nach GORNALL, BARDAWILL und DAVID[8] und die RNS nach WEBB[9] bestimmt.

Ergebnisse. Die prozentuale Verteilung der Brenzcatechinamine, der RNS und der RN-ase-Aktivität wurde pro mg Eiweiss berechnet und in der Figur 2 dargestellt. Die Fraktion 1 ist nach den biochemischen und elektronenmikroskopischen Untersuchungen von HAGEN und BARNETT[5] partikelfrei; das Vorkommen von Brenzcatechinaminen, RNS, Eiweiss und RN-ase-Aktivität in dieser Fraktion ist wahrscheinlich auf die während des Trennungsverfahrens aus geschädigten Partikeln freiwerdenden Substanzen zurückzuführen. Die Fraktion 2 besteht aus Mitochondrien und endoplasmatischem Reticulum, die Fraktion 3 aus Mitochondrien. Beide enthalten fast keine Brenzcatechinamine, wohl aber RNS (16,3% bzw. 17,3%) und RN-ase-Aktivität (15,3% bzw. 27,1%). Die Fraktion 4 enthält einen grossen Anteil an Brenzcatechinaminen (33,5%), 33,6% des RNS-Gehaltes und 23,0% der RN-ase-Aktivität, da sie in der Hauptsache chromaffine Granula enthält und ausserdem 10–15% der Mitochondrien. Die Fraktion 5 besteht nur aus chromaffinen Granula und enthält mit 49,8% die meisten Brenzcatechinamine, fast soviel RNS wie die Mitochondrienfraktion 3 (15,7%) sowie 18,7% der gesamten RN-ase-Aktivität.

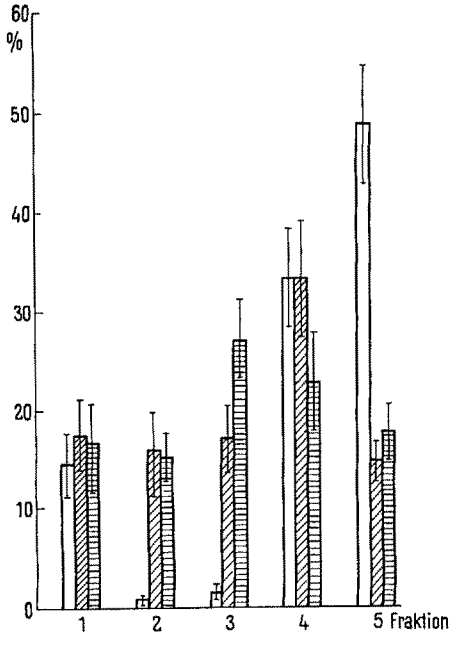

Fig. 2. Prozentuale Verteilung von Brenzcatechinaminen, RNS und RN-ase-Aktivität (pH 5,0) pro mg Eiweiss in den einzelnen Fraktionen. ☐ Amin, ▨ RNS, ▤ RN-ase-Aktivität. Mittelwerte von 4 Versuchen und deren Standardabweichungen.

Die chromaffinen Granula des Nebennierenmarks enthalten demnach eine erhebliche RN-ase-Aktivität, die für die bei 37°C spontan auftretende RNS-, Brenzcatechinamin- und ATP-Freisetzung verantwortlich sein könnte.

Summary. Homogenates from suprarenal medulla of cattle were fractionated by density gradient centrifugation. The catecholamine and RNA content of all fractions obtained, as well as their RN-ase activity, were determined and expressed as % per mg protein. The chromaffin granules contain 49.8% of the catecholamines, 15.7% of the RNA, and 18.7% of the RN-ase activity of all fractions. The demonstration of RN-ase activity in the chromaffin granules is in favour of the hypothesis that this enzyme could catalyse the spontaneous release of RNA, catecholamines, and ATP at 37°C.

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[8] A. G. GORNALL, C. J. BARDAWILL und M. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[9] J. M. WEBB, J. biol. Chem. *221*, 635 (1956).
[10] Ausgeführt mit Unterstützung der Deutschen Forschungsgemeinschaft.

Effect of Carbon Tetrachloride Intoxication on the Liver of Toad (*Bufo melanostictus*)

Fatty infiltration with degenerative changes in liver has been observed after carbon tetrachloride intoxication in rats[1], rabbits[2], guinea-pigs[3] and mice[4]. OPPENHEIMER[5] observed that the intoxication produced hepatic damage in some of the hens but not in roosters. To our knowledge the effect of this hepatotoxic agent has not been studied in lower vertebrates. In the present investigation, the effect of carbon tetrachloride administration on the liver of toad (*Bufo melanostictus*) has been described.

50 toads were used in the experiment. They were collected from natural environment during non-hibernating (May-June) and hibernating (Jan.-Feb.) seasons and kept for 2 days in the laboratory prior to experiment. The animals were given a single intraperitoneal injection of carbon tetrachloride in coconut oil; the maximum dose was 0.6 ml/100 g and the minimum 0.03 ml/100 g. Control animals were injected with coconut oil only. Some of the toads were injected with adrenaline-ephedrine solution at a dose level of 5 μ/100 g body weight 1 h after carbon tetrachloride administration and were killed after 24 h. The livers of all the animals were fixed in formol. Sections

[1] C. BAISCH and K. SCHREIER, Nuclear Med. *1*, 182 (1959).
[2] G. T. PAVLOV, Arkh. Patol. *13*, 39 (1951).
[3] V. CALI, Bull. Soc. ital. Biol. sper. *31*, 948 (1955).
[4] H. BORNIG, K. STADE, H. FRUNDER, and G. RICHTER, Z. physiol. Chem. *310*, 232 (1958).
[5] E. H. OPPENHEIMER, Bull. Johns Hopkins Hosp. *102*, 313 (1958).

were prepared by the freezing method and stained for neutral lipid by Sudan III and IV. For histological study, paraffin sections were stained with Masson's Trichrome stain[6]. Succinic dehydrogenase[7], unsaturated lipid[8], plasmal[9] and ceroid pigments[10] were also localized histochemically.

Mortality rate after carbon tetrachloride injection was found to be very high in toads. Some of them died within 2 h, even after a single injection of 0.3 ml/100 g. However, we were able to keep a few of them alive in the same condition for 8 days, injecting daily with the above dose.

In none of the non-hibernating toads could any fatty degeneration in liver be observed. In a similar type of experiment fatty degeneration was observed in rats within 6 h after a single injection of 0.03 ml/100 g. Slight cirrhotic changes in liver and a higher amount of ceroid pigment were, however, localized in the liver of toad after carbon tetrachloride intoxication. No marked change in plasmal, unsaturated lipid and succinic dehydrogenase activity was localized histochemically in the liver of the above animals after the hepatotoxic agent. A considerable change in all the above cytoplasmic constituents has previously been observed in the liver of rat damaged by the same drug (CHAKRAVARTY and DEB, unpublished). It has, however, been observed that carbon tetrachloride produced slight fatty degeneration in the liver of toad when injected together with adrenaline. It also caused similar damage in the liver of hibernating animals even when injected singly.

Several investigators are of the opinion that carbon tetrachloride produces disturbances in intralobular circulation. Ischemia and anoxia of the central zone cause nutritional deficiency in that part which results in centrolobular necrosis[11,12]. But recently[13] it has been observed that some mitochondrial enzymes are destroyed after carbon tetrachloride administration. The absence of fatty degeneration in liver of toad in spite of a very high mortality rate cannot be properly explained. It has been observed previously that transection of the spinal cord at the cervical region markedly reduces the hepatotoxic action of carbon tetrachloride in rats[14,15]. LARSON and PLAA[16] have recently demonstrated that the livers of carbon tetrachloride treated spinal cord sectioned rats kept at 24°C showed minimal changes in their livers compared to the severe necrosis observed in those kept at 34°C. They have attributed this difference to the lower metabolic rate by hypothermia. Although this can explain the failure of the hepatotoxic agent to produce damage to the liver of toad as observed in the present experiment, it fails to explain how carbon tetrachloride produced necrosis of liver during hibernation. In hibernation the rectal temperature of the toads dropped to 20–23°C from 27–32°C observed in non-hibernation (BORAL, unpublished). A drop in the metabolic rate of toads has also been noted during hibernation[17].

There is another possible explanation as regards the comparative resistance of toad's liver to the damaging action of carbon tetrachloride. BERNHEIM[18] obtained a correlation between ascorbic acid content of liver and the hepatotoxic agent, as he noted a fall in the particular vitamin after tetrachloride intoxication. A class difference as regards the biosynthesis of ascorbic acid has been observed in the toad and the rat. The site of biosynthesis in the toad is the kidney, and in the rat the liver; the rate in the former case is, however, much higher[19]. It might be possible that this higher ascorbic acid status of liver in the toad might have some protective role as to the damaging action of tetrachloride. This postulation obtained further experimental support from the observation that there was a fall in the rate of biosynthesis during hibernation[19], when the hepatotoxic agent was able to produce a damaging action.

CALVERT and BRODY[14] on the other hand have noted a decrease in the amount of adrenaline and noradrenaline in the adrenal medulla, 20 h after a single dose of this hepatotoxic agent in both rat and rabbit. According to the authors, the toxic action of carbon tetrachloride might be an indirect one (on hepatic blood vessels), mediated *via* release of catecholamines. BRODY et al.[15] observed that adrenalectomy has little protective effect on the central necrosis of liver. They have also demonstrated that the centrolobular necrosis of the liver was protected by some adrenergic blocking agents when administered simultaneously or prior to carbon tetrachloride. Absence of fatty degeneration in liver of toad after carbon tetrachloride intoxication as observed in the present experiment might possibly be related to the low adrenaline level of the animal compared to the liver of those in which this drug acts as a toxic agent. In a preliminary experiment (unpublished), we have noted a lower adrenaline level in the blood of the toad compared to that of the rat when tested on cat's blood pressure. It is probably for this reason that administration of an extra amount of adrenaline together with tetrachloride was able to produce fatty degeneration in liver. There is also a possibility that the level of adrenaline in hibernating animals is higher than in non-hibernating ones.

From the above evidence no definite conclusion as regards the comparative ineffectiveness of the hepatotoxic agent to produce damage in toads can be arrived at[20].

Résumé. Les auteurs constatent que le tétrachloride de carbone ne cause pas de dommage au foie du Crapaud non hibernant, mais provoque une dégénérescence de cet organe pendant l'hibernation de l'animal. Ils émettent des suggestions quant au mécanisme auquel seraient due cette différence.

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Department of Physiology, University College of Science and Technology, Calcutta (India), May 11, 1964.

[6] M. P. MASSON, J. Tech. Methods *12*, 75 (1929).
[7] M. M. NACHLAS, K.-C. TSOU, E. DESOUZA, C.-S. CHENG, and A. M. SELIGMAN, J. Histochem. Cytochem. *4*, 420 (1957).
[8] M. MUKHERJI, C. DEB, and P. B. SEN, J. Histochem. Cytochem. *8*, 189 (1960).
[9] E. R. HAYES, Stain Technol. *24*, 19 (1949).
[10] C. S. LEE, J. Nat. Cancer. Inst. *11*, 339 (1950).
[11] L. E. GLYNN and H. P. HIMSWORTH, Clin. Sci. *6*, 235 (1948).
[12] M. V. PATWARDHAN, V. RAMALINGASWAMY, S. SRIRAMACHARY, and V. N. PATWARDHAN, Ind. J. Med. Sci. *8*, 15 (1954).
[13] J. D. JUDAH and K. P. REES, Fed. Proc. *18*, 1013 (1959).
[14] D. N. CALVERT and T. M. BRODY, Am. J. Physiol. *198*, 669 (1960).
[15] T. M. BRODY, D. N. CALVERT, and A. F. SCHNEIDER, J. Pharm. exp. Therap. *131*, 341 (1961).
[16] R. E. LARSON and G. L. PLAA, Exper. *19*, 604 (1963).
[17] C. DEB and M. MUKHERJI, Bull. Nat. Inst. Sci. Ind. No. 19, 155 (1962).
[18] M. L. C. BERNHEIM, Biochem. Pharmacol. *7*, 59 (1961).
[19] R. N. ROY and B. C. GUHA, Nature *182*, 319 (1958).
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