A strong inhibition of the synthetase reaction by Cbz-derivatives with an additional aromatic ring was also observed in the case of rat liver glutamine synthetase (Table II).

Table III shows the effect of Cbz-derivatives of amino acids on ovine brain γ-glutamyl transferase activity. Although in this case the inhibition by Cbz-derivatives without an additional aromatic ring is more pronounced than in the synthetase reaction, here, too, the derivatives with an additional aromatic ring are considerably stronger inhibitors.

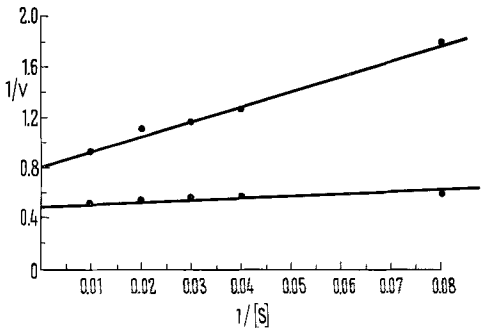


Fig. 1. Inhibition of ovine brain glutamine synthetase by Cbz-L-phenylalanine. (I) L-glutamic acid; (II) L-glutamic acid and Cbz-L-phenylalanine (75 µmoles). [S] is the concentration of L-glutamic acid (µmoles) in 5.5. ml reaction mixture. Velocity (V) is expressed in µmoles inorganic phosphate liberated in 15 min at 30 °C.

Figures 1 and 2 show that the inhibitions of ovine brain glutamine synthetase and γ-glutamyl-transferase by Cbz-L-phenylalanine are of a mixed type. Similar results, for both reactions, were obtained with Cbz-L-tyrosine and N-Cbz-S-benzyl-L-cysteine.

In the case of ovine brain glutamine synthetase, the extent of inhibition exerted by Cbz-L-phenylalanine (125 µmoles) or N-Cbz-S-benzyl-L-cysteine (75 µmoles) was the same when the respective concentrations of ATP, hydroxylamine or Mg^{++} were increased three-fold.

Discussion. The strong inhibitory activity of the derivatives containing 2 aromatic groups may be explained as follows. The 2 aromatic rings interact through hydrophobic bonds with 2 suitable sites on the enzyme molecule. This causes inhibition either by blocking the active site *per se* or by exerting an allosteric effect. It remains to be shown, by use of appropriate compounds, whether the carboxyl groups of the inhibitors play a role in the inhibition. Cbz-derivatives of amino acids bearing an additional aromatic group also strongly inhibit rat liver asparaginase[7] and rat liver glutaminase[8]. It was also reported[9] that Cbz-L-phenylalanine inhibits the proteolytic and the esterolytic activity of chymotrypsin. On the other hand, Cbz-L-phenylalanine does not inhibit the glutamine-requiring carbamyl phosphate synthetase of *E. coli*[8].

It is of interest to note that i.p. injections of the sodium salts of Cbz-L-phenylalanine and N-Cbz-S-benzyl-L-cysteine markedly inhibit the growth of Ehrlich ascites carcinoma in mice[10]. Since both compounds affect a multitude of enzymes, the exact nature of these tumour inhibitions require further elucidation.

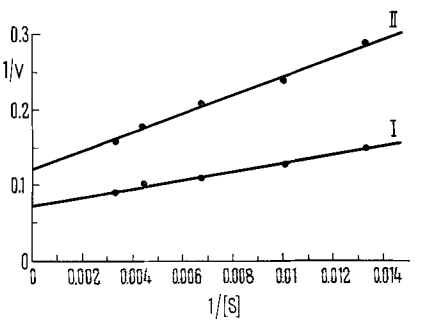


Fig. 2. Inhibition of ovine brain γ-glutamyl transferase by Cbz-L-phenylalanine. (I) L-glutamine; (II) L-glutamine and Cbz-L-phenylalanine (50 µmoles). [S] is the concentration of L-glutamine (µmoles) in 5.3 ml reaction mixture. Velocity (V) is expressed in µmoles hydroxamic acid formed in 15 min at 30 °C.

Zusammenfassung. Carbobenzoxyderivate von aromatischen Aminosäuren mit einer zusätzlichen aromatischen Gruppe hemmen die Glutaminsynthetase und γ-Glutamyl-Transferase aus Schafshirn sowie die Glutaminsynthetase aus Rattenleber stark.

H. SPIEGELSTEIN-KLARFELD, N. LUSTIG and N. LICHTENSTEIN

Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem (Israel), 4 July 1969.

[7] G. MOR and N. LICHTENSTEIN, Fedn. Europ. Biochem. Letters *3*, 313 (1969).
[8] G. MOR and N. LICHTENSTEIN, to be published.
[9] M. MURUMATU, Y. HAYAKUMO and S. FUJII, J. Biochem., Japan, *62*, 408 (1967).
[10] M. SCHLESINGER, N. GROSSOWICZ and N. LICHTENSTEIN, to be published.

Purines and Cortisone in Lipid Mobilization

Lipolysis in adipose tissue is under a complex control system[1]. A number of hormones including epinephrine, glucagon, ACTH and adrenal cortical steroids have been shown to affect the release of fatty acids from adipose tissue in several species[2–6], and a variety of purines and purine derivatives have a similar affect[7–9]. Some of these substances, epinephrine and ACTH for instance, apparently act on the cell membrane in such a way as to activate adenyl cyclase, thus leading to increase in cyclic 3′5′-AMP[10,11]. Other compounds such as purine, theophylline and caffeine appear to have their effect by inhibiting the phosphodiesterase[7,12], thus increasing the level of cyclic AMP by a different mechanism. Cortisone has been shown to act by a third, entirely different mechanism which has been called the 'permissive effect', since it permits the lipolytic action of epinephrine to be expressed[6,13]. This is thought to occur through a decrease in uptake of glucose by the fat cell, which in turn causes a decrease in

Serum free fatty acids (FFA) in rats treated with cortisone and purine derivatives

Purine derivative	No cortisone			Cortisone		
	No. of animals	FFA μEq/l ± 1 S.D.	p[a]	No. of animals	FFA μEq/l ± 1 S.D.	p[a]
Purine	7	282 ± 50	0.02	7	282 ± 55	0.02
Caffeine	7	345 ± 70	0.002	7	304 ± 27	0.005
Adenine	7	175 ± 35	N.S.	7	199 ± 40	N.S.
Control (saline)	17	200 ± 38	–	15	399 ± 40	0.001

[a] Comparison with saline, no cortisone, controls. Purine (50 mg/0.5 ml saline), caffeine (5 mg/0.5 ml saline) or adenine sulfate (40 mg/4 ml saline) was given 2 h prior to sacrifice. Control animals received saline. Cortisone was given as 6.25 mg of cortisone acetate in saline s.c. the day before and on the day of treatment with the other drug.

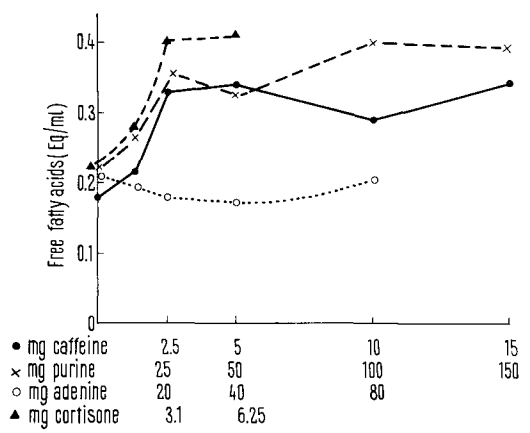
the amount of glycerophosphate available for esterification with fatty acids[14,15]. Larger doses of cortisone may cause lipolysis by still another, poorly understood, mechanism[16].

We have performed experiments using purine, caffeine and adenine, alone and combined with cortisone, which show that the lipid-mobilizing property of cortisone is antagonized by adenine.

Materials and methods. White, female, 170–300 g Sprague-Dawley rats were maintained on a liquid diet administered by tube feeding 3 times a day throughout the experiment and for a day prior[17]. During the course of the experiment at intervals detailed in the Table, cortisone, free purine, caffeine or adenine sulfate were given i.p. or s.c. At the time of sacrifice (always at the same time of day), rats were decapitated and the blood collected. Free fatty acid (FFA) determinations were performed in duplicate on 0.25 ml serum by the method of TROUT et al.[18], or by a modification of the colorimetric method of MASSION and SELIGSON[19]. The dosages of cortisone, purine and caffeine were selected after dose-response curves were obtained (Figure), and provide maximal responses. Drug solutions were adjusted to between pH 5–8 before use.

Results and discussion. Cortisone, purine base and caffeine all produced equivalent maximal elevations of serum FFA whether used alone or in combination (Table). Adenine produced no elevation of serum FFA and was in fact able to reverse the cortisone-induced elevation.

Caffeine, theophylline, xanthine, 6-mercaptopurine, purine base, and other purines have been shown previously to have a lipolytic action in rat adipose tissue in vitro, apparently through inhibition of the phosphodiesterase which inactivates cyclic 3′, 5′-AMP (cAMP)[7,10,12], a cofactor for adipose tissue lipase. Caffeine has also been shown to cause lipid mobilization in vivo in the mouse[8]. The lipid mobilizing effect of caffeine and purine base has been shown in these experiments in vivo in the rat. Adenine, by contrast, did not cause elevation of FFA when given parenterally in the rat, which is consistent with previous experience suggesting that it does not inhibit phosphodiesterase[10].



Dose-response curves of serum FFA 2 h after various doses of caffeine, purine and adenine, and 28 h after various doses of cortisone. Each point represents the average of 3 rats, each determined in duplicate.

Adenine, moreover, reversed the cortisone-induced fatty acidemia. While not giving specific information regarding the mechanism of the cortisone effect, this points clearly to an active role of adenine in adipose tissue metabolism, well beyond its known lack of effect on phosphodiesterase[10,20].

Zusammenfassung. Purin-Basen, Kaffein und Cortison führen zu einer Vermehrung der Fettsäuren im Rattenserum. Adenin vermindert im Gegensatz zu den anderen Purinen die Lipolyse.

R. B. HILL JR., JOAN N. STOCK and J. H. MABRY

Department of Pathology, State University of New York, Upstate Medical Center, 766 Irving Avenue, Syracuse (New York 13210, USA), 25. September 1969.

[1] J. K. GRANT, *The Control of Lipid Metabolism* (Academic Press, New York 1963).
[2] J. N. FAIN, R. O. SCOW and S. S. CHERNIK, J. biol. Chem. *238*, 54 54 (1963).
[3] R. B. HILL JR., W. E. DROKE and A. P. HAYS, Expl. molec. Path. *4*, 320 (1965).
[4] R. L. JUNGAS and E. G. BALL, Biochemistry *2*, 38 (1963).
[5] B. JEANRENAUD and A. E. RENOLD, J. biol. Chem. *235*, 2217 (1960).
[6] L. RESHEF and B. SHAPIRO, Metabolism *9*, 551 (1960).
[7] V. P. DOLE, J. biol. Chem. *256*, 3125 (1961).
[8] C. J. ESTLER and H. P. T. AMMON, Experientia *15*, 589 (1966).
[9] M. S. RABEN and F. MATSUZAKI, J. biol. Chem. *241*, 4781 (1966).
[10] M. A. RIZAK, Ann. N.Y. Acad. Sci. *131*, 250 (1965).
[11] E. W. SUTHERLAND and G. A. ROBINSON, Pharmac. Rev. *18*, 145 (1966).
[12] R. W. BUTCHER and E. W. SUTHERLAND, J. biol. Chem. *237*, 1244 (1962).
[13] E. SHAFRIR and D. STEINBERG, J. clin. Invest. *39*, 310 (1960).
[14] A. MUNCK, Biochem. biophys. Acta *57*, 318 (1962).
[15] E. SHAFRIR and S. KERPEL, Arch. Biochem. *105*, 237 (1964).
[16] R. F. MAHLER and W. L. STAFFORD, *The Control of Lipid Metabolism* (Academic Press, New York 1963), p. 155.
[17] R. B. HILL JR. and W. E. DROKE, Proc. Soc. exp. Biol. Med. *114*, 766 (1963).
[18] D. L. TROUT, E. H. ESTES JR. and S. J. FRIEDBERG, J. Lipid Res. *1*, 199 (1960).
[19] C. B. MASSION and D. SELIGSON, Am. J. clin. Path. *48*, 301 (1967).
[20] Supported by Grant No. AM-10651 from the USPHS.