activity, DNA synthesis and proliferation of HeLa cells. This includes daunomycin, adriamycin and daunosaminyl-daunomycin. When CO group of acetyl side chain is alterated, the derivatives preserve the ability to bind to DNA and a reduced antimitotic activity, but are inactive or only slightly active in inhibiting DNA synthesis and cellular proliferation. Examples are daunomycin-thiosemicarbazone, daunomycin-oxime, daunomycin-semicarbazone and 13-dihydro-daunomycin.

When daunosamine is exchanged for α-D-glucosamine or if amino group is masked, the derivatives are found to bind to DNA very weakly and to lack biological activity.

The results once again suggest that the ability of these compounds to bind to DNA is closely connected with the special chemical structure of the amino sugar.[2]

Work is proceeding in this laboratory to elucidate further possible correlations between the chemicophysical properties of the complex formed by daunomycin derivatives and their biological activity.

Experimental Chemotheraphy Section,
National Cancer Institute,
20133 *Milano, Italy*

A. Di Marco
F. Zunino
R. Silvestrini
C. Gambarucci
R. A. Gambetta

REFERENCES

1. A. Di Marco, in *Antibiotics* (Eds. D. Gottlieb and P. D. Shaw), Vol. 1, p. 190, Springer-Verlag, Berlin (1967).
2. E. Calendi, A. Di Marco, M. Reggiani, B. M. Scarpinato and L. Valentini, *Biochim. biophys. Acta* **103**, 25 (1965).
3. A. Rusconi, *Biochim. biophys. Acta* **123**, 627 (1966).
4. L. S. Lerman, *J. Molec. Biol.* **3**, 18 (1961).
5. W. Muller and D. M. Crothers, *J. Molec. Biol.* **35**, 251 (1968).
6. G. Cohen and H. Eisenberg, *Biopolymers* **8**, 45 (1969).
7. L. D. Zeleznick, J. A. Crim and G. D. Gray, *Biochem. Pharmac.* **18**, 1823 (1969).
8. N. H. Kurnick, *Methods Biochem. Anal.* **9**, 15 (1962).

Biochemical Pharmacology, Vol. 20, pp. 1328–1331. Pergamon Press, 1971. Printed in Great Britain

Effect of phenobarbital treatment on lysosomal enzyme activity in rat liver

(*Received* 10 *October* 1970; *accepted* 28 *November* 1970)

Phenobarbital is known to stimulate the formation of smooth-surfaced endoplasmic reticulum in the liver cell[1] and to induce a number of microsomal drug metabolizing enzyme systems.[2] In the course of studies on the effect of phenobarbital on the submicrosomal distribution of the enzyme UDP glucuronyltransferase from rat liver[3] a reduced acid phosphatase (EC 3.1.3) activity per gram of liver was found after this pretreatment during 4 days. Acid phosphatase was used as marker enzyme for the lysosomal fraction in which it is known to be located.[4] A decrease in the activity of four lysosomal enzyme activities is reported in the present study.

Materials and methods

Male rats (TNO, Zeist, The Netherlands) weighing 190–250 g who had free access to food and water were used. The animals received intraperitoneal injections of phenobarbital (90 mg/kg) at 4 p.m. on each of 4 consecutive days. About 9 a.m. on the day after the last injection the rats were decapitated, the livers were excised and removed into ice-cold 0·154 M KCl. A 20% (w/v) homogenate in 0·154 M KCl was made with a Potter–Elvehjem homogenizer with teflon pestle at 0–4°.

All enzyme activities were measured in the diluted homogenates during incubations at 37°. UDP glucuronyltransferase was assayed as described before[3] with *p*-nitrophenol as substrate; the enzyme preparations were activated by the detergent Triton X-100 *in vitro*.[3,5] Cathepsin B and C were determined by the microdiffusion method of Conway as described by Bouma.[6] Glucose-6-phosphatase

was assayed by the method of de Duve *et al.*[4] as modified by Bouma;[6] acid phosphatase by that of Gianetto and de Duve[7] with β-glycerolphosphate as substrate.

β-Glucuronidase was assayed with phenolphthalein-β-D-glucuronide as substrate.[8] The incubation mixture was 0·75 mM in the substrate in 75 mM acetate buffer pH 5·0. After 30 min incubation the reaction was terminated by the addition of 0·4 M glycine buffer pH 10·4, the suspension was filtered and the extinction at 555 nm was measured spectrophotometrically. In the rats used there seemed to be two populations in respect to β-glucuronidase. About 30 per cent of the rats had a low β-glucuronidase activity per gram of liver (Table 1). There was a slight difference in the K_m for phenolphthalein glucuronide, which was in the high-activity animals 0·41 (0·44; 0·48; 0·39; 0·35)

TABLE 1. β-GLUCURONIDASE ACTIVITY PER GRAM OF LIVER

Units/g of liver	Number of rats
0·00–0·10	0
0·10–0·20	0
0·20–0·30	14
0·30–0·40	4
0·40–0·50	0
0·50–0·60	4
0·60–0·70	9
0·70–0·80	13
0·80–0·90	6
0·90–1·00	5
1·00–1·10	1
	56

and in the low activity animals 0·65 mM (0·65; 0·63; 0·68). As it seemed not to be possible to attribute these different levels of β-glucuronidase to any artifact, it was tentatively attributed to a genetic difference. For the results in the present study those values that were from high-activity animals were used and all results from low-activity animals were rejected. None of the other measured enzyme activities seemed to be connected with this tentative genetic difference.

The activities are expressed in units representing the conversion of 1 μmole of substrate per minute. The lysosomal enzymes and β-glucuronidase were activated by adjusting the homogenates to 0·25% (v/v) of Triton X-100 after which they were further diluted for the enzyme estimations. Only in the case of the microsomal glucose-6-phosphatase no Triton X-100 was added.

Results and discussion

After 4 days treatment with phenobarbital the liver weight–body weight ratio was markedly increased. The two microsomal enzymes glucose-6-phosphatase and UDP glucuronyltransferase which were used as reference enzymes for the phenobarbital effect, as expected gave opposite responses to the phenobarbital treatment.[9,10] Table 2 shows the effect of the pretreatment on 3 lysosomal enzymes (cathepsin B and C; acid phosphatase), on one enzyme that is partly lysosomal and partly microsomal (β-glucuronidase),[4] and on the two microsomal enzymes. Phenobarbital decreased the activities per gram of liver of the lysosomal enzymes significantly to about the same extent. When the enzyme activity is related to the body weight of the rats, however, there was no change in the enzyme activity per 100 gram of rat after phenobarbital (Table 3). This suggests that the decrease in lysosomal enzyme activity per gram of liver results from an increase in liver weight without a concomitant increase in the total amount of lysosomal enzymes during the treatment.

It is known that phenobarbital treatment stimulates the formation of smooth-surfaced endoplasmic reticulum in the liver cell[1] and thereby increases liver weight. The present results suggest that this increase in endoplasmic reticulum causes a "dilution" of lysosomal enzyme activity in the liver. This suggestion seems to be contradicted by the results of the morphometric studies of Stäubli *et al.*[1] who found an increase in the volume of liver occupied by lysosomes in 5 days phenobarbital treated rats. This difference may perhaps be due to the use of different strains of rats or to the difference in the parameters measured: lysosomal volume and lysosomal enzyme activity may not be parallel. It is not clear from their data whether the number of lysosomes has increased after phenobarbital of whether the volume of the existing lysosomes has increased; in the latter case it need not be accompanied by an increase in enzyme content.

TABLE 2. EFFECT OF PHENOBARBITAL ON SOME LYSOSOMAL AND MICROSOMAL ENZYMES

	Activity per gram of liver			Activity per 100 g of rat		
	Untreated	Treated	Untreated %	Untreated	Treated	Untreated rats %
Acid phosphatase	2·89 ± 0·09 (8)	2·49 ± 0·04 (8)*	86	12·07 ± 0·39	12·97 ± 0·35†	107
Cathepsin B	0·98 ± 0·03 (4)	0·78 ± 0·03 (4)*	81	4·12 ± 0·18	4·14 ± 0·12	101
Cathepsin C	5·65 ± 0·35 (8)	4·56 ± 0·28 (8)*	81	23·76 ± 1·74	23·76 ± 1·53	100
β-Glucuronidase	0·77 ± 0·05 (5)	0·58 ± 0·02 (6)*	75	3·21 ± 0·24	2·99 ± 0·64	93
Glucose-6-phosphatase	11·8 ± 0·2 (8)	8·30 ± 0·3 (8)*	70	45·26 ± 1·35	39·82 ± 1·30*	88
UDP-glucuronyltransferase	0·50 ± 0·02 (8)	0·80 ± 0·02 (8)*	157	1·95 ± 0·12	3·83 ± 0·12*	197
Liver weight/body weight × 100	4·18 ± 0·09 (8)	5·21 ± 0·11 (8)*	125	—	—	

Activities of some lysosomal and microsomal enzymes, expressed as units per gram of liver and per 100 g of rat, were determined in untreated rats and rats created for 4 days with phenobarbital (each day 90 mg/kg intraperitoneally). Activities were assayed in diluted homogenates in 0·154 M KCl and are expressed as units catalyzing the conversion of 1 μmole/min. The number in parenthesis indicates the number of rats used. The values given are means ± the standard error of the mean. Statistical analysis was carried out according to the method of Wilcoxon.[12]

* $P < 0.05$.

† $P < 0.10$.

Recently Platt and Katzemeier[11] reported an increase in the activity of some lysosomal enzyme activities (β-glucuronidase, β-acetylglucosaminidase and collagenase) per mg of protein in the lysosomal fraction after phenobarbital treatment during 6 days. They also found that the liability to induction subsides with growing age of the rats. Thus in their 26-week-old rats they could find only an induction effect of phenobarbital on β-glucuronidase activity whereas collagenase and β-acetylglucosaminidase did not respond any more. This seems largely in agreement with the present work in which no induction effect of phenobarbital treatment on four lysosomal enzymes in adult rats was found though the difference between the present results and those of Platt and Katzenmeier[11] about β-glucuronidase remains to be explained.

Hornef[13] found an increase of kathepsin D activity per gram of liver after phenobarbital treatment, whereas acid phosphatase activity per gram of liver in his experiments was unchanged.

At present it seems difficult to assess the precise factors which cause these differences in the findings on the effects of phenobarbital on lysosomal enzyme activity.

Acknowledgements—I am much indebted to Mr. R. Makken and Mr. A. Kiewiet for technical assistance and to Dr. J. M. W. Bouma of the department of Biochemistry for assistance in the determination of Kathepsin activities.

State University of Groningen,
Department of Pharmacology,
Bloemsingel 1,
Groningen, The Netherlands

G. J. MULDER

REFERENCES

1. W. STÄUBLI, R. HESS and E. R. WEIBEL, *J. Cell Biol.* **42,** 92 (1969).
2. R. KUNTZMAN, *Ann. Rev. Pharmac.* **9,** 21 (1969).
3. G. J. MULDER, *Biochem. J.* **117,** 319 (1970).
4. C. DE DUVE, B. C. PRESSMAN, R. GIANETTO, R. WATTIAUX and F. APPELMANS, *Biochem. J.* **60,** v, 604 (1955).
5. K. K. LUEDERS and E. L. KUFF, *Archs. Biochem. Biophys.* **120,** 198 (1967).
6. J. M. W. BOUMA and M. GRUBER, *Biochim. biophys. Acta* **113,** 350 (1966).
7. R. GIANETTO and C. DE DUVE, *Biochem. J.* **59,** 433 (1955).
8. W. H. FISHMAN in *Methods of Biochemical Analysis* (ed. D. GLICK), Vol. 15, p. 77, Interscience, New York (1967).
9. S. ORRENIUS, J. L. E. ERICSSON and L. ERNSTER, *J. Cell Biol.* **25,** 627 (1965).
10. P. ZEIDENBERG, S. ORRENIUS and L. ERNSTER, *J. Cell Biol.* **32,** 528 (1967).
11. D. PLATT and U. KATZEMEIER, *Arzneimittel-Forsch.* **20,** 258 (1970).
12. F. WILCOXON, *Biometrics* **1,** 80 (1945).
13. W. HORNEF, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmac.* **266,** 361 (1970).

Biochemical Pharmacology, Vol. 20, pp. 1331–1333. Pergamon Press, 1971. Printed in Great Britain

Influence of methamphetamine on incorporation of glucose into brain glycogen

(*Received* 28 *September* 1970; *accepted* 28 *November* 1970)

WE FOUND[1] earlier that after administration of methamphetamine to mice the glycogen content of the brain falls continuously over a period of 2 hr. Until now it has not been decided what mechanism caused this effect: Acceleration of glycogen breakdown due to the sympathomimetic effect of methamphetamine or reduced glycogen synthesis in order to make a greater part of the glucose taken up into the brain available for energy yielding processes. To clarify the cause of the methamphetamine-induced drop of the glycogen content the incorporation of glucose into brain glycogen has now been examined.