of esterases with substrate and does not act on the synthesis of studied esterases. This is supported also by further facts:

(1) Esterolytic activity does not alter even during starvation (Table II) where a rapid decline in the level of insulin has been demonstrated by YALOW and BERSON[16], not even in the tissue of alloxandiabetic animals (Figure 3).

(2) Alloxan-diabetic rats treated with insulin in comparison with those treated with actinomycin D (a substance which specifically affects the DNA-dependent

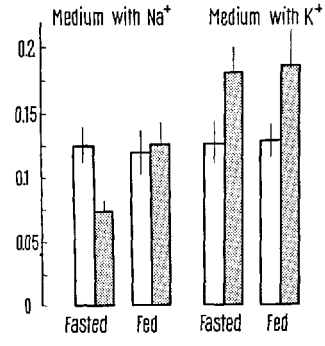

Fig. 1. Esterolytic activity of samples of adipose tissue homogenates preincubated in the presence of KCl or NaCl in the medium with insulin (black columns) or without insulin (white columns). Esterolytic activity was expressed in μmol of 2-naphtol liberated from naphtolacetate/mg tissue protein/h.

Table I. Influence of insulin on the esterolytic activity of adipose tissue homogenates from rats fasted for 48 h before experiment

Incubation medium (with NaCl)	Esterolytic activity (μmol liberated naphthol/mg tissue proteins/h)	S.E. ±	No.	p
Control	0.108	0.009	14	
+ insulin	0.076	0.007	11	< 0.001

Table II. Adipose tissue esterolytic activity in fed and starved rats

Rats	Esterolytic activity (μmol liberated naphthol/mg tissue protein/h	S.E. ±	No.	p
Fed	0.112	0.009	10	–
Starved for 48 h	0.107	0.011	12	–
Starved for 96 h	0.109	0.013	11	–

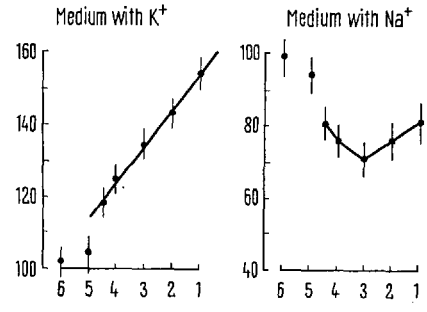

Fig. 2. Influence of different doses of insulin on the adipose tissue homogenate esterolytic activity. The samples were taken from rats fasted for 48 h before experiment. Abscissa – log of insulin concentration (μU/ml). Ordinate – esterolytic activity (% of control values).

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RNA synthesis[17] and insulin did not show any substantial difference in the tissue esterolytic activity (Figure 3).

From these results we concluded that this new insulin effect occurred so rapidly that the mechanism of suppression or induction of esterases by insulin can be excluded. On one hand, it contrasts with the results of many authors who found that the synthesis of a number of key enzymes of lipid and saccharide metabolism was directly governed by insulin[9,18–22]. On the other hand, as far as the rapidity of the response is concerned, the effect described above is similar to the antilipolytic effect of insulin[23] as well as to its hypoglycemic one[9], which is, moreover, also known not to be modified by actinomycin D[9].

The interaction of insulin and esterases, however, does not seem to be quite direct, as apparently in this interaction also ions take a share. The most important is the presence of K^+ or Na^+ (Table I, Figure 1), and also H^+ concentration the significance of which we have discussed elsewhere[15]. Also the differences in reaction of tissue esterases from fed and starved rats show that the interaction is not simple. During starvation we have found the shift of ions especially K^+ from intracellular to extracellular space in rat adipose tissue[24]. The K^+/Na^+ ratio, significance of which was recently demonstrated for lipolysis[25], is apparently also important for the response of tissue esterase on insulin.

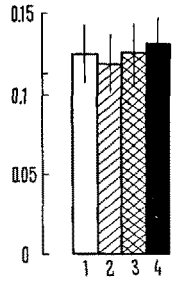

Fig. 3. Esterolytic activity of adipose tissue samples taken from control rats (1), alloxan-diabetic untreated rats (2), diabetic rats treated with insulin (3) and diabetic rats treated with insulin and actinomycin D (4). Esterolytic activity was expressed in μmol of 2-naphtol liberated/mg tissue protein/h.

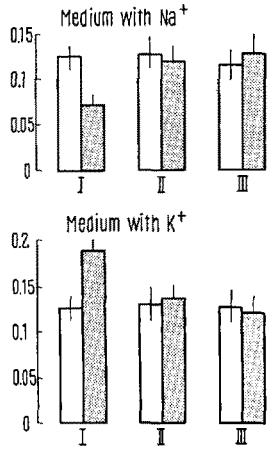

Fig. 4. Esterolytic activity of samples of adipose tissue homogenates preincubated in the presence of KCl or NaCl with insulin (black columns) or without insulin (white columns). I, control determination; II, insulin antibodies; III, 0.001 *M* cystein. Adipose tissue was taken from rats fasted for 48 h before experiment. Esterolytic activity was expressed in μmol of 2-naphtol liberated/mg tissue protein/h.

The different reaction of tissue esterases on insulin in the presence of K^+ or Na^+ could be explained by 2 components of the insulin-sensitive esterolytic activity both of them being K^+ and 1 also Na^+ dependent[24]. In this way it would be possible also to explain the fact that in the presence of K^+ there was a linear dependence of insulin effect on its doses which was not demonstrable in the presence of Na^+ (Figure 2).

The independence of the effect on the kind of insulin preparation (Table III) and the fact that cystein and antiinsulin serum removed the described effect (Figure 4) demonstrated that we are dealing with a genuine insulin activity. Although for the physiological interpretation of the results it is important that tissue homogenates should react even on the physiological concentrations of insulin (Figure 2), the application of non-physiological substrate does not permit definite conclusions. It is, however, interesting that we have found the insulin-sensitive esterases predominantly in so-called insulin-sensitive tissues, i.e. in adipose tissue, muscle and myocardium.

Table III. Influence of insulin on the esterolytic activity of adipose tissue homogenates from fed rats (in the presence of KCl in medium)

Insulin preparation	Insulin effect on esterolytic activity in % (control value = 100%)	S.E. ±	No.	*p*
Insulin Spofa pro inj.	126.9	4.5	10	< 0.01
Crystalline bovine insulin Organofarma	128.8	3.7	9	< 0.01
Crystalline bovine insulin Organofarma labelled with fluoresceinisothiocyanate	122.8	4.6	8	< 0.02

Zusammenfassung. Die in vitro-Wirkung des Insulins (Konzentrationsbereich von 50–100000 μU/ml des Inkubationsmediums) auf die esterolytische Aktivität der Fettgewebe, Homogenate von Myokard, Lunge und Muskel wurde bei Ratten beschrieben. Es ergibt sich eine von der Enzym-Synthese unabhängige schnelle Insulinwirkung.

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