of 6 min in either case. The emesis elicited through both routes of administration was eliminated after chronic ablation of the chemoreceptor trigger zone in the area postrema of the medulla oblongata. The emetic response to intravenous cassaine was blocked by pretreatment with intraventricular cassaine. Intravenous deslanoside only partially blocked the emetic activity of intraventricular cassaine. These results indicate that the emetic receptors of the chemoreceptor trigger zone are accessible to cassaine from the blood as well as from the cerebrospinal fluid, and that identical receptor elements are stimulated by the drug through both routes of administration. This lack of directional selectivity of cassaine stands in contrast to the known selectivity of the cardiac glycosides.

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1. Borison, H. L., J. Pharmacol. Exptl. Therap. **104**, 396 (1952).
2. Borison, H. L., McCarthy, L. E., Clark, W. G., and Radhakrishman, N., Toxicol. Appl. Pharmacol. **5**, 350 (1963).
3. Borison, H. L., J. Physiol. (London) **147**, 172 (1959).
4. Gaitondé, B. B., McCarthy, L. E., and Borison, H. L., J. Pharmacol. Exptl. Therap. **147**, 409 (1965)
5. Bonting, S. L. and Canady, M. R., Am. J. Physiol. **207**, 1005 (1964).
6. Koefoed-Johnsen, V., Acta Physiol. Scand., Suppl. **145**, 87 (1957).
7. Caldwell, P. C. and Keynes, R. D., J. Physiol., London **148**, 8P (1959).
8. Bonting, S. L., Hawkins, N. M., and Canady, M. R., Biochem. Pharmacol. **13**, 13 (1964).
9. Vates, Jr., T. S., Bonting, S. L., and Oppelt, W. W., Am. J. Physiol. **206**, 1165 (1964).
10. Feldberg, W. and Sherwood, S. L., J. Physiol., (London) **120**, 3P (1953).
11. McCarthy, L. E. and Borison, H. L., Anat. Record **155**, 305 (1966).
12. Shimizu, N. and Ishii, S., Z. Zellforsch. **64**, 462 (1964).
13. Rivera-Pomar, J. M., Z. Zellforsch. **75**, 542 (1966).
14. Rall, D. P. and Patlak, C. S., Federation Proc. **21**, 324 (1962).
15. Hoffman, H. J. and Olszewski, J., Neurology **11**, 1081 (1961).
16. Kao, C. Y., Pharmacol. Rev. **18**, 997 (1966).
17. Kao, C. Y. and Fuhrman, F. A., J. Pharmacol. Exptl. Therap. **140**, 31 (1963).

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Effect of Experimental Portacaval Shunt on Hepatic Drug Metabolizing Enzymes* (32711)

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Previous experiments in our laboratory have demonstrated that end-to-side portacaval shunts in rats produce hepatic injury. Diversion of the portal blood from the liver leads to hepatic siderosis (1), increased DNA synthesis, and ultrastructural changes in hepatocytes (2). However, in view of the large reserve functional capacity of the liver, these changes by themselves should not necessarily be considered evidence of a hepatic functional deficit. Since clinical portacaval shunts are often followed by an encephalopathy (3), which may result from interference with the detoxifying ability of the liver in addition to the bypass of ammonia from the liver, we thought it interesting to study this facet of liver function by assaying the effects of the experimental portacaval shunts on hepatic detoxifying enzymes.

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Material and Methods. End-to-side porta-

caval shunts were constructed in 10 male Sprague-Dawley rats, of 200 gm initial weight, by microsurgical techniques. In 10 control rats, the abdominal cavity was entered and the portal vein was mobilized, but otherwise left intact. The animals were then pair-fed for the duration of the experiment. After 26 days the rats were divided into two groups of five pairs each. The rats in one group received daily subcutaneous injections of 60 mg of phenobarbital for 4 days, while the animals in the other group were injected with an equivalent amount of saline solution. All the animals were fasted for 24 hours after the last injection and then killed, 30 days after construction of the portacaval shunt. The livers were immediately removed, weighed, and a 10% homogenate in sucrose was prepared. Aliquots of the homogenate were analyzed for the activities of aniline hydroxylase by the method of Imai and Sato (4) and for nitroreductase by the method of Fouts and Brodie (5). To estimate the amount of enzyme activity per cell, DNA content was also measured. Portions of liver were fixed in 10% formalin. Paraffin sections were prepared and stained with hematoxylin and eosin. Small blocks of liver were fixed in ice-cold 1% osmic acid in a phosphate buffer at pH 7.4. The tissue was embedded in epon, and ultrathin sections were stained with lead citrate and examined with a Hitachi HS-7 electron microscope.

Results. The shunted and pair-fed sham operated rats either remained at the initial weight or lost up to 10% of this value, after 30 days. In the shunted rats not treated with phenobarbital, the mean ratio of liver weight to body weight was 37% lower than their sham operated controls. The ratio of liver weight to body weight was increased in shunted animals treated with phenobarbital by 35%, but the mean was still 36% lower than their pair-fed phenobarbital treated controls. Sections of liver showed no difference between shunted and control animals by light microscopy.

The mean activity of aniline hydroxylase in shunted rats not treated with phenobarbital was about half that of their controls, whether using dry weight or DNA content as a ref-

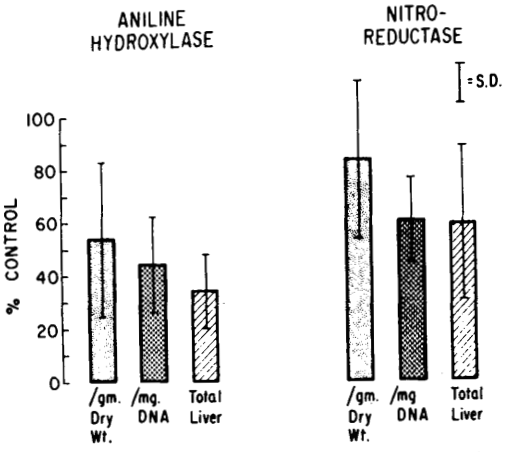

FIG. 1. Mean hepatic activities of aniline hydroxylase and nitroreductase in rats subjected to portacaval shunts, expressed as percentage of control values. Mean control values: *Aniline hydroxylase* (measured as μmoles of *p*-aminophenol) 1.62 ± 0.25/gm dry wt., 0.17 ± 0.04/mg of DNA, 1.45 ± 0.24 per total liver per 100 gm body wt. *Nitroreductase* (measured as μmoles of *p*-aminobenzoic acid) 10.03 ± 2.27/gm dry wt., 1.02 ± 0.22/mg of DNA, 9.03 ± 2.29/total liver.

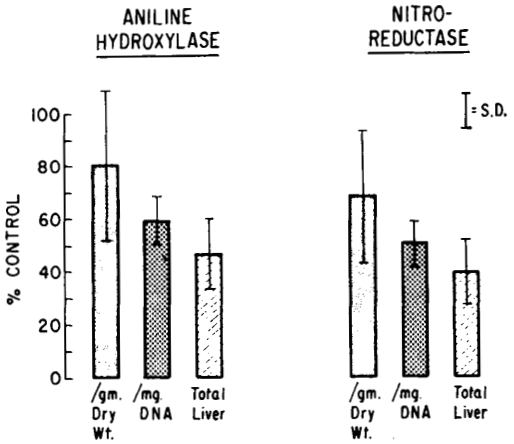

FIG. 2. Mean hepatic activities of aniline hydroxylase and nitroreductase in rats subjected to portacaval shunt after treatment with phenobarbital, 60 mg/kg body wt., for 4 days, expressed as percentage of control values. Mean control values: *Aniline hydroxylase* (measured as μmoles of *p*-amino phenol) 2.54 ± 0.53/gm dry wt., 0.40 ± 0.07/mg DNA, 3.54 ± 0.82 per total liver per 100 gm body wt. *Nitroreductase* (measured as μmoles of *p*-aminobenzoic acid) 19.02 ± 4.88/gm dry wt., 2.96 ± 0.51/mg of DNA, 26.01 ± 4.23 per total liver per 100 gm body wt.

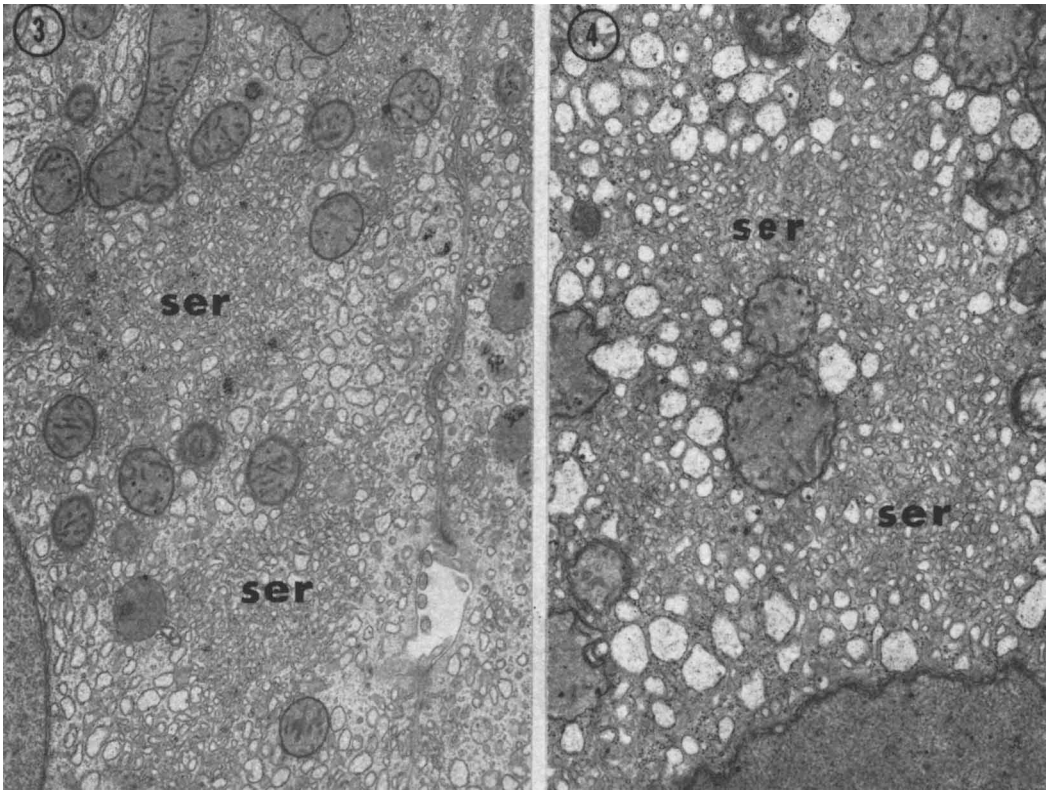

FIG. 3. Electron micrograph of liver from control rat after phenobarbital, 60 mg/kg body wt. for 4 days. Note hypertrophied smooth endoplasmic reticulum (SER). × 13,600.

FIG. 4. Electron micrograph of liver from rat subjected to portacaval shunt, after phenobarbital, 60 mg/kg body weight, for 4 days. Smooth endoplasmic (SER) reticulum is hypertrophied. × 13,600.

erence point (Fig. 1). The mean total hepatic activity in these shunted animals was only about a third that of their controls. The aniline hydroxylase activity in shunted rats treated with phenobarbital was more than triple that in untreated shunted rats when referred to DNA or total liver (Fig. 2). In the phenobarbital treated controls, the activity referred to DNA or total liver was somewhat more than twice that in untreated controls. Compared to their controls the phenobarbital treated shunted rats still had considerably lower activities of aniline hydroxylase (Fig. 2).

The levels of hepatic nitroreductase activity were more variable than those of aniline hydroxylase, and changes referred to dry weight were not statistically significant. The results using DNA and total liver as reference points were statistically significant, and were generally similar to those found for aniline hydroxylase (Figs. 1,2). In the case of nitroreductase the increase in activity after phenobarbital treatment was greater in controls than in shunted rats (Fig. 2).

Electron microscopically, as previously described (2), the hepatocytes of shunted animals displayed small lipid droplets and an increased variability of cytoplasmic density—so-called "light" and "dark" cells. Their controls appeared normal. After phenobarbital treatment the hepatocytes of both control (Fig. 3) and shunted animals (Fig. 4) revealed a striking hypertrophy of the smooth endoplasmic reticulum, without any obvious difference between them.

Discussion. Diversion of portal blood flow from the liver of the normal rat leads to de-

creased activities of the detoxifying enzymes aniline hydroxylase and nitroreductase. The amount of enzyme activity per cell is reduced, and because of the partial atrophy of the liver following diversion of the portal flow, the total enzyme activity of the liver is even more drastically decreased. In normal animals treatment with phenobarbital results in hypertrophy of the smooth endoplasmic reticulum (6) and increased activities of hepatic drug metabolizing enzymes (7). After treatment with phenobarbital, the liver deprived of portal blood can still respond with a hypertrophy of smooth endoplasmic reticulum and an increase in enzyme synthesis. Although the relative increase in the levels of drug metabolizing enzymes is comparable in both experimental and control animals, the total increase is greater in controls, since their initial levels are higher. No quantitative correlation between the degree of hypertrophy of smooth endoplasmic reticulum and the increased levels of enzymes can be made. The cause for the decreased levels of detoxifying enzymes is not clear. This defect may be a manifestation of liver cell injury, possibly anoxic. An oxygen deficit may interfere with microsomal electron transport mechanisms. Another possibility is that these enzymes are normally induced by products of bacterial flora or of digestion, which are brought to the liver from the intestine via the portal vein. Diversion of the portal flow decreases the concentration of these products and thus may reduce the concentration of an endogenous inducer. The liver does not lose its potential for reacting to an inducer, as manifested by its response to phenobarbital.

It has been suggested that portasystemic encephalopathy after portacaval shunts in man may be caused by a toxic factor which is not detoxified by the liver because of the diversion of the portal flow (3). The data here presented indicate that under these conditions a defect in the detoxifying ability of the liver itself may be an additional factor.

Summary. Diversion of the portal flow from the liver in rats results in a reduction of the activities of hepatic drug metabolizing enzymes. Such animals still respond to phenobarbital with an increase in hepatic enzyme levels and hypertrophy of smooth endoplasmic reticulum in hepatocytes. It is suggested that a defect in the detoxifying ability of the liver itself may play an additional role in portasystemic encephalopathy in man.

1. Rubin, E., Kohan, P., Fusayoshi, T., and Jacobson, J. H., II, Proc. Soc. Exptl. Biol. Med. **115**, 350 (1964).

2. Rubin, E., Gervirtz, N. R., Kohan, P., Tomita, F., and Jacobson, J. H., II, Proc. Soc. Exptl. Biol. Med. **118**, 235 (1965).

3. Sherlock, S., Am. J. Med. **24**, 805 (1958).

4. Imai, Y. and Sato, R., Biochem. Biophys. Res. Commun. **25**, 80 (1967).

5. Fouts, J. R. and Brodie, B. B., J. Pharmacol. Exptl. Therap. **119**, 197 (1957).

6. Jones, A. L. and Fawcett, D. N., J. Histochem. Cytochem. **14**, 215 (1966).

7. Remmer, H. and Merker, H. J., Klin. Wochschr. **41**, 276 (1963).

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Inhibition of the Esterase Activity of Thrombin by Na^+* (32712)

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Although it is well known that Na^+ and K^+ have opposing effects on the activities of several enzymes, to our knowledge this has not been reported to be the case with any of the proteins involved in the clotting of the blood. We are now reporting that Na^+ inhibits the hydrolysis of TAME(*p*-toluenesulfonyl-L-arginine methyl ester) by both human and bovine thrombin, while K^+ does not.

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Materials and Methods. Four preparations