"LA CICLOHEXIMIDA COMO UN ARMA PARA EL ESTUDIO DE LA REGULACION DEL PROCESO DE ESTERIFICACION EN TEJIDO ADIPOSO".

T E S I S

que presenta el Médico Cirujano Jesús Adolfo ~~García~~ Sáinz, para obtener el grado de Maestro en Ciencias Químicas (Esp. Bioquímica)

U. N. A. M.

México, D. F.

1978

Universidad Nacional
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No me es posible iniciar esta Tésis sin agradecer a la Dra. Victoria Chagoya de Sánchez y al Dr. Enrique Piña su ayuda y ejemplo que tanto contribuyeron en la realización de este trabajo y en mi formación profesional.

Puesto que vivimos en pleno misterio,
luchando contra fuerzas desconocidas,
tratemos en lo posible de esclarecerlo.
No nos desaliente la consideración de
la pobreza de nuestro esfuerzo ante los
magnos e innumerables problemas de la
vida. Concluida la ardua labor, seremos
olvidados, como la semilla en el surco;
pero algo nos consolará el considerar
que nuestros descendientes nos deberan
una pequeña parte de su dicha y que
gracias a nuestras iniciativas aquella
minúscula parte de la Naturaleza, objeto
de nuestros afanes, resultará un poco
mas agradable e inteligible.

Santiago Ramón y Cajal.

INTRODUCCION

Cuando por primera vez entramos a un laboratorio de bioquímica y observamos sobre uno de sus muros uno de esos impresionantes mapas metabólicos, nos sentimos aplastados: los bioquímicos que nos precedieron, lograron esclarecer el metabolismo y no nos queda nada por hacer. Sin embargo, nada mas equivocado que esta primera impresión. Observamos con mayor cuidado y vemos que estos mapas nos señalan los principales caminos metabólicos y las enzimas involucradas, pero nada nos dicen acerca de la regulación de las vias, sus flujos relativos y sobre todo no nos explican cómo la célula, el tejido o el organismo íntegro, logran adaptarse a los cambios en los medios interno y externo, manteniendo un estado de equilibrio aparente (homeostasis). El panorama ha cambiado: nos llenamos de optimismo y muchas son las dudas y pregun-

tas que nos asaltan. La investigación se inicia.

Uno de los capitulos mas apasionantes del metabolismo, es el metabolismo de los lípidos. Su estudio se ha visto obstaculizado por los problemas inherentes a la naturaleza hidrofóbica de sus metabolitos. Sin embargo, importantes logros se han realizado y la mayoria de las vias han podido ser esclarecidas. La comprensión de la regulación del metabolismo de los lípidos es importante no solo por si misma, ciencia básica, sino que tiene gran proyeccion a problemas biomédicos: obesidad, diabetes, cetosis, hiperlipidemias, ateroesclerosis, etc.

Sin duda, uno de los tejidos mas importantes en el metabolismo de los lípidos es el tejido adiposo. El tejido adiposo es un constituyente del tejido conectivo y está formado por la reunión de células grasas o adipocitos, en lobulillos. El tejido adiposo es por lo tanto una variante especializada del tejido conectivo. Las células del lobulillo se encuentran rodeadas por fibras reticulares y se hallan provistas de abundantes capilares, que permiten el intercambio metabólico constante entre ellas y el resto de la economía y por ende su participación en la homeostasis.

El tejido adiposo se encarga de la síntesis y depósito de triacilgliceroles, como fuente potencial de energía y de su liberación en forma de ácidos grasos y glicerol. El acúmulo en forma de triacilgliceroles, es conveniente por su alto valor ca-

lórico y porque por su naturaleza profundamente hidrofóbica no requieren iones y agua de solvatación como el glucógeno, para su depósito, sino que lo hacen en forma anhidra. Se ha calculado conservadoramente que si un individuo de 70 kilogramos, transformara a glucógeno, la energía depositada como triacilgliceroles en el tejido adiposo, su peso facilmente se duplicaría.

Hace aproximadamente 40 años el tejido adiposo se consideraba como inerte. Se suponía que los procesos de depósito y movilización de grasa se realizaban independientemente del adipocito. En los últimos veinte años, el estudio del tejido adiposo ha tenido un desarrollo explosivo. Con el esfuerzo de muchos investigadores, ha sido posible demostrar que el adipocito posee una maquinaria enzimática respetable y una gran actividad metabólica. Además de estos atributos, este tejido tiene un exquisita sensibilidad ante cualquier cambio en el medio que lo rodea. Esta sensibilidad ha permitido su uso como modelo experimental y con él se han logrado importantes avances en el campo de la regulación metabólica hormonal. Interesantes revisiones han aparecido a este respecto (1-4).

A pesar de este importantísimo avance, persisten una serie de problemas de regulación metabólica, de trascendencia fisiológica. El tejido adiposo posee un depósito de triacilgliceroles que en condiciones normales se encuentra en un equilibrio dinámico, es decir, que está en contínua síntesis y degradación.

La hidrólisis de los triacilgliceroles proporciona ácidos grasos y glicerol. El glicerol no es reutilizado por el tejido adiposo y es liberado al medio interno, donde es usado principalmente por el hígado. Los ácidos grasos por el contrario, pueden ser reutilizados. Durante los estadios postprandiales, el tejido adiposo debe, no solamente reesterificar los ácidos grasos liberados por la lipólisis basal, sino esterificar los provenientes de la dieta, liberados por acción de la lipasa lipoproteica y los sintetizados _de novo_. Por el contrario, durante los periodos de ayuno, el adipocito proporciona a la economía ácidos grasos para la obtención de energía. Ello necesariamente significa que la célula adiposa debe poseer un sistema que le permita regular el proceso de esterificación, para adaptarse a las condiciones nutricionales y cumplir con su fin biológico.

En los trabajos que adjunto a esta introducción, presento el uso de la cicloheximida como un arma para conocer la regulación del proceso de esterificación en tejido adiposo (5-8). Resumiré a continuación los principales hallazgos y su trascendencia.

La cicloheximida es uno de los inhibidores de la biosíntesis de proteínas mas empleados en investigación (9), sin embargo, los efectos a los cuales me referiré, no están relacionados con esta propiedad del compuesto (Referencia 5, Fig 4 y Ref. 6, Fig 2).

La administración *in vivo* del compuesto a ratas macho ayunadas produce un aumento en la incorporación de glucosa radioactiva a lípidos totales de aproximadamente 10 veces (Ref. 5, Fig. 1). La mayoría de la marca se localiza en la fracción de glicerol de los glicéridos (Ref. 5, Tabla 5) y concomitantemente existe una disminución en los niveles de ácidos grasos libres en suero (Ref. 5, Tabla 6). Todos estos datos sugieren que la cicloheximida está favoreciendo el proceso de esterificación en tejido adiposo. Además, no fue posible detectar un efecto significativo en ratas alimentadas *ad libitum* (Ref. 5, Tabla 1) en las cuales el proceso de esterificación está notablemente activo en relación con las ayunadas (10).

La medición directa del proceso de esterificación, es imposible en el animal íntegro; por lo cual, se trató de reproducir el efecto *in vitro*, incubando panículos adiposos de ratas ayunadas, en un medio semisintetico. Se observo que el antibiótico produce en estas condiciones un efecto cualitativamente identico al descrito, pero de menor magnitud, esto es: la cicloheximida incrementó la incorporación de glucosa radioactiva a lípidos en un 100% y la mayor parte de la marca se localizó en la fracción de glicerol de los glicéridos (Ref 6, Fig 1 y Tabla 4). Al medir directamente la esterificación de los ácidos grasos, se logró demostrar que la cicloheximida estimula este proceso (Tabla 2).

Estudiando el metabolismo de la glucosa, se observó que la cicloheximida incrementa no solo su incorporación a lípidos sino también su oxidación por glucólisis y ciclo de Krebs (Ref 6, Tablas 3 y 5). Es mas, la captación de la hexosa por el tejido también se encontró aumentada (Ref 6, Tabla 3). Todos estos hechos sugerían la posibilidad de que el efecto primario de la cicloheximida fuese sobre la captación de la glucosa y que el aumento en la oxidación de la hexosa y en la esterificación de los ácidos grasos fueran secundarios. Es más, se ha propuesto que la disponibilidad de alfa-glicerofosfato es uno de los factores que regulan la esterificación de los ácidos grasos tanto en hígado como en tejido adiposo (11,12) y la cantidad de este metabolito se pudiera ver incrementada al aumentarse el flujo glucolítico.

Sin embargo, se realizaron experimentos <u>in vitro</u> con tejido adiposo de animales alimentados <u>ad libitum</u> y se observó que bajo estas condiciones la cicloheximida no incrementa ni la captación de glucosa ni su oxidación en forma significativa, pero sí su incorporación a lípidos, encontrándose éstos preferentemente marcados en la fracción de glicerol de los glicéridos (Ref 7, Tablas I y II). Ello se debe a un incremento en el proceso de esterificación de los ácidos grasos (Ref 7, Tabla III). Este hecho tiene un significado especial: la cicloheximida es capaz de modificar en forma primaria el proceso de esterificación

y probablemente algunas de las acciones observadas con anterioridad sean efectos secundarios.

Para tratar de comprobar este hecho se usó el siguiente enfoque: se usó lactato radioactivo y se midió tanto su incorporación a grasa total, como la distribución de la marca. Dado que el lactato se incorpora a glicerol de los glicéridos por via gliceroneogénica, una mayor incorporación indica que el proceso de esterificación está utilizando mas alfa-glicerofosfato, sea por via glicerogénica (glucólisis) o por via gliceroneogénica (gluconeogénesis). La cicloheximida incrementó la incorporación de lactato a lípidos y desvió su distribución hacia glicerol de los glicéridos. Estos resultados señalan la importancia del proceso de esterificación en tejido adiposo, ya que como se aprecia es capaz de desviar los flujos metabólicos. Este hecho podemos considerarlo como teleológicamente adecuado, ya que la principal función del tejido adiposo es satisfacer las demandas de ácidos grasos y modular su concentración en sangre.

Una vez localizado el efecto de la cicloheximida al proceso de esterificación, se decidió estudiar cual era la enzima afectada, ya que esto no solamente nos señalaría en forma precisa donde actúa en antibiótico para producir este efecto, sino que de acuerdo a las consideraciones hechas anteriormente en relación al efecto según las condiciones nutricionales del animal experimental, sugeriría cual es la enzima reguladora de este pro

ceso. Se estudiaron los niveles de metabolitos precursores de los triacilglicéridos y cómo se presenta en la Fig 1 de la referencia 8, los niveles de glicerol-3-fosfato y acil-CoA bajan, mientras que los de fosfolípidos (ácido fosfatídico), diacil y triacilglicéridos se encuentran aumentados. De acuerdo a los postulados del "crossover theorem" (13,14), la enzima involucrada es la glicerol-3-fosfato aciltransferasa. Esta enzima se ha demostrado que juega un papel regulador en hígado (15): pero no ha sido posible demostrar su participación en la regulación en tejido adiposo (15,16). Sin embargo se ha logrado detectar una pequeña estimulación con insulina (17), lo cual está totalmente de acuerdo con nuestros resultados.

Es bien conocido el hecho de que la lipólisis está mediada por la actividad de la lipasa sensible a hormonas, dependiente de AMP cíclico. Al estimularse la lipolisis, por una catecolamina, por ejemplo, se elevan inicialmente mucho los niveles de AMP cíclico, disminuyendo a los pocos minutos a niveles discretamente superiores a los basales. Este hecho a sido explicado por la acumulación de un "regulador fisiológico de la adenilato ciclasa" (18,19). Recientemente el grupo de Fain, ha demostrado que los ácidos grasos libres son el regulador fisiológico (20,21). La cicloheximida estimula la lipólisis (5,6) y eleva los niveles de AMP cíclico (22,23). Sin embargo se ha observado que la caida en los niveles de AMP cíclico es lenta (23). Esto

se correlaciona bien con los datos presentados, ya que al aumentar la esterificación y disminuir por lo tanto el nivel de ácidos grasos libres, se evita la represión fisiológica de la adenilato ciclasa. Un hecho semejante ha sido demostrado con la insulina (24).

Por último, se ha postulado que el mecanismo regulador de los niveles de ácidos grasos en sangre, dependiendo de las condiciones nutricionales es la lipólisis (25). Sin embargo es posible observar que en condiciones basales la lipólisis es similar en tejidos obtenidos de animales ayunados o alimentados _ad libitum_, pero el tejido de un animal ayunado reesterifica solamente una pequeña fracción de los ácidos grasos liberados, mientras que el tejido de un animal comido lo hace con la casi totalidad (Ref 6, Tabla 2 y Ref 7, Tabla III). Estos datos claramente señalan la importancia del proceso de esterificación en la regulación de la liberación neta de ácidos grasos por el tejido adiposo y señalan que el considerar a la lipólisis como único mecanismo regulador es una simplificación excesiva.

En esta tésis, se presenta el análisis de un fenómeno detectado en el animal íntegro y su disección hasta llegar a la explicación básica a un nivel molecular. Considero que este es el camino menos peligroso para explicar los fenómenos a los que nos enfrentamos. Aunque es largo y riesgoso, nos puede evitar el problema de luchar contra enemigos que nosotros mismos crea-

mos en el tubo de ensayo y hacer extrapolaciones que nada tienen

que ver con la realidad.

REFERENCIAS

1.-Renold, A. E. y Cahill, G. E., Jr., (editores de la sección) Handbook of Physiology, Section 5, Adipose Tissue, American Physiological Society. Washington (1965).

2.-Jeanrenaud, B. y Hepp, D. (editores) Adipose Tissue. Regulation and Metabolic Functions. Academic Press. New York (1970).

3.-Fain, J. N. Pharmacological Reviews 25, 67 (1973)

4.-Hollember, C. H., Vost, A y Patten, R. L., Recent Progr. Hormone Res. 26, 463 (1970).

5.-García-Sáinz, J. A., Amador, A. G., Piña, E. y Chagoya de Sánchez, V. Biochem. Pharmacol. 24, 891 (1975).

6.-García-Sáinz, J. A., Piña, E. y Chagoya de Sánchez, V., J. Lipid Res. 18, 93 (1977)

7.-García-Sáinz, J. A., Piña, E. y Chagoya de Sánchez, V., Biochem. Pharmacol., en prensa.

8.-García-Sáinz, J. A. y Chagoya de Sánchez, V., Biochem. Pharmacol. enviado a revisión.

9.-Verbin, R. S., Longnecker, D. S., Liang, H. y Farber, E., Amer. J. Pathol. 62, 111 (1971).

10.-Vaughan, M. y Steimberg, D. J. Lipid Res. 4, 193 (1963)

11.-Fritz, I. B. Physiol. Reviews 42, 52 (1961)

12.-Shapiro, B. en Handbook of Physiology, Section 5, Adipose Tissue American Physiological Society. Washington. p 217. (1965)

13.-Chance, B., Holmes, W., Higgins, J. J. y Connelly, C. M.,

Nature 182, 1190 (1958).

14.-Rolleston, F. S. en Current Topics in Cellular Regulation, Vol. 5, p 47, Academic Press, New York. (1972).

15.-Bremer, J., Bjerve, K. S. y Borreback B., Mol. Cell. Biochem. 12, 113 (1976).

16.-Aas, M. y Daae, L. N. W., Biochim. Biophys. Acta 239, 208 (1971)

17.-Sooranna, S. R. y Saggerson, E. D., FEBS Letters 64, 36 (1976).

18.-Ho, R. J. y Sutherland, E. W., J. Biol. Chem., 246, 6822 (1971)

19.-Manganiello, V. Murad, F. y Vaughan, M., J. Biol. Chem., 246, 2195 (1971)

20.-Fain, J. N. y Shepherd, R. E., J. Biol. Chem., 250, 6586 (1975)

21.-Malgieri, J. A., Shepherd, R. E y Fain, J. N., J. Biol. Chem., 250, 6593 (1975).

22.-Moskowitz, J. y Fain, J. N. J. Biol. Chem., 245, 1101 (1970)

23.-Wititsuwannakul, D. y Kim K.-H., Biochem. Biophys. Res. Commun. 76, 86 (1977).

24.-Fain, J. N., J. Cyclic Nucleotide Res. 1, 359 (1975).

25.-Fain, J. N., Shepherd, R. E., Malbon, C. C. y Moreno, F. J. en The Physiology of Lipids and Lipoproteins in Health and in Disease. American Physiological Society. en prensa.

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 < 0·01; 60 and 120 min, P < 0·001; and 180 min, P < 0·005). 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].

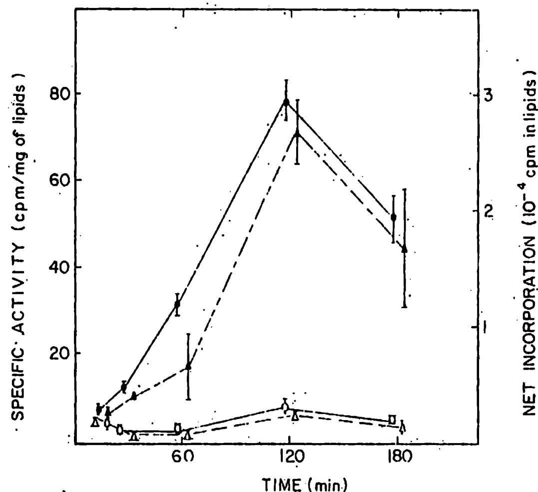

Fig. 1. Effect of cycloheximide on the incorporation of radioactive glucose into lipids of the epididymal fat pad as a function of time. Specific activity in control rats (○—○), net incorporation in control rats (△—△); specific activity in rats injected with cycloheximide (●- - -●), and net incorporation 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

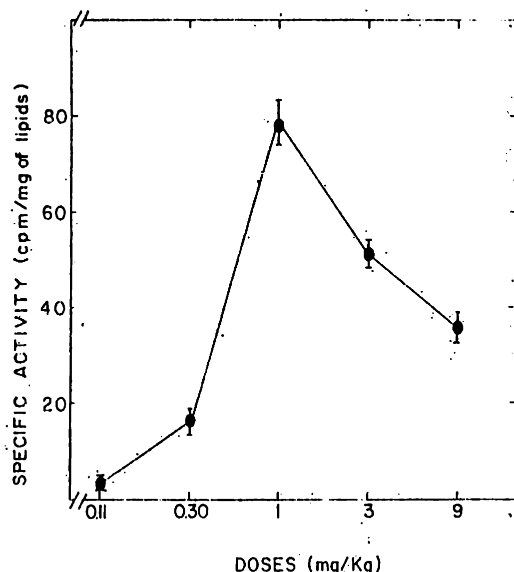

Fig. 2. Dose-response curve of cycloheximide on the transformation of [UL-^{14}C-D] glucose into lipids of the epididymal fat pad. The value at 1 mg/kg of body weight is the same as that at 120 min in Fig. 1. Other specifications as in Fig. 1.

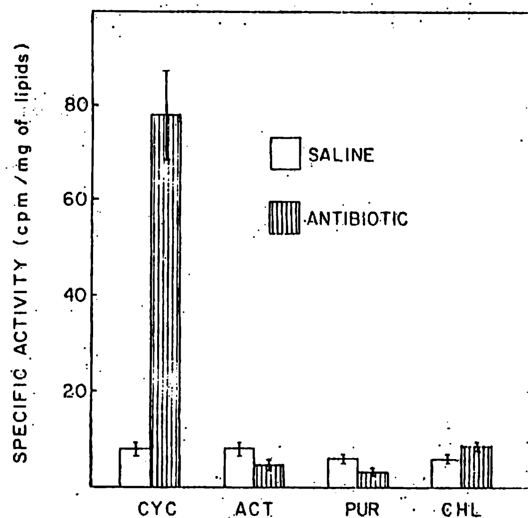

Fig. 4. Comparative effect of different inhibitors of protein synthesis on the conversion of labeled glucose into lipids of the epididymal fat pad. All the inhibitors were injected intraperitoneally: actinomycin-D, 1 mg/kg of body weight [33]; puromycin, 50 mg/kg of body weight [34]; and chloramphenicol, 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].

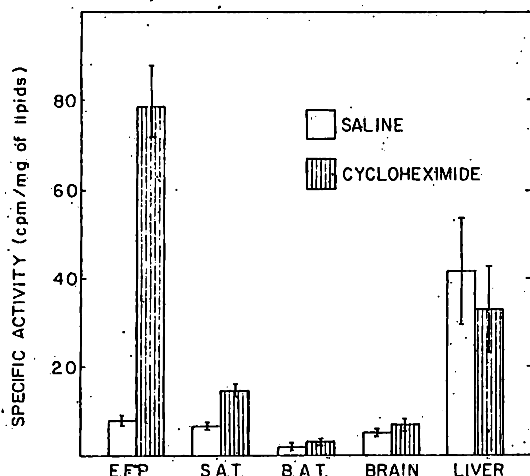

Fig. 3. Effect of cycloheximide on different tissues with active lipid metabolism. The animals were sacrificed 2 hr after injection of the antibiotic. The values for the epididymal fat pad are the same as in Fig. 1. E. F. P. = epididymal fat pad; S. A. T. = subcutaneous adipose tissue; B. A. T. = brown 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 fed *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 ± 4·81 (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 ± 25·40 (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		
	Saline	Cycloheximide	P
^{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 ± 0·34 (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. Felicetti 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-Treviño, *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. Gielic, 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. Mentendick, 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).

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Stimulatory action of cycloheximide on glucose metabolism in the rat epididymal fat pad

J. Adolfo García-Sáinz, Enrique Piña, and Victoria Chagoya de Sánchez
Departmento de Biología Experimental, Instituto de Biología, Universidad Nacional Autónoma de México. México 20, D.F.

Abstract The action of cycloheximide on some parameters of glucose and lipid metabolism was studied in vitro in epididymal fat pads from fasted rats. Incubation of fat pads with cycloheximide (1 μg/ml) for 2 hours resulted in a two-fold increase in glucose uptake, glucose oxidation, incorporation of glucose into lipids, and reesterification of free fatty acid. The increase in glucose oxidation was evident in experiments in which [U-^{14}C], [1-^{14}C], or [6-^{14}C]glucose was added to the media, but it was absent when the media were supplemented with pyruvate. Incorporation of glucose into glycogen and accumulation of lactate in the medium were not seriously modified by the presence of cycloheximide. The stimulatory effect of cycloheximide on incorporation of glucose into lipids was absent when insulin or cortisol was added to the medium. A cycloheximide-mediated increase in glucose uptake seems to be responsible for the subsequent changes in glucose metabolism, and would seem to be independent of an inhibition in protein synthesis; puromycin and actinomycin-D did not mimic the cycloheximide action on glucose incorporation into lipids.

Supplementary key words adipose tissue metabolism · glucose uptake · glucose incorporation into lipids · free fatty acid esterification

Cycloheximide (CHM) is an antibiotic widely used as an inhibitor of protein synthesis in vivo (1) and in vitro (2). In a previous paper (3) we reported that 2 hr after an intraperitoneal injection of CHM into fasted male rats, there was a 10-fold increase in the incorporation of ^{14}C from ^{14}C-labeled glucose into epididymal fat pad lipids. Other inhibitors of protein synthesis did not produce this effect on lipid metabolism.

The aim of the present paper is to characterize the in vitro effects of CHM on adipose tissue metabolism in order to gain further insight on the mechanism of action of the antibiotic in a system that is less complex than the whole animal.

MATERIALS AND METHODS

Cycloheximide, puromycin, bovine serum albumin (fraction V) and glycerokinase were obtained from Sigma Chemical Company (St. Louis, MO). Actinomycin-D was purchased from Nutritional Biochemical Corporation (Cleveland, OH). D-[U-^{14}C]Glucose (200 mCi/mmole), D-[1-^{14}C]glucose (5 mCi/mmole), D-[6-^{14}C]glucose (51.2 mCi/mmole) and L-[U-^{14}C]leucine (180 mCi/mmole) were obtained from International Chemical and Nuclear Corporation (Cleveland, OH). Hexokinase, glucose 6-phosphate dehydrogenase, and α-glycerophosphate dehydrogenase were obtained from Behringer und Soehne (Mannheim, Germany).

Experiments were performed with male Wistar rats weighing between 120 and 170 g and fasted for 16–20 hr. Rats were killed by decapitation and exsanguinated. The epididymal fat pads were removed as fast as possible with minimal handling, rinsed in 0.85% NaCl, and incubated in a Dubnoff metabolic shaker at 37°C in stoppered 25-ml Erlenmeyer flasks. Usually one of the two tissues obtained from each animal served as control; the medium in which the contralateral tissue was incubated contained CHM. The amount of tissue per flask ranged between 120 and 150 mg (133.06 ± 5.4 mg control; 138.77 ± 1.96 mg experimental; an average of 30 samples ± SEM in each case). In addition to the tissue, each flask contained 3 ml of Krebs-Ringer bicarbonate buffer, pH 7.3, which contained: 115.38 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 24.88 mM $NaHCO_3$, 150 mg of bovine serum albumin (fraction V, filtered through a millipore filter according to Dole (4) and containing

Abbreviations: CHM, cycloheximide; FA, fatty acids; FFA, free fatty acids; cyclic AMP, cyclic 3′,5′ adenosine monophosphate.

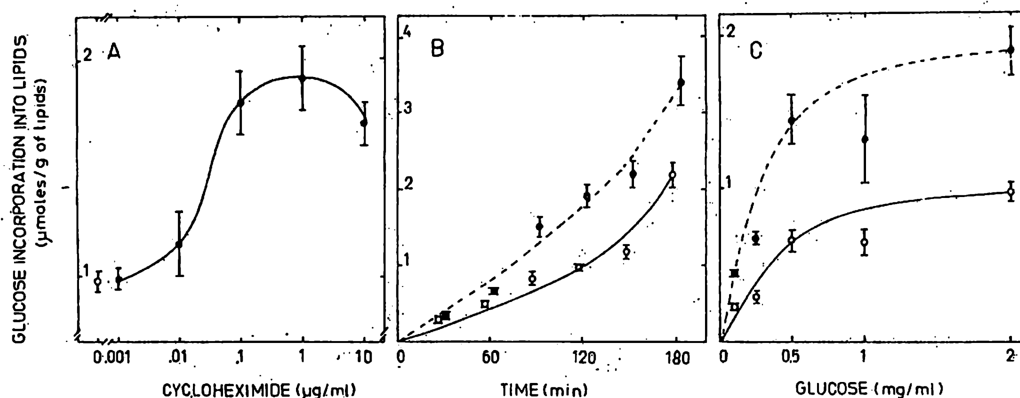

Fig. 1. Effect of cycloheximide on the incorporation of ^{14}C from [^{14}C]glucose into lipids in epididymal fat pads. A) Dose-response, B) time-course and C) medium glucose concentration ([^{14}C]glucose specific activity was maintained constant). Panels B and C, control (O —— O); CHM (● - - - ●). Vertical lines represent the standard error of at least five independently incubated pads.

0.9 μeq of FFA assayed by the method of Dole and Meinertz (5)), and 11.1 mM (2 mg/ml) glucose. In some experiments 1 μCi of ^{14}C-labeled glucose was in the incubation medium. The incubation medium was heated to 37°C and gassed with 5% CO_2–95% O_2 before the donor animals were killed, and the flasks were flushed with the same gas mixture after the tissue was added.

Lipids were extracted according to the method of Folch, Lees, and Sloane Stanley (6). In some experiments the lipid extracts were saponified and the distribution of radioactive carbon from glucose between glyceride-glycerol and fatty acids was studied by the method of Kornacker and Ball (7) adapted as previously described (3).

When lipolysis and esterification were studied, one pad from each rat was used to determine the initial concentration of glycerol and FFA, and the other one was incubated. The epididymal fat pads were homogenized in 3 ml of cold glass-distilled water. One ml each was taken from both medium and homogenates; FFA was determined by the method of Dole and Meinertz (5) and glycerol by the procedure of Wieland (8). The rate of FFA esterification was calculated according to Vaughan (9). Glucose uptake was measured as disappearance of hexose from the medium and was determined by the method of Slein (10).

Glycogen was quantified by the anthrone (11) method. The [^{14}C]glucose incorporated into glycogen was measured according to Hassid and Abraham (11). Lactic acid was measured by the method of Hohorst (12). Oxidation of radioactive glucose was measured by its conversion to $^{14}CO_2$, which was absorbed in 0.2 ml of 1 M Hyamine dissolved in methanol according to the method of Del Boca and Flatt (13). Protein synthesis was studied by measuring the incorporation of [^{14}C]leucine (0.2 μCi per flask) into protein. Proteins were isolated according to Feigelson, Feigelson, and Fancher (14), and suspended in formic acid for measurement of radioactivity. The ^{14}C radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL) as previously described (3). Special conditions used in some particular experiments are given in the figures, tables, or in the text.

The results are presented as per gram of lipid or wet weight of tissue. Both measurements are comparable since it was found that under our experimental conditions 70.69 ± 2.23% of the wet weight of the pads was lipid (mean ± SEM of 11 determinations). Statistical comparisons between groups of data were performed by the Student's *t* test.

RESULTS

Fig. 1 shows the results of preliminary studies that defined experimental conditions used in subsequent incubations. CHM increased incorporation of [^{14}C]glucose into lipids at doses between 0.1 μg/ml and 10 μg/ml ($P < 0.001$) (panel A, Fig. 1); a dose of 1 μg/ml was selected for all subsequent studies including those of panels B and C. The incorporation of [^{14}C]glucose into lipids did not follow a linear pattern either in control or in CHM-treated tissues (panel B, Fig. 1). There was a lag period in the stimulatory action of CHM and the highest CHM/control ratio was obtained after 2 hr of incubation; this was the incubation time used for all the experiments including those represented in panels A and C. The maximum response to CHM in vivo was also found 2 hr after its administration (3).

The effect of increasing glucose concentration on the conversion of [^{14}C]glucose to lipids followed a

simple saturation kinetics (panel C, Fig. 1). The difference between control and CHM-treated tissues was statistically significant in all cases. A saturating glucose concentration (2 mg/ml) was used for all the experiments.

Modifications of the incubation medium

The concentrations of several components of the incubation mixture were modified in an effort to magnify the action of the antibiotic and to obtain the optimal conditions to study its action.

It has been reported that glucose uptake by epididymal fat pads is related to the concentration of FFA in the medium (15) and that the mobilization of FFA is related to the calcium concentration in the extracellular fluids (16). In our system, the incubation mixture contained 50 mg/ml of albumin and 0.3 μeq/ml of FFA. In some experiments the concentration of both substances was changed independently: albumin concentrations of 12.5–75 mg/ml, and FFA concentrations of 0–0.6 μeq/ml were used. The calcium concentration usually (2.54 mM) was also modified in a series of experiments from 0 to 5.8 mM. Changes in the concentrations of these components in the incubation mixture did not modify the CHM-mediated stimulation of lipid metabolism (data not shown).

Additions to the incubation medium

Some actions of CHM have been correlated to hormones (3, 17, 18). Thus, the effect of CHM on lipid metabolism reported in vivo was substantially decreased by orchiectomy or adrenalectomy (3). Because of this, several hormones were tested for their ability to magnify the CHM effect.

Epinephrine (10 μg/ml), corticosterone-21-acetate (0.1–1 mg/ml) and testosterone (0.01–0.1 mg/ml) added to the incubation medium did not affect the response to CHM. However, in the presence of cortisol (1 mg/ml) or insulin (1 mU/ml) the effect of CHM was not observed (results not shown).

In the presence of serum from saline-treated animals, the conversion of [^{14}C]glucose into lipids was decreased in the epididymal fat pads incubated either with or without CHM. Opposite results were observed when serum from rats treated with CHM was used (**Table 1**). Of interest is the response obtained in experiments in which the medium was supplemented with CHM and with serum from CHM-treated rats. The higher incorporation obtained in these pads cannot be attributed to an extra dose of CHM, since an optimal dose was used (panel A, Fig. 1). Additional volumes of serum produced results quite similar to those reported in Table 1 (results not shown).

TABLE 1. Influence of cycloheximide on the incorporation of radioactive glucose into lipids in epididymal fat pads incubated with serum from control or cycloheximide-injected rats

Addition	Control	CHM	CHM/Control	P
	μmoles/g of lipids			
None	0.98 ± 0.06[a] (8)	1.91 ± 0.16 (6)	1.95	<0.001
Serum[b] from saline-treated animals	0.74 ± 0.08 (3)	1.20 ± 0.12 (3)	1.62	<0.05
Serum[b] from CHM-treated animals	1.77 ± 0.10 (3)	2.65 ± 0.07 (3)	1.50	<0.005

[a] The results are expressed as the mean ± SEM with the number of observations in parentheses.
[b] Serum was obtained from a blood sample collected 2 hr after the administration of saline or cycloheximide dissolved in saline at a dose of 1 mg/kg of body weight. Each flask containing the incubation mixture described in Materials and Methods was supplemented with 0.15 ml of serum.

Lipolysis and reesterification

The action of CHM on lipolysis and reesterification was investigated to determine the metabolic pathway

TABLE 2. Effects of cycloheximide on lipolysis and free fatty acid reesterification

	Rate of Lipolysis (Glycerol Production)	Net Change in FFA	Rate of Esterification
	μmole/g wet weight	*μeq/g wet weight*	*μeq FFA/g wet weight*
Control	4.94 ± 0.20[a] (7)	11.66 ± 0.98 (7)	3.16
Cycloheximide	6.65 ± 0.75 (7) $P < 0.05$	13.66 ± 1.71 (7) $P < 0.3$	6.29

[a] Mean ± SEM with the number of observations in parentheses.

TABLE 3. Effects of cycloheximide on glucose metabolism

	Control	CHM	CHM/Control	P
	μmoles/g wet weight			
Glucose uptake	3.03 ± 0.31[a] (11)	6.78 ± 0.41 (12)	2.24	<0.001
Glucose incorporation into glycogen	0.067 ± 0.014 (6)	0.102 ± 0.026 (6)	1.52	<0.3
CO_2 formation	1.04 ± 0.11 (5)	1.82 ± 0.24 (6)	1.75	<0.01

[a] Mean ± SEM. The number of observations is in parentheses.

TABLE 4. Relative distribution of radioactive carbon from glucose in different fractions of lipid extracts of epidydimal fat pads

Additions	Nonsaponifiable Lipids	Fatty Acids	Glyceride–Glycerol
		%	
None (4)[a]	4.35 ± 1.30[b]	5.60 ± 1.05	88.97 ± 2.14
CHM (5)	2.86 ± 1.63	9.18 ± 3.32	89.66 ± 3.36

[a] Number of experiments.
[b] Mean ± SEM.

that was preferentially stimulated by the antibiotic. CHM enhanced the net production of glycerol by 34.6% and that of FFA by 17.1% (**Table 2**). The rate of reesterification was calculated from the data of the table. Twice as much FFA was esterified in pads incubated with CHM. Similar results had been previously obtained in vivo (3).

Effect of CHM on glucose metabolism

A general study of the influence of CHM on glucose metabolism in the epididymal fat pads was carried out. CHM mainly stimulated both glucose uptake and glucose oxidation (**Table 3**). The magnitude of stimulation was parallel to the increase in the conversion of [^{14}C]glucose into lipids (Fig. 1). A slight but statistically insignificant increase in glucose incorporation into glycogen was also noted.

Since the differential response in glucose metabolism shown in Table 3 could have been due to utilization of endogenous glycogen or to the release of lactate into the incubation mixture, both of these parameters were examined. After 2 hr of incubation, the glycogen content decreased from 2.11 ± 0.22 μmoles of glucose/g wet weight to 0.66 ± 0.06 in control pads and to 1.00 ± 0.11 when the antibiotic was added to the medium (mean ± SEM of 4,6, and 6 determinations respectively). Lactate accumulation in the medium was equivalent in the absence or presence of CHM (control 4.73 ± 0.62 μmoles/g and CHM-treated 4.40 ± 0.54 μmoles/g; mean ± SEM of 6 determinations in each case).

Table 4 shows data on the effect of CHM on conversion of [^{14}C]glucose to lipid in the epididymal fat pads. Twice as much radioactivity was present in lipid extracts from tissues treated with CHM, but the relative distribution was not affected.

In order to determine if the stimulation of glucose oxidation by CHM (Table 3) increased Krebs cycle activity or pentose phosphate cycle activity, experiments with [1-^{14}C]glucose and with [6-^{14}C]glucose were performed. In both cases the stimulatory action of CHM on hexose oxidation was present (**Table 5**). The addition of pyruvate masked the action of the antibiotic, suggesting that the stimulation of oxidation occured via the Krebs cycle.

TABLE 5. Effect of cycloheximide on CO_2 formation from glucose

^{14}C-Labeled Glucose	Pyruvate 5 mM	Control	CHM	CHM/Control	P
		µmoles/g wet weight			
[1-^{14}C]	–	0.73 ± 0.08[a] (6)	1.41 ± 0.16 (5)	1.93	<0.005
	+	0.80 ± 0.07 (6)	0.96 ± 0.06 (6)	1.20	<0.1
[6-^{14}C]	–	0.52 ± 0.06 (6)	0.84 ± 0.10 (6)	1.62	<0.05
	+	0.21 ± 0.03 (5)	0.25 ± 0.03 (6)	1.19	<0.3

[a] Mean ± SEM. The number of observations is in parentheses.

Inhibition of protein synthesis

An attempt was made to correlate the inhibition of protein synthesis due to CHM with its effect on lipid metabolism in epididymal fat pads. Other inhibitors of protein synthesis, such as actinomycin-D and puromycin, did not share with CHM the ability to increase the conversion of glucose into lipids of the fat pads (**Fig. 2**). Furthermore, puromycin decreased this process in agreement with the report of Fain (19).

DISCUSSION

Our present results resemble the in vivo effect of CHM on epididymal fat pads (3). The qualitative

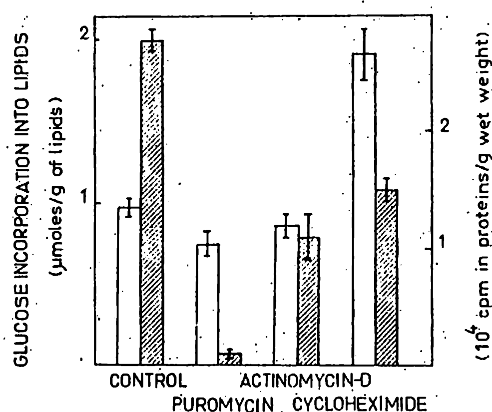

Fig. 2. Effect of several inhibitors of protein synthesis on the incorporation of [^{14}C]glucose into lipids (open bars) and [^{14}C]leucine into proteins (cross hatched bars). Doses: puromycin 10^{-4} M actinomycin-D 10^{-7} M, and cycloheximide 3.55×10^{-6} M (1 μg/ml). Vertical lines represent the standard error of at least five independently incubated pads.

nature of the response to CHM was similar in vivo and in vitro in the following aspects: *a*) most of the label from glucose was in the glyceride–glycerol moiety of the lipid extracts (Table 4); *b*) the actions on lipolysis and reesterification (Table 2); *c*) the time required to show a maximum effect (Fig. 1, Panel B); and *d*) the absence of correlation with an inhibition in protein synthesis (Fig. 2). Nevertheless, the quantitative stimulation in conversion of glucose into lipids produced by the antibiotic was increased 10-fold in vivo (3) and only 2-fold in vitro (Fig. 1).

Some humoral factor appears to be involved in the effect of CHM on lipid metabolism in adipose tissue. A further increase in the incorporation of glucose into lipids was detected when serum from CHM-treated animals was added to the incubation mixture containing the optimal dose of the antibiotic (Table 1). In addition, some actions of CHM have been shown to be hormone-mediated (17, 18). Thus the 10-fold increase in the incorporation of glucose into lipids of the epididymal fat pads from male rats fasted for 16–20 hr was lower in orchiectomized animals (2-fold), adrenalectomized animals (3-fold), and in the parametrial adipose tissue from female rats (4.6-fold) (3). Therefore the differences between our results and those of other authors who have studied the effect of CHM on adipose tissue under different experimental conditions are not surprising.

Fain (2) and Caldwell and Fain (20), in studies with isolated fat cells from starved female rats, and Goodman (21), in studies with epididymal fat pads from hypophysectomized, fed male rats, reported no change in the basal rates of glycerol and FFA production due to CHM.

The CHM-inhibition of lipid metabolism in rat adipose tissue reported by Jomain-Baum and Hanson (22) is not related to the present finding since these authors used the antibiotic at a dose 1000 times higher than that employed in the present study.

The increase in glucose uptake produced by the in vitro addition of CHM to epididymal fat pads might explain most of the other effects on glucose and lipid metabolism in the pads. The stimulation in glucose uptake was mainly reflected in glucose oxidation (Table 3) and FFA reesterification (Table 2). Glycogen synthesis, the hexose monophosphate shunt, and lactate accumulation in the medium were affected to a lesser extent.

The results in Table 5 suggest that there was an increased oxidation of the entire hexose molecule, presumably via the Krebs tricarboxylic acid cycle. Furthermore, the addition of pyruvate, in order to dilute glucose metabolites entering the tricarboxylic acid cycle (15), masked the stimulatory action of the antibiotic (Table 5). The stimulation in glucose oxidation by CHM is consistent with the observed increase in fatty acid reesterification, since the latter process depends on an energy source (23) and enhances oxygen consumption (24).

The esterification of FFA depends on glucose concentration in the medium (9) and considering that the CHM action was observed at all the concentrations of glucose tested (Fig. 1, panel C), it might be assumed that the increase in reesterification produced by the antibiotic was a consequence of the enhanced glucose uptake. The pattern of distribution of label between fatty acids and glyceride–glycerol was not modified by CHM (Table 4) and is further evidence in favor of an increase in FFA reesterification. The basal rate of lipolysis was slightly stimulated by CHM (Table 2). This increase probably is related to the accumulation of cyclic AMP reported in isolated fat cells treated with the antibiotic (25). Experiments are in progress to elucidate the mechanism of these effects of CHM in adipose tissue.

Manuscript received 21 May 1976 and accepted 14 September 1976.

REFERENCES

1. Verbin, R. S., D. S. Longnecker, H. Liang, and E. Farber. 1971. Some observations on the acute histopathologic effects of cycloheximide in vivo. *Amer. J. Pathol.* **62:** 111–120.
2. Fain, J. N. 1967. Studies on the role of RNA and protein synthesis in the lipolytic action of growth hormone in isolated fat cells. *Adv. Enzyme Reg.* **5:** 39–51.
3. García-Sáinz, J. A., A. G. Amador, E. Piña, and V. Chagoya de Sánchez. 1975. Lipogenic action of cycloheximide on the rat epididymal fat pad. *Biochem. Pharmacol.* **24:** 891–897.
4. Dole, V. P. 1961. Effect of nucleic acid metabolites on lipolysis in adipose tissue. *J. Biol. Chem.* **236:** 3125–3130.
5. Dole, V. P., and H. Meinetz. 1960. Microdetermination of long chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235:** 2595–2599.
6. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
7. Kornacker, M. S., and E. G. Ball. 1968. Respiratory processes in brown adipose tissue. *J. Biol. Chem.* **243:** 1638–1644.
8. Wieland, O. 1965. Glycerol. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, New York. 211–214.
9. Vaughan, M. 1962. The production and release of glycerol by adipose tissue incubated in vitro. *J. Biol. Chem.* **237:** 3354–3358.

0. Slein, M. W. 1965. D-Glucose. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, New York. 117–123.

1. Hassid, W. Z., and S. Abraham. 1957. Chemical procedures for analysis of polysaccharides. *In* Methods in Enzymology. S. P. Colowick and N. O. Kaplan, editors. Academic Press, New York. Vol III, 34–50.

2. Hohorst, H. J. 1965. L-Lactate. *In* Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, New York. 266–270.

3. Del Boca, J., and J. P. Flatt. 1969. Fatty acid synthesis from glucose and acetate and the control of lipogenesis in adipose tissue. *Eur. J. Biochem.* **11:** 127–134.

4. Feigelson, O., N. Feigelson, and C. Fancher. 1959. Kinetics of liver nucleic acid turnovers during enzyme induction in the rat. *Biochim. Biophys. Acta.* **32:** 133–139.

5. Cahill, G. F., B. Leboeuf, and R. B. Flinn. 1960. Studies on rat adipose tissue. VI. Effect of epinephrine on glucose metabolism. *J. Biol. Chem.* **235:** 1246–1250.

6. Akgun, S., and D. Rudman. 1969. Relationship between mobilization of free fatty acids from adipose tissue and the concentration of calcium in the extracellular fluid and in the tissue. *Endocrinol.* **84:** 926–930.

7. Jondorf, W. R., D. C. Simon, and M. Aunimelech. 1966. The stimulation of amino acid incorporation in a mammalian system with phenobarbital, 3-methylcholantrene and cycloheximide. *Biochem. Biophys. Res. Commun.* **22:** 644–649.

18. Fiala, S., and E. Fiala. 1965. Hormonal dependence of actidione action. *Biochim. Biophys. Acta.* **103:** 699–701.

19. Fain, J. N. 1964. Effect of puromycin on incubated adipose tissue and its response to dexamethasone, insulin and epinephrine. *Biochim. Biophys. Acta.* **84:** 636–642.

20. Caldwell, A. B., and J. N. Fain. 1970. Inhibition by cycloheximide and actinomycin of the activation of lipolysis due to the growth hormone in white fat cells. *Horm. Metab. Res.* **2:** 3–5.

21. Goodman, H. M. 1968. Effects of growth hormone on the lipolytic response of adipose tissue to theophylline. *Endocrinol.* **81:** 1027–1034.

22. Jomain-Baum, M., and R. W. Hanson. 1975. The inhibition by cycloheximide of lipid metabolism by rat adipose tissue in vitro. *Life Sci.* **16:** 345–352.

23. Ho, R. J. 1970. Dependence of hormone stimulated lipolysis on ATP and cyclic AMP level in fat cells. *In* Adipose Tissue. B. Jeanrenaud and D. Hepp, editors. Academic Press, New York. 83–87.

24. Ball, E. G., and R. L. Jungas. 1961. On the action of hormones which accelerate the rate of oxygen consumption and fatty acid release in rat adipose tissue in vitro. *Proc. Natl. Acad. Sci. USA.* **47:** 932–941.

25. Moskowitz, J., and J. N. Fain. 1970. Stimulation by growth hormone and dexamethasone of label cyclic adenosine 3′,5′ monophosphate accumulation by white fat cells. *J. Biol. Chem.* **245:** 1101–1107.

W29184 PBP

MS. No. 558

Biochemical Pharmacology, Vol. 00, pp. 0000-0000. Pergamon Press, 0000. Printed in Great Britain.

IMPORTANCE OF THE ESTERIFICATION PROCESS IN ADIPOSE TISSUE METABOLISM AS EVIDENCED BY CYCLOHEXIMIDE

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

Departamento de Biología Experimental, Instituto de Biología, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F.

(*Received 10 August 1977; accepted 12 October 1977*)

Abstract—The addition *in vitro* of cycloheximide (1 μg/ml) produces an increase in the incorporation of isotopic glucose or lactate into total lipids of epididymal fat pads obtained from fed rats. This enhancement in the incorporation of glucose proceeds via the glyceride-glycerol moiety. The synthesis of fatty acids is slightly decreased by the antibiotic which affects neither the uptake of glucose nor its oxidation. The regulation of the esterification process in adipose tissue is discussed.

Aside from the inhibitory effect of cycloheximide (CHM) on protein synthesis, intraperitoneal administration to fasted rats produced a 10-fold increase in the incorporation of [^{14}C]glucose into the lipids of epididymal fat pads, mainly through an increase in esterification of free fatty acids (FFA) [1]. By contrast, a small increase, without statistical significance, in the incorporation of glucose into lipids was found when the antibiotic was injected into fed rats [1]. It was rationalized that, in the pads from fasted animals, CHM might increase a metabolic process normally stimulated by food intake. The physiological consequences of food intake play an important role in the metabolism of adipose tissue as evidenced by lower rates of glucose uptake [2] and FFA esterification [3] observed in fasting rats compared to fed ones. Recently, we reported that CHM, added *in vitro* to epididymal fat pads from fasted rats, increases 2-fold the uptake, oxidation and incorporation of glucose into lipids, concomitently with an increase in esterification of FFA [4]. Since these parameters are increased by feeding, we have studied whether the antibiotic, added *in vitro* to epididymal fat pads from fed rats, elicits further stimulation, in an effort to elucidate the prime pathway affected by CHM.

MATERIALS AND METHODS

Cycloheximide, bovine serum albumin (fraction V), and glycerokinase were obtained from Sigma Chemical Co. (St. Louis, MO). D[U-^{14}C]glucose (200 mCi/m-mole) and L[U-^{14}C]lactic acid as the sodium salt (60 mCi/m-mole) were purchased from International Chemical and Nuclear Corp. (Cleveland, OH). Hexokinase, glucose 6-phosphate dehydrogenase, and alpha-glycerophosphate dehydrogenase were obtained from Boehringer & Soehne (Mannheim).

The experiments were performed using male Wistar rats weighing between 120 and 170 g. The animals were fed *ad lib.* with Purina rat chow; they were killed by decapitation and then exsanguinated. The epididymal fat pads were removed, rinsed in 0.85% NaCl and incubated in a Dubnoff metabolic shaker at 37° for 2 hr in a 25-ml Erlenmeyer stoppered flask. In addition to the tissue, each flask contained 3 ml of Krebs–Ringer bicarbonate buffer adjusted to pH 7.3 and supplemented with 150 mg of bovine serum albumin and 11.1 mM glucose. Some variability in the results was observed when different batches of albumin were used. To avoid this problem, albumin was systematically purified according to the procedure of Chen [5]. When radioactive glucose was used, it was added to a final concentration of 1 μCi/11.1 μmoles. Where indicated, glucose was substituted for lactate at a concentration of 1 mM, and 0.4 μCi [^{14}C]lactate was added to each flask. The incubation medium was heated to 37° and gassed with 5% CO_2–95% O_2 before the donor animals were killed; the flasks were flushed with the same gas mixture before sealing. Usually one of the two pads obtained from each animal served as the control while the other pad was in medium which contained CHM at a dose of 1 μg/ml.

Lipids were extracted according to Folch *et al.* [6]. In some experiments the lipids from the extracts were saponified and the distribution between glyceride–glycerol and fatty acids of radioactive carbon from glucose or lactate was studied by the method of Kornacker and Ball [7], with minor modifications [1]. When lipolysis and esterification were studied, one pad from each rat was used to determine the initial concentration of glycerol and FFA. The net changes in glycerol and FFA are presented. Free fatty acids were determined according to Dole and Meinertz [8] and glycerol was determined according to Wieland [9]. Glucose uptake was measured by the disappearance of the hexose from the medium according to the method of Slein [10]. Oxidation of [^{14}C]glucose was measured by its conversion to $^{14}CO_2$, using the method of Del Boca and Flatt [11]. Other experimental details have been reported elsewhere [1, 4].

Table 1. Effects of cycloheximide on glucose metabolism*

	Control (μmoles/g)	Cycloheximide (μmoles/g)	P
Uptake (μmoles/g wet wt)	9.42 ± 1.07 (6)	11.61 ± 1.15 (6)	< 0.2
Incorporation into lipids (μmoles/g lipids)	1.55 ± 0.18 (7)	2.32 ± 0.18 (7)	< 0.02
Oxidation (μmoles/g lipids)	3.21 ± 0.39 (5)	3.09 ± 0.36 (6)	< 0.9

* The results are expressed as the mean ± S.E.M., with the number of observations in parentheses.

RESULTS AND DISCUSSION

The incorporation of [^{14}C]glucose into lipids of epididymal fat pads from fed rats was found to be increased 50 per cent by cycloheximide. The drug also produced a statistically insignificant increase in the uptake of glucose without promoting its oxidation (Table 1). To explore the metabolic pathway modified by CHM, the lipid extracts were fractionated and it was found that the antibiotic produced a marked shift in the distribution of the label from the fatty acid moiety to the glyceride-glycerol moiety (Table 2), showing a pattern of distribution similar to that found in fasted animals [4]. By combining the data from Tables 1 and 2 it is possible to calculate the amount of glucose incorporated into fatty acids or glyceride-glycerol. In control pads ≈ 0.85 μmole was incorporated into fatty acids and ≈ 0.64 μmole was channeled toward the synthesis of glycerophosphate. In pads incubated with CHM, ≈ 0.59 and ≈ 1.66 μmoles glucose/g of lipids were incorporated into fatty acids and glyceride-glycerol respectively. Therefore, the antibiotic stimulated the incorporation of glucose into lipids by ≈ 0.77 μmole/g of lipids through the synthesis of the glyceride-glycerol moiety of the neutral fats (1.02 μmoles/g of lipids) and decreased its incorporation into the fatty acids (0.26 μmole/g of lipids). The action of CHM on the esterification of FFA, reported in Table 3, is a consequence of the previous findings. The antibiotic stimulated this pathway 47 per cent; this is quantitatively identical to the CHM-mediated stimulation in the incorporation of [^{14}C]glucose into lipids (Table 1). The decrease in the incorporation of glucose into fatty acids is in agreement with the paper by McNamara *et al.* [12], who reported that CHM causes a dramatic decrease in the conversion of isotopic acetate to fatty acids in rat liver homogenates.

The increase in glucose uptake produced by the addition *in vitro* of CHM to epididymal fat pads from fasted rats [4] seemed to explain most of its actions on glucose and lipid metabolism. In pads obtained from fed rats, the slight stimulation of the uptake

Table 2. Relative distribution of radioactive carbon from glucose in different fractions of lipid extracts of epididymal fat pads*

Fraction	Control (%)	Cycloheximide (%)	P
Non-saponifiable lipids	3.53 ± 0.55 (4)	3.04 ± 0.13 (4)	< 0.05
Fatty acids	55.13 ± 3.70 (4)	25.53 ± 1.12 (4)	< 0.001
Glyceride-glycerol	41.08 ± 3.77 (4)	71.43 ± 1.73 (4)	< 0.001

* Specifications are as in Table 1. Per-cent values are given on the basis of the results presented in Table 1, i.e. 1.55 μmoles glucose/g incorporated into lipids for the controls, and 2.32 μmoles/g for the experiments with cycloheximide.

Table 3. Effects of cycloheximide on lipolysis and free fatty acid esterification*

Net change	Control	Cycloheximide	P
Glycerol (μmoles/g)	4.16 ± 0.63 (5)	5.49 ± 0.61 (5)	< 0.2
FFA (μEq/g)	0.58 ± 0.16 (5)	−1.00 ± 0.26 (5)	< 0.001
Rate of esterification (μEq/g)	11.90	17.47	—

Table 4. Effect of cycloheximide on isotopic lactate incorporation into lipids and relative distribution of the label*

	Control	Cycloheximide	P
	(cpm/mg of lipids)		
Incorporation into lipids	45.27 ± 3.26 (4)	65.77 ± 7.05 (4)	< 0.05
Distribution	(per cent)		
Non-saponifiable lipids	3.73 ± 1.38 (4)	2.35 ± 0.61 (4)	< 0.4
Fatty acids	40.10 ± 3.54 (4)	25.79 ± 2.06 (4)	< 0.02
Glyceride–glycerol	56.18 ± 4.59 (4)	71.86 ± 1.93 (4)	< 0.05

* Specifications are as in Table 1. Per cent values are given on the basis of the incorporation of lactate into lipids presented in the first line of the table.

of glucose appears to be more a consequence of satisfying the demands of metabolites to increase the production of glycerophosphate than a direct action of the antibiotic on the uptake of the hexose. CHM enhanced the uptake of glucose 23 per cent (Table 1) and although this increase is more than enough to account for the 50 per cent stimulation of the incorporation of glucose into lipids (Table 1), this must take place specifically via glycerophosphate (Table 2) to subsequently raise the esterification of FFA (Table 3).

The results of Table 4 give support to this point. The antibiotic increased the incorporation of lactate into lipids, and the distribution of the label showed a shift to the glyceride–glycerol moiety. Furthermore, the magnitude of the stimulation (45 per cent) was quite similar to that observed with glucose (Table 1). The enhancement produced by CHM in the incorporation of glucose or lactate into lipids is of special significance since these metabolites are incorporated into lipids either through glycerogenesis (Tables 1 and 2) or glyceroneogenesis (Table 4) in order to satisfy the demands of the esterification process. These results indicate that the prime action of CHM should be localized on the esterification pathway.

The CHM-mediated increase in glucose uptake and oxidation observed in tissues from fasted animals [4] might be a consequence of the stimulation of the esterification process by the antibiotic. The lack of effect on these parameters in tissue from fed rats (Table 1) may be due to the fact that they are already stimulated by the food intake, and probably by the same mechanism that is a result of the antibiotic action in tissues from fasted animals, so that no additive action is observed. The results of this paper focus on the importance of the esterification process as a point of regulation of adipose tissue metabolism.

Acknowledgement—The authors wish to thank Dr. Antonio Peña for the careful revision of the manuscript.

REFERENCES

1. J. A. García-Sáinz, A. G. Amador, E. Piña and V. Chagoya de Sánchez, *Biochem. Pharmac.* 24, 891 (1975).
2. M. Vaughan, *J. Lipid Res.* 2, 293 (1961).
3. B. Shapiro, in *Handbook of Physiology, Adipose Tissue* (Eds A. E. Renold and G. F. Cahill, Jr.), Sect. 5, p. 217. American Physiological Society, Washington, D.C. (1965).
4. J. A. García-Sáinz, E. Piña and V. Chagoya de Sánchez, *J. Lipid Res.* 18, 93 (1977).
5. R. F. Chen, *J. biol. Chem.* 242, 173 (1967).
6. J. Folch, M. Lees and G. H. Sloane Stanley, *J. biol. Chem.* 226, 497 (1957).
7. M. S. Kornacker and E. G. Ball, *J. biol. Chem.* 243, 1638 (1968).
8. V. P. Dole and H. Meinertz, *J. biol. Chem.* 235, 2595 (1960).
9. O. Wieland, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 211. Academic Press, NY (1965).
10. M. W. Slein, in *Methods in Enzymatic Analysis* (Ed. H. U. Bergmeyer), p. 117. Academic Press, NY (1965).
11. J. Del Boca and J. P. Flatt, *Eur. J. Biochem.* 11, 127 (1969).
12. D. J. McNamara, F. W. Quackenbush and V. M. Rodwell, *Lipids* 5, 146 (1970).

UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO
INSTITUTO DE BIOLOGIA
DEPARTAMENTO DE BIOLOGIA EXPERIMENTAL
APARTADO POSTAL 70-600
MEXICO 20, D. F.

May 11, 1978.

Prof. Alan C. Sartorelli
Regional Editor
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Dear Prof. Sartorelli:

I am enclosing the manuscript entitled "Cycloheximide as a tool for studying the regulation of the esterification process in adipose tissue". I would greatly appreciate having this paper considered for publication in Biochemical Pharmacolog

I would also be grateful for any information you can give me concerning the paper "Importance of the esterification proces in adipose tissue metabolism as evidenced by cycloheximide", which was accepted for publication in Biochemical Pharmacology on October 12, 1977. Since then I have not received any further communications and I am afraid that it might have been lost in the mail.

Thank you very much for your kind attention to these matters.

Sincerely yours,

J. Adolfo García-Sáinz, MD

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333 Cedar Street
New Haven, Conn. 06510

Tel: (203) 436-4595
436-4654

19 May 1978

Dr. J. Adolfo García-Sáinz
Institúto de Biologia
Dept. de Biologia Experimental
Apartado Postal 70-600
Mexico 20, D.F. Mexico

Dear Dr. García-Sáinz:

This note acknowledges receipt of your manuscript (#4031) with Victoria Chagoya de Sánchez, entitled "Cycloheximide as a Tool for Studying the Regulation of the Esterification Process in Adipose Tissue". In regard to your manuscript entitled "Importance of the Esterification Process in Adipose Tissue Metabolism as Evidenced by Cycloheximide" (#3798), we received the galley proofs on 12 May 1978 and hope that you have also received your proofs by now; if this is not the case, please advise our office and we will arrange to have another set sent to you.

Sincerely,

Carolyn Wilcox

Carolyn G. Wilcox
Editorial Assistant

"CYCLOHEXIMIDE AS A TOOL FOR STUDYING THE REGULATION OF THE ESTERIFICATION PROCESS IN ADIPOSE TISSUE"

J. Adolfo García-Sáinz
and
Victoria Chagoya de Sánchez.

Departamento de Biología Experimental, Instituto de Biología;
Departamento de Bioquímica, Facultad de Medicina;
Universidad Nacional Autónoma de México México 20, D.F.

INTRODUCTION

The importance of adipose tissue in the metabolism and homeostasis of mammals is well established: it is second only to the liver in the maintenance of fuel levels in the blood. The most important function of the adipocyte is to serve as a metabolically active depot of lipids which is undergoing a continuous turnover. In fat cells the hydrolysis of triacylglycerols is hormonally controlled by the cyclic AMP-dependent activation of hormone sensitive lipase (1). The free fatty acids (FFA) produced through lipolysis may be released non-covalently bound to plasma albumin or re-esterified within the adipocyte with alpha-glycerol-3-phosphate (aGP) and then resynthesize triacylglycerols. Therefore, the net release of free fatty acids from adipose tissue is the result of a balance between lipolysis and re-esterification. Considerable information about the regulation of lipolysis in this tissue is available (2,3). On the contrary, knowledge of the regulation of the esterification process remains fragmentary and incomplete (4-6).

Cycloheximide (CHM), aside from and unrelated to its well known property of inhibiting protein synthesis, produces a marked increase in the esterification of free fatty acids both *in vivo* (7) and *in vitro* (8,9). Furthermore, this effect of the antibiotic is strongly influenced by the nutritional

condition of the animals, being much more clearly observable in fasted rats (7-9). This point suggests that CHM may affect the esterification process in a way similar to that produced physiologically.

Once the main action of CHM was localized in the esterification process (9), our interest focused on studying the metabolic step(s) affected by the antibiotic. The aim of the present paper is to present the localization of the action of CHM at the enzyme level which may give some insight into the regulation of this important pathway in adipose tissue metabolism.

MATERIAL AND METHODS

Cycloheximide, bovine serum albumin (Fraction V) lactic dehydrogenase, glycerophosphate dehydrogenase and lipid standards were obtained from the Sigma Chemical Company (St. Louis, Mo.). D- U-^{14}C glucose (200 mCi/mmole) was purchased from the International Chemical and Nuclear Corporation (Cleveland, O.). Coenzymes were obtained from Boehringer und Soehne (Mannheim). All the other chemicals used were of the best quality available.

The experiments were performed using male Wistar rats weighing between 120 and 170 g. which had fasted for 16-20 hours. The animals were killed by decapitation and exsanguinated. The epididymal fat pads were removed. rinsed in 0.85% NaCl and incubated in 25-ml stoppered Erlenmeyer flasks in a Dubnoff metabolic shaker at 37°C for 2 hours. In addition to the tissue, each flask contained 3 ml

of Krebs-Ringer bicarbonate buffer adjusted to pH 7.3 and suplemented with 150 mg of bovine serum albumin purified according to Chen (10) and 11.1 mM glucose. When radioactive glucose was used it was adjusted to a final specific activity of 1 µCi/11.1 µmoles. The incubation medium was heated to 37°C and gassed with 5% CO_2 - 95% O_2 before the donor animals were killed and the flasks were flushed with the same gas mixture before sealing. Usually one of the two pads obtained from each animal served as control, while the contralateral tissue was incubated in a medium which contained CHM at a concentration of 1 µg/ml.

Lipids from the tissue were extracted according to Folch, Lees and Sloane Stanley (11). The separation of triacylglycerols diacylglycerols, and phospholipids was achived by thin-layer chromatography using silica gel G plates with a solvent system, containing petroleum ether (b.p. 30-60°C)-diethyl ether-glacial acetic acid (80:20:1, v/v/v). The fractions were visualized by iodine vapours and were identified according to reference standards. The separated lipid classes were scraped into counting vials and 10 ml of a toluene solution of 2,5-p-phenylene-bis-(5 phenyloxazole) were added to each vial. The radioactivity was counted with a Packard liquid scintillation spectrometer. Free fatty acids were quantified by the method of Dole and Meinertz (12); lactate, pyruvate, aGP and dihydroxyacetone phosphate (DHAP)

were determined enzymatically according to the methods of Wieland (13), Bucher et al. (14), Hohorst (15) and Bucher and Hohorst (16), respectively. Acyl-CoA was quantified by the method of Garland (17). Enzyme activities were assayed in a defatted homogenate prepared as follows: After incubation, the epidydimal fat pads were rinsed in 0.85% NaCl and homogenized in a medium containing 0.24 M sucrose, 1 mM EDTA and 10 mM Tris/HCl, pH 7.4. The homogenate was centrifuged at 700 x g for 10 min at 0-4°C, the fat cake was discarded and the internatant straw-colored aqueous liquid was used as the enzyme source. Lactic dehydrogenase was assayed according to the method of Kornberg (18), glycerophosphate dehydrogenase following Beisenherz *et al.* (19), acyl-CoA synthetase using that of Pande and Mead (20) and aGP-acyltransferase according to Schlossman and Bell (21). Protein was determined according to the method of Lowry *et al.* using bovine serum albumin as the standard (22). Cytoplasmic NAD^+/NADH ratios were calculated as in Saggerson and Greenbaum, (23) assuming the apparent equilibrium constants for lactate dehydrogenase and aGP dehydrogenase reported by Williamson *et al.* (24) and Hohorst *et al.* (25). Statistical comparisons of groups of data were performed by the Student's *t* test.

RESULTS AND DISCUSSION

Cycloheximide increased by 2-fold the incorporation of radioactive glucose into lipids (Table I) as previously reported

(8). However, the distribution of the label percentagewise, among phospholipids, diacylglycerols and triacylglycerols (Table I) was not modified by the antibiotic. In other words, the amount of radioactivity present in phospholipids and acylglycerols was also increased by 2-fold.

Glycerol-3-phosphate and long chain fatty acyl-CoA are the recognized precursors of acylglycerols and the suggestion has been made by several authors that the concentration of either or both of these substrates may be critical in determining the rate of triacylglycerol synthesis (26). In addition, it has also been suggested that the activity of Acyl-CoA synthetase serves to promote the capture of fatty acids as CoA derivatives and thereby promotes the synthesis of triacylglycerols (27). Therefore, the concentrations of Acyl-CoA and aGP and their precursors FFA and DHAP were determined.

Cycloheximide produced a significant decrease in aGP and a slight increase in DHAP. The decrease in aGP may reflect an enhanced utilization of this metabolite by the esterification process which was strongly evident in the cytoplasmic redox state (Table II).

Another cytoplasmic redox couple was studied: lactate and pyruvate. The antibiotic decreased the content of lactate and insignificantly increased pyruvate (Table II). Therefore, the modification of the redox state was confirmed with this redox

couple (Table II). Neither the amount of free fatty acids within the cell nor the net content of acyl-CoA were significantly affected by the antibiotic. (Table III).

The use of change in flux and steady-state levels of intermediates to identify positions of regulation or inhibition was first discussed in detail by Chance _et al_. (28). From then on the concept of the crossover theorem has been applied in the identification of regulatory steps (29). Using the data of Tables I, II and III, a crossover plot was constructed and is presented in Fig. 1. In this figure, it may be observed that the amount of phospholipids (phosphatidic acid and derivatives) and acylglycerols are nearly 2-fold increased whereas the amount of aGP is decreased. It has been suggested that the affected step or regulatory step may be found at the level where the substrate of the reaction changes in direction opposite to that of the flux (29). Thus, the activity of glycerol-3-phosphate acyltransferase in defatted homogenates of the control and treated tissues was assayed. No modification of its activity was detected (Table IV) even by the direct addition of CHM to the assay medium (data not shown). The activity of tiokinase was also unaffected by the antibiotic (Table IV). In an effort to explain the effect on the redox state, the activity of lactic dehydrogenase and aGP dehydrogenase were also assayed but no significant modification of their activities was detected (data not shown).

Within the limitations discussed in regard to the crossover theorem (29) and in spite of the lack of stimulation of aGP acyltransferase activity (Table IV), our results strongly suggest that this is the step affected (Fig. 1). The failure to detect any activation may be related to the dilution of some ligand inestability of the enzyme or even to the conditions of the assay. Bremer et al. (30) have shown that these enzymes regulate the esterification process in the liver and that their activities vary depending on the nutritional conditions of the animals. These authors and others (30,31) have not been able to detect this regulatory property of this enzyme in adipose tissue. However, a slight stimulation by insulin of fat cell acyltransferase activity has been reported (32).

Our results are in agreement with the work of Denton and Halperin (33) and Saggerson and Greenbaum (23) in concluding that the rate of triacylglycerol synthesis can not be correlated with the concentration of either aGP or fatty acyl-CoA. Furthermore, the decreases in aGP and Acyl-CoA suggest that they are being acti used for esterification and that their net concentrations do not play a regulatory role (Tables II, and III and Fig. 1).

The results herein are also in agreement with the suggestion that a near equilibrium between lactate dehydrogenase and aGP dehydrogenase exists in the cytoplasm of the adipocyte (23) and that therefore the concentration of aGP appears to depend on the

cytoplasmic NAD/NADH ratio in adipose tissue (23) as it is well stablished in the liver cell (34).

Since the antibiotic did not increase the activity of the aGP acyltransferases when added to the assay medium, the possibility of a direct action of CHM on it may be discarded. The results also suggest that the response may be secondary to another action promoted by the antibiotic. This point is further supported by the slow onset of the effect of CHM both in vivo (7) and in vitro (8).

It has been previously shown that cycloheximide increased the release of pyruvate in Neurospora. (35). In addition, a report that CHM inhibits alcohol dehydrogenase and lactic dehydrogenase has been published (36). However, we were unable to detect any inhibition in homogenates, in agreement with other authors (37). Nevertheless the possibility of an inhibition of NAD-dependent dehydrogenases can not be completely ruled out.

In summary, CHM increases the incorporation of glucose into phospholipids and acylglycerols and decreases the amount of aGP, suggesting that it affects the glycerophosphate acylation process. (Table I, Table II and Fig. 1). Since this effect of the antibiotic varies depending upon the feeding conditions of the animals (7-9), the results also indicate that this may be the regulatory step in the biosynthesis of triacylglycerols in adipose tissue.

Summary

The *in vitro* addition of cycloheximide (1 µg/ml) produced a 2-fold increase in the incorporation of isotopic glucose into phospholipids and acylglycerols of epidydimal fat pads obtained from fasted rats. In addition, while the antibiotic diminished the concentration of glycerophosphate modifying the cytoplasmic redox state, it affected neither the level of free fatty acids nor of fatty acyl-CoA within the adipocytes. The results suggest that the acylation of glycerophosphate is the metabolic step affected by cycloheximide. The implications of this finding in the physiological regulation of the esterification process in adipose tissue are discussed.

TABLE I

EFFECT OF CYCLOHEXIMIDE ON THE INCORPORATION OF ^{14}C-GLUCOSE INTO DIFFERENT LIPID FRACTIONS

	Control	CHM	P
Specific Activity (cpm/mg lipids)	175.73 ± 20.05 (6)	378.40 ± 15.02 (6)	<0.001
	% Distribution of radioactivity:		
Phospholipids	21.71 ± 4.24 (6)	20.64 ± 5.32 (6)	<0.8
Diacylglycerols	23.10 ± 5.81 (6)	22.94 ± 4.48 (6)	<0.9
Triacylglycerols	52.88 ± 9.44 (6)	53.02 ± 10.57 (6)	<0.9

The results are expressed as the mean ± the standard error of the mean with the number of observations in parenthesis.

TABLE II

EFFECT OF CYCLOHEXIMIDE ON THE CYTOPLASMIC REDOX STATE

	Control	CHM	p
aGP (nmol/g)	175.30 ± 19.66 (11)	114.04 ± 8.13 (9)	<0.01
DHAP (nmol/g)	24.39 ± 2.35 (11)	32.80 ± 5.20 (11)	<0.2
aGP/DHAP	7.19	3.48	
$NAD^+/NADH$	1562.72	3228.72	
lactate (μmol/g)	3.24 ± 0.21 (12)	2.25 ± 0.18 (12)	<0.001
pyruvate (μmol/g)	0.37 ± 0.04 (12)	0.51 ± 0.07 (12)	<0.10
lactate/pyruvate	8.76	4.41	
$NAD^+/NADH$	1028.43	2042.86	

Indications as in Table I

TABLE III

EFFECT OF CYCLOHEXIMIDE ON THE AMOUNT OF FREE FATTY ACIDS AND LONG CHAIN ACYL-CoA WITHIN THE FAT PADS.

	Control	CHM	P
FFA (μEq/g)	4.94 $\pm$ 0.50 (7)	4.58 $\pm$ 0.69 (7)	$<$ 0.7
Acyl-CoA (nmol/g)	6.61 $\pm$ 1.05 (5)	4.30 $\pm$ 0.63 (5)	$<$ 0.1

Indications as in Table I

TABLE IV

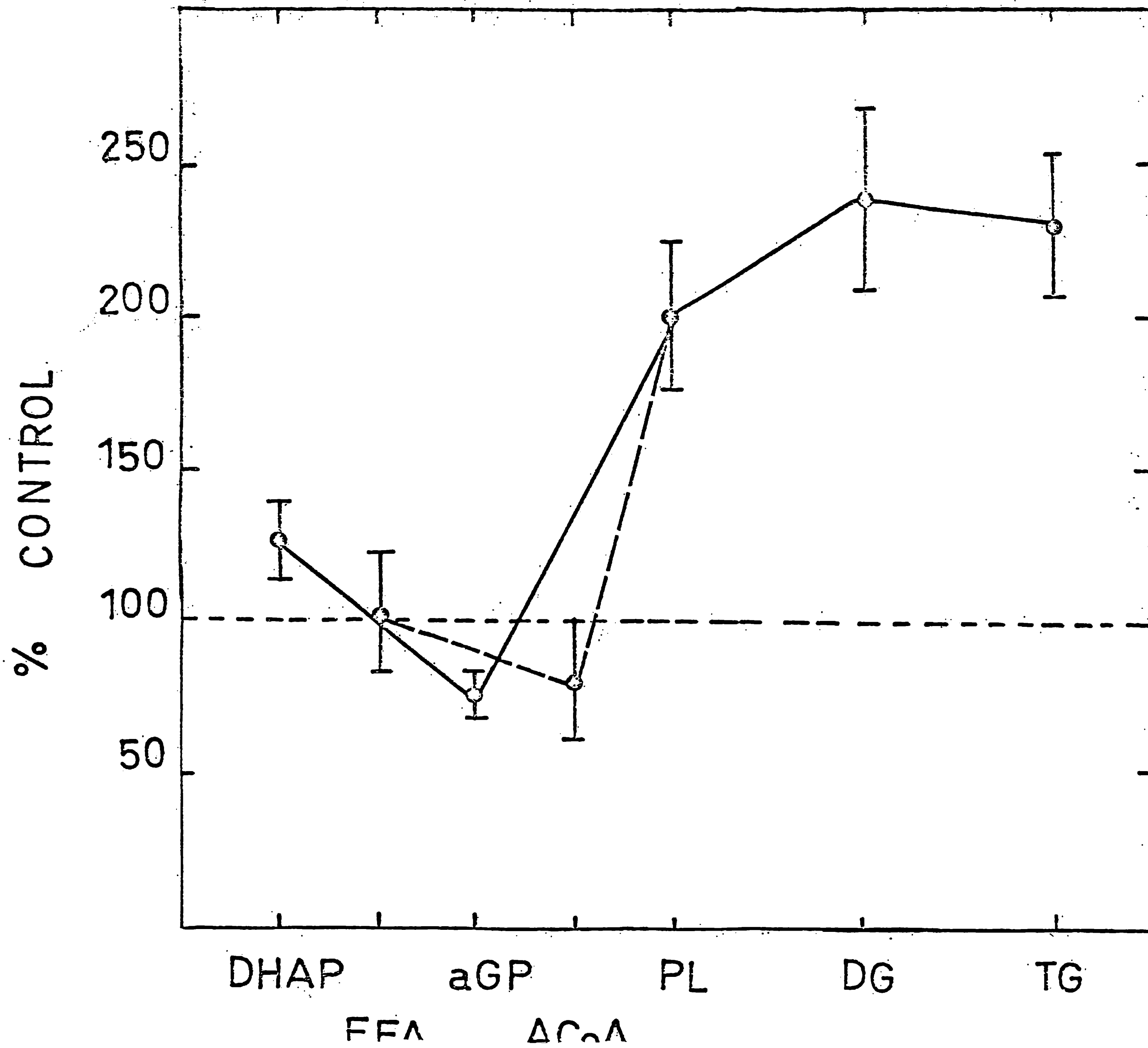
EFFECT OF CYCLOHEXIMIDE ON THE ACTIVITY OF ACYL-CoA SYNTHETASE AND GLYCEROL-3-PHOSPHATE ACYL-TRANSFERASE

	Control	CHM	p
	n mol /min /mg protein		
Acyl-CoA synthetase	9.41 ± 0.97 (6)	10.43 ± 0.70 (6)	<0.5
Glycerol-3-phosphate Acyl-transferase	0.94 ± 0.11 (6)	0.97 ± 0.09 (6)	<0.9

Indications as in Table I

Fig. 1

Crossover plot of the effect of cycloheximide on the esterification process. Values are taken from Tables I, II and III and the results are expressed as per cent of the control value. Vertical bars represent the standard error of the mean. DHAP, dihydroxyacetone phosphate; aGP, alpha-glycerophosphate; PL, phospholipids; DG, diacylglycerols; TG, triacylglycerols; FFA, free fatty acids; ACoA, long chain acyl-CoA.


250
200
150
100
50
% CONTROL
DHAP
aGP
PL
DG
TG

References

(1) D. Steimberg, Adv. Cyclic Nucleotide Res. 7, 157 (1976)

(2) D. Steimberg and M. Vaughan 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)

(3) R.W. Butcher in Adipose tissue, Regulation and Metabolic Functions (Eds. B. Jeanrenaud and D. Hepp) p 5, Academic Press, New York, (1970)

(4) R.M. Denton and B.R. Martin in Adipose tissue, Regulation and Metabolic Functions (Eds. B. Jeanrenaud and D. Hepp) p 143, Academic Press, New York (1970)

(5) D.A.K. Roncari and C.H. Hollemberg, Biochim. Biophys. Acta 137, 446 (1967)

(6) E.A. Newsholme and C. Start in Regulation of Metabolism, p.195, John Wiley and Sons, London (1973)

(7) J.A. García-Sáinz, A.G. Amador, E. Piña and V. Chagoya de Sánchez, Biochem Pharmacol 24, 891 (1975)

(8) J.A. García-Sáinz, E. Piña and V. Chagoya de Sánchez, J. Lipid Res. 18, 93 (1977)

(9) J.A. García-Sáinz, E. Piña and V. Chagoya de Sánchez, Biochem Pharmacol. in press

(10) R.F. Chen, J. Biol. Chem. 242, 173 (1967)

(11) J. Folch, M. Lees and G.H. Sloane Stanley J. Biol. Chem. 226, 497 (1957)

(12) V.P. Dole and H. Meinertz J. Biol. Chem. 235, 2595 (1960)

(13) O. Wieland in Methods of Enzymatic Analysis (Ed. H. U. Bergmeyer) p 271, Academic Press, New York (1965)

(14) T. Bucher R. Czok, W. Lamprecht and E. Latzko in Methods of Enzymatic Analysis (Ed H. U. Bergmeyer) p 253, Academic Press, New York (1965)

(15) H.J. Hohorst in Methods of Enzymatic Analysis (Ed. H.U. Bergmeyer) p 215 Academic Press New York (1965)

(16) T. Bucher and H.J. Hohorst in Methods of Enzymatic Analysis (Ed. H.U. Bergmeyer) p 246, Academic Press, New York (1965)

(17) P.B. Garland in Methods of Enzymatic Analysis (Ed. H.U. Bergmeyer) Vol. 4, p 2015) Academic Press, New York (1974)

(18) A. Kornberg in Methods of Enzymology (Eds. S.P. Colowick and N.O. Kaplan) Vol 1, p 441, Academic Press, New York (1955)

(19) G. Beisenherz, T. Bucher and K.H. Garbade in Methods in Enzymology (Eds. S.P. Colowick and N.O. Kaplan) Vol 1, p 391, Academic Press, New York (1955)

(20) S.V. Pande and J.F. Mead, J. Biol. Chem. 243, 352 (1968)

(21) D.M. Schlossman and R.M. Bell, J. Biol. Chem. 257, 5738 (1976)

(22) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193, 265 (1951)

(23) E.D. Saggerson and A.L. Green baum, Biochem. J. 119, 193 (1970)

(24) D.H. Williamson, P. Lund and H.A. Krebs (1967) Biochem J. 103,

514 (1967)

(25) H.J. Hohorst, F.H. Kreutz and M. Rein Biochem Biophys Res. Commun 4, 159 (1961)

(26) B. Shapiro, in Handbook of Physiology, Adipose tissue (Eds A. E. Renold and G. F. Cahill, Jr) Section 5, p 217, Am. Physiol Soc., Washington, D.C. (1965)

(27) C.J. Janson, M.A. Polokoff and R.M. Bell J. Biol. Chem. 251, 1488 (1976)

(28) B. Chance, W. Holmes, J.J. Higgins and C.M. Connelly, Nature 182, 1190 (1958)

(29) F.S. Rolleston in Current Topics in Cellular Regulation (Ed B.L. Horecker and E. W. Stadtman) Vol 5, p 47 Academic Press, New York (1972)

(30) J. Bremer, K.S. Bjerve B. Borreback, Mol. Cell. Biochem 12, 113 (1976)

(31) M. Aas and L.N.W. Daae, Biochim Biophys Acta 239, 208 (1971)

(32) S.R. Sooranna and E.D. Saggerson FEBS Lett 64, 36 (1976)

(33) R.M. Denton and M.L. Halperin Biochem J. 110, 27 (1968)

(34) H.A. Krebs in Adv. Enzyme Reg. (Ed G. Weber) Vol 5, p 409 Pergamon Press London (1967)

(35) J. Seydoux and G. Turian, Path Microbiol. 25, 752 (1962)

(36) H.E. Latuasan and W. Berends, Recueil 77, 416 (1958)

(37) A.G. Garber, M. Jomain-Baum, L. Salganicoff, E. Barber and R.W. Hanson, J. Biol. Chem. 248, 1530 (1973)