one being stimulated, the other being kept as the control. The neurovascular pedicle was stimulated, using a Palmer stimulator, for 1 hr, frequency 25 impulses/sec, 5 volts, 1·0 msec square wave pulses, duration 1/1000 second. At the end of this incubation FFA in the medium have been titrated according to Dole.[11]

The results, summarized in Fig. 1, clearly show that epididymal fat from MTU-fed rats is insentitive to the direct stimulation of the sympathetic fibers, as far as FFA release is concerned. The basal FFA release is not significantly affected. When T_3 is administered to normal and MTU-fed rats, as shown in Fig. 2, an increased sensitization of adipose tissue to electrical stimulation appears evident. Both normal and MTU-fed rats response with a similar increase of FFA release, after stimulation, when compared with non-stimulated controls. T_3 treatment completely antagonizes the effect of MTU-feeding.

Our data clearly indicate that the metabolic activities of the sympathetic system at the level of adipose tissue are influenced and probably require normal thyroid activity.

Acknowledgement—Investigation supported by the U.S. Atomic Energy Commission, Washington D.C., Contract No. AT (30-1)-3097.

Institute of Pharmacology,
University of Milan,
Italy

F. BERTI
M. M. USARDI

REFERENCES

1. F. X. HAUSBERGER and Z. MIKROSKOP, *Anat. Fortschr.* **36**, 231 (1934).
2. G. CLÉMENT, *Arch. Sci. Physiol.* **4**, 13 (1950).
3. R. L. SIDMAN and D. W. FAWCETT, *Anat. Rec.* 118, 487 (1954).
4. R. PAOLETTI, R. L. SMITH, R. P. MAICKEL and B. B. BRODIE, *Biochem. Biophys. Comm.* **5**, 424 (1961).
5. R. L. SMITH, R. PAOLETTI and B. B. BRODIE, *Biochem. J.* **82**, 19 P (1962).
6. R. L. SIDMAN, M. PERKINS and N. WEINER, *Nature Lond.* **193**, 36 (1962).
7. A. F. DEBONS and I. L. SCHWARTZ, *J. Lipid Research* **2**, 86 (1961).
8. D. DEYKIN and M. VAUGHAN, *J. Lipid Research* **4**, 200 (1963)√
9. J. W. CORREL, *Science* **140**, 387 (1963).
10. F. BERTI, M. M. USARDI, *Giorn. Aterosclerosi* **2**, (1964), in press.
11. V. P. DOLE, *J. Clin. Invest.* **35**, 150 (1956).

Biochemical Pharmacology, 1965, Vol. 14, pp. 359–363. Pergamon Press Ltd., Printed in Great Britain.

Evidence for the function of D-glucaric acid as an indicator for drug induced enhanced metabolism through the glucuronic acid pathway in man

(*Received* 16 *August* 1964; *accepted* 13 *October* 1964)

IN ANIMAL experiments the barbiturate induced stimulation of drug metabolism appears to be accompanied by a variety of phenomena:[1]

1. A profound influence of the drug on the smooth surfaced endoplasmatic reticulum in liver cells[2] accompanied by an enhanced microsomal capacity to oxidyze NADPH.[1–3]

2. An increased activity of androgen hydroxylases in rat-liver microsomes.[4] It has been found by Orrenius *et al.*[3, 5] and by Reichert and Remmer[6] that the level of the CO—cytochrome[7] connected with the oxidation of drugs in rat-liver microsomes is increased after pretreatment with phenobarbital. These facts suggest a relationship between the increase of the level of the CO—cytochrome and the increased activity of androgen hydroxylases, since the CO—cytochrome[8] in the adrenal cortex microsomes is connected with the hydroxylation of steroids.

3. A stimulation of the synthesis of free D-glucuronic, L-gulonic and L-ascorbic acid through the glucuronic acid pathway in rat-liver,[9] which is interpretable on the basis of the enhanced activity

of UDP-glucose:NAD-oxidoreductase in the soluble fraction of liver homogenates.[10] Non microsomal D-glucose-6-phosphate:NADP-oxidoreductase and 6-phospho-D-gluconate:NADP-oxidoreductase (decarboxylating) also show enhanced activity after phenobarbital treatment.[11] Experiments by Winegrad and Shaw[12] gave a strong indication that in adipose tissue of rats the glucose utilization via the glucuronic acid pathway is stimulated by barbital.

It has been found that drug administration in therapeutic doses is or may be the reason for an accelerated drug metabolism in man, because of an influence on enzymatic level.[13–19] It is of importance therefore to investigate to what extent the effects of barbiturates, which accompany the drug-induced enhanced drug metabolism in animal experiments, occur in man dosed with therapeutic quantities of drug.

The first evidence for the effect of drugs on metabolism through the glucoronic acid pathway came from studies by Enklewitz and Lasker[20] who found that amidopyrine and antipyrine—stimulators of L-ascorbic acid synthesis in the rat—elevate the urinary excretion of L-xylulose in human subjects with essential pentosuria. The possibility of getting an indication for an enhanced metabolism through the glucuronic acid pathway in man, with the aid of L-ascorbic acid as indicator,[21] naturally fails.

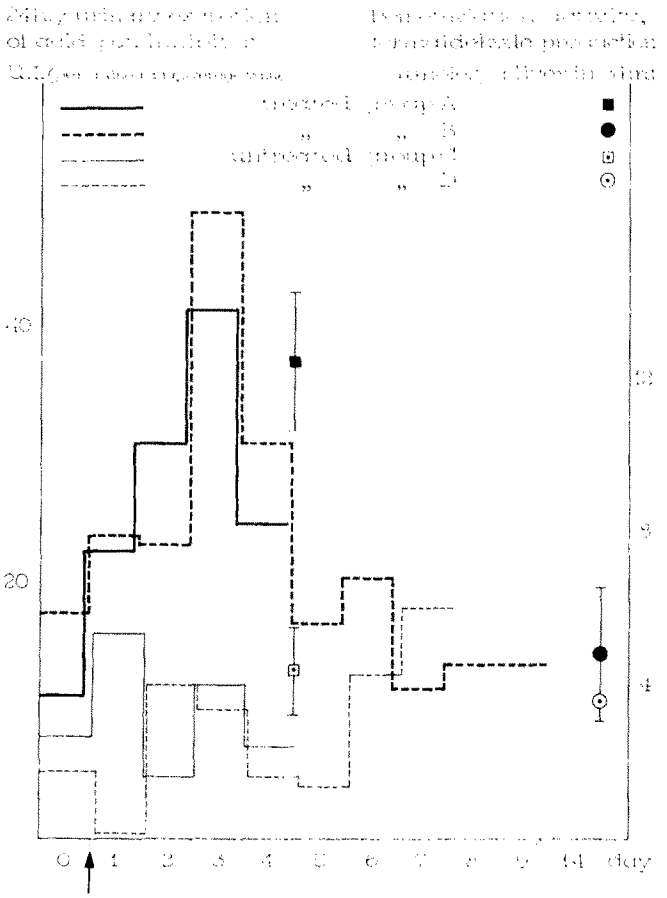

FIG. 1. Urinary excretion of acid-potentiated inhibitor of β-glucuronidase by guinea pigs during 24 hour periods, and demethylation activity of homogenates of guinea pig-livers centrifugated at 9000 g tested with amidopyrine. Animals of each group A and B were injected intraperitoneally with 16 mg sodium barbital in 4 ml physiological saline twice daily during days 1, 2 and 3; animals of group C and D 5 and 3 respectively were injected with physiological saline at the same times. Group A and C were sacrificed after day 4, group B and D after day 14 and the livers were homogenized for estimation of demethylation activity in each liver supernatant. Vertical lines represent the standard deviations.

This fact, the identification by Marsh[22] of D-glucaric acid—also formed through the glucuronic acid pathway—as a normal constituent in urine of mammals and the observation by Marsh and Reid[23] that pretreatment of rats with barbital elevates the urinary level of acid-potentiated inhibitor of β-glucuronidase, accepted by these investigators as an enhanced D-glucaric acid excretion, led us to the examination of the acid-potentiated inhibitor in urine of man and guinea pigs treated with drugs. In this communication experiments are described which show an enhanced acid-potentiated inhibition of β-glucuronidase in urine of man and guinea pigs dosed with drugs.

MATERIALS AND METHODS

Experimental animals: Female guinea pigs weighing 230 to 280 g were used. The animals were maintained on Hope Farms Laboratory Diet for guinea pigs, with vegetables and free access to water. Urines were stored at 4°.

Determination of acid-potentiated inhibitor of β-glucuronidase: Crude urine was kept at 100° for 20 min after adjustment to pH 7. After readjustment of pH, if necessary, the urine was incubated with bacterial β-glucuronidase (Sigma type I, active at pH 7, 1000 U./ml urine) for 48 hr to eliminate possible interfering glucuronides. After inactivation of the β-glucuronidase by heating for 5 min at 100° the urine was extracted twice with a double volume of ethyl-acetate and assayed by the procedure of Marsh.[22] As a basis for the comparison of the acid-potentiated inhibition the "unit of inhibitor" (U.I.) defined by Marsh[22] was chosen.

Demethylation of amidopyrine was determined by the procedure described by Smith *et al.*[24] Moreover glucose-6-phosphate-dehydrogenase (5 Kornberg units of G-6PDH 15303 Boehringer) was added to the incubation mixture.

RESULTS

Guinea pigs (5) with an acid-potentiated inhibitor concentration of 3·7 U.I. (S.D. 3·5) in 24 hr urine before treatment were given 75 mg/kg barbital intraperitoneally per day for 3 days. A 24 hourly excretion of 31 U.I. (S.D. 19) was found for the fourth day. In another experiment the acid-potentiated inhibitor excretion was measured before, during and after dosage with barbital, and in addition the demethylation activity of guinea pig-liver microsomes was measured at times of enhanced and normal acid-potentiated inhibitor excretion in urine (see Fig. 1). An enhanced acid-potentiated inhibitor excretion was observed simultaneously with an enhanced drug-metabolism. The acid-potentiated inhibitor excretions by patients under treatment with drugs are shown in table 1. A pronounced enhancement is observed for patients with phenobarbital diphantoine or amidopyrine. In two out of three cases of barbiturate treatment the increased urinary excretion of acid-potentiated inhibitor over a number of days could be followed, starting with a normal value before treatment. In one of these experiments a healthy individual was treated with 100 mg sodium phenobarbital three times a day for a week and the daily acid-potentiated inhibitor excretions were measured (see Fig. 2).

TABLE 1. ACID-POTENTIATED INHIBITOR (IN U.I.) OF β-GLUCURONIDASE IN 24 HR URINE OF PATIENTS UNDER TREATMENT WITH DRUGS.

Drug administered per day	No. of patients Male	and female	Time of drug administration	U.I.	± S.D.
Amidopyrine 1800–2400 mg	—	10	two weeks or longer	7680	4530
Phenobarbital-diphantoine 100–300 mg	9	2	two months or longer	12200	7870
Phenylbutazone 400–600 mg	6	5	two weeks or longer	2226	630
Salicylic acid 1500 mg	5	—	10 days	1090	400
Tolbutamide 500–2000 mg	4	7	two months or longer	985	670
None	11	—		1400	850
,,	16	—		2197*	425
,,		14		1698*	337

* values according to Marsh[22]

DISCUSSION

Marsh[25] has shown that the oxidation of D-glucuronolactone to D-glucaric acid by D-glucurono-γ-lactone:NAD-oxidoreductase represents an alternative pathway of glucuronic acid conversion. in addition to the conversion to L-ascorbic acid or to L-xylulose. Marsh and Reid[23] showed that there was no significant difference between the activity of liver-D-glucurono-γ-lactone:NAD-oxidoreductase

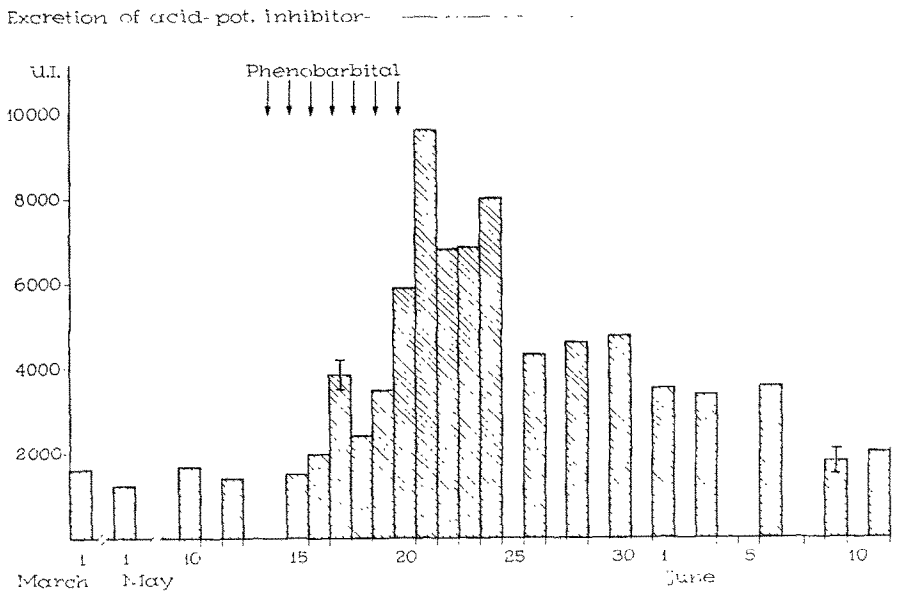

FIG. 2. Urinary excretions (24 hourly) of acid-potentiated inhibitor of β-glucuronidase by an individual treated with 300 mg sodium phenobarbital daily for a week (see arrows). Vertical lines represent the standard deviations calculated from 5 determinations.

of drug-treated rats and those of controls. This means that the elevated excretion of acid-potentiated inhibitor—assuming it is D-glucaric acid—can be explained by an increased availability of the precursor D-glucuronic acid or its lactone[23] in connection with the enhanced level of activity of UDPG-dehydrogenase as found by Hollmann and Touster[10] in barbital-treated rats.

From a 24 hr urine (containing 17600 U.I.) of a patient chronically under treatment with phenobarbital diphantoine, D-glucaric acid could be isolated by means of ion exchange chromatography and determined after conversion to D-glucaric acid bisphenylhydrazide. Moreover measurement by the enzymatic method of Marsh and measurement as an alpha hydroxycarboxylic acid[26] gave the same values for the isolated D-glucaric acid. The quantity isolated (67 mg) covered 75 per cent of the D-glucaric acid calculated on basis of the acid potentiated inhibition in the 24 hr urine used, while the normal 24 hr excretion is about 10 mg calculated on the same basis by Marsh[22].

The acid-potentiated inhibitor—D-glucaric acid—can therefore be an indicator for the intensivity and duration of drug-induced enhanced metabolism through the glucuronic acid pathway in man (see Fig. 2).

Acknowledgements—The technical assistance of Miss H. Asselbergs, Miss M. van Mierlo and Mr. J. Chappin is acknowledged.

Department of Pharmacology,
University of Nijmegen,
Nijmegen,
Holland.

E. M. AARTS.

REFERENCES

1. A. H. CONNEY, *Proc. 1st. Internat. Pharmacol. Meeting* **VI**, p. 250, Pergamon, New York (1962).
2. H. REMMER, H. J. MERKER, *Science* **142**, 1657 (1963).
3. S. ORRENIUS, L. ERNSTER, *Biochem. J.* **92**, 37P (1964).
4. A. H. CONNEY, A. KLUTCH, *J. Biol. Chem.* **238**, 1611 (1963).
5. S. ORRENIUS, G. DALLNER, L. ERNSTER, *Biochem. Biophys. Res. Communs.* **14**, 329 (1964).
6. R. REICHERT, H. REMMER, *Arch. exp. Path. Pharmak.* **247**, 374 (1964).
7. M. KLINGENBERG, *Arch. Biochem. Biophys.* **75**, 376 (1958).
8. R. W. ESTABROOK, D. Y. COOPER, O. ROSENTHAL, *Biochem. Z.* **338**, 741 (1963).
9. C. EVANS, A. H. CONNEY, N. TROUSOF, J. J. BURNS, *Biochim. biophys. Acta* **41**, 9 (1961).
10. S. HOLLMANN, O. TOUSTER, *Biochem. biophys. Acta* **62**, 338 (1962).
11. E. BRESNICK, HU-YU YANG, *Biochem. Pharmacol.* **13**, 497 (1964).
12. A. I. WINEGRAD, W. N. SHAW, *Amer. J. Physiol.* **206**, 165 (1964).
13. J. J. BURNS, R. K. ROSE, T. CHENKIN, A. GOLDMAN, A. SCHULERT, B. B. BRODIE, *J. Pharmacol.* **109**, 346 (1953).
14. H. REMMER, M. SIEGERT, F. LIEBENZCHÜTZ, *Klin. Wochenschr.* **39**, 490 (1961).
15. W. CHEN, P. A. VRINDTEN, P. G. DAYTON, J. J. BURNS, *Life Sci.* **2**, 35 (1962).
16. P. G. DAYTON, J. TARCAN, T. CHENKIN, M. WEINER, *J. clin. Invest.* **40**, 1797 (1961).
17. J. F. DOUGLAS, B. J. LUDWIG, N. SMITH, *Proc. Soc. Exp. Biol. Med.* **112**, 346 (1963).
18. K. SCHMID, F. CORNU, P. IMHOF, H. KEBERLE, *Schweiz. Med. Wschr.* **94**, 235 (1964).
19. D. BUSFIELD, K. J. CHILD, R. M. ATKINSON, E. G. TOMICH, *Lancet* **2**, 1042 (1963).
20. M. ENKLEWITZ, M. LASKER, *J. Biol. Chem.* **110**, 443 (1935).
21. J. J. BURNES, A. H. CONNEY, R. KOSTER, *Ann. N.Y. Acad. Sci.* **104**, 881 (1963).
22. C. A. MARSH, *Biochem. J.* **86**, 77 (1963).
23. C. A. MARSH, L. M. REID, *Biochem. biophys. Acta* **78**, 726 (1963).
24. J. A. SMITH, W. J. WADDELL, T. C. BUTLER, *Life Sci.* **7**, 486 (1963).
25. C. A. MARSH, *Biochem. J.* **87**, 82 (1963).
26. R. M. MATULIS, J. C. GUYON, *Anal. Chem.* **36**, 118 (1964).

Biochemical Pharmacology, 1965, Vol. 14, pp. 363–365. Pergamon Press Ltd., Printed in Great Britain.

Evidence for the presence of monoamine oxidase in sympathetic nerve endings

(*Received* 8 *September* 1964; *accepted* 7 *October* 1964)

RECENT studies have emphasized the role of monoamine oxidase (MAO) activity in sympathetic nervous function.[1] It is thought that MAO activity is responsible for the catabolism of a portion of norepinephrine which is released intraneuronally and then leaves the nerve in the form of inactive metabolites. An important supposition for this point of view is the presence of MAO in sympathetic nerves in the region of the nerve endings. In attempting to establish a role for MAO in the inactivation of neurohumors at sympathetic nerve endings, several workers have measured MAO activity in such tissues of the cat as the nictitating membrane, iris, and blood vessels, after sympathetic denervation.[2, 3] No consistent reduction in MAO activity after sympathetic denervation could be demonstrated. If MAO is present extraneurally as well as intraneurally, it is possible that in the tissues examined in these studies the proportion of neural to extraneural MAO was so small that even a complete loss of the neural enzyme after denervation would not be reflected in measurements performed on the whole tissue.

A considerable portion of the total mass of the pineal gland is composed of sympathetic nerve endings.[4] Accordingly, studies were undertaken to examine the effect of sympathetic denervation on MAO activity in the rat pineal gland, and our results show that there is a 50% reduction in the MAO activity of this organ. Sympathetic denervation of the submaxillary gland results in a smaller but significant decrease in MAO activity.