Excerpt from
ACTA DIABETOLOGICA LATINA Vol. XII - March-April 1975 - No. 2

CÁTEDRA DE FISIOLOGÍA GENERAL, FACULTAD DE BIOLOGÍA
UNIVERSIDAD DE BARCELONA, BARCELONA

EFFECTS OF EPINEPHRINE AND GLUCOSE ON THE IN VITRO RESPONSE OF ADIPOSE TISSUE TO SULFONYLUREAS

EMILIO HERRERA

CÁTEDRA DE FISIOLOGÍA GENERAL, FACULTAD DE BIOLOGÍA
UNIVERSIDAD DE BARCELONA, BARCELONA

EFFECTS OF EPINEPHRINE AND GLUCOSE ON THE IN VITRO RESPONSE OF ADIPOSE TISSUE TO SULFONYLUREAS

EMILIO HERRERA

We have shown recently that sulfonylureas reduce lipolysis and also inhibit the utilization of glycerol by adipose tissue when incubated in basal conditions [11]. Some lipolytic agents have been found to enhance the antilipolytic effects of sulfonylureas [1, 7]. In the present investigation, we have determined whether epinephrine, a wellknown lipolytic agent, also affects the action of sulfonylureas on the uptake and utilization of glycerol in adipose tissue.

It has been suggested that the effect of sulfonylureas on adipose tissue metabolism could be mediated by a reduction of the availability of ATP which is needed for the activation of the hormone sensitive lipase [7]. ATP is also required for the phosphorylation of glycerol and its further metabolization [2, 18]. Thus, in the present study we have investigated whether glucose, the main metabolic substrate in adipose tissue, affects the action of sulfonylureas on these parameters.

Sulfonylureas selected for the present study were tolbutamide and two potent hypoglycemic sulfonylureas of the socalled second generation [17], glibenclamide, N-4-[2-(5-chloro-2-methoxybenzamido)-ethyl]phenyl-sulfonyl-N'-cyclohexyl-urea, and glipentide, N-4-[β(o-anisamide)-ethyl]-benzenesulfonyl-N'-cyclopentylcarbamide.

MATERIALS AND METHODS

Female Wistar rats weighing 150-185 g were used. They were killed without anesthesia by cervical fracture. Pieces of the left and right lumbar pads (18 ± 3 mg) were removed from each rat and were placed in incubation vials containing 1 ml of Krebs Ringer bicarbonate pH 7.4 [20] supplemented with 0.5 μCi of 1-^{14}C-glycerol (32.5 mCi/mM, from the Radiochemical Center, Amersham, England), 10 mg of bovine albumin purified by the method of CHEN [5] and the appropriate amount of the sulfonylurea. Glibenclamide and glipentide were kindly supplied by *Hoechst Ibérica* and *Laboratorios Uriach*, Spain, respectively.

Pooled tissues from two rats were used in each incubation vial. In each experiment pieces of tissues from the same animals were incubated under the different conditions studied.

Drugs were diluted in chloroform. The proper amount of drug was added to each vial in a volume of 100 μl. Chloroform alone (100 μl) was added to control vials. The

Key-words: Adipose tissue glycerol metabolism; Epinephrine; Glucose; Sulfonylureas.
Received: February 12, 1975.
Acta diabet. lat. *12*, 106, 1975.

vials were blown to dryness under N_2 at 37 °C and shaken intensely with the media for 90 min before introducing the tissue. These were incubated for 180 min at 37 °C while being shaken at 100 cycles/min as already described [12, 14]. $^{14}CO_2$ was determined [12, 14]. The tissues were placed in chloroform-methanol (2:1) for the extraction and purification of lipids [8] and subsequent fractionation [12]. Aliquots of the media were used for the determination of glycerol by an enzymatic method [9]. Statistical analysis was carried out by paired comparison.

RESULTS

Dose-response curves for the effects of tolbutamide, glipentide and glibenclamide on parameters of glycerol metabolism in adipose tissue *in vitro*, in the presence of epinephrine and the absence of glucose, are shown in figs 1, 2 and 3. The production of glycerol was reduced significantly by tolbutamide, glipentide and glibenclamide at a concentration of 1 mg/ml (fig. 1).

Glibenclamide reduced glycerol production to 25% of the basal level while the decrease induced by glipentide and tolbutamide was to 53 and 55% of the basal level. The uptake of 1-^{14}C-glycerol by the tissue (fig. 2) and its conversion to ^{14}C-glyceride-glycerol (fig. 3) were reduced by glibenclamide and glipentide while tolbutamide failed to affect these parameters of glycerol metabolism significantly.

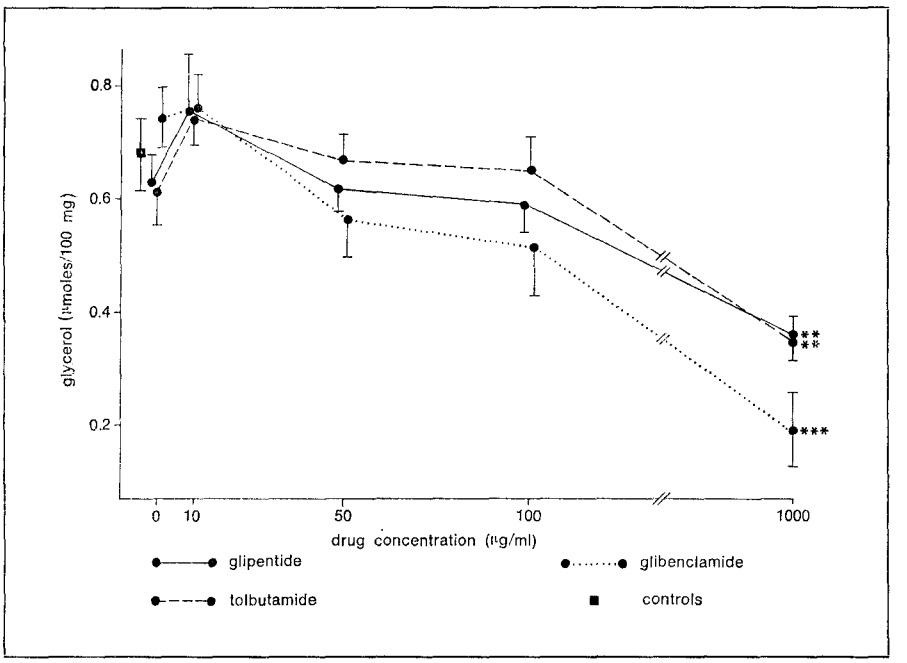

Fig. 1 - Dose-response curves for the effects of tolbutamide, glipentide and glibenclamide on the *in vitro* formation of glycerol by adipose tissue from fed rats in the presence of epinephrine (2.8 µM) in the incubation medium. Statistical comparison to controls are shown by astherisks: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Mean values ± SEM for 5 rats/group.

None of the sulfonylureas significantly altered the formation of $^{14}CO_2$ from 1-^{14}C-glycerol in the presence of epinephrine (data not shown).

The effects of the sulfonylurea drugs at a concentration of 1 mg/ml on parameters of glycerol metabolism *in vitro* in the presence of glucose with or without epinephrine in the media are presented in tab. 1. In the presence of glucose and in the presence or the absence of epinephrine, only glibenclamide leads to a significant decrease in glycerol production. This indicates that the effect of tolbutamide and glipentide on glycerol production is more pronounced in the absence (fig. 1) than in the presence (tab. 1) of glucose in the incubation media.

As previously observed [13], epinephrine significantly increases the level of glycerol production and reduced the uptake of 1-^{14}C-glycerol by the tissue under basal conditions (tab. 1). In the presence of glucose, tolbutamide and glipentide failed to affect the uptake of 1-^{14}C-glycerol. Glibenclamide significantly reduced the level of 1-^{14}C-glycerol uptake in the absence of epinephrine. Because of this, epinephrine failed to have a significant effect on the uptake of 1-^{14}C-glycerol in the presence of glibenclamide (tab. 1).

The formation of $^{14}CO_2$ and of ^{14}C-total lipids from 1-^{14}C-glycerol under basal conditions was significantly reduced by epinephrine (tab. 1). Neither tolbutamide nor glipentide had significant effects on these two parameters

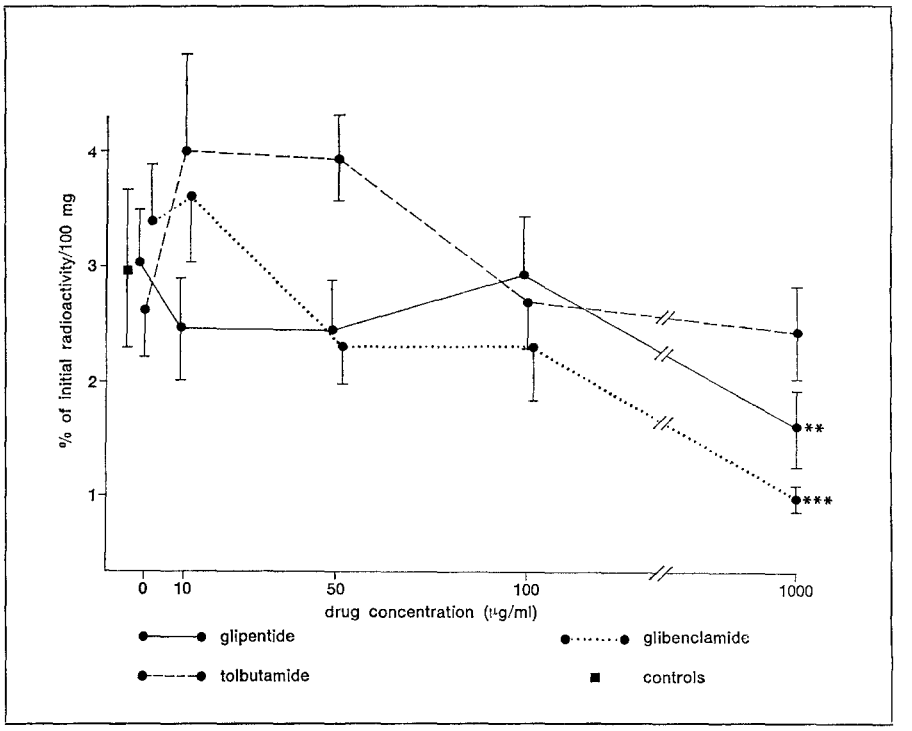

Fig. 2 - Dose-response curves for the effects of tolbutamide, glipentide and glibenclamide on the *in vitro* uptake of 1-^{14}C-glycerol by adipose tissue from fed rats in the presence of epinephrine (2.8 µM) in the incubation medium. Symbols as in fig. 1.

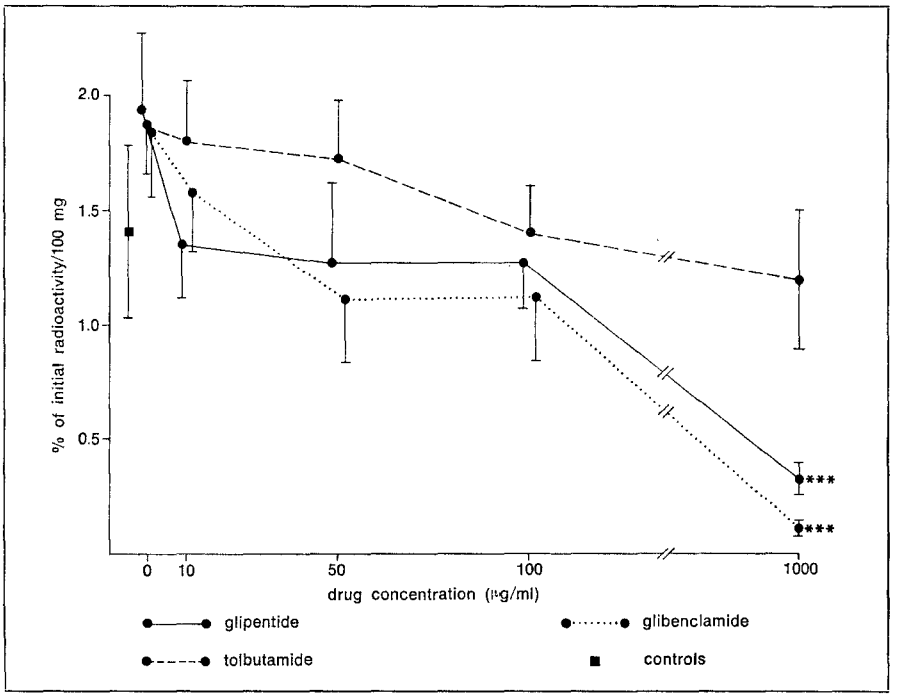

Fig. 3 - Dose-response curves for the effects of tolbutamide, glipentide and glibenclamide on the *in vitro* formation of ^{14}C-glyceride-glycerol from 1-^{14}C-glycerol by adipose tissue from fed rats in the presence of epinephrine (2.5 µM) in the incubation medium. Symbols as in fig. 1.

while glibenclamide significantly reduced them to the levels observed under basal conditions in the presence of epinephrine. As a result, the difference in the formation of $^{14}CO_2$ and ^{14}C-total lipids observed in the presence versus the absence of epinephrine under basal conditions failed to occur when the tissues were incubated in the presence of glibenclamide.

In the presence of glucose, a considerable amount of the 1-^{14}C-glycerol converted to ^{14}C-total lipids appeared in the form of ^{14}C-fatty acids. Epinephrine significantly decrease the formation of ^{14}C-fatty acids as well as the formation of ^{14}C-glyceride-glycerol (tab. 1). Tolbutamide and glipentide were without significant effect compared to the basal conditions on ^{14}C-fatty acid and ^{14}C-glyceride-glycerol formation in the presence and absence of epinephrine. Glibenclamide, on the other hand, very significantly decreased fatty acid and glyceride-glycerol formation compared to basal conditions in the absence of epinephrine in the medium. Because of this, epinephrine failed to reduce significantly the formation of fatty acids and glyceride-glycerol in adipose tissue treated with glibenclamide.

DISCUSSION

The present study shows that tolbutamide inhibits epinephrine-stimulated lipolysis. While tolbutamide typically lacks an effect on glycerol

	basal level		tolbutamide		glipentide		glibenclamide	
	—	+	—	+	—	+	—	+
glycerol production	0.355±0.120[1]	1.242±0.360*[3]	0.304±0.81	1.345±0.440*	0.228±0.057	0.715±0.089**	0.151±0.026	0.362±0.177
p[2]			n.s.	n.s.	n.s.	n.s.	< 0.05	< 0.05
utilization of 1-^{14}C-glycerol (uptake)	6.84±1.58	1.99 ±0.40**	6.63±1.02	2.03±0.56**	5.54±2.23	2.43±0.77*	2.22±0.72	1.33±0.69
p			n.s.	n.s.	n.s.	n.s.	< 0.05	n.s.
formation of $^{14}CO_2$	2.63±0.58	0.81±0.20*	2.53±0.53	0.72±0.19**	2.58±0.73	1.14±0.27*	1.23±0.49	0.47±0.21
p			n.s.	n.s.	n.s.	n.s.	< 0.05	n.s.
formation of ^{14}C-total lipids	4.19±1.02	1.17±0.24*	3.91±0.55	0.96±0.23***	2.93±1.36	1.28±0.56*	0.99±0.37	0.85±0.50
p			n.s.	n.s.	n.s.	n.s.	< 0.05	n.s.
formation of ^{14}C-fatty acids	1.45±0.37	0.24±0.13*	1.31±0.26	0.15±0.11**	0.73±0.36	0.76±0.33	0.14±0.04	0.04±0.02
p			n.s.	n.s.	n.s.	n.s.	< 0.001	n.s.
formation of ^{14}C-glyceride-glycerol	2.69±1.02	0.78±0.18**	2.35±0.68	0.72±0.23**	2.50±1.30	1.19±0.68*	0.61±0.28	0.48±0.36
p			n.s.	n.s.	n.s.	n.s.	< 0.01	n.s.

[1] Mean values ± SEM for 6 rats/group

[2] Statistical comparison to basal level, n.s. = not significant ($p > 0.05$)

[3] Statistical comparison to drug-treated tissue without epinephrine in the medium: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Table 1 - Effect of sulfonylurea drugs (1 mg/ml) on *in vitro* glycerol metabolism in adipose tissue from fed rats carried out in the presence of glucose, with (+) or without (—) epinephrine (0.5 µg/ml) in the incubation medium.

production in the absence of epinephrine [1, 11], STONE et al. [19] observed an antilipolytic effect of tolbutamide in adipose tissue incubated under basal conditions. But the use of fasted rats in which lipolytic activity in adipose tissue is intense [12, 16], may account for the results obtained by STONE et al. [19]. Thus, the antilipolytic action of tolbutamide may be said to appear only when tissue lipolysis is activated.

We have previously observed [11] that 50 µg of glipentide and 100 µg of glibenclamide/ml were enough significantly to decrease the *in vitro* production of glycerol by adipose tissue incubated in basal conditions. In the present study we have observed that doses of 1 mg/ml of either sulfonylurea are needed to obtain the same effect in tissues incubated in the presence of epinephrine. Thus, contrary to tolbutamide, both glipentide and glibenclamide have a less marked antilipolytic effect in the presence than in the absence of epinephrine. The possibility exists that while tolbutamide does not affect the amount of glycerol taken up by the tissue either in the absence or in the presence of epinephrine, this parameter is much lower in the tissues incubated with glipentide and glibenclamide in the presence of epinephrine than in the absence of this hormone [11]. This means that in the presence of epinephrine and glipentide or glibenclamide a smaller fraction of the glycerol from the medium returns to the tissue and the differences in the net production of glycerol are less apparent.

The mechanisms by which tolbutamide, glipentide and glibenclamide exert their effects on the metabolism of glycerol in adipose tissue is not yet clear. It has been suggested that the antilipolytic effect is exerted by a direct action at the level of hormone-sensitive triglyceride lipase [1]. Sulfonylurea drugs might decrease the availability of ATP needed for activation of the enzyme by acting as uncoupling agents in white adipose tissue as they do in brown fat cells [14]. Support for this notion comes from the fact that these drugs were found in the present study to reduce the uptake and utilization of glycerol via pathways directly dependent upon ATP availability [2, 18]. Tolbutamide reduced the production of glycerol without affecting the utilization of 1-^{14}C-glycerol. This observation does not contradict the above notion as the specific activity of the tracer is higher in the tissues incubated with tolbutamide than in the controls. Consequently, the actual amount of glycerol taken up by the tolbutamide-treated tissue would be lower when this dilution of the isotope is taken into account. When this consideration is applied to the effects of glipentide and glibenclamide, their effects on the utilization of glycerol are higher than that derived directly from the raw data. The differences in the effects of the sulfonylurea drugs on lipolysis and glycerol utilization is not unexpected since the degree of ATP reduction can be different with each drug as is the case with other metabolic inhibitors [15].

Our results with glucose in the incubation media also support these points. We have observed here that the antilipolytic effect of tolbutamide in both basal conditions and under the stimulation of epinephrine is completly abolished by the presence of glucose which could compensate the reduction in the availability of ATP produced by the drug. These results differ from those of BROWN et al. [3], who failed to observe a decrease in the antilipolytic effect of tolbutamide in the presence of glucose, but they incubated their specimens in the presence of theophylline which has intense

effects on the metabolism of glycerol in adipose tissue that are quite different from those produced by epinephrine [7]. Glucose also abolished the antilipolytic effect of glipentide as well as the inhibitory effect of this drug on the utilization of glycerol in both the absence and the presence of epinephrine. Glibenclamide however maintains its antilipolytic effect and its inhibitory effect on the utilization of glycerol in the presence of glucose. The effects of epinephrine on the production and utilization of glycerol are completely abolished in the presence of glibenclamide and glucose. These effects of glibenclamide are very similar to those of oligomycin [6], a potent uncoupling agent known to be active in adipose tissue [10].

In agreement with other authors [1, 3, 19], we observed that the concentrations of the sulfonylureas needed to obtain *in vitro* effects on adipose tissue metabolism are higher than those observed in human fluids during treatment with these hypoglycemic drugs. The poor solubility of these drugs could decrease their actual concentration in contact with the tissue pieces. In any event, although the antilipolytic effect of the sulfonylurea drugs has been well documented both *in vitro* and *in vivo*, the extrapolation of their effects on the *in vitro* glycerol utilization by adipose tissue here observed to the *in vivo* situation remains to be established.

CONCLUSIONS

The data presented here show once more that the utilization of glycerol by rat adipose tissue incubated *in vitro* is considerably higher than previously thought. Besides their antilipolytic effect, the sulfonylureas decrease the uptake and metabolization of glycerol by the tissue, and this effect is seen both in the absence and the presence of epinephrine.

As both lipolysis and glycerol utilization in adipose tissue depend on ATP, the possibility exists that either effect of these drugs could be mediated by reducing the availability of ATP in the tissue.

Our results with glucose support this possibility. Glucose, the main metabolic fuel for adipose tissue, decreases both the antilipolytic effect of sulfonylureas and their inhibitory effect on glycerol utilization by the tissue.

ACKNOWLEDGEMENT

The present work was partly supported by *Laboratorios Uriach* and *Hoechst Ibérica*, Spain.

SUMMARY

Pieces of lumbar fat pads from fed female rats were incubated in the presence of 1-^{14}C-glycerol. Epinephrine (2.8 μM), glucose (5 mM), tolbutamide, glipentide or glibenclamide were added to the incubation media. The sulfonylurea drugs reduced the lipolytic effect of epinephrine in the absence of glucose; the effect of glibenclamide was greatest. In the absence of epinephrine, both glipentide and glibenclamide reduced the uptake of 1-^{14}C-glycerol and its conversion to glyceride-glycerol while tolbutamide had no effect on these parameters. In the presence of glucose, the lipolytic effect of epinephrine was retained but tolbutamide and glipentide no longer affected glycerol metabolism. Glibenclamide still inhibited the production of glycerol and the utilization of 1-^{14}C-glycerol by the tissues in the presence of glucose. The possibility that sulfonylureas exert their effect on adipose tissue glycerol metabolism by acting as uncoupling agents is discussed.

REFERENCES

1) ALLEN D. O., LARGIS E. E., ASHMORE J.: Antilipolytic Action of Tolbutamide in Isolated Fat Cells of the Rat - Diabetes *23*, 51, 1974.
2) ANTONY G., WHITE L. W., LANDAU B. R.: Metabolism of D- and L-Glyceraldehyde in Adipose Tissue: a Stereochemical Probe for Glycerokinase Activity - J. Lipid Res. *10*, 521, 1969.
3) BROWN J. D., STEELE A. A., STONE D. B., STEELE F. A.: The Effect of Tolbutamide on Lipolysis and Cyclic AMP Concentration in White Fat Cells - Endocrinology *90*, 47, 1972.
4) CHAN S. S., FAIN J. N.: Uncoupling Action of Sulfonylureas on Brown Fat Cells - Molec. Pharmacol. *6*, 513, 1970.
5) CHEN R. F.: Removal of Fatty Acids from Serum Albumin by Charcoal Treatment - J. biol. Chem. *242*, 173, 1967.
6) DOMINGUEZ M. C., HERRERA E.: Effects of 2-Deoxy-D-Glucose, Oligomycin and Theophylline on in Vitro Glycerol Metabolism in Rat Adipose Tissue: Response to Insulin and Epinephrine - Horm. Metab. Res. (In press).
7) FAIN J. N.: Biochemical Aspects of Drug and Hormone Action on Adipose Tissue - Pharmacol. Rev. *25*, 67, 1973.
8) FOLCH J., LEES M., SLOANE-STANLEY G. H.: A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues - J. biol. Chem. *226*, 497, 1957.
9) GARLAND P. B., RANDLE P. J.: A Rapid Enzymatic Assay for Glycerol - Nature (Lond.) *196*, 987, 1962.
10) HEPP D., CHALLONER D. R., WILLIAMS R. H.: Respiration in Isolated Fat Cells and the Effects of Epinephrine - J. biol. Chem. *243*, 2321, 1968.
11) HERRERA E.: Effects of Sulfonylureas (Tolbutamide, Glipentide and Glibenclamide) on in Vitro Glycerol Metabolism in Adipose Tissue from Rats - Life Sci. *16*, 645, 1975.
12) HERRERA E., AYANZ A.: Calculation of Lipolysis and Esterification from Glycerol Metabolism in Rat Adipose Tissue - J. Lipid Res. *13*, 802, 1972.
13) HERRERA E., DOMINGUEZ M. C.: Effects of Epinephrine and Insulin on Glycerol Utilization by Adipose Tissue - In: MALAISSE W. J., PIRART J. (Eds): Diabetes. Proc. of the 8th Congress of the International Diabetes Federation, Bruxelles 1973. Excerpta Medica Foundation, Amsterdam, 1974; p. 100.
14) HERRERA E., LAMAS L.: Utilization of Glycerol by Rat Adipose Tissue in Vitro - Biochem. J. *120*, 433, 1970.
15) HOLLEMBERG C. H., PATTEN R. L.: Relation of Fat Cell ATP Content to Lipolysis Induced by Dibutyril-3,5-Cyclic AMP - Metabolism *19*, 856, 1970.
16) KNOPP R. H., HERRERA E., FREINKEL N.: Carbohydrate Metabolism in Pregnancy. VIII. Metabolism of Adipose Tissue Isolated from Fed and Fasted Pregnant Rats during Late Gestation - J. clin. Invest. *49*, 1438, 1969.
17) LOUBATIÈRES A., MARIANI M.-M., RIBES G., ALRIC R.: Pharmacological Comparison between Tolbutamide and Two Second Generation Hypoglycemic Sulfonylureas (Glibenclamide and Glisoxepide) - Acta diabet. lat. *10*, 261, 1973.
18) ROBINSON J., NEWSHOLME E. A.: Glycerolkinase Activities in Rat Heart and Adipose Tissue - Biochem. J. *104*, 2c, 1967.
19) STONE D. B., BROWN J. D., COX C. P.: Effect of Tolbutamide and Phenformin on Lipolysis in Adipose Tissue in Vitro - Amer. J. Physiol. *210*, 26, 1966.
20) UMBREIT W. W., BURRIS R. H., STAUFER S. F.: Manometric Techniques - 4th ed. Publishing Co., Minneapolis, 1964; p. 132.

Requests for reprints should be addressed to:

EMILIO HERRERA
Cátedra de Fisiología General
Facultad de Biología, Universidad de Barcelona
Av. José Antonio 585, Barcelona 7 - Spain