Biochemical Pharmacology, Vol. 24, pp. 891–897. Pergamon Press, 1975. Printed in Great Britain.

LIPOGENIC ACTION OF CYCLOHEXIMIDE ON THE RAT EPIDIDYMAL FAT PAD

J. ADOLFO GARCÍA-SÁINZ, ARMANDO G. AMADOR, ENRIQUE PIÑA and VICTORIA CHAGOYA DE SÁNCHEZ

Departamento de Bioquímica, Facultad de Medicina, Departamento de Biología Experimental, Instituto de Biología; Universidad Nacional Autónoma de Mexico, México

(*Received* 22 *May* 1974; *accepted* 4 *October* 1974)

Abstract—Cycloheximide produced a 10-fold increase in the incorporation of glucose into lipids of the rat epididymal fat pad and a 43 per cent decrease in plasma free fatty acids. These changes were observed 2 hr after the intraperitoneal injection of 1 mg/kg of the antibiotic to male rats fasted for 16–20 hr and weighing between 120 and 170 g. Under the same conditions, subcutaneous adipose tissue showed a 2-fold increase, while brain, liver and brown adipose tissue did not give any response. The 10-fold increase was absent in fed rats and was lower (4-fold) in male animals weighing over 250 g and in 120–170 g female rats when the parametrial adipose tissue was studied. The higher incorporation of glucose into lipids produced by cycloheximide was also smaller in the epididymal fat pad from orchiectomized (2-fold), adrenalectomized (3-fold) and alloxan diabetic (7-fold) rats. Hormonal substitutive treatment with testosterone in orchiectomized animals and with cortisol, corticosterone or epinephrine in adrenalectomized animals did not elicit the response obtained in intact rats. The relative distribution of the label from radioactive glucose into lipid extracts between glycerol and fatty acids after cycloheximide treatment resembles that found in control rats, but differs from that observed after insulin administration. Actinomycin-D, chloramphenicol and puromycin did not mimic the action of cycloheximide on lipid metabolism. Epididymal fat pads obtained from fasted male rats injected 1 hr earlier with cycloheximide showed, after 60 min of incubation, a 5-fold increase in the incorporation of glucose into lipids, a 37 per cent increase in the release of glycerol and a 36 per cent diminution in the release of free fatty acids into the incubation mixture, when compared to the values obtained with the tissues of the control animals. It is postulated that in the rat epididymal fat pad cycloheximide has a marked lipogenic effect together with an accelerated fatty acid re-esterification which is independent of both insulin secretion and inhibition of protein synthesis.

Cycloheximide (Actidione), an antibiotic isolated by Whiffen *et al.* [1] and Leach *et al.* [2] from *Streptomyces griseus*, is a widely used inhibitor of protein synthesis [3–7]. A metabolic response impaired by administration of this antibiotic is usually interpreted as dependent on *de novo* protein synthesis. Thus, in lipid metabolism, it has been reported that the induction of fatty acid biosynthesis in L cells deprived of exogenous fatty acids [8] and the lipolysis stimulated by growth hormone in fat cells [9] are blocked by the presence of Actidione. In addition, it has been observed that in rat liver cycloheximide inhibits the activity of diglyceride acyl transferase [10], favors the accumulation of triglycerides [11] and decreases the conversion of [^{14}C]acetate into cholesterol, CO_2 and fatty acids [12]. All these effects have been attributed to the inhibition of protein synthesis produced by the antibiotic. Paradoxically, it has been observed that cycloheximide produces a stimulation of amino acid incorporation in liver microsomes [13] and an increase in the RNA content of regenerating liver and of the adrenal glands [14] in rats.

During study of the action of adenosine on lipid metabolism of rat epididymal fat pad [15], cycloheximide was used to explore the role of *de novo* protein synthesis in the effect of the nucleoside. Unexpectedly, cycloheximide alone produced a more drastic effect on lipid metabolism than did adenosine. The aim of the present paper is to give a preliminary characterization of this effect.

MATERIALS AND METHODS

Cycloheximide, chloramphenicol, alloxan monohydrate and corticosterone-21-acetate were obtained from Sigma Chemical Co., puromycin dihydrochloride from Maror Chemical LTD; epinephrine from Servet Laboratories; depot-testosterone from Schering and actinomycin-D from Calbiochem. Hydrocortisone hemisuccinate was a generous gift from Upjohn de México, S. A., [U-^{14}C]glucose was obtained from International Chemical and Nuclear Corp., and α-glycerophosphate dehydrogenase and glycerokinase from Boehringer und Soehne, Mannheim.

The experiments were generally performed with male Wistar rats weighing between 120 and 170 g and fasted for 16–20 hr. Other animal conditions used are indicated in the figures and tables.

Bilateral orchiectomy was performed according to usual techniques, conserving the epididymus and the epididymal fat pad. The animals were used at least 4 days after surgical treatment in order to minimize any testosterone effect [16]. Where indicated, an intramuscular injection of 5 mg depot-testosterone in vegetable oil was used as androgen replacement therapy [17]

and these animals were used 7 days after both orchiectomy and testosterone administration.

Bilateral adrenalectomy was performed according to usual techniques. Adrenalectomized rats were maintained at constant room temperature (20°) with 0·85% NaCl instead of drinking water. Animals were used at least 72 hr after adrenalectomy. Where indicated, hydrocortisone hemisuccinate, 25 mg/kg [18], corticosterone-21-acetate, 10 mg/kg [19], or epinephrine, 1 mg/kg [20], was administered intraperitoneally 120 min before sacrifice as substitutive therapy.

Rats fasted for 30 hr were made diabetic by the intraperitoneal injection of 120 mg/kg of alloxan monohydrate [21] dissolved in 0·001 N HCl [22]. Alloxan-treated rats were used only when they had blood glucose levels between 200 and 460 mg/100 ml under fed conditions. The animals were injected intraperitoneally with saline or with 1 mg/kg of cycloheximide suspended in saline and were sacrificed by decapitation and exsanguination at different times after the injection. Although the animals were not subject to strict feeding and lighting schedules, all of them were kept under the same conditions. The rats were selected randomly. Five min prior to sacrifice, [U-^{14}C]-glucose (sp. act., 180 mCi/m-mole) was administered intraperitoneally at a dose of 20 μCi/kg.

For epididymal fat pad incubations, groups of rats were decapitated 60 min after injection of saline or cycloheximide. The epididymal fat pads were removed as fast as possible with minimal handling, rinsed in 0·85% NaCl, and incubated for 1 hr in a metabolic shaker at 37° in 25-ml stoppered flasks containing, in a 3 ml final volume: Krebs–Ringer bicarbonate buffer, pH 7·3; 150 mg bovine serum albumin (fraction V); 3 μCi of labeled glucose and 33·34 μmoles of nonradioactive hexose. The same experimental design was used to investigate possible changes in glycerol and fatty acids production in response to administration of cycloheximide *in vivo*.

Lipids were extracted according to Folch *et al.* [23]. The distribution of label in the lipids was studied by the method of Kornacker and Ball [24], with several modifications: nonsaponifiable material was extracted with petroleum ether and the glyceride–glycerol estimation was done directly by counting the sample after fatty acid extraction. Plasma was prepared from heparinized blood and all samples showing traces of hemolysis were discarded. Radioactive measurements were made in a Packard Tri-Carb liquid scintillation spectrometer in a toluene solution of 2,5-*p*-phenylene-bis-(5-phenyloxazole); glyceride–glycerol radioactivity was counted in Bray's solution [25]. Blood glucose concentration was determined according to the method of Nelson and Somogyi [26]; free fatty acids and glycerol were estimated by the methods of Dole and Meinertz [27] and Wieland [28] respectively. Total purines and pyrimidines were quantified by the method of McIntire and Smith [29].

Statistical significance of the differences between comparable groups was determined by the Student *t*-test.

RESULTS

Time, doses and tissues

The administration of cycloheximide at a dose of 1 mg/kg of body weight produced a significant increase in the incorporation of [^{14}C]-glucose into the lipids of the epididymal fat pad. The results are presented in Fig. 1 and, whether expressed as specific activity or as net incorporation, a parallel response can be observed. The maximum effect was found 120 min after cycloheximide administration; however, the difference from the control was statistically significant even at 30 min after administration of the antibiotic (30 min, $P < 0{\cdot}01$; 60 and 120 min, $P < 0{\cdot}001$; and 180 min, $P < 0{\cdot}005$). In a different set of experiments, [^{14}C]-glucose was injected simultaneously with the antibiotic 2 hr before the sacrifice of the animals. Under these experimental conditions, incorporation of radioactive glucose into lipids of the epididymal fat pad was 4·30 ± 1·84 cpm/mg of lipids in four control animals and, in six rats injected with the antibiotic, 16·45 ± 7·19 cpm/mg of lipids. (Values are means ± S. E.) Therefore the "pulse type" of experiment was preferred to study this action of the antibiotic. Different doses were tested at 120 min and the best action was detected with the dose initially used (1 mg/kg) (Fig. 2), which is within the range usually employed to obtain inhibition of protein synthesis *in vivo* [10, 11]. This inhibition of the synthesis of proteins is observed 10 min after administration of the antibiotic [30] and is still present 3 hr later [10].

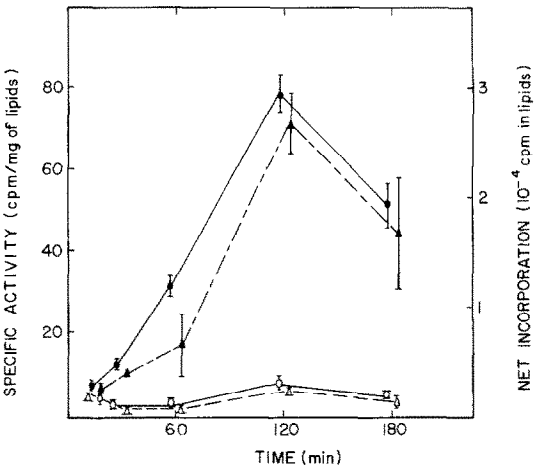

Fig. 1. Effect of cycloheximide on the incorporation of radioactive glucose into lipids of the epididymal fat pad as a function of time. Specific activity in control rats (○——○), net incorporation in control rats (△- - - -△); specific activity in rats injected with cycloheximide (●——●), and net incorporation in rats injected with cycloheximide (▲- - - -▲). Vertical lines represent the standard error of the mean of at least five animals.

A study of the action of cycloheximide on the transformation of radioactive glucose into lipids in tissues with an active lipid metabolism is presented in Fig. 3. In the epididymal fat pad, a 10-fold increase was detected; the subcutaneous adipose tissue responded with a 2-fold increase. Under the conditions described in this experiment, no effect was detected in brain, brown adipose tissue or liver. It has been shown that the epididymal fat pad has a more active metabolism than does the subcutaneous adipose tissue [31]; therefore, the higher response obtained in this tissue is not surprising.

Other inhibitors of protein synthesis

A possible effect of other inhibitors of protein synthesis on lipid metabolism of the epididymal fat pad

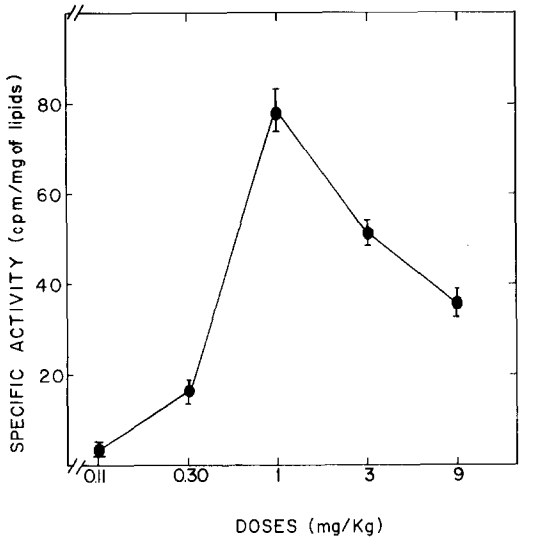

Fig. 2. Dose–response curve of cycloheximide on the transformation of [UL-^{14}C-D] glucose into lipids of the epididymal fat pad. The value at 1 mg/kg of body weight is the same as that at 120 min in Fig. 1. Other specifications as in Fig. 1.

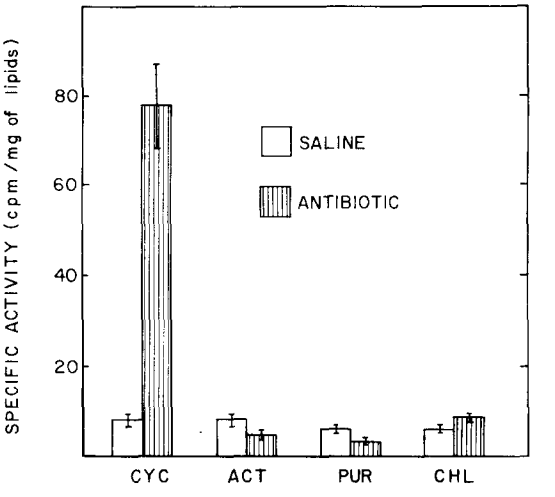

Fig. 4. Comparative effect of different inhibitors of protein synthesis on the conversion of labeled glucose into lipids of the epididymal fat pad. All the inhibitors were injected intraperitoneally: actinomycin-D, 1 mg/kg of body weight [33]; puromycin, 50 mg/kg of body weight [34]; and chloramphenicol, 750 mg/kg of body weight [11]. The values for cycloheximide and its saline control were taken from Fig. 1. CYC = cycloheximide; ACT = actinomycin-D; PUR = puromycin; CHL = chloramphenicol. Other indications as in Fig. 1.

was also explored. Since puromycin has a rapid and transient inhibitory effect on protein synthesis [32], its action was studied 1 hr after administration. For actinomycin-D and chloramphenicol, the effect was explored 2 hr after injection. Under the conditions described in Fig. 4, none of the tested compounds shared with cycloheximide the capacity for increasing the transformation of labeled glucose into lipids.

Age and feeding conditions

Two factors that influence the lipogenic action of Actidione on the rat epididymal fat pad are the weight and feeding conditions of the experimental animals (Table 1). A total of 15 rats was used in each of the saline and cycloheximide-treated groups. These rats were used in several experiments performed on different days; therefore this response was not an isolated phenomenon. As shown in Table 1, rats treated with cycloheximide and weighing more than 250 g present only a 4-fold increase in incorporation of ^{14}C from glucose into lipids. The antibiotic treatment in fed rats produced a very slight effect without statistical significance. In addition, the conversion of [U-^{14}C]-glucose into lipids was greater in saline-treated fed animals than in fasted rats, in agreement with the results of Boxer and Stetten [34].

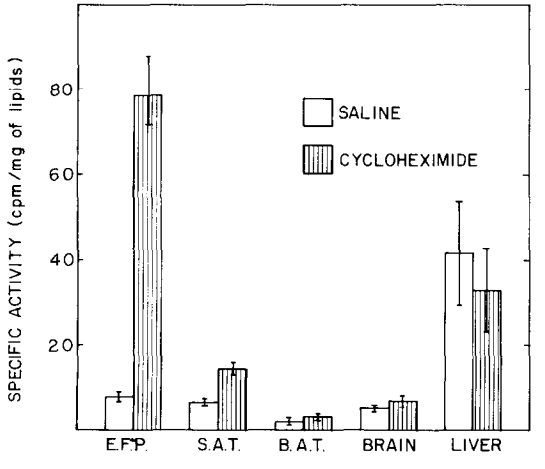

Fig. 3. Effect of cycloheximide on different tissues with active lipid metabolism. The animals were sacrificed 2 hr after injection of the antibiotic. The values for the epididymal fat pad are the same as in Fig. 1. E. F. P. = epididymal fat pad; S. A. T. = subcutaneous adipose tissue; B. A. T. = brown adipose tissue. Other indications as in Fig. 1.

Role of hormones

Sexual hormones. The effect of cycloheximide was tested in the parametrial adipose tissue of fasted female rats. The response elicited was lower than that observed in the epididymal fat pad of male rats (Table 2). Due to the different magnitude of response according to sex, the effect of the antibiotic was explored in orchiectomized and in orchiectomized testosterone-treated rats (Table 2). The 10-fold increase in the incorporation of labeled glucose into lipids was lowered to a 2-fold increase by orchiectomy, susequent testosterone treatment of the orchiectomized rats, as described under Methods, was unable to reverse this diminution.

Adrenal hormones: Since some of the effects of Actidione have been attributed to adrenal secretions [13, 14], the action of this drug on epididymal fat pad was explored in adrenalectomized rats. Adrenalectomy decreased the magnitude of the cycloheximide effect, with only a 3-fold increase in the incorporation of radioactive glucose into lipids (Table 3). Substitutive treatment with cortisol, corticosterone or epinephrine in adrenalectomized rats failed to restore the cycloheximide effect to the 10-fold level observed in intact animals. Furthermore, corticosterone lowered the response to a 2-fold increase (Table 3). The increase in

Table 1. Role of physiological conditions, weight and feeding on the response to cycloheximide in the epididymal fat pad*

Feeding conditions	Weight (g)	Substance injected	Specific activity (cpm/mg of lipids)	Ratio†	P value
Fasted	120–170	Saline	7·62 ± 1·05 (15)	10·10	< 0·001
		Cycloheximide	77·48 ± 9·67 (15)		
	250	Saline	6·47 ± 0·66 (3)	4·22	< 0·02
		Cycloheximide	27·33 ± 7·18 (7)		
Fed	120–170	Saline	52·86 ± 10·44 (5)	1·19	< 0·6
		Cycloheximide	63·10 ± 11·33 (5)		

* The conversion of radioactive glucose into lipids in the epididymal fat pad was studied in rats red *ad lib.* and in rats fasted for 16–20 hr. The results are expressed as the mean ± S. E. with the number of observations in parentheses. The data for fasted animals, 120–170 g, were taken from Fig. 1.
† Cycloheximide/saline.

the incorporation of [^{14}C]-glucose into lipids produced by epinephrine alone is in agreement with the findings of Leboeuf *et al.* [35].

Insulin. Insulin is one of the hormones with considerable influence on lipid metabolism in adipose tissue. To determine whether the action of cycloheximide was insulin-dependent, the effect of the antibiotic was assayed in alloxan-diabetic rats. Cycloheximide produced the expected effect in fasted diabetic animals, but failed to elicit a response in fed rats (Table 4).

A characteristic of the lipogenic action of insulin when [^{14}C]-glucose is used is unequal distribution of the label between fatty acids and glyceride-glycerol, favoring the former moiety [36]. Cycloheximide behaved differently from insulin in that the antibiotic increased the label equally in both parts of the triglyceride molecule without producing changes in its distribution (Table 5).

Changes in circulating fluids

Blood glucose and glycerol, free fatty acids and total purines and pyrimidines of the plasma were studied in an attempt to correlate these parameters with the effect of cycloheximide. It has been shown that cycloheximide produces depletion of liver glycogen and no change in blood glucose levels in fed rats injected with the antibiotic from 2–10 hr previously [37]. In agreement with these results, no differences in blood sugar levels were detected in fasted animals treated for 2 hr with cycloheximide (Table 6).

It has been reported that Actidione produces an accumulation of RNA in yeast [38, 39], in regenerating

Table 2. Role of sexual hormones on the response to cycloheximide in the conversion of glucose into lipids*

Sex of the rats	Experimental conditions	Substance injected	Specific activity (cpm/mg of lipids)	Ratio†	P value
Males	Normal	Saline	7·62 ± 1·05 (15)	10·10	< 0·001
		Cycloheximide	77·48 ± 9·67 (15)		
	Orchiectomy	Saline	9·02 ± 1·08 (6)	2·31	< 0·005
		Cycloheximide	20·84 ± 3·17 (10)		
	Orchiectomy plus testosterone	Saline	11·95 ± 2·01 (6)	2·27	< 0·10
		Cycloheximide	27·22 ± 8·21 (6)		
Females	Normal	Saline	8·21 ± 2·03 (9)	4·62	< 0·001
		Cycloheximide	37·94 ± 5·50 (12)		

* The data of the normal male rats are from Fig. 1. Other specifications as in Table 1.
† Cycloheximide/saline.

Table 3. Effect of cycloheximide on the epididymal fat pad of adrenalectomized rats and adrenalectomized rats with substitutive hormonal treatment*

Treatment	Substance injected	Specific activity (cpm/mg of lipids)	Ratio†	P value
Adrenalectomy	Saline	9·71 ± 1·87 (6)	3·38	< 0·01
	Cycloheximide	32·84 ± 6·69 (7)		
Adrenalectomy plus cortisol	Saline	5·04 ± 1·78 (3)	3·32	< 0·1
	Cycloheximide	16·76 ± 4·12 (3)		
Adrenalectomy plus corticosterone	Saline	8·82 ± 3·21 (3)	2·35	< 0·05
	Cycloheximide	20·76 ± 2·09 (3)		
Adrenalectomy plus epinephrine	Saline	21·64 ± 6·68 (3)	1·10	< 0·9
	Cycloheximide	23·83 ± 4·02 (3)		

* Specifications as in Table 1.
† Cycloheximide/saline.

rat liver and in rat adrenal glands [14]. On the other hand, an intraperitoneal injection of RNA, AMP or adenosine increases the synthesis of lipids in adipose tissue [15]. Although RNA liberation into the bloodstream has not been reported in animals treated with cycloheximide, a possible increase of RNA or its hydrolysis products in the circulating fluids of animals treated with this agent was explored. The decrease in the total amount of purines and pyrimidines in plasma, which has been considered by McIntire and Smith [29] to be equivalent to the content of nuclei acids, observed after cycloheximide administration (Table 6) is against the idea that the effect of actidione on lipid metabolism is mediated through this mechanism.

Plasma glycerol and free fatty acids were quantified as an index of lipolysis *in vivo*. Cycloheximide produces a slight decrease in plasma glycerol (Table 6) and, in agreement with the findings of other authors [10, 40], a significant fall in plasma free fatty acids (Table 6).

Epididymal fat pad incubations

Epididymal fat pads from animals treated for 1 hr with saline or cycloheximide were incubated *in vitro* as

Table 4. Effect of cycloheximide on the transformation of [U-^{14}C]-glucose into lipids of the epididymal fat pad from alloxan-diabetic rats*

Feeding conditions	Substance injected	Specific activity (cpm/mg of lipids)	Ratio†	P value
Fasted	Saline	7·91 ± 0·88 (3)	6·91	< 0·05
	Cycloheximide	54·70 ± 15·07 (4)		
Fed	Saline	16·13 ± 5·04 (3)	0·44	< 0·20
	Cycloheximide	7·17 ± 2·10 (4)		

* Specifications as in Table 1.
† Cycloheximide/saline.

Table 5. Relative distribution of radioactive carbon from uniformly labeled [^{14}C]-glucose in different lipid fractions*

Substance injected	Non-saponifiable (%)	Fatty acids (%)	Glyceride–glycerol (%)	Glyceride–glycerol/fatty acids
Saline	1·36 ± 0·46 (3)	9·36 ± 7·16 (3)	88·93 ± 7·77 (3)	9·5
Cycloheximide	0·91 ± 0·33 (3)	9·07 ± 4·86 (3)	90·01 ± 4·67 (3)	10·0

* Specifications as in Table 1.

Table 6. Effect of cycloheximide on blood glucose and on plasma total purines and pyrimidines, glycerol and free fatty acids*

	Time after injection (min)	Saline	Cycloheximide	P value
Glucose (mg/100 ml)	60	66·67 ± 2·30 (5)	69·72 ± 1·81 (5)	< 0·4
	120	57·75 ± 3·59 (5)	60·15 ± 2·49 (5)	< 0·6
TPP† (equivalent to μg DNA/ml of plasma)	60	319·22 ± 25·10 (9)	210·55 ± 37·56 (8)	< 0·1
Glycerol (μmoles/l.)	120	275·21 ± 29·70 (11)	215·12 ± 15·71 (11)	< 0·1
Free fatty acids (μequiv./l.)	120	399·98 ± 20·17 (7)	229·26 ± 22·62 (8)	< 0·001

* Specifications as in Table 1.
† Total of purines and pyrimidines.

described under Materials and Methods. The epididymal fat pads from rats injected with cycloheximide presented a 5-fold increase in the incorporation of [U-^{14}C]-glucose into lipids, an enhancement in the release of glycerol and a diminution in the release of free fatty acids into the medium (Table 7).

DISCUSSION

A stimulation in fatty acid re-esterification in the epididymal fat pad is one of the metabolic events elicited by cycloheximide. The following responses to the antibiotic support this point of view: (a) the enhanced release of glycerol from epididymal fat pad (Table 7); (b) the diminished release of free fatty acids in the same tissue (Table 7); (c) the decrease in plasma free fatty acids *in vivo* (Table 6); (d) the absence of response in fed rats (Table 1) where 97 per cent of free fatty acids are re-esterified [20], mading undetectable any further stimulation in this process; and (e) the limited 4-fold increase of radioactive carbon into lipids when [^{14}C]-glucose was injected 2 hr earlier compared to the increase obtained in the "pulse-type" experiment (see Results).

According to the data of Table 5, either in control or in cycloheximide-treated rats, ≈90 per cent of the label from ^{14}C-glucose was localized in the glycerol moiety and ≈ 10 per cent in the fatty acids of the triglycerides. Since cycloheximide increases 10-fold the incorporation of glucose into lipids (reported both as specific activity and as net incorporation in "pulse-type" experiments; Fig. 1), it means that the antibiotic, in addition to stimulating re-esterification, is very active in accelerating the utilization of glucose to form α-glycerophosphate and fatty acids. Summarizing, cycloheximide behaves like a lipogenic compound, increasing the synthesis of triglycerides in fasted rats, thus mimicking what happens in the fed ones. This lipogenic action of cycloheximide appears to be independent of an inhibition of protein synthesis, since the other inhibitors tested failed to produce the effect described here. However, mediation of some metabolic disturbances resulting from inhibition of protein synthesis produced by cycloheximide cannot be ruled out. The glycogenolytic action of cycloheximide [37] does not seem to be responsible for the effect described here, since it is not present in fed animals with high liver glycogen levels and it was observed in fasted animals with low levels of liver glycogen. In addition, puromycin, which is also a glycogenolytic compound [32], does not exhibit the same action as cycloheximide on lipid metabolism.

The increase in glucose incorporation into lipids due to cycloheximide is probably independent and distinct from the lipogenic action of insulin, since it is present in alloxan-diabetic rats (Table 4) and the distribution of radioactive carbon from [^{14}C]-glucose between glycerol and fatty acids after antibiotic treatment (Table 5) is different from that produced by insulin [36]. It also seems to be independent of the action of epinephrine, since this hormone enhances the release of free fatty acids while cycloheximide decreases it (Table 7). The effect of cycloheximide on lipid metabolism is probably related to some other hormonal requirements not yet clearly established. The smaller lipogenic response to cycloheximide in female rats and in

Table 7. Incubation of epididymal fat pads from saline and cycloheximide-treated animals*

	Treatment		P
	Saline	Cycloheximide	
^{14}C from glucose incorporated into lipids (cpm/mg of lipids)	81·47 ± 8·88 (6)	395·50 ± 32·60 (6)	< 0·001
Glycerol released (μmoles/g wet weight)	3·59 ± 0·18 (8)	4·94 ± 0·31 (8)	< 0·005
Free fatty acids released (μequiv./g wet weight)	5·69 ± 0·46 (8)	3·63 ± 0·49 (8)	< 0·01

* Aliquots of the medium before and after incubation were taken for the determination of free fatty acids and glycerol. Other specifications as in Table 1.

orchiectomized or adrenalectomized males, together with the inability of various hormones to restore a complete response to the antibiotic, did not allow any definite conclusion.

Finally, the findings described in this paper indicate that great care must be taken in the use of cycloheximide as an inhibitor of protein synthesis in studies of lipid metabolism. On the other hand, its use as a tool in the investigation of the regulation of lipid biosynthesis must be considered.

Acknowledgement—The authors wish to thank Dr. Saúl Villa-Treviño for his generous gift of puromycin and for help and advice. They also thank Dr. Aurora Brunner and Dr. Antonio Peña for careful revision of the manuscript.

REFERENCES

1. A. J. Whiffen, N. Bohonos and R. L. Emerson, *J. Bact.* **52,** 610 (1946).
2. B. E. Leach, J. H. Ford and A. H. Whiffen, *J. Am. chem. Soc.* **69,** 474 (1947).
3. D. Kerridge, *J. gen. Microbiol.* **19,** 497 (1958).
4. B. Colombo, L. Feliceti and C. Baglioni, *Biochem. biophys. Res. Commun.* **18,** 389 (1965).
5. A. C. Trakatellis, M. Montjar and A. E. Axelrod, *Biochemistry, N.Y.* **4,** 2065 (1965).
6. A. Korner, *Biochem. J.* **101,** 627 (1966).
7. B. S. Baliga, A. W. Pronczuk and H. N. Munro, *J. biol. Chem.* **244,** 4480 (1969).
8. R. A. Raff, *J. Cell. Physiol.* **75,** 341 (1970).
9. A. B. Cadwell and J. N. Fain, *Hormone Metab. Res.* **2,** 3 (1970).
10. H. Bar-On, O. Stein and J. Stein, *Biochim. biophys. Acta* **270,** 444 (1972).
11. S. Jaczilevich and S. Villa-Trevino, *Lab. Invest.* **23,** 590 (1970).
12. D. J. McNamara, F. W. Quackenbush and J. W. Rodwell, *Lipids* **5,** 146 (1970).
13. W. R. Jondorf, D. C. Simon and M. Aunimelech, *Biochem. biophys. Res. Commun.* **22,** 644 (1966).
14. S. Fiala and E. Fiala, *Biochim. biophys. Acta* **103,** 699 (1965).
15. V. Chagoya de Sánchez and E. Piña, *FEBS Lett.* **19,** 331 (1972).
16. E. S. West, W. R. Todd, H. S. Manson and J. T. Van Bruggen, in *Textbook of Biochemistry*, 4th Edn, p. 1153. Editorial Interamericana, S. A. México, D. F. (1969).
17. R. B. Gielie, A. Ruhmann-Wendhold, L. R. Johnson and D. H. Nelson, *Proc. Soc. exp. Biol. Med.* **142,** 54 (1973).
18. V. Chagoya, A. Hamabata and J. Laguna, in *International Symposium Enzymatic Aspects of Metabolic Regulation* (Ed. M. P. Stulberg), p. 61. National Cancer Institute Monograph 27 (1966).
19. H. A. León, *Endocrinology* **78,** 481 (1966).
20. S. P. Mukherjee and C. Mukherjee, *Am. J. Physiol.* **224,** 898 (1973).
21. G. Weber, R. L. Singhal, N. B. Stam, E. A. Fisher and M. A. Mentendiek, in *Advances in Enzyme Regulation* (Ed. G. Weber), Vol. 2, p. 1. Pergamon Press, New York (1964).
22. H. J. Honorat, F. H. Krents and M. Reim, *Biochem. biophys. Res. Commun.* **4,** 163 (1961).
23. J. Folch, M. Lees and G. H. Sloane Stanley, *J. biol. Chem.* **226,** 497 (1957).
24. M. S. Kornacker and E. G. Ball, *J. biol. Chem.* **243,** 1638 (1968).
25. G. A. Bray, *Analyt. Biochem.* **1,** 279 (1960).
26. N. Nelson and M. Somogyi, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol III, p. 85. Academic Press, New York (1958).
27. V. P. Dole and H, Meinertz, *J. biol. Chem.* **235,** 2595 (1960).
28. O. Wieland, in *Methods of Enzymatic Analysis* (Ed. H. V. Bergmeyer), p. 211. Academic Press, New York (1965).
29. F. C. McIntire and M. I. Smith, *Proc. Soc. exp. Biol. Med.* **98,** 76 (1958).
30. M. Maramatsu, N. Shimada and H. Nakagawa, *J. molec. Biol.* **53,** 91 (1970).
31. E. Schafrir and E. Wertheimer, in *Handbook of Physiology, Adipose Tissue* (Eds. A. E. Reynold and G. F. Cahill Jr.), Section 5, p. 417. Am. Physiol. Soc., Washington, D.C. (1965).
32. J. Hofert, J. Gorski, G. C. Mueller and R. K. Boutwell, *Archs Biochem. Biophys.* **97,** 134 (1962).
33. P. O. Ray, D. O. Foster and H. A. Lardy, *J. biol. Chem.* **239,** 3396 (1964).
34. G. E. Boxer and D. Stetten, *J. biol. Chem.* **153,** 607 (1944).
35. B. Leboeuf, R. B. Flinn and G. F. Cahill Jr., *Proc. Soc. exp. Biol. Med.* **102,** 527 (1959).
36. V. P. Dole, in *Handbook of Physiology, Adipose Tissue* (Eds. A. E. Renold and G. F. Cahill Jr.), Section 5, p. 15. Am. Physiol. Soc., Washington, D.C. (1965).
37. H. Cecil and J. Bitman, *Archs Biochem. Biophys.* **119,** 105 (1966).
38. H. Fukuhara, *Biochem. biophys. Res. Commun.* **18,** 297 (1965).
39. R. L. Taber Jr. and W. S. Vicent, *Biochem. biophys. Res. Commun.* **34,** 488 (1969).
40. E. Graveta, P. Pani, A. Ferrari and C. Mazzarino, *Biochem. Pharmac.* **20,** 3423 (1971).