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## Effect of rat serum on glycerol metabolism in adipose tissue *in vitro*

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It has been proposed that the lipolytic effect of serum is based on the presence of either lipoproteins or catecholamines. To test these hypotheses, pieces of epididymal fat pads from fed rats were incubated in the presence of albumin and glucose for 120 min. The addition of rat serum (5  $\mu$ l/vial) enhanced the rates of both glycerol release to the media and [ $^{14}$ C] glycerol utilization by the tissue. Heparin did not alter these parameters or the response produced by serum. VLDL from rat plasma also enhanced glycerol release and utilization for the formation of CO<sub>2</sub> and lipids, and heparin significantly augmented these effects. Neither of the conditions studied affected the percentual distribution of  $^{14}$ C-lipid fractions in the tissues. It is known that in similar conditions to those used in the present study, adrenaline produces a decrease in the utilization of glycerol. Thus our findings do not support the proposed hypothesis explaining the fat-mobilizing action of serum, the mechanism of which remains to be determined.

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### Introduction

The lipolytic effect of serum when incubated *in vitro* in the presence of adipose tissue has been reported by various investigators (RECANT *et al.*, 1960; BURNS *et al.*, 1967; CURTIS-PRIOR & HANLEY, 1973; FEDERSPIL *et al.*, 1975; TUTTEROVÁ & MOSINGER, 1975). Although the nature of the fat mobilizing factor (or factors) is still unknown, it has been proposed that the mechanism may be based either on the interaction of lipoprotein lipase of adipose tissue and the lipoproteins present in the serum (TUTTEROVÁ & MOSINGER, 1975) or on the presence of catecholamines in the serum (CURTIS-PRIOR, 1973; CURTIS-PRIOR & HANLEY, 1973; FEDERSPIL *et al.*, 1975; TUTTEROVÁ & MOSINGER, 1975) which by themselves have an intense lipolytic action (FAIN, 1973). It is known that the lipoprotein lipase activity of adipose tissue is enhanced by the presence of heparin in the incubation medium (PERSSON *et al.*, 1966; STEWART & SCHOTZ, 1974), whereas catecholamines markedly affect the ability of adipose tissue *in vitro* to metabolize glycerol (DOMINGUEZ & HERRERA, 1976a & b). To test these proposals, the production of glycerol and the utilization of [ $^{14}$ C] glycerol by rat epididymal fat-pad pieces were studied *in vitro* in the presence or not of rat serum, rat very low density lipoproteins (VLDL) and heparin.

### Materials and Methods

Male Wistar rats weighing 160-180 g and fed standard rat chow were maintained in a temperature ( $22 \pm 2$  °C) and light cycle (12 h on-off) controlled room. They were sacrificed by cervical fracture without anaesthesia and four pieces of epididymal fat pads ( $19.2 \pm 0.6$  mg) from each rat were placed in vials containing Krebs-Ringer bicarbonate buffer pH 7.4 with suitable salt content to yield the desired final concentration (UMBREIT *et al.*, 1964), supplemented with bovine serum albumin purified by the method of CHEN (1967) and glucose (final concentrations were 0.8 % and 4 mM, respectively). Fresh solutions of [ $U-^{14}C$ ] glycerol (46 mCi/mmol, 0.3  $\mu$ Ci) in addition to serum from 24-h fasted rats (5  $\mu$ l), heparin (3 IU) or VLDL from rats prepared as described below and corresponding to 0.4 ml of initial plasma were pipetted into the vials which had a final volume of 1.25 ml. Incubations were performed for 120 min at 37 °C after gassing for 5 min with  $O_2$ - $CO_2$  (95 : 5) in a Dubnoff shaking incubator at 100 cycles/min. The incubations were terminated by addition of  $HClO_4$ , and  $CO_2$  was trapped in Hyamine 10-X hydroxide by gentle shaking at room temperature for 60 min (HERRERA & AYANZ, 1972). The medium was processed (HERRERA & AYANZ, 1972) and glycerol was determined enzymatically (GARLAND & RANDLE, 1962). Lipids were extracted from the incubated tissue (FOLCH *et al.*, 1957) and purified and fractionated as previously described (DOMINGUEZ & HERRERA, 1976a; HERRERA & AYANZ, 1972). VLDL were isolated from the blood of 3-h fasted female Wistar rats collected under light ether anaesthesia from the aorta into a syringe containing 100 mg of  $Na_2$ -EDTA. Plasma obtained after centrifugation at  $1\ 000 \times g$  for 30 min at 4 °C was centrifuged under 0.15 M NaCl for 18 h at  $143\ 000 \times g$  at 15 °C. The supernatant was recovered by tube slicing and was designated VLDL, although it also contained some chylomicrons, as shown by electromicroscopic study (LASUNCIÓN & HERRERA, unpublished results). This supernatant was further purified by dialysis against 5 litres of 0.9 % NaCl-1 mM EDTA- $Na_2$  (3 times) and finally against 0.9 % NaCl only for a total of 18 h at 4 °C. Aliquots of this purified VLDL preparation were used directly for adding to the incubation vials. Proteins were measured (WANG & SMITH, 1975) in aliquots of the tissue preparations.

The rates of glycerol utilization by the tissues were calculated as previously described (HERRERA, 1973; CHAVES & HERRERA, 1978). Statistical comparison of the groups was determined by means of the Student *t* paired test.

### Results

Addition of tiny amounts of serum (5  $\mu$ l/vial) from 24-h fasted rats to pieces of epididymal fat pads incubated for 120 min resulted in increased glycerol release (Table I). Heparin in the media did not alter the release of glycerol or the response to serum. When the incubations were performed in the presence of rat VLDL, there was an enhancement in glycerol release which was not further augmented by serum (Table I). In the presence of both heparin and VLDL, the production of glycerol was greater than with either of these substances alone ( $P < 0.01$  and  $P < 0.05$  respectively) and serum did not produce any other change.

The incubations were performed in the presence of trace amounts of [ $U-^{14}C$ ] glycerol. During the incubation period, this tracer was diluted with the unlabelled glycerol released to the media. The change of specific activity of the glycerol in the media was taken into account to calculate the rates of glycerol uptake by the tissues (HERRERA, 1973; CHAVES & HERRERA, 1978) (Table I). Serum and VLDL signifi-

TABLE I. Effect of serum on glycerol release and [ $U-^{14}C$ ] glycerol uptake by pieces of epididymal fat pad incubated in vitro.

	Glycerol release ( $\mu\text{mol}/100 \mu\text{g protein}$ )			[ $U-^{14}C$ ] glycerol uptake ( $\text{pmol min}^{-1} 100 \mu\text{g protein}^{-1}$ )		
	Without serum	With serum	<i>P</i>	Without serum	With serum	<i>P</i>
Basals .....	16.3 $\pm$ 2.3	24.0 $\pm$ 3.9	<0.05	1.78 $\pm$ 0.19	2.34 $\pm$ 0.09	<0.05
+ Heparin..... <i>P'</i> .....	15.0 $\pm$ 3.3 NS	27.5 $\pm$ 4.2 NS	<0.05	1.62 $\pm$ 0.35 NS	2.51 $\pm$ 0.32 NS	NS
+ VLDL .....	30.7 $\pm$ 5.3 <0.01	30.2 $\pm$ 4.8 NS	NS	2.93 $\pm$ 0.28 <0.01	3.40 $\pm$ 0.69 NS	NS
+ Heparin + VLDL <i>P'</i> .....	44.0 $\pm$ 5.4 <0.001	45.1 $\pm$ 4.5 <0.05	NS	3.65 $\pm$ 0.78 <0.05	3.80 $\pm$ 0.53 <0.05	NS

Incubations were carried out for 120 min with 19.2  $\pm$  0.6 mg of fat-pad pieces (corresponding to 263  $\pm$  11  $\mu\text{g}$  proteins) and a final volume of 1.25 ml/vial of media containing bovine albumin (0.8 %) and glucose (4 mM). Additions : serum from 24-h fasted rat (5  $\mu\text{l}$ /vial), heparin (3 IU/vial) and or VLDL from plasma of 3-h fasted rat (VLDL contained in 0.4 ml of initial plasma/vial). Results are expressed as means  $\pm$  SEM of 5/group.

TABLE II. Effect of serum on the rates of [ $U-^{14}C$ ] glycerol conversion to  $CO_2$  and lipids by pieces of epididymal fat pad incubated in vitro.

	Formation of $CO_2$ ( $\text{pmol min}^{-1} 100 \mu\text{g protein}^{-1}$ )			Formation of total lipids ( $\text{pmol min}^{-1} 100 \mu\text{g protein}^{-1}$ )		
	Without serum	With serum	<i>P</i>	Without serum	With serum	<i>P</i>
Basals .....	0.70 $\pm$ 0.32	0.67 $\pm$ 0.33	NS	1.01 $\pm$ 0.13	1.66 $\pm$ 0.09	<0.001
+ Heparin..... <i>P'</i> .....	0.47 $\pm$ 0.13 NS	0.71 $\pm$ 0.10 NS	NS	1.14 $\pm$ 0.23 NS	1.78 $\pm$ 0.24 NS	NS
+ VLDL .....	0.87 $\pm$ 0.13 NS	1.03 $\pm$ 0.29 NS	NS	2.05 $\pm$ 0.15 <0.001	2.35 $\pm$ 0.39 NS	NS
+ Heparin + VLDL <i>P'</i> .....	1.00 $\pm$ 0.29 NS	1.09 $\pm$ 0.24 NS	NS	2.64 $\pm$ 0.50 <0.05	2.69 $\pm$ 0.30 <0.05	NS

Incubations were carried out for 120 min with 19.2  $\pm$  0.6 mg of fat-pad pieces (corresponding to 263  $\pm$  11  $\mu\text{g}$  proteins) and a final volume of 1.25 ml/vial of media containing bovine albumin (0.8 %) and glucose (4 mM). Additions : serum from 24-h fasted rat (5  $\mu\text{l}$ /vial), heparin (3 IU/vial) and or VLDL from plasma of 3-h fasted rat (VLDL contained in 0.4 ml of initial plasma/vial). Results are expressed as means  $\pm$  SEM of 5/group.

cantly enhanced the rate of glycerol uptake while heparin did not affect this parameter.

The glycerol being taken up by the tissue corresponded to its conversion to CO<sub>2</sub> and lipids. As shown in Table II, the rate of CO<sub>2</sub> formation from glycerol was not altered in the tissues incubated with serum, heparin or VLDL, and it was slightly but not significantly enhanced in these tissues with VLDL plus serum and with VLDL plus heparin. Greater differences were found in the formation of lipids, the rate of which was enhanced by serum and VLDL and unchanged by heparin. The effect of serum was not altered by heparin or VLDL while that of VLDL was augmented by heparin.

The percentage of radioactivity found in the lipids was  $45.9 \pm 6.7\%$  in fatty acids and  $53.6 \pm 6.7\%$  in glyceride glycerol, but neither condition affected this distribution.

### Discussion

Our findings confirm previous observations (REcant *et al.*, 1960; BURNS *et al.*, 1967; CURTIS-PRIOR & HANLEY, 1973; FEDERSPIL *et al.*, 1975; TUTTEROVÁ & MOSINGER, 1975) showing that rat adipose tissue incubated *in vitro* in the presence of normal plasma or serum exhibited increased lipolysis. The amount of serum used for the incubations in the present work is much lower than that used by other investigators and shows the high sensitivity of the effect. The present results do not support the proposal of some investigators that the mechanism is based on the interaction of lipoprotein lipase from the tissue and lipoproteins present in the serum (TUTTEROVÁ & MOSINGER, 1975) for two reasons: (i) The small amount of serum present in the incubation medium did not contain enough lipoprotein-glycerides to cause any measurable change in the amount of glycerol in the medium even when the lipoprotein glycerides were totally hydrolysed by the lipoprotein lipase present in the tissue preparation; and (ii) The addition of heparin to the vials with serum did not modify the appearance of glycerol in the medium although it is well known that heparin enhances the lipoprotein lipase action on the hydrolysis of lipoprotein-triglycerides (PERSSON *et al.*, 1966; STEWART & SCHOTZ, 1974; LASUNCIÓN & HERRERA, unpublished observations). The explanation may be valid, however, when high amounts of serum are used, as it was seen here that when purified VLDL from rat plasma were added to the medium, there was an increase of glycerol in the medium which was further enhanced by heparin. The presence of catecholamines or some other hormonal activator of  $\beta$ -adrenergic receptors has also been proposed as the fat-mobilizing factor of plasma (CURTIS-PRIOR & HANLEY, 1973). Our results of glycerol utilization by the tissue do not support this theory as it was observed that serum enhanced the uptake and conversion of glycerol to lipids while we have reported that these specific parameters are greatly reduced by adrenaline (DOMINGUEZ & HERRERA, 1976a & b) and analogues of sympathomimetic amines (HERRERA & PASCUAL, 1973) when incubated with adipose tissue in conditions similar to those used in the present study. More research should be done to establish the nature of the fat-mobilizing factor of plasma, the high sensitivity of which may have some physiological role in the continuous turnover of lipid stores in the organism.

Whether the enhanced release of glycerol by VLDL is due only to their hydrolysis by the lipoprotein lipase action or whether it also reflects a lipolytic action of these lipoproteins cannot be determined from present results. Either of these possibilities may be correct as it has been shown here that heparin, an activator of the lipoprotein lipase action, also enhances the effect of VLDL on glycerol release, while it has been

reported that these lipoproteins activate adipocyte adenylate cyclase (PAIRAULT *et al.*, 1977) which could result in augmented lipolysis. Our findings do, however, demonstrate that at least part of the free glycerol released to the medium in these conditions (either by endogenous lipolysis or by the actual hydrolysis of VLDL triglycerides) was taken up and metabolized by the tissue. This result is in agreement with the recent report of the formation of labelled fatty acids in adipose tissue in incubations performed with  $^{14}\text{C}$ -labelled glyceride glycerol of VLDL (LASUNCIÓN, 1979).

In all experimental conditions used in the present study, the rate of glycerol being taken up by the tissue seemed to parallel the actual amount of glycerol present in the incubation medium. This may indicate that, in situations where the glycerokinase reaction is not inhibited (either by inhibition of the enzyme activity or decreased availability of ATP), the main factor which determines the rate of glycerol utilization is the actual amount of available glycerol. Thus the effect of serum and VLDL enhancing glycerol utilization by the tissue is probably a secondary consequence of their enhancement of glycerol release and not a direct action on glycerokinase activity.

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