Effect of glucocorticoids on the oxidative desaturation of fatty acids by rat liver microsomes

Irma N. T. de Gómez Dumm,[1] María J. T. de Alaniz,[1] and Rodolfo R. Brenner[1]

Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, Calle 60 y 120, 1900-La Plata, Argentina

Abstract The effect of glucocorticoids on the oxidative desaturation of fatty acids by liver microsomal preparations of rats has been studied. Hydrocortisone produced a significant decrease in the conversion of [1-^{14}C]linoleic acid to γ-linolenic acid and [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid. Triamcinolone and dexamethasone were more active than hydrocortisone in depressing Δ6 and Δ5 fatty acid desaturating activity in liver microsomes. The glucocorticoids evoked a maximal response approximately 24 hr after admission. Palmitic acid conversion to palmitoleic acid showed no statistically significant changes by any of the glucocorticoids. The mechanism of action of glucocorticoids is apparently different from other hyperglycemic hormones that produce similar effects.—**Gómez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner.** Effect of glucocorticoids on the oxidative desaturation of fatty acids by rat liver microsomes. *J. Lipid Res.* 1979. **20:** 834–839.

Supplementary key words palmitic acid · linoleic acid · eicosa-8,11,14-trienoic acid · hydrocortisone · triamcinolone · dexamethasone

In previous work it was demonstrated that the oxidative desaturation of fatty acids is under hormonal control (1–8). Several studies have revealed that Δ9 desaturation activity is depressed in the liver microsomes of alloxan diabetic rats (1–4). This defect was overcome by parenteral injection of insulin (2–4). The administration of thyroxine to normal rats for several days produces an increase of Δ9 desaturation activity of liver microsomal preparations (5).

Another series of studies has dealt with the Δ6 desaturase. It was shown that Δ6 desaturase activity is also depressed in diabetes (3, 4) and that low doses of insulin produced an enhancement of linoleic acid desaturation, an effect that is probably mediated through an activation of protein synthesis (6). On the other hand, hyperglycemic hormones such as glucagon or epinephrine significantly depress conversion of linoleic acid to γ-linolenic acid. This effect would appear to be mediated through an enhancement of the intracellular levels of cyclic AMP (7, 8), because dibutyryl cyclic AMP administered to rats (7) or to cultured cells (9) also produced a depression of Δ6 desaturation activity. In addition, thyroxine treatment of normal rats also caused a decrease of γ-linolenic acid biosynthesis (5).

In the present work we have investigated the effect of glucocorticoids on desaturating activities of enzymes, because the mechanism of action of these hyperglycemic hormones is different from the other hormones mentioned above.

MATERIALS AND METHODS

Chemicals

[1-^{14}C]Palmitic acid (58 mCi/mmol, 99% radiochemical purity) was purchased from the Radiochemical Centre, Amersham, England. [1-^{14}C]Linoleic acid (60 mCi/mmol, 99% radiochemical purity) and [1-^{14}C]-eicosa-8,11-14-trienoic acid (58.9 mCi/mmol, 98% radiochemical purity) were purchased from New England Nuclear Corp., Boston, MA. NADH, ATP, CoA, and other cofactors were provided by Boehringer, Argentina. The glucocorticoids used were hydrocortisone (Solucortril, Pfizer), triamcinolone acetate (Kenacort A, Squibb), and dexamethasone (Decadron, Merck, Sharpe, and Dohme).

Animals and treatment of animals

Adult female Wistar rats, weighing 180–220 g and maintained on standard Purina chow were used.

Several experiments were performed. Experiment 1 was designed to show the effects of hydrocortisone administration on the oxidative desaturation of palmitic, linoleic, and eicosa-8,11,14-trienoic acids by liver microsomes of normal rats. The rats were placed in two groups of four animals each. One group was injected with hydrocortisone (10 mg/rat per 12 hr) and the other group, used as control, was injected

[1] Member of the Carrera del Investigador of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

with 0.9% saline solution. All the rats were killed 24 hr after the first injection.

Experiment 2 was designed to compare the effect of different glucocorticoids on microsomal desaturation activities. The rats (divided in groups of four animals each) received daily injections of hydrocortisone (10 mg/rat), triamcinolone (2.5 mg/rat), or dexamethasone (1 mg/rat) for 3 days. A control group was injected with saline solution.

In experiment 3 the effect of a single dose of hydrocortisone (10 mg/rat) or dexamethasone (1 mg/rat) was investigated. The rats (four per group) were injected with the hormones and were killed 8, 12, 24, and 48 hr after the injection.

All compounds were injected intraperitoneally. In order to avoid individual differences among the rats, in all the experiments the animals were fasted for 24 hr, refed for 2 hr, and then killed 12 hr after the end of the feeding period.

Isolation of microsomes

The rats were killed by decapitation; the blood was allowed to drain and it was collected for determinations of glucose and free fatty acids. Livers were rapidly excised and immediately placed in ice-cold homogenizing medium (10). After homogenization, samples were taken to measure protein and glycogen content. Microsomes were separated by differential centrifugation at 100,000 *g* as described elsewhere (10).

Assay procedures

Desaturation of the fatty acids by liver microsomes was measured by estimation of the percentage conversion of [1-^{14}C]palmitic acid to palmitoleic acid, [1-^{14}C]linoleic acid to γ-linolenic acid, and [1-^{14}C]-eicosa-8,11,14-trienoic acid to arachidonic acid. Three nmol of the labeled acid and 97 nmol of unlabeled acid were incubated with 5 mg of microsomal protein in a Dubnoff shaker at 35°C for 20 min, in a total volume of 1.5 ml of 0.15 M KCl–0.25 M sucrose solution. The medium contained 4 μmol of ATP, 0.1 μmol of CoA, 1.25 μmol of NADH, 5 μmol of $MgCl_2$, 2.25 μmol of glutathione, 62.5 μmol of NaF, 0.5 μmol of nicotinamide, and 62.5 μmol of phosphate buffer (pH 7). The reaction was stopped by addition of 2 ml of 10% KOH in methanol. The fatty acids were recovered after saponification of the incubation mixture (45 min at 85°C), acidification, and extraction with petroleum ether (bp 30–40°C). The fatty acids were esterified with methanolic 3 M HCl (3 hr at 68°C), and the distribution of the radioactivity in substrate and product was measured by gas–liquid radiochromatography in an apparatus equipped with a Packard proportional counter as described elsewhere (11). A column containing 10% diethylene glycol succinate on Chromosorb W (80–100 mesh) was used. Palmitic, linoleic, and eicosa-8,11,14-trienoic acids were desaturated to palmitoleic, γ-linolenic, and arachidonic acids, respectively. Percentage conversion was calculated from the distribution of radioactivity between substrate and product measured directly on the radiochromatogram (11).

Protein content in the different fractions was determined by the biuret method of Gornall, Bardawill, and David (12) using crystalline bovine albumin as a standard. Blood glucose was measured by the *o*-toluidine method (13), free fatty acids in plasma were determined according to the procedure of Itaya and Ui (14), and liver glycogen was determined by the method of Van Handel (15).

RESULTS

The results obtained after 24 hr of hydrocortisone treatment are shown in **Table 1.** In this experiment 10 mg of the hormone were injected every 12 hr. Under these conditions the hormone caused a marked depression of Δ6 desaturation of linoleic acid and Δ5 desaturation of eicosa-8,11,14-trienoic acid, while no significant differences on Δ9 desaturation of palmitic acid were observed, compared to the controls. Hydrocortisone produced an increase in blood glucose and liver glycogen levels, but these results were not statistically significant.

TABLE 1. Modification of fatty acid desaturation and glucose metabolism by 24-hr hydrocortisone treatment (10 mg/rat/12 hr) of normal rats[a]

Treatment	Conversion			Plasma Glucose	Liver Glycogen
	16:0 → 16:1	18:2 → 18:3	20:3 → 20:4		
	%	%	%	*mg/dl*	*mg/mg protein*
Control	6.7 ± 0.6[b]	26.5 ± 4.6	44.7 ± 2.8	58 ± 8	0.046 ± 0.006
Hydrocortisone	10.6 ± 3.6	13.7 ± 1.2	37.9 ± 1.6	65 ± 8	0.067 ± 0.022
	$P < 0.1$	$P < 0.001$	$P < 0.01$		

[a] For experimental conditions, see Materials and Methods.
[b] Averages of the analysis of four rats ±one standard deviation of the mean.

TABLE 2. Comparative effect of different glucocorticoids on liver microsomal fatty acid desaturation activities and other metabolic parameters[a]

Treatment	Conversion 16:0 → 16:1	Conversion 18:2 → 18:3	Conversion 20:3 → 20:4	Plasma Glucose	Plasma Free Fatty Acids	Liver Glycogen
	%	%	%	*mg/dl*	*μmol/l*	*mg/ml prot.*
Control	8.4 ± 1.2[b]	19.7 ± 2.4	45.8 ± 8.2	30 ± 12	356 ± 184	0.049 ± 0.160
Hydrocortisone	8.9 ± 0.6	17.5 ± 1.6	28.2 ± 2.2[c]	39 ± 16	653 ± 372	0.042 ± 0.022
Triamcinolone	8.2 ± 2.4	2.4 ± 2.4[d]	19.5 ± 3.8[c]	112 ± 18[c]	509 ± 180	0.035 ± 0.014
Dexamethasone	15.1 ± 9.4	5.8 ± 6.8[c]	12.2 ± 9.2[d]	40 ± 8	404 ± 100	0.043 ± 0.022

[a] Three days of treatment with a daily intraperitoneal dose. For details, see Materials and Methods.
[b] Averages of the analyses of four rats (each analysis performed in duplicate) ±one standard deviation of the mean.
[c] and [d] Results statistically significant compared to the control. [c]$P < 0.01$; [d]$P < 0.001$.

To determine whether the effect on the microsomal fatty acid desaturating activities was also found in other glucocorticoids and was not specific for hydrocortisone, two synthetic glucocorticoids, triamcinolone and dexamethasone were studied. **Table 2** illustrates the effect produced by the three different glucocorticoids on the Δ9, Δ6, and Δ5 desaturation of fatty acids. In this study the treatment was prolonged for 72 hr, but the frequency of hydrocortisone injection was reduced to 10 mg/rat every 24 hr. As before, palmitic acid desaturation activity showed no differences between the control and the treated groups. The desaturation of linoleic acid to γ-linolenic acid was significantly decreased in liver microsomes of the rats treated with triamcinolone or dexamethasone, but not with hydrocortisone, whereas the desaturation of eicosa-8,11,14-trienoic acid to arachidonic acid was significantly reduced by all the corticoids. Liver glycogen, plasma free fatty acids, and glucose were not significantly modified in this experiment, except for the glucose concentration that was significantly increased by triamcinolone administration.

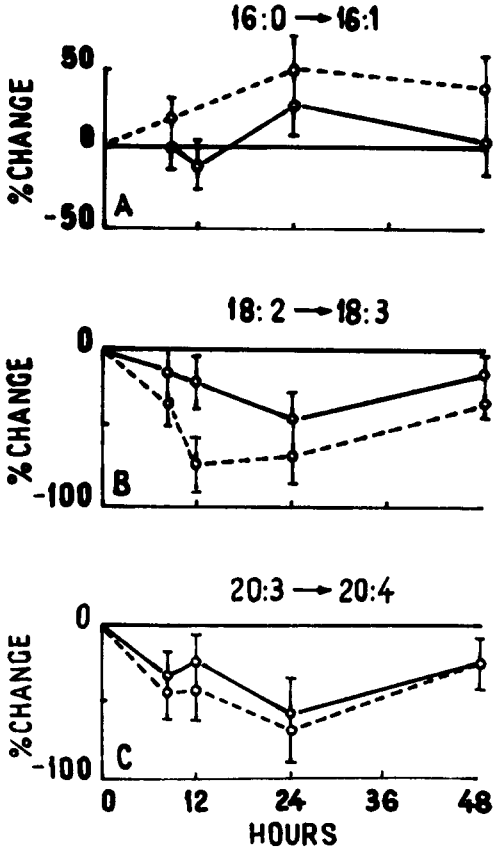

Fig. 1. Effect of a single injection of hydrocortisone (○ —— ○) and dexamethasone (○ - - - ○) on the change of palmitic acid conversion to palmitoleic acid (*A*), linoleic acid to γ-linolenic acid (*B*), and eicosa-8,11,14-trienoic acid to arachidonic acid (*C*). Zero point corresponds to the percentage conversion (*A*, 7.5 ± 0.8; *B*, 30.9 ± 1.7; *C*, 51.6 ± 4.5) of normal animals injected with a single dose of saline solution. Each point represents the average of four rats. Bars represent ±one standard deviation.

The three desaturases Δ9, Δ6, and Δ5, responded in different ways to the glucocorticoids. Whereas the Δ9 desaturase activity was apparently unaffected under the conditions of our experiment, the Δ6 and Δ5 desaturases were both inactivated. Moreover, triamcinolone and dexamethasone were more potent and had longer residual effects than hydrocortisone. The modification of the Δ6 and Δ5 desaturase activities was shown even when there was no longer an effect on glucose metabolism. In addition, by comparing results of Table 1 and 2 it would appear that the Δ5 desaturase activity was altered for a longer period than that of the Δ6 enzyme.

This effect of several injections of hydrocortisone on the Δ6 desaturation of fatty acids (shown in Tables 1 and 2) prompted an investigation of the effect of a single injection of hydrocortisone or dexamethasone on Δ9, Δ6, and Δ5 desaturation by rat liver microsomes. The results are shown in **Fig. 1.** Both glucocorticoids markedly depressed linoleic acid and eicosa-8,11,14-trienoic acid desaturating activities. Hydrocortisone was again shown to be less effective in depressing Δ6 and Δ5 desaturating activity than dexamethasone; moreover the times curves for both hormones and substrates were similar. In this experiment it was demonstrated that both hydrocortisone and

dexamethasone evoked an effect that increased with time and exhibited a maximal level after 24 hr. In addition, Fig. 1 also shows that the residual effect is longer for dexamethasone than for hydrocortisone, as suggested in the preceeding paragraph (Table 2). This is apparently the result of a more potent effect that therefore remains statistically significant for a longer period.

The Δ9 desaturase activity showed a slight increase after 24 hr, but the results obtained after hydrocortisone or dexamethasone treatment were not significantly different from those obtained from untreated animals. **Table 3** shows the changes of liver glycogen, and plasma glucose and free fatty acids, 8, 12, 24, and 48 hr after a single injection of hydrocortisone or dexamethasone. No statistically significant differences among the treated groups and the control were observed except for the increase in liver glycogen levels 12 hr after hydrocortisone and 8 hr after dexamethasone treatment.

DISCUSSION

The results obtained in these experiments demonstrate that glucocorticoids strongly depress the activity of Δ6 and Δ5 desaturases of liver microsomes. This effect was clearly shown for all the glucocorticoids studied, natural or synthetic. However, triamcinolone and dexamethasone were more effective than hydrocortisone (Fig. 1). Moreover both synthetic corticoids showed a longer residual effect than hydrocortisone, but this effect was apparently due to their greater potency since the decay curves showed similar slopes (Fig. 1). In this regard, it has been shown (16) that the half time of the biological effect for hydrocortisone is about 3 hr and is 7 hr for dexamethasone; however both dexamethasone and triamcinolone were also more effective than hydrocortisone and they caused a greater gluconeogenic enzyme response than hydrocortisone with markedly smaller doses (16, 17).

The inverse ratio between the Δ6 desaturase activity and the hyperglycemia effect of hormones was stressed in the introduction of this report. These experiments demonstrate that glucocorticoids can also evoke a similar effect. However, the changes in liver glycogen or blood glucose levels evoked by the hormones were less significant than the modification on the Δ6 desaturase activity at the moment of sample collection. Similarly, as it was already stated for epinephrine (8), the difference might be due to a different time of response for each parameter. However, in spite of the similarity of effect found for epinephrine, glucagon, and glucocorticoids, the mechanism of action is not necessarily the same. Moreover, glucagon and epinephrine are glycogenolytic and they would produce their effects by increasing the intracellular levels of cyclic AMP which would produce, directly or indirectly, an inhibition on the Δ6 desaturating activity (7, 8). Glucocorticoids are also involved in glucose metabolism but they evoke an increase of gluconeogenesis and glycogen stores in liver (17); moreover cortisol produces an inhibition of the enzymes involved in glucose degradation (18, 19). Therefore, it is difficult to speculate that a glucose metabolite is involved in the glucocorticoid mechanism of action in the present experiments and probably it is evoked through an enhancement of the synthesis of specific proteins rather than through cyclic AMP (17). This hypothesis is supported by the observation that the depression of Δ6 desaturase activity produced by glucocorticoids was about 30%, 8 hr after the injection, while epinephrine produced a 40% decay 90 min after the administration of the hormone (8).

TABLE 3. Changes of liver glycogen, blood glucose level, and plasma free fatty acids during a 48-hr period after hydrocortisone and dexamethasone injection[a]

	% Change[b]					
	Liver Glycogen		Plasma Glucose		Plasma Free Fatty Acids	
Time	Hc	Dx	Hc	Dx	Hc	Dx
hr						
8	+35.5 ± 12.8	+101.6 ± 30.6[c]	+1.5 ± 8.4	+2.4 ± 13.8	+5.2 ± 9.0	−5.1 ± 3.8
12	+72.6 ± 32.0[c]	+42.2 ± 34.4	−4.2 ± 13.8	+6.6 ± 23.4	+1.3 ± 23.0	−7.1 ± 22.6
24	+5.3 ± 25.0	+13.4 ± 23.2	0.0 ± 6.8	−3.4 ± 10.4	−24.2 ± 26.6	+5.2 ± 27.6
48	+16.1 ± 17.8	−1.0 ± 32.0	−10.3 ± 13.8	−10.3 ± 13.8	−23.8 ± 34.1	+11.9 ± 24.0

[a] For details, see Materials and Methods.
[b] The results were expressed as the percentage changes of the values compared with the control. Each point represents the average of four rats ±one standard deviation of the mean. The mean of control values corresponds to 0.112 mg of liver glycogen/mg protein; plasma glucose, 72 mg/dl; and free fatty acids, 603 μmol/l.
[c] Results statistically significant compared to the controls. $P < 0.05$.
Hc, hydrocortisone; Dx, dexamethasone.

Free fatty acids have also been considered as putative factors on the hormonal control of Δ6 desaturases (20). However, dexamethasone affected neither tissue levels of free fatty acids nor the basal release of free fatty acids (21). Moreover, under our experimental conditions, plasma free fatty acids were not modified by glucocorticoid treatment (Tables 2 and 3) and it has been published that hormones of the cortisol type prevent the mobilization of fats in starved rats (22).

Data presented in this report also define a distinct role for glucocorticoids in the regulation of Δ9 desaturation of fatty acids. Tables 1 and 2 and Fig. 1 show that whereas the activity of Δ5 and Δ6 desaturases was decreased, the microsomal conversion of palmitic acid to palmitoleic acid increased under dexamethasone and hydrocortisone treatment; however this increase was not statistically significant under the conditions of our experiments. These results parallel the behavior of the Δ9 desaturase in animals treated with other hyperglycemic hormones. Neither glucagon nor epinephrine decreased the Δ9 desaturation of stearic or palmitic acids (7, 8). Moreover, Jeffcoat and James (23) have recognized that a remarkable parallelism exists in the changes of the Δ9 desaturase and fatty acid synthetase activity. This parallelism is stressed again by the present results; Volpe and Marasa (24) have revealed that the glucocorticoids evoke no changes of fatty acid synthetase and carboxylase activities of liver.

Although further studies are necessary to clarify the mechanism of action of glucocorticoids on fatty acid desaturating activities, it is evident that linoleic and eicosa-8,11,14-trienoic acid desaturases are sensitive to these hormones. According to the results reported in the literature, we presume that the effect of glucocorticoids could be produced through an induction of protein synthesis which directly or indirectly would inhibit Δ6 and Δ5 desaturating activity in rat liver microsomes.

This work was supported in part by the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina and Comisión de Investigaciones Científicas of the Province Buenos Aires. Technical assistance was provided by Mrs. María C. P. de Stringa.

Manuscript received 16 November 1978; accepted 24 April 1979.

REFERENCES

1. Imai, J. 1961. Studies on the lipogenesis in animal tissues under pathological conditions. *J. Biochem.* **49:** 642–648.
2. Gellhorn, A., and W. Benjamin. 1964. The intracellular localization of an enzymatic defect of lipid metabolism in diabetic rats. *Biochim. Biophys. Acta.* **84:** 167–175.
3. Mercuri, O., R. O. Peluffo, and R. R. Brenner. 1966. Depression of microsomal desaturation of linoleic to γ-linolenic acid in the alloxan diabetic rat. *Biochim. Biophys. Acta.* **116:** 409–411.
4. Mercuri, O., R. O. Peluffo, and R. R. Brenner. 1967. Effect of insulin on the oxidative desaturation of γ-linolenic, oleic and palmitic acids. *Lipids.* **2:** 284–285.
5. Gómez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner. 1977. Effect of thyroxine on Δ6 and Δ9 desaturation activity. *In* Function and Biosynthesis of Lipids. Advances in Experimental Medicine and Biology. Vol. 83. N. Bazán, R. R. Brenner, and N. Guisto, editors. Plenum Press, New York. 609–616.
6. Peluffo, R. O., I. N. T. de Gómez Dumm, M. J. T. de Alaniz, and R. R. Brenner. 1971. Effect of protein and insulin on linoleic acid desaturation of normal and diabetic rats. *J. Nutr.* **101:** 1075–1084.
7. Gómez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner. 1975. Effect of glucagon and dibutyryl adenosine 3′5′-cyclic monophosphate on the oxidative desaturation of fatty acids in the rat. *J. Lipid Res.* **16:** 264–268.
8. Gómez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner. 1976. Effect of epinephrine on the oxidative desaturation of fatty acids in the rat. *J. Lipid Res.* **17:** 616–621.
9. Alaniz, M. J. T. de, I. N. T. de Gómez Dumm, and R. R. Brenner. 1976. The action of insulin and dibutyryl cyclic AMP on the biosynthesis of polyunsaturated acids of γ-linolenic acid family in HTC cells. *Mol. Cell. Biochem.* **12:** 3–8.
10. Castuma, J. C., A. Catalá, and R. R. Brenner. 1972. Oxidative desaturation of eicosa-8,11,14-dienoic acid to eicosa-5,8,11-trienoic acid: comparison of different diets on the oxidative desaturation at the 5,6 and 6,7 positions. *J. Lipid Res.* **13:** 783–789.
11. Brenner, R. R., and R. O. Peluffo. 1966. Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acids. *J. Biol. Chem.* **241:** 5213–5219.
12. Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177:** 751–766.
13. Dubowsky, K. M. 1962. An *o*-toluidine method for the body fluid glucose determination. *Clin. Chem.* **8:** 215–235.
14. Itaya, K., and M. Ui. 1965. Colorimetric determination of free fatty acids in biological fluids. *J. Lipid Res.* **6:** 16–20.
15. Van Handel, E. 1965. Estimation of glycogen in small amounts of tissue. *Anal. Biochem.* **11:** 256–265.
16. Meikle, A. W., and F. H. Tyler. 1977. Potency and duration of action of glucocorticoids. Effects of hydrocortisone prednisone and dexamethasone on human pituitary-adrenal function. *Am. J. Med.* **63:** 200–207.
17. Weber, G., R. L. Singhal, N. B. Stamm, E. A. Fischer, and M. A. Mentendiek. 1964. Regulation of enzymes involved in gluconeogenesis. *In* Advances in Enzyme Regulation. Vol. 2. G. Weber, editor. Pergamon Press, London. 1–38.
18. Weber, G., R. L. Singhal, N. B. Stamm, M. A. Lea, and E. A. Fischer. 1966. Synchronous behavior pattern of key glycolytic enzymes: glucokinase, phosphofructo-

kinase and pyruvate kinase. *In* Advances in Enzyme Regulation. Vol. 4. G. Weber, editor. Pergamon Press, London. 59–81.
19. Seubert, W., H. V. Henning, W. Schoner, and M. L'Age. 1968. Effects of cortisol on the levels of metabolites and enzymes controlling glucose production from pyruvate. *In* Advances in Enzyme Regulation. Vol. 6. G. Weber, editor. Pergamon Press, London. 153–187.
20. Gómez Dumm, I. N. T. de, M. J. T. de Alaniz, and R. R. Brenner. 1978. Effect of catecholamines and β-blockers on linoleic acid desaturation activity. *Lipids.* **13:** 649–652.
21. Goodman, H. M. 1970. Permissive effects of hormones on lipolysis. *Endocrinology.* **86:** 1064–1074.
22. Levy, A. C., and E. R. Ramey. 1959. The effect of adrenal steroids on peripheral fat mobilization in the rat. *Endocrinology.* **64:** 586–591.
23. Jeffcoat, R., and A. T. James. 1977. Interrelationship between the dietary regulation of fatty acid synthesis and the fatty acyl-CoA desaturases. *Lipids.* **12:** 469–474.
24. Volpe, J. J., and J. C. Marasa. 1975. Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. *Biochim. Biophys. Acta.* **380:** 454–472.