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.

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Influence of methamphetamine on incorporation of glucose into brain glycogen

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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.

As in our previous experiments[1] mice from a NMRI-strain bred in our own laboratory* were injected s.c. with 3 μg/g methamphetamine HCl. Fifteen min later 0·1 μc/g ^{14}C-labelled glucose (specific activity 288 mc/m-mole) was given i.v. Thirty min after the methamphetamine injection the animals were killed by immersion in liquid air. Untreated controls received the same amount of ^{14}C-glucose 15 min before sacrifice.

Glycogen was isolated from brain samples according to Isselhard *et al.*[2] An aliquot was used for the enzymatic determination of the cerebral glycogen content according to the principles of Keppler and Decker.[3] In this test glycogen is degraded by amyloglucosidase, and the glucose formed is measured enzymatically by means of the hexokinase and glucose-6-phosphate dehydrogenase reactions. The original procedures were modified as follows: Samples of frozen brain tissue were finely ground in a mortar chilled with liquid air. The powder was extracted with 6 vol. of 0·33 M perchloric acid in the cold using an Ultra-Turrax homogenizer. One ml of the homogenate was mixed with 3 ml 28% NaOH and heated for 25 min in a boiling water bath. 4·4 ml 95% ethanol were added, the mixture was brought to boiling again and then cooled and left overnight at room temperature to allow sedimentation of glycogen. After centrifugation at 4000 *g* for 20 min the supernatant was discarded. The precipitated glycogen was washed twice with 2 ml of a chloroform–methanol (2:8) mixture and then dissolved in 1 ml distilled water. 0·2 ml of this solution was incubated with 0·1 ml 0·1 M acetate buffer, pH 4·6 and 0·02 ml amylo-α-1,4-α-1,6-glucosidase (EC 3.2.1.—) (20 mg protein/ml) for 15 min at room temperature. The mixture was then neutralized with 2·8 ml triethanolamine buffer, pH 7·6 containing 0·1% $MgSO_4.7H_2O$. One mg NADP and 5 mg ATP in 0·2 ml distilled water, 0·01 ml hexokinase (10 mg protein/ml) (EC 2.7.1.1) and 0·02 ml glucose-6-phosphate dehydrogenase (1 mg protein/ml) (EC 1.1.1.49) were added and the increase of the optical density at 366 mμ was registered.† The amount of glycogen is calculated as μmoles glucose equivalents/g fresh tissue.

TABLE 1. CEREBRAL GLYCOGEN CONTENT AND ITS SPECIFIC ACTIVITY AFTER METHAMPHETAMINE

	Glycogen content (μmoles glucose equiv./g)	Specific activity (counts/min/μmole)
Control	2·25±0·21	2·10±0·11
	$n = 10$	$n = 9$
Methamphetamine	1·59±0·19	3·78±0·58
3 μg/g s.c.	$n = 9$	$n = 8$
30 min	$P < 0·05$	$P < 0·01$

Mean values ±S.E.

Incorporation of [^{14}C]glucose into glycogen was measured in another aliquot of the precipitated glycogen after hydrolization with 1 N HCl and subsequent neutralization using the liquid scintillation technique. Quench effect was corrected by means of internal standards.

Pilot studies revealed that 15 min after the injection of [^{14}C]glucose an equilibrium was reached and that the labelling of the cerebral glycogen remained constant for about 30 min. The effect of methamphetamine on the cerebral glycogen content and its specific activity is presented in Table 1.

Our results show that at a time when the glycogen content of the brain has declined by 29 per cent the specific activity of the cerebral glycogen has increased by 80 per cent. The increase of the specific activity of the glycogen can, therefore, not only be the result of the reduced size of the glycogen pool, which could account for an increase of the specific activity by not more than 40 per cent. It must reflect an enhanced rate of incorporation of glucose into glycogen possibly due to the greater amount of glucose present in the brain.[1] As we know, that, nevertheless, the glycogen content continues to decline we must assume that glycogen breakdown is also stimulated under our experimental conditions and even more than glycogen synthesis. Thus, the conclusion may be drawn from our results that the decrease of the glycogen content of the brain produced by methamphetamine cannot be attributed to an inhibition of glycogen synthesis but must be deduced to enhanced glycogenolysis.

* The strain was originally obtained from Zentralinstitut für Versuchstierzucht, Hannover, Germany.

† Chemicals and reagents were obtained from Boehringer Mannheim GmbH, Mannheim, Germany, or from E. Merck A. G., Darmstadt, Germany, at the highest purity available.

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Some species differences in the rates of reaction of diaphragm particulate acetylcholinesterases with tetraethyl pyrophosphate and pralidoxime

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PRETREATMENT of guinea pigs with non-toxic doses of TEPP (tetraethyl pyrophosphate) raised the LD_{50} of Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) by a factor of about 7, provided that atropine and P2S (2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate) were given shortly after Soman.[1] This treatment was ineffective in rats, 2 LD_{50}'s being fatal.[1] The postulated mechanism of action[1] is that TEPP inhibits most or all of the acetylcholinesterase (AChE; EC 3.1.1.7) of vital organs which is in excess of that required to support normal function, and that at least part of the TEPP-inhibited fraction is later reactivated by P2S. This simple hypothesis does not explain why the procedure is effective in the guinea pig but not in the rat, nor why TMB-4 (1,3-di(4-hydroxyiminomethylpyridinium) propane dihalide) given to guinea pigs instead of P2S is therapeutically ineffective in spite of being a better reactivator *in vitro*.

Parenteral administration of quaternary aldoximes fails adequately to reactivate brain AChE[2–4] because of poor penetration.[5,6] The action of these oximes is therefore mainly peripheral, and the diaphragm has been chosen as representing a vital peripheral organ.[7] Berry and Rutland[8] showed that there are two forms of AChE in diaphragm muscle of guinea pig or rat, a soluble form not sedimented by centrifuging at 100,000 *g* for 60 min, and a particulate form. The latter is almost certainly directly associated with neuromuscular function, and it is this fraction which has been studied. The "short method", by centrifuging at 500 *g*,[8] was used in the present study. The purpose of the experiments was to look for species differences in the kinetic properties of the particulate AChE, which might explain the observed toxicological findings.

The velocity of hydrolysis of 5·5 mM acetylcholine, in an initial volume of 10 cm^3 was measured by automatic continuous titration,[8] using a twin syringe assembly to keep the concentration of substrate constant. The general procedure was first to record a normal velocity, then to add 2–10 mm^3 of a stock solution of 1 mM-TEPP in propan-1-ol (to a final concentration of 0·2–1 μM). When a steady inhibited rate was established, 0·5 cm^3 of oxime solution was added, and recording continued until a new steady rate was noted. Control experiments showed that neither the dilution with titrants or oxime nor the propanol caused any appreciable departure from linearity in prolonged experiments.

It appeared that inhibition by TEPP was pseudo-reversible, i.e. that the inhibitor was stable during the period before adding oxime, and that at the steady state the rates of phosphorylation and dephosphorylation were equal. Rigorous proof of this hypothesis is difficult with preparations of low specific activity such as these.

Inhibition of guinea-pig preparations by TEPP in the range stated gave final steady rates of 15–20 per cent in 40–50 min. The final concentration of P2S then added was 0·1 mM, approximately that found in the diaphragm *in vivo* 30–60 min after intramuscular injection of the therapeutic dose of