Biochemical Pharmacology, Vol. 18, pp 2053–2060. Pergamon Press. 1969. Printed in Great Britain

THE HYPERSENSITIVITY OF ADIPOSE TISSUE TO NOREPINEPHRINE AND OTHER LIPOLYTIC AGENTS DURING BLOCKADE OF FREE FATTY ACIDS (FFA) MOBILIZATION*

A. BIZZI, A. M. CODEGONI and S. GARATTINI

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62, 20157 Milano, Italy

(*Received* 31 *December* 1968; *accepted* 22 *February* 1969)

Abstract—Adipose tissue of rats pretreated with 5-carboxy-3-methylpyrazole (5C3MP) or nicotinic acid released less glycerol and FFA (basal lipolysis) in their incubation medium. However, they were more sensitive to the lipolytic action of norepinephrine or theophylline. The maximum release of FFA and glycerol due to norepinephrine addition was increased in adipose tissue from 5C3MP pretreated rats. Moreover, the sensitivity to cyclic 3′5′ AMP (dibutyryl ester) was not significantly increased. The hypersensitivity to lipolytic agents already appeared at times when plasma FFA were lowered, but was present also during the rebound phase.

Adrenalectomy did not prevent the inhibition of lipolytic activity exerted by these compounds, but almost completely inhibited the hypersensitivity of adipose tissue of treated rats to lipolytic agents. The possibility that these changes in the responsiveness of adipose tissue were correlated with the increased level of plasma corticosterone observed after the administration of 5C3MP or nicotinic acid are taken into consideration. A possible role of the hypersensitivity in the onset of rebound effect is discussed.

IT HAS been recently demonstrated that compounds such as nicotinic acid,[1] 5-carboxy-3-methylpyrazole[2, 3] and its amide derivative[4] decrease plasma free fatty acid (FFA) levels by inhibiting the lipolytic activity of adipose tissue.

However, it was observed that after the period of lipolytic inhibition there was an increase of plasma FFA above the level of control animals (rebound effect).[3, 5]

The purpose of this investigation was to establish if the rebound effect was related to changes in sensitivity of adipose tissue to lipolytic agents such as noradrenaline, theophylline and cyclic 3′, 5′ AMP.

MATERIAL AND METHODS

Overnight fasted male Sprague–Dawley rats weighing about 150 g were used in all the experiments.

The *in vivo* treatment consisted of intraperitoneal (i.p.) administration of 5-carboxy-3-methyl-pyrazole (5C3MP), 7·5 mg/kg body wt. or nicotinic acid 50 mg/kg body wt. Control rats received an equal amount of saline.

At different time intervals, the animals were killed, plasma was collected and epididymal adipose tissue rapidly excised, minced, pooled and distributed (200 mg ± 10) to flasks containing 5 ml of Krebs phosphate buffer with 3% bovine albumin (fraction V Pentex).

* Supported by NIH Grant No. HE09971-01.

Details of the incubation technique have previously been described.[4] Free fatty acids (FFA) were determined in the incubation medium, in adipose tissue and in plasma according to Dole[6] with minor modifications. Washing of the heptane phase was carried out according to Trout.[7] Glycerol was measured according to Wieland.[8]

Determinations of plasma corticosterone were carried out according to Guillemin with minor modifications.[9] Some experiments were also carried out on isolated fat cells prepared according to Rodbell[10] and using Krebs phosphate Ringer containing glucose and albumin. Chemicals: 5-carboxy-3-methylpyrazole was obtained through the courtesy of Dr. G. Gerritsen (Upjohn Co., Kalamazoo, Mich.); norepinephrine, as bitartrate salt, and theophylline monohydrate were a gift of Recordati Co. (Milano) and 3′5′ cyclic AMP, as dibutyryl ester, was kindly supplied by Prof. M. Carissimi (Maggioni, Milano).

RESULTS

The i.p. administration of a single dose (7·5 mg/kg) of 5C3MP to fasted rats resulted in a marked decrease of plasma FFA, which lasted about 2 hr. Within 6 hr after the administration of 5C3MP, the plasma FFA values were always somewhat elevated above the control levels (Table 1).

TABLE 1. EFFECT OF 5C3MP AND NICOTINIC ACID ON PLASMA FFA AND ON THE BASAL LIPOLYSIS OF ADIPOSE TISSUE

Treatment (mg/kg)	Time (min)	FFA (μequiv./l ± S.E.)	Plasma Corticosterone μg/100 (ml ± S.E.)	Adipose tissue FFA (μequiv./g ± S.E.)	*In vitro* release FFA (μequiv./g/hr ± S.E.)	*In vitro* release Glycerol (μM/g/hr ± S.E.)
controls	—	717 ± 31	26 ± 4	6·5 ± 0·2	7·4 ± 0·1	2·7 ± 0·3
5C3MP 7·5	30	262 ± 24*	50 ± 2*	4·1 ± 0·1*	3·0 ± 0·1*	1·7 ± 0·1*
,,	60	211 ± 14*	53 ± 5*	3·6 ± 0·1*	3·2 ± 0·1*	1·5 ± 0·1*
,,	120	289 ± 33*	47 ± 3*	3·3 ± 0·1*	5·1 ± 0·2*	1·6 ± 0·2*
,,	360	783 ± 71	17 ± 4	5·9 ± 0·2	11·1 ± 0·2*	3·9 ± 0·2*
controls	—	673 ± 73	21 ± 4	11·6†	6·2 ± 0·3	2·5 ± 0·2
Nicotinic acid 50	30	193 ± 22*	60 ± 5*	6·1	2·2 ± 0·4*	1·6 ± 0·3*
,,	60	193 ± 21*	71 ± 3*	8·0	4·3 ± 0·1*	2·2 ± 0·3*
,,	120	199 ± 23*	63 ± 5*	7·3	4·6 ± 0·1*	1·5 ± 0·2*
,,	360	877 ± 20*	18 ± 3	8·4	7·6 ± 0·2*	2·4 ± 0·2*

Rats were fasted overnight.
Time represents minutes between treatment and sacrifice. Afterwards adipose tissues were incubated.
* $P < 0·01$ in respect to controls.
† These figures were obtained from pooled adipose tissues.

The FFA concentrations in adipose tissue were in good agreement with the changes observed in plasma FFA. A rise of plasma corticosterone was observed concomitantly with a decrease of plasma FFA.

When the epididymal adipose tissue of 5C3MP pretreated rats was incubated in Krebs phosphate albumin buffer, the basal lipolysis measured as release of FFA and glycerol in the medium was decreased at times when plasma and adipose tissue FFA were low.

Similar data were obtained when nicotinic acid was administered to fasted rats (Table 1), indicating a relationship between the rate of spontaneous lipolysis and plasma FFA levels.

Experiments were then undertaken in an attempt to compare the hormonal stimulated lipolysis of rats pretreated with 5C3MP or nicotinic acid against that of normal rats. Epididymal fat of 5C3MP or nicotinic acid pretreated rats (from 30 to 360 min) were considerably more sensitive to the lipolytic action of norepinephrine (Table 2) and theophylline (Table 3).

TABLE 2. EFFECT OF A PRETREATMENT WITH LIPOLYTIC INHIBITORS ON NE (NOREPINEPHRINE BITARTRATE 0·06 μg/ml), STIMULATED LIPOLYSIS

Treatment (mg/kg i.p.)	Time (min)	*In vitro* release			
		FFA (μequiv./g/hr ± S.E.)		Glycerol (μmoles/g/hr ± S.E.)	
		total	Δ	total	Δ
controls	—	11·3 ± 0·4	6·3	4·8 ± 0·0	2·5
5C3MP 7·5	30	12·6 ± 0·4	9·2	5·8 ± 0·2*	3·9
,,	60	15·9 ± 0·3*	13·2	8·4 ± 0·3*	6·7
,,	120	19·3 ± 1·9*	15·4	8·9 ± 0·6*	5·8
,,	360	18·7 ± 0·3*	12·9	9·8 ± 0·5*	7·6
controls	—	11·2 ± 0·4	4·9	4·4 ± 0·3	1·9
Nicotinic acid 50	30	16·8 ± 0·3*	14·6	8·0 ± 0·3*	6·4
,,	60	18·5 ± 0·6*	14·2	8·4 ± 0·3*	6·1
,,	120	24·6 ± 0·3*	20·0	10·4 ± 0·5*	8·9
,,	360	24·4 ± 0·6*	16·8	8·8 ± 0·2*	7·4

In vivo experimental conditions as in Table 1. Δ represents the net release due to stimulation (total release minus basal release).
* $P < 0·01$ in respect to controls.

TABLE 3. EFFECT OF A PRETREATMENT WITH 5C3MP ON THEOPHYLLINE AND CYCLIC 3′5′ AMP STIMULATED LIPOLYSIS OF ADIPOSE TISSUE

Treatment (mg/kg i.p.)	Time (min)	Additions to the medium (μg/ml)	*In vitro* release			
			FFA (μequiv./g/hr)		Glycerol (μmoles/g/hr)	
			total	Δ	total	Δ
controls	—	theophylline 75	13·2 ± 0·2	4·7	4·6 ± 0·1	1·9
5C3MP 7·5	30	,,	8·2 ± 0·3	4·8	3·7 ± 0·1	1·8
,,	60	,,	14·4 ± 0·4	9·5	6·8 ± 0·1*	4·1
,,	120	,,	20·5 ± 0·5*	15·1	8·6 ± 0·2*	6·5
,,	360	,,	22·5 ± 0·6*	14·1	9·5 ± 0·1*	6·8
controls	—	3′5′ AMP 250	17·4 ± 0·2	11·0	8·9 ± 0·6	6·4
5C3MP 7·5	30	,,	15·2 ± 0·4	12·4	7·7 ± 0·6	6·2
,,	60	,,	19·2 ± 0·5	15·6	10·3 ± 0·4	8·1
,,	120	,,	19·7 ± 0·0*	14·1	11·0 ± 0·2*	6·3
,,	360	,,	19·3 ± 0·3*	13·2	9·5 ± 0·4	7·1

Theophylline = theophylline monohydrate. 3′5′ AMP = cyclic 3′5′ AMP dibutyryl ester. Experimental conditions as in Table 2. * $P < 0·01$ in respect to controls.

In fact, when the release of FFA and glycerol due to the action of lipolytic agents, was measured both as total or net release, it was considerably higher in 5C3MP treated than in controls animals.

The hypersensitivity to the adipokinetic compounds was already present at times when plasma FFA were very low (30–60 min) and the basal lipolysis was depressed, but it was still present when the plasma FFA values were again normal or above normal.

However, when 3′5′ cyclic AMP, as the dibutyryl ester, was added to the incubation medium, the lipolysis was only slightly increased in adipose tissue of pretreated rats (Table 3).

When epididymal adipose tissue of rats pretreated with 5C3MP 1 hr before, were incubated with graded doses of norepinephrine, it was observed that adipose tissue from 5C3MP pretreated rats was much more sensitive to the lipolytic action of norepinephrine than that of control rats (Table 4). In this case the maximal lipolysis

TABLE 4. THE LIPOLYTIC RESPONSE OF 5C3MP PRETREATED RATS' ADIPOSE TISSUES TO GRADED DOSES OF NOREPINEPHRINE

Additions to the medium (μg/ml)	*In vivo* release			
	FFA (μequiv./g/hr)		Glycerol (μmoles/g/hr)	
	C	T	C	T
—	6·5 ± 0·6	4·0 ± 0·7	1·7 ± 0·2	2·0 ± 0·2
NE 0·006	6·7 ± 0·6	6·7 ± 0·6	1·1 ± 0·1	2·6 ± 0·6
0·012	7·8 ± 0·8	10·1 ± 0·6*	2·2 ± 0·1	4·2 ± 0·4*
0·025	11·2 ± 1*	16·1 ± 0·8*	3·7 ± 0·2*	6·5 ± 0·1*
0·05	12·9 ± 0·3*	23·6 ± 0·2*	5·9 ± 0·1*	10·3 ± 0·4*
0·12	20·9 ± 0·7*	31·5 ± 1*	7·8 ± 0·3*	12·9 ± 0·3*
0·25	23·8 ± 0·3*	35·2 ± 1*	9·9 ± 0·3*	13·7 ± 0·3*
0·5	27·1 ± 1*	35·8 ± 9*	9·7 ± 0·4*	14·8 ± 0·3*
1	29·0 ± 0·4*	37·5 ± 1·3*	10·7 ± 0·1*	15·7 ± 0·3*
2	28·6 ± 1·2*	36·2 ± 0·3*	10·7 ± 0·3*	15·8 ± 0·5*

C = controls (overnight fasted). T = Rats pretreated with 5C3MP 7·5 mg/kg i.p. Animals were sacrificed 60 min after the treatment and pooled adipose tissues were incubated with graded doses of norepinephrine bitartrate (NE). * $P < 0·01$ compared with the same dose in controls.

was elicited by the same doses of norepinephrine in both groups, however the amount of FFA and glycerol released by the tissue obtained from 5C3MP group always exceeded that released by the control group (Fig. 1).

Graded concentrations of cyclic 3′5′ AMP added to the medium failed to increase the rate of lipolysis in the 5C3MP group (Table 5). In order to avoid the fact that the FFA basal release was different in controls and 5C3MP treated groups a number of experiments were repeated with lipolytic agents by using isolated fat cells, a preparation which does not show a basal release.

Results concerning the stimulation induced by norepinephrine, summarized in Table 6, were consistent with those obtained with minced adipose tissue. In other experiments not reported here in details, cyclic 3′5′ AMP was equally effective in controls and 5C3MP treated groups.

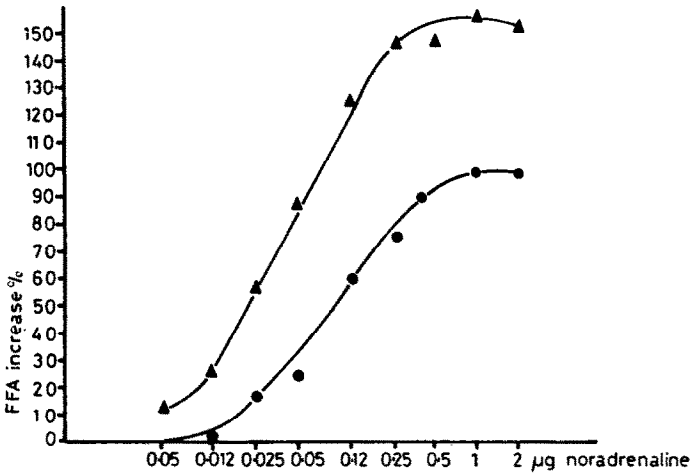

FIG. 1. Lipolytic effect of norepinephrine on epididymal adipose tissue of normal and 5C3MP treated rats. ▲——— controls. ●——— 5C3MP treated (7·5 mg/kg i.p. after 60 min). All values are referred to the maximal release of FFA obtained in the control group (= 100).

TABLE 5. LIPOLYTIC RESPONSE TO GRADED DOSES OF CYCLIC 3′5′ AMP

Addition to the medium (µg/ml)		*In vitro* release			
		FFA (µequiv./g/hr ± S.E.)		Glycerol (µmoles/g/hr ± S.E.)	
		C	T	C	T
saline		6·9 ± 0·3	3·3 ± 0·1	3·0 ± 0·5	3·1 ± 0·1
3′5′ AMP	62	7·4 ± 0·2	6·6 ± 0·3	3·4 ± 0·4	3·6 ± 0·6
,,	125	8·3 ± 0·3	7·8 ± 0·7	3·8 ± 0·1	5·1 ± 0·2*
,,	250	16·6 ± 0·4	16·9 ± 0·4	8·9 ± 0·2	11·1 ± 0·5
,,	500	22·8 ± 0·4	21·2 ± 0·4	10·9 ± 0·2	9·7 ± 0·4
,,	750	31·6 ± 0·6	33·1 ± 1·0	15·4 ± 0·3	17·0 ± 0·3*
,,	1000	32·4 ± 1·0	38·1 ± 0·5	16·7 ± 0·6	19·8 ± 0·4

Experimental conditions as in Table 4. 3′5′ AMP = cyclic 3′5′ AMP dibutyryl ester. * $P < 0·01$ in comparison to the same dose for controls.

TABLE 6. LIPOLYTIC RESPONSE OF ISOLATED FAT CELLS, OBTAINED FROM CONTROLS OR 5C3MP PRETREATED RATS, TO GRADED DOSES OF NOREPINEPHRINE

Addition to the medium (µg/ml)	*In vitro* release			
	FFA (µequiv./mM Tg/hr ± S.E.)		Glycerol µmoles/mM Tg/hr ± S.E.	
	C	T	C	T
NE 0·0062	5·6 ± 0·6	19 ± 1*	8 ± 1	9·5 ± 1
0·0125	40 ± 3	79 ± 9*	19 ± 1	36 ± 1*
0·062	195 ± 4	268 ± 4*	78 ± 1	108 ± 2*
0·125	218 ± 4	257 ± 4*	100 ± 1	117 ± 1*
0·250	228 ± 2	269 ± 4	107 ± 1	129 ± 2*
0·5	232 ± 2	277 ± 3*	111 ± 1	124 ± 2
1	236 ± 2	273 ± 4	112 ± 1	126 ± 1*

NE = norepinephrine bitartrate. *In vivo* experimental conditions as in Table 4. * $P < 0·01$ in comparison to the same dose of controls.
Tg = triplyceride.

TABLE 7. PLASMA FFA AND *IN VITRO* BASAL LIPOLYSIS OF ADRENALECTOMIZED RATS TREATED WITH 5C3MP

Treatment (mg/kg)	Plasma FFA (μequiv./l. ± S.E.)	*In vitro* release	
		FFA (μequiv./g/hr ± S.E.)	Glycerol (μmoles/g/hr ± S.E.)
controls	604 ± 93	7·07 ± 0·6	2·54 ± 0·2
5C3MP 7·5	222 ± 8*	2·23 ± 0·5*	1·71 ± 0·0*
Adx (1 day)	536 ± 21	3·04 ± 0·2	1·39 ± 0·3
Adx (1 day) + 5C3MP 7·5	218 ± 7*	1·31 ± 0·3*	0·9 ± 0·1
controls	675 ± 57	9·58 ± 0·30	3·02 ± 0·18
5C3MP 7·5	187 ± 7*	3·07 ± 0·20*	2·25 ± 0·10*
Adx (3 days)	491 ± 35	4·53 ± 0·09	1·98 ± 0·07
Adx (3 days) + 5C3MP 7·5	158 ± 5*	1·30 ± 0·04*	1·09 ± 0·10*

Adx = adrenalectomized rats. Adx rats received saline instead of drinking water. On the day of the experiment, overnight fasted animals were pretreated with 5C3MP 7·5 mg/kg and were sacrificed 60 min later. Pooled adipose tissues were incubated.
* $P < 0·1$ in respect to their controls.

The observation that plasma corticosterone values were elevated after treatment with nicotinic acid or 5C3MP, prompted us to study the role of adrenals on the development of hypersensitivity.

One and 3 day adrenalectomized rats were injected with 5C3MP, 7·5 mg/kg i.p., and were sacrificed 60 min later. Results in Table 7 indicate that the decrease of plasma FFA rats resembled that of controls. Also the basal lipolysis already low in adrenalectomized animals was further decreased with the treatment.

When adipose tissue of adrenalectomized rats and 5C3MP treated-adrenalectomized rats were stimulated *in vitro* with norepinephrine, the total release was similar in both

TABLE 8. NOREPINEPHRINE (NE)—STIMULATED LIPOLYSIS OF ADRENALECTOMIZED RATS PRETREATED WITH 5C3MP

Treatment (mg/kg)	Additions to the medium	*In vitro* release			
		FFA (μequiv./g/hr ± S.E.)		Glycerol (μmoles/g/hr ± S.E.)	
		total	Δ	total	Δ
Adx (1 day)	saline	3·0 ± 0·2		1·4	
	NE 0·06	6·3 ± 0·3	3·26	2·7 ± 0·1	1·36
	NE 0·50	7·2 ± 1·1	4·15	3·5 ± 0·1	2·15
Adx (1 day) + 5C3MP 7·5	saline	1·31 ± 0·1		0·9 ± 0·1	
	NE 0·06	6·84 ± 0·3	5·53	3·19 ± 0·2	2·29
	NE 0·50	6·21 ± 0·4	5·90	3·88 ± 0·1	2·98
Adx (3 days)	saline	4·53 ± 0·09		1·98 ± 0·07	
	NE 0·06	4·95 ± 0·3	0·42	2·92 ± 0·1	0·94
	NE 0·50	8·43 ± 0·4	3·90	3·94 ± 0·1	1·96
Adx (3 days) + 5C3MP	saline	1·30 ± 0·04		1·09	
	NE 0·06	4·89 ± 0·3	3·59	3·05 ± 0·1	1·96
	NE 0·50	7·72 ± 0·4	6·42	4·72 ± 0·1	3·63

Experimental conditions as in Table 7.

groups (Table 8). These data indicate that the hypersensitivity due to 5C3MP was much lower in adrenalectomized than in intact rats.

DISCUSSION

The plasma FFA level of rats treated with 5C3MP was lower than that of controls, 1 or 2 hr after 5C3MP administration. The adipose tissue of these rats released, during *in vitro* incubation, FFA and glycerol to a lesser extent than adipose tissue obtained from normal fasted rats.

However, when lipolytic agents, such as norepinephrine and theophylline, were added to the incubation medium, the adipose tissue of 5C3MP pretreated animals became definitely more sensitive.

The hypersensitivity to norepinephrine was already apparent 30 min after the *in vivo* treatment, when the antilipolytic activity of 5C3MP reached its maximum effect. The lipolytic response of adipose tissue of 5C3MP treated rats to graded doses of norepinephrine was increased by about 50 per cent. The observation that the sensitivity to 3′5′ cyclic AMP was almost similar in treated and in control animals indicates that phosphodiesterase was not affected by the 5C3MP treatment.

The hypersensitivity to norephinephrine could be observed only in the particular conditions of these experiments. It is in fact known that nicotinic acid and pyrazole derivatives may counteract the stimulation of lipolysis induced by norepinephrine when the amine is given *in vivo* simultaneously with these agents.[1, 11, 12] Futhermore, when added in suitable concentrations to the adipose tissue *in vitro*, nicotinic acid and 5C3MP are able to block the stimulation of lipolysis induced by norepinephrine or theophylline.

The mechanism responsible for the onset of such hypersensitivity is not yet clear.

The large reduction of the availability of FFA in 5C3MP treated rats could be responsible for the stimulation of some homeostatic mechanisms which tend to overcome the inhibition of lipolysis.

The development of a hypersensitivity to norepinephrine in adipose tissues of hyperthyroid rats[13] as well as a hypersensitivity to theophylline in hypophysectomized rats treated with growth hormone[14] has been recently described.

It was also demonstrated that the administration of nicotinic acid induces a rise in the level of growth hormone.[15] The observed rise of plasma corticosterone after the administration of nicotinic acid or 5C3MP could also have some role in the development of the hypersensitivity. The observation that adrenalectomized rats treated with 5C3MP are less hypersensitive than intact animals, would support this hypothesis. It is interesting in this respect that Shafrir *et al.*[16] found an increased release of FFA and glycerol in rats pretreated with cortisol. Although in our experiments the level of plasma corticosterone returned to normal values when the hypersensitivity was still present, it could not be excluded that the concentration of corticosterone in the adipose tissue was still elevated.

In evaluating the possible role that hormonal changes could play in the control of lipolysis, it is interesting to note that the hypersensitivity to lipolytic agents observed in adipose tissue of 5C3MP or nicotinic acid treated animals was longer lasting than the block of lipolysis. This may suggest that the rebound[3, 5] observed after the fall of plasma FFA is, at least in part, connected to the hypersensitivity here described.

REFERENCES

1. L. A. CARLSON, *Acta Med. Scand.* **173**, 719 (1963).
2. G. C. GERRITSEN and W. E. DULIN, *J. Pharmac. exp. Ther.* **150**, 491 (1965).
3. A. BIZZI, In, *Progress in Biochemical Pharmacology* 4, (Eds. C. J. MIRAS, A. N. HOWARD and R. PAOLETTI) 573–77 (1968).
4. A. BIZZI, A. M. CODEGONI and S. GARATTINI, *Il Farmaco* (*Sci.*) **22**, 709 (1967).
5. J. N. PEREIRA, *J. Lipid Res.* **8**, 239 (1967).
6. V. P. DOLE, *J. Clin. Invest.* **35**, 150 (1956).
7. D. L. TROUT, E. H. ESTES, JR. and S. J. FRIEDBERG, *J. Lipid Res.* **1**, 199 (1960).
8. O. WIELAND, *Biochem. Z.* **329**, 313 (1954).
9. R. GUILLEMIN, G. W. GLAYTON and H. S. LIPSCOMB, *J. Lab. Clin. Med.* **53**, 830 (1959).
10. M. RODBELL, *J. biol. Chem.* **239**, 375 (1964).
11. L. A. CARLSON and L. ORÖ, *Acta Med. Scand.* **172**, 641 (1962).
12. S. GARATTINI, A. BIZZI, *Pharmac. Rev.* **18**, 243 (1966).
13. G. KRISHNA, S. HYNIE and B. B. BRODIE, *Proc. natn. Acad. Sci., Wash.* **59**, 884 (1968).
14. H. M. GOODMAN, *Endocrinology* **82**, 1027 (1968).
15. M. IRIE, M. SAKUMA, T. TSUSHIMA, K. SHIZUME and K. NAKAO, *Proc. Soc. exp. Biol. Med., N.Y.* **126**, 708 (1967).
16. E. SHAFRIR and S. KERPEL, *Archs Biochem. Biophys.* **105**, 237 (1964).