ouabain is less toxic than in intact rats. This curious phenomenon is now under investigation. The LD_{100} of intact animals has been determined previously as about 17 mg/kg[10] and the LD_{50} as 5.5 mg/kg[11]. Variations in the toxicity of ouabain in different strains of rats is not unusual.

In the experiments with chlorothiazide[3], nephrectomy was necessary in order to eliminate interference by the diuretic action of the dosage applied. However, pronounced oligodipsia is found in nephrectomized rats with doses of ouabain only about $^1/_{25}$–$^1/_{50}$ of the LD_{50} (see Figure). Such doses are unlikely to exert any diuretic action. Therefore it appeared possible that the oligodipsic effect of the glycoside may be demonstrated also in intact animals. The Figure shows that indeed normal rats drink less when small doses of ouabain are administered. The dose-response curve is less steep than the curve for nephrectomized animals, and in the range of 0.25 to 2.0 mg/kg the effect on normal animals is weaker.

The present observations reveal a new extrarenal effect of ouabain, antagonistic to its diuretic action, but do not yet permit safe conclusions as to its localization. Further experiments with implantation of ouabain into various parts of the brain are necessary to establish beyond doubt a central mechanism for the oligodipsia evoked by this drug.

Table I. Effect of ouabain on water intake after subcutaneous injection of hypertonic sodium chloride

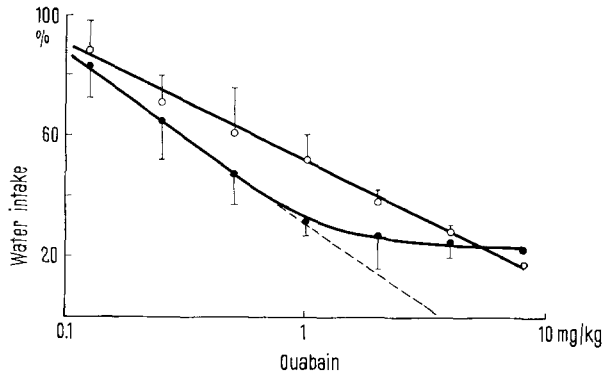
Ouabain mg/kg	Water consumption (ml/kg) during the period (h)				
	0–2	2–4	4–20	Total	% of control
A. Nephrectomized rats					
Control	49	25	53	127	100
0.25	36	31	54	121	95
0.5	26	21	50	97	76
1.0	16	8	40	64	50
B. Normal rats					
Control	58	11	75	144	100
0.25	45	9	93	147	102
0.5	46	9	74	129	90
1.0	28	7	65	100	69

Groups of 4 rats received 50 ml/kg of 3% NaCl by subcutaneous injection and ouabain, dissolved in 0.5 ml isotonic saline, intravenously. The controls received intravenous saline only

Table II. Acute toxicity of ouabain in normal and nephrectomized rats

Ouabain mg/kg	Death rate	
	Normal rats	Nephrectomized rats
9	0/4	
10	2/8	
11	2/8	0/4
12	7/9	1/8
13		2/4
14		6/7

Groups of 4 rats of 200-300 g body weight received i.v. injections of ouabain in 0.5 ml isotonic saline.



Dose-response curves for the depression of water intake by intravenous ouabain. Groups of 4 rats received 50 ml/kg of 3% NaCl s.c. and the dose of ouabain shown on the abscissa. o—o Normal rats; •—• nephrectomized animals. Each point, with the exception of 8 mg/kg, represents the mean of 3 experiments. Vertical bars indicate standard deviation.

Résumé. L'ouabain diminue la consommation d'eau, stimulée par l'injection d'une solution sallée hypertonique, chez les rats normaux ou néphrectomisés.

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Chronic Hepatic Injury Following Experimental Viral Hepatitis in the Dog

Experimental production of chronic hepatic disease in animals has been possible only by withdrawal of essential nutrients, administration of toxic chemicals or a combination of both. Although much useful information has been obtained by these methods, the relation of hepatic injury produced in this way to the chronic liver disease seen following viral infection remains doubtful. The availability of an experimental model bearing a closer resemblance to chronic hepatic disease in man would be desirable. This report describes a spontaneously occurring form of prolonged hepatic injury in dogs following experimental infection with the virus of hepatitis canis. This disease pattern, when contrasted with the course of acute canine hepatitis, appears to be distinctly different.

In the course of a study on the correlation of viral invasion with hepatic dysfunction[1] during hepatitis in 12–16-week-old pure-bred Beagle dogs, at least 16 examples of subacute and chronic progressive hepatic damage have been encountered. These puppies of 5–7 kg

[1] R. Preisig, D. Gocke, J. Sweeting, T. Morris, and S. E. Bradley, Gastroenterology *48*, 511 (1965).

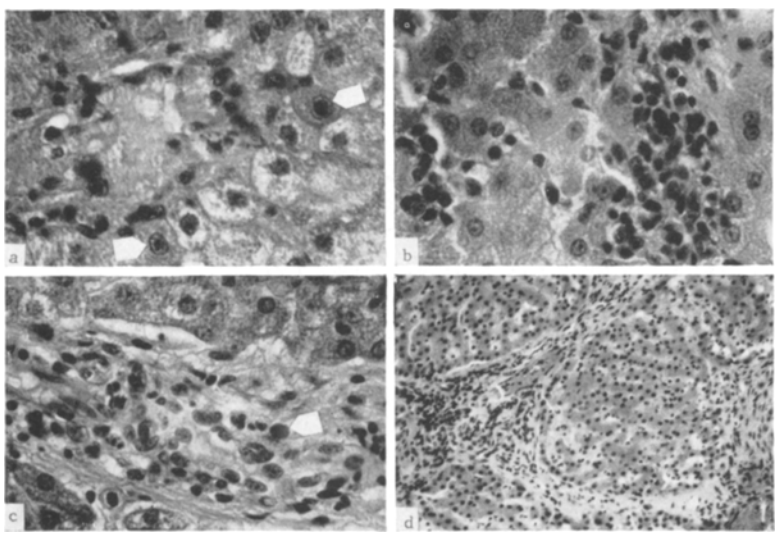
body weight had been reared on an isolated farm and were pre-immunized against distemper and leptospirosis. Upon arrival in the laboratory, they were infected intraocularly with 300 $TCID_{50}$ of canine hepatitis virus. In contrast to animals with acute disease which died in 4–6 days with extensive hepatic necrosis, these dogs survived the initial phase even though there was definite evidence of viral involvement of the liver. Foci of necrosis with characteristic viral intranuclear inclusion bodies, which stained with fluorescein labelled antiviral antibody, could be demonstrated in the first week after infection (Figure a).

6 of these dogs died 8–21 days after infection. At autopsy, severe hepatic necrosis with dense collections of round cells and plasma cells was found, but the virus could not be demonstrated any longer. The remaining 10 dogs survived up to 8 months.

Serial hepatic biopsies demonstrated the evolution of a pattern characterized by absence of intranuclear inclusions or fluorescent staining, and by the appearance of lymphocytes and plasma cells, predominantly localized in dense collections about the portal regions (Figure b and c). Eventually, fibrosis appeared in these areas and progressed to 'bridge-formation' (Figure d).

It was observed that all the dogs with this chronic disease pattern had low levels of neutralizing antiviral antibody prior to infection. Since animals dying with the accute illness never had detectable antibody, it was suspected that partial immunity may have modified the course of the infection. Consequently, attempts were made to modulate the disease by intravenous administration of a dose of immune serum calculated to yield serum titers between 1/4 and 1/100 in the animal. Although sufficient time has not yet elapsed for adequate observation of all of these dogs, in at least 5 of 8 animals sequential hepatic biopsies have shown the evolution of the same chronic pattern described above.

Thus, it appears that partial immunity may modify the course of canine hepatitis, with resulting chronic hepatic injury. This modification of viral hepatitis in the dog may provide a model suitable for the study of liver injury resembling chronic hepatitis in man.



Sequential hepatic biopsies demonstrating the evolution of progressive hepatic damage following infection with canine hepatitis virus (dog 'Tally'; pre-infection neutralization titer 1/100; 300 $TCID_{50}$ of virus intraocularly; hematoxylin and eosin stain). (a) Day 5 after infection: small focus of hepatic necrosis with characteristic intranuclear viral inclusions (arrow). × 430. (b) Day 27 after infection: accumulation of round cells and plasma cells in hepatic parenchyma. × 430. (c) 2 months after infection: portal tract infiltrated with round cells and plasma cells (arrow). × 430. (d) 7 months after infection, postmortem: section showing chronic inflammation, fibrosis and bridging. × 100.

Zusammenfassung. 20 junge, nicht immune Beagle Hunde (Neutralisationstiter < 1/4) wurden mit 300 ID_{50} (Gewebskultur) des Hepatitis-canis-Virus infiziert. Alle Tiere starben innerhalb von 4–6 Tagen an ausgedehnter Lebernekrose. Im Gegensatz dazu überlebten 16 partiell immune Tiere (Neutralisationstiter 1/4 bis 1/100) die akute Erkrankung. Im Verlaufe einer Überlebenszeit bis zu 8 Monaten entwickelte sich eine der menschlichen Hepatitis chronica ähnliche Lebererkrankung.

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A Useful New Stain for Electron Microscopy

The use of aqueous salts of *dodeca*-tungstoboric acid for negative contrast examination of protein molecules has been suggested by ROWE[1]; it was said to be preferable to phosphotungstate because it caused less molecular aggregation. I thought that tungstoborate might also be a good direct stain, that is, useful for the electron microscopic examination of ultra-thin sections of embedded tissues. This has proved to be so.

Tungstoboric acid crystals (Hopkin and Williams) are very soluble both in water and in alcohol. For staining they have been used at 10% concentration w/v in ethanol. In this form the intensity of staining seems of the order obtained with 2% uranyl acetate in methanol, but with the advantage that no contaminating precipitate is obtained by exposure to the atmosphere.

[1] A. J. ROWE, Proc. R. Soc. B *160*, 437 (1964).