TABLE 1. CATION DEPENDENCE OF RAT SERUM LACTONASE ACTIVITY

	μmoles of GBL hydrolyzed/min/ml serum ± S.D.	Number of determinations
Blanks		
GBL + isotonic saline	0*	4
Serum + isotonic saline	0*	4
Control		
GBL + serum + isotonic saline	26·2 ± 4·4	16
Additions to controls (Experimental)		
1. EDTA	0*	16
2. EDTA + Ca^{2+}	26·7 ± 3·3	5
3. EDTA + Ba^{2+}	0*	4
4. EDTA + Zn^{2+}	0*	4
5. EDTA + Mg^{2+}	0*	4
6. EDTA + Mn^{2+}	1·7 ± 0·3	4
7. EDTA + Co^{2+}	0*	4

* No detectable acid production observed over at least a 15-min period.

Note: EDTA was used in a concentration of 1×10^{-4}M throughout and was preincubated with serum for 5 min before the addition of the substrate, GBL. All the cations were tested in a concentration of 2×10^{-4}M and were preincubated with serum–EDTA mixture for another 5 min before the addition of GBL.

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Hydroxylation of cinchophen by microsomal enzymes

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HYDROXYLATION is a common primary metabolic fate of exogenous aromatic substances. The extent of hydroxylation and the orientation of the substituting group have been the subjects of some previous studies with intact animals and with cell-free systems.[1–3] In previous studies on cinchophen detoxication we have found that cinchophen *in vivo* is mainly hydroxylated to 4′- and 8-hydroxycinchophen in dog. This paper describes an enzyme system which hydroxylates cinchophen to 4′-hydroxycinchophen in rabbit and dog.

Male rabbits and dogs were chosen for test animals. In the rabbit the liver and in the dog both the liver and duodenum were used. The tissue samples were homogenized with Ultra-Turrax (TP 18/2) for 5–10 sec on 0·25 M sucrose solution in 0°C (40 g tissue/100 ml 0·25 M sucrose). The homogenate was centrifuged at 9000 *g* for 10 min in 0°C (Servall, SS-1). The supernatant was then centrifuged at 105,000 *g* for 30 min, 0°C (Spinco, L). The precipitate was washed once with cold 0·25 M sucrose

solution. The obtained microsome preparation was finally suspended in cold 0·1 M K-phosphate buffer, pH 7·4. The liver microsomes were suspended into 70 ml and duodenal microsomes in 10 ml of the buffer. The protein concentration of duodenal microsome preparation was about 20 mg/ml whereas the same concentration of the liver preparation was 40–50 mg/ml.

Incubation

The incubation mixture was as follows:

5 mg NADP (Boehringer et Soehne);
25 mg Nicotinic acid amide (Hoffman La Roche);
0·05 M $MgCl_2$ 0·25 ml;
0·5 M *tris*-HCl buffer, pH 7·4, in which 5 mg Na-cinchophen/ml 2·0 ml;
Microsome preparation 5·0 ml.

In experiments on the cofactor requirements NADP was replaced with NADPH-generating system which consisted of 5 mg NADP, 14 mg glucose-6-phosphate and about 50 Kornberg units of glucose-6-phosphate dehydrogenase (Sigma), 5 mg NAD and 5 mg NADH (Sigma).

The incubation period was 5 hr at 37°C in air with intermittent shaking.

Isolation and determination of 4'-hydroxycinchophen

The proteins were denatured by boiling (15 min) and removed by centrifugation. Into the precipitate was added 5 ml of 1 N HCl and kept in a boiling water bath for 15 min. The washing was added to the supernatant, into which another ml of concentrated HCl was added. The mixture was kept in a boiling water bath for 10–15 min and cooled thereafter. The incubate was extracted twice with 10 ml of butanol saturated with water. The extracts were concentrated to 2 ml by distillation. The residue was once more washed with 5 ml of water for elimination of the salts. The washed butanol extract was further concentrated to 200 μl, and pipetted into a Whatman No. 1 paper.

Paper chromatographic runs were carried out by the descending technique at room temperature with propanol-2 N NH_4OH (2+1) as the solvent The running time was 18–20 hr. After the run the papers were dried and sprayed with methanol containing 1–5 per cent NaOH. In ultraviolet light 4'-hydroxycinchophen was seen as yellow spot. The spot was cut out, eluted with water and measured spectrophotometrically at 375 mμ in an acid solution. The 4'-hydroxycinchophen amounts corresponding to the readings obtained were taken from the standard lines (1–10 μl/ml).

The results obtained with rabbit liver microsome in the cofactor study are presented in Fig. 1. It appears that the hydroxylation is activated in the presence of NADP and nicotinic acid amide.

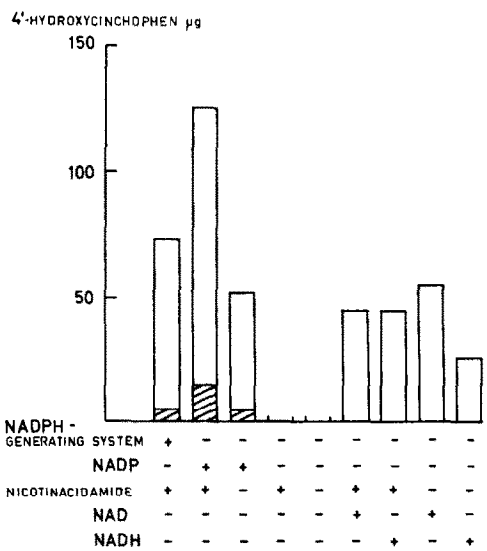

FIG. 1. The effect of various cofactors to the hydroxylation of cinchophen *in vitro*. The columns represent the results obtained with a microsomal enzyme preparation isolated from rabbit liver (white columns) and dog liver (striated columns).

Elimination of nicotinic acid amide reduces the hydroxylation activity by 60 per cent when NADP acted as the coenzyme. When NAD acted as the coenzyme removal of nicotinic acid amide did not cause any significant change whereas when NADH was used the hydroxylation activity was slightly reduced.

The microsomal hydroxylation activity of rabbit liver seemed to be about ten times greater than the activity of dog liver. The cofactor studies gave the same results in dog and rabbit so that the hydroxylation was more effective in the presence of NADP and nicotinic acid amide. In dog the hydroxylation capacity of the duodenum was found to be approximately the same as in the liver.

In previous studies we have found that cinchophen is hydroxylated *in vivo* also to 8-hydroxycinchophen in addition to 4′-hydroxycinchophen. In these *in vitro* studies no 8-hydroxycinchophen was found.

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The effect of diphenylhydantoin on sodium-, potassium-, magnesium-stimulated adenosine triphosphatase activity of rat brain*

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IT HAS been reported that diphenylhydantoin has a specific effect upon mechanisms in the neural membrane by increasing the rate of transport of Na^+ from brain cells.[1, 2]

There is evidence that Na- K- Mg-activated ATPase (Na K Mg-ATPase) is either the carrier mechanism involved in the active transport of Na^+ and K^+ or is closely related to it.[3] The present study was designed to ascertain whether or not diphenylhydantoin has any effect upon the Na K Mg-ATPase of rat brain *in vivo* or *in vitro*.

Preparation of brain tissue. Albino rats were decapitated and the entire brain, including cerebellum and medulla, was removed, weighed, and homogenized at 0° in 9 volumes of 0·32 M sucrose solution.

Adenosine triphosphatase assay. The reaction mixture contained 30 mM KCl, 3 mM $MgCl_2$, 3 mM ATP (Sigma), and 100 mM NaCl buffered at pH 7·4 with 25 mM Tris-HCl in a 1·0 ml volume. At times NaCl was omitted, and ouabain in varying concentrations was added. The mixture was incubated for 10 min at 37°. The reaction was stopped by the addition of 5·0 ml of cold 10% $HClO_4$. Reaction blanks were prepared by adding $HClO_4$ without incubation. The mixture was centrifuged, and inorganic phosphate was measured in 1·0 ml of supernatant fraction by the method of Fiske and Subbarow.[4] The Na K Mg-ATPase activity was estimated by subtracting non-Na K Mg-ATPase values from total ATPase values. Non-Na K Mg-ATPase activity was estimated by phosphate

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