functions as a neuroendocrine conduit (*12*), transporting various peptides made in one brain region to sites of action in distant brain areas. A possible function of vasopressin in CSF is regulating the behavioral processes of learning and memory. This conjecture is based primarily on studies of the vasopressin-deficient Brattleboro rat, which has deficits in acquisition and retention of information necessary for success in active and passive avoidance training (*13*). The deficit in retention can be corrected by vasopressin administration, the intraventricular route of delivery being several hundred times more effective than the systemic route (*14*). Also, inactivation of endogenous CSF vasopressin by intraventricular administration of vasopressin antiserum to normal rats induces severe impairment of memory (*15*); systemic administration of the antiserum elicits no behavioral effects despite inducing profound alterations in water balance.

An interesting aspect of memory consolidation is evidence that the circadian timekeeping system plays an important role in that behavior (*16–18*). For example, rats manifest a repetitive daily variation in retention performance after one-trial passive-avoidance training (*17*). Also, disrupting circadian organization in the rat results in a long-term loss of memory (*18*). Thus, our finding of a daily vasopressin rhythm in mammalian CSF provides a potential link between the reported effects of CSF vasopressin on memory and the circadian character of memory processes and thus strengthens the argument that vasopressin in the CSF may function physiologically to modulate memory.

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4. Cats weighing 3.5 to 5.0 kg were individually housed in cages under an automated daily light-dark cycle (12:12), with lights on from 0600 to 1800; food and water were freely available, and the time of day that daily care was provided was randomized. An indwelling styleted metal cannula was inserted into the cisternal CSF space of anesthetized animals. The exposed end of the implanted cannula was sleeved with a hollow brass screw and both were anchored to the skull with dental cement. Two to six weeks after the operation, the stylet was removed, polyethylene tubing was attached to the cannula, threaded through a protective stainless steel spring, and connected to the inlet port of a single-channel cannula swivel (Alice King Chatham Medical Arts, Los Angeles) located at the top of the cage. A nut affixed to the proximal end of the steel spring was then threaded over the brass screw on the head, and the distal end was attached to the base of the cannula swivel. From the outlet port of the swivel, polyethylene tubing led to a peristaltic pump in an adjoining room, whereby CSF was continously withdrawn for periods up to 10 days at a constant rate of 600 μl/hour and collected as 2-hour fractions by an automated fraction collector. The CSF was collected for a 24-hour period preceding the start of an experiment. The collected CSF samples remained in the fraction collector for up to 8 hours at room temperature (20° to 22°C) and were then stored at −40°C until analysis. We determined that vasopressin is stable in CSF for at least 24 hours at room temperature.
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8. Continuous CSF samples and intermittent blood samples were withdrawn from free-ranging cats over a 24-hour period in constant light; the period of constant light was accomplished on the experimental day by leaving the lights on throughout the normal 12-hour dark period of diurnal lighting. Blood samples were collected from an indwelling venous catheter, surgically placed through the external jugular vein into the superior vena cava, and by use of a two-channel swivel incorporated into the tethering system described for continuous CSF withdrawal (*4*). Withdrawn blood samples (2 ml) were transferred to chilled tubes containing 10 μl of 15 percent K_2EDTA and centrifuged at 4°C. Vasopressin was extracted from 1 ml of plasma by use of the bentonite extraction technique [W. R. Skowsky, A. A. Rosenbloom, D. A. Fisher, *J. Clin. Endocrinol. Metab.* **38**, 278 (1978)]. For each plasma sample, vasopressin content of duplicate portions, each equivalent to 400 μl of plasma, of the resuspended extract was determined by radioimmunoassay (*5*). The lower limit of assay sensitivity for plasma vasopressin was 0.1 μU/ml.
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Toxic Injury to Isolated Hepatocytes Is Not Dependent on Extracellular Calcium

Abstract. *Freshly isolated hepatocytes from phenobarbital-treated rats were incubated in the presence or absence of extracellular calcium with three differently acting liver cell toxins, namely carbon tetrachloride, bromobenzene, and ethylmethanesulfonate. In the absence of extracellular calcium these three compounds were far more toxic to the cells than in its presence. This result is inconsistent with the hypothesis that an influx of extracellular calcium is required as the final step in toxic liver cell injury.*

Liver cell death and its morphological expression as necrosis have been the subject of many clinical and experimental studies. Since liver cell death may be the ultimate result of a number of different hazards in the cell's environment, including toxic chemicals, anoxia, and viruses, various experimental models have been designed to investigate the morphological and biochemical changes associated with liver cell injury and death. As a result, morphological and biochemical changes of increasing severity have been described and attempts have been made to define "a point of no return" at which the process becomes irreversible (*1–3*). Moreover, several hypotheses have been proposed to explain the underlying biochemical lesion [for example, see (*3*)]. One such hypothesis is that the plasma membrane is the primary target in the pathogenesis of liver cell death and that the functional consequence of this membrane injury is a lethal influx of a high concentration of Ca^{2+} ions into the liver cells (*4*). The influx of extracellular Ca^{2+} has therefore been proposed as the final common pathway in toxic liver cell death (*4*, *5*).

Although it has been known for many years that calcium accumulates in necrotic tissues, such accumulations of

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Ca^{2+} could always be explained as simply a passive equilibration of Ca^{2+} concentration along a very steep electrochemical gradient, because the extracellular Ca^{2+} concentration is approximately 1000 times higher than the cytosolic Ca^{2+} concentration (*6*). However, recent studies involving the use of primary cultures of adult rat hepatocytes have apparently shown an absoute requirement for extracellular Ca^{2+} in the killing of these cells by a variety of different toxins, not requiring metabolic activation to a proximate or ultimate toxin, such as phalloidin and ethylmethanesulfonate (EMS) (*5, 7*). In direct contrast to this finding, the results of the present study show that extracellular Ca^{2+} is not required for the killing of freshly isolated hepatocytes by three well-known liver cell toxins, namely carbon tetrachloride (*8*), bromobenzene (*2, 9*) and EMS (*7*).

Suspensions of isolated hepatocytes are frequently used for studies on the metabolism and toxicity of foreign compounds (*2, 9, 10*). The viability of the freshly isolated cells is invariably high and immediately after isolation the cells exclude both trypan blue and reduced nicotinamide adenine dinucleotide (NADH) (Fig. 1) and contain concentrations of adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), reduced NADP (NADPH), and glutathione (GSH) similar to those observed in the isolated perfused liver (*11*). On incubation of the hepatocytes in a balanced salt solution containing 2.6 m*M* Ca^{2+}, for up to 5 hours, there is a slight decrease in ATP and GSH concentrations but no measurable change in the total level of NADP, NADPH, or in the NADPH/$NADP^+$ ratio. Trypan blue exclusion reveals a small decrease, and NADH penetration a corresponding increase (Figs 1, a and c). However, after 4 hours of incubation the hepatocytes are still approximately 80 percent viable (Figs 1, a and c). Moreover, since freshly isolated hepatocytes are able to metabolize foreign compounds at rates comparable to those observed in vivo or with the isolated perfused liver (*12*), it is possible to study the toxicity of foreign compounds requiring metabolic activation to a proximate or ultimate toxin, such as carbon tetrachloride (*8*) and bromobenzene (*2, 9*). This is not possible when primary cultures of adult rat hepatocytes, maintained in complete media, are used as the experimental model because these cultured hepatocytes rapidly lose their ability to metabolize foreign compounds (*13*). We have previously described the cell isolation and incubation procedures and the various characteristics of the liver cells before and after long-term incubation (*9, 10*).

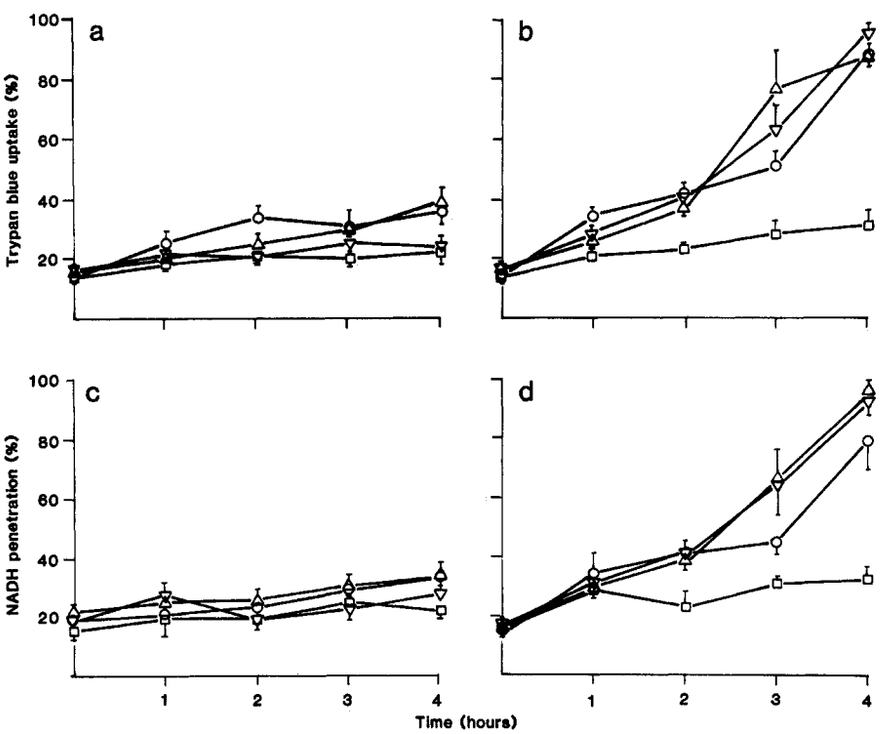

Fig. 1. Toxic liver cell death is not dependent on extracellular calcium. Isolated hepatocytes were prepared from the livers of phenobarbital-treated male Sprague-Dawley rats (200 to 250 g) by the method of collagenase perfusion as described (*10*). Sodium phenobarbital was administered intraperitoneally at a daily dose of 80 mg/kg for 3 days in order to induce the microsomal monooxygenase enzyme system. The yield of each preparation was 2×10^8 to 4×10^8 cells, as measured by counting the final cell suspension in a Buerker chamber. Immediately after isolation the cells excluded both trypan blue and NADH (90 to 95 percent). Incubations of hepatocytes were performed at 37°C in rotating round-bottom flasks (*17*) under an atmosphere of 95 percent O_2 and 6.5 percent CO_2 at a cell concentration of 10^6 cells per milliliter in either Ca^{2+}-free (b and d) or normal (2.6 m*M* Ca^{2+}) Krebs-Henseleit buffer, *p*H 7.4 (a and c) supplemented with 25 m*M* Hepes (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid). Cell viability was assayed by both trypan blue exclusion (a and b) (*10*) and NADH penetration with use of the lactate dehydrogenase latency test (c and d) (*17*). The amount of cell death in the absence (□) and presence of either (△) carbon tetrachloride, (○) bromobenzene, or (▽) EMS is expressed as the percentage of stained cells (a and b) or as the percentage of cells permeable to NADH (c and d). Values represent the means and standard errors of four separate experiments.

Both carbon tetrachloride and bromobenzene are well-known hepatotoxins requiring metabolic activation in order to become cytotoxic (*2, 8, 9*). Carbon tetrachloride is known to be metabolized by the hepatic microsomal monooxygenase system to highly reactive free radicals which interact with cellular constituents and initiate peroxidation of the membrane lipids (*8, 14*). This, in turn, alters cellular membrane structure and function and consequently causes disturbances in electrolyte distribution, swelling, and the appearance of intracellular enzymes in the plasma, which eventually leads to cell death (*3, 4, 8, 14*).

Bromobenzene produces centrilobular liver necrosis when administered in sufficient doses to laboratory animals (*2, 9*). Hepatocytes isolated from phenobarbital-treated rats metabolize bromobenzene to a reactive intermediate, which has been tentatively identified as bromobenzene-3,4-epoxide (*15*). This metabolite may subsequently become rearranged nonenzymatically to yield the corresponding phenol, act as a substrate for epoxide hydrolase to produce a transdihydrodiol, or react with GSH to form the corresponding conjugate (*2, 9*). The last reaction is the main pathway for inactivation of the epoxide. Thus, in the absence of GSH, the reactive intermediate can accumulate intracellularly and interact with various low molecular weight and macromolecular nucleophiles (*2, 9*). The final result of these interactions is cell death.

Ethylmethanesulphonate is different from both carbon tetrachloride and bromobenzene in that it does not require metabolic activation in order to become cytotoxic. It is a widely used mutagen and its reactivity results in the alkylation of many tissue macromolecules including membrane constituents.

From Fig. 1, a and c, it can be seen

that isolated hepatocytes from phenobarbital-treated rats are relatively resistant to the toxic effects of carbon tetrachloride (1.0 m*M*), bromobenzene (0.6 m*M*), and EMS (8 m*M*) for up to 4 hours when they are incubated at 37°C in Krebs-Henseleit buffer, *p*H 7.4, containing 2.6 m*M* Ca^{2+}. However, when similarly prepared hepatocytes are incubated at 37°C in Ca^{2+}-free Krebs-Henseleit buffer, *p*H 7.4, in the presence of either 1.0 m*M* carbon tetrachloride, 0.6 m*M* bromobenzene, or 8 m*M* EMS, approximately 90 percent of the cells are permeable to both NADH and trypan blue after 4 hours (Fig. 1, b and d), whereas control hepatocytes incubated in Ca^{2+}-free Krebs-Henseleit buffer alone are still approximately 70 percent viable after 4 hours (Fig. 1, b and d). It therefore appears that these three compounds are far more toxic to isolated hepatocytes in the absence of extracellular Ca^{2+} than in its presence. This result is inconsistent with the notion that an influx of extracellular Ca^{2+} is required as the final step in toxic injury of liver cells caused by carbon tetrachloride, bromobenzene, and EMS. Moreover, similar results are obtained when 0.1 m*M* EGTA, a highly specific Ca^{2+} chelator (*16*), is added to the Ca^{2+}-free Krebs-Henseleit buffer, *p*H 7.4. Thus, if carbon tetrachloride, bromobenzene, and EMS are toxic to isolated liver cells in Ca^{2+}-free buffer containing EGTA, it is apparent that the toxic liver cell injury caused by these compounds, as measured by two well-established parameters of liver cell viability, namely, trypan blue exclusion (*10*) and NADH penetration (*17*), is not dependent on extracellular calcium.

We therefore conclude that the influx of extracellular Ca^{2+} is not the final common pathway for the toxic death of isolated liver cells. We do not know why an influx of extracellular Ca^{2+} appears to be required for the toxic killing of cultured hepatocytes (*5, 7*) but not for freshly isolated hepatocytes. However, this discrepancy may be due to different effects of extracellular Ca^{2+} on attached and suspended liver cells. Moreover, our findings are still compatible with the hypothesis that the plasma membrane is a primary target in toxic liver cell injury, and that changes in its permeability to other ions, such as Na^+ and K^+, may be a crucial part of events that lead to cell death.

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Competition for Dispersal in Ant-Dispersed Plants

Abstract. *Two closely related and coexisting plants (Chenopodiaceae) of the Australian arid zone are adapted for seed dispersal by ants. These facultatively perennial shrubs persist in saltbush communities largely as a result of highly directional dispersal to ant mounds, where conditions are favorable for establishment and growth. The two species grow predominantly on mounds and compete for dispersal to these favorable microhabitats.*

Active seed dispersal by animals is characteristic of many land plants and of most plants in certain habitats (*1*). Ecologists have speculated that these plants compete for dispersal services, but the evidence has been inferential. Fruits are often superabundant; when unharvested, they decline in quality and attractiveness, and many seeds are lost to seed predation and decay (*2, 3*). Differences among coexisting species in fruit type and fruiting phenology have been interpreted as evolutionary adaptations to minimize simultaneous demand for limited dispersal agents (*2, 4*). We provide the first quantitative data showing that competition for dispersal services affects plant population dynamics.

Myrmecochory is the dispersal of plant propagules by ants. In exchange for dispersal services, myrmecochorous plants provision their diaspores (dispersal units) with ant-attractive food bodies. Often, the advantage to the plant is a reduction in the rate of parasitism or the intensity of competition as seeds are removed from the vicinity of the parent (*5*). Myrmecochores are remarkably common in the flora of Australia; an estimated 1500 species occur in dry heathlands and sclerophyll forests alone (*6*). Our studies (*7, 8*) suggest that in the Australian arid zone, myrmecochory functions primarily to position seeds in favorable microhabitats for establishment and growth. We report on investigations of arid zone myrmecochores in two closely related genera, *Sclerolaena* and *Dissocarpus* [united until recently (*9*) in *Bassia* (Chenopodiaceae)]. We demonstrate that (i) myrmecochores are differentially abundant on ant mounds, where diaspores are concentrated by the foraging activities of ants; (ii) ant mounds represent favorable microhabitats for myrmecochorous and nonmyrmecochorous plants alike; (iii) myrmecochores are relatively poor competitors when growing away from mounds; and (iv) mound populations of one myrmecochore are reduced significantly in the presence of a second species of ant-dispersed plant.

Sclerolaena diacantha and *Dissocarpus biflorus* var. *biflorus* are common ant-dispersed shrubs in the saltbush communities of arid central Australia (*7*). The diaspores of both species consist of

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