It is suggested that, under the conditions of the experiments reported, the phenothiazine derivatives decrease the permeability of erythrocytes to water by altering cell membrane structure. However, the possibility must also be considered that chlorpromazine may affect the exit of intracellular ions, or act on the membrane in such a manner as to increase the critical hemolytic volume of the cells.

Work is presently under way testing these possibilities. Preliminary experiments employing techniques other than the one used in this study have already indicated that 10^{-5} chlorpromazine, the addition of which produces no pH change, can cause red blood cell membrane permeability changes. This low concentration affecting a cell membrane compares favorably with that needed to influence a subcellular (mitochondrial) membrane.[4]

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A mechanism for the induction by carbon tetrachloride of fatty liver in the rat

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THE occurrence of a fatty liver following poisoning with chlorinated hydrocarbons, such as chloroform or carbon tetrachloride, has long been established.[1] The mechanism by which the fatty infiltration of the liver is produced, however, has not been understood. It has been demonstrated recently that, following the administration of CCl_4 to intact animals, there is a decrease in the concentration of plasma triglycerides and a simultaneous increase in liver triglycerides.[2] This observation is suggestive in part either of an inhibition of release of triglyceride by the liver or increased uptake of triglyceride by the liver, or both. We have investigated these possibilities using the isolated perfused rat liver. It is clear from our observations that the release of triglyceride by the liver into the perfusate is completely inhibited, whereas uptake proceeds at normal or moderately increased rates when the livers are obtained from CCl_4-poisoned animals.

EXPERIMENTAL

Male rats weighing 250–350 g, maintained on a balanced ration *ad libitum*, were given by stomach tube, per 100 g of body weight, 0·5 ml of a 1 : 1 (v/v) mixture of CCl_4 and mineral oil.* After 3·5 hr, the liver was isolated and perfused through the portal vein *in vitro*. The perfusion procedure, substrates and analytical methods have been reported previously.[3, 4]

Triglycerides were estimated by the method of Van Handel and Zilversmit.[5] The results presented in Table 1 demonstrate the inhibition of release of triglyceride by the liver induced by administration of CCl_4. All animals treated with CCl_4 exhibited a significant decrease in the concentration of serum triglycerides. The release experiments measured net changes in total perfusate triglyceride in the absence of an exogenous source of triglyceride. The negative number observed after treatment with

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CCl_4, in contrast to that of control animals which secreted triglyceride into the medium, implies a net uptake of endogenous triglycerides from the circulating diluted blood. Release was measured over a 3-hr period. Uptake was estimated by the rate of removal of triglyceride radioactivity from a perfusate containing a synthetic neutral fat emulsion (Lipomul) 0·075% (w/v) labeled with tripalmitin-1-^{14}C. Uptake for the initial 30-min period is recorded.

Since the liver is a major source of plasma triglyceride, this inhibition of release of triglyceride may account, in part, for the decreased levels of plasma triglycerides seen in poisoning with CCl_4.

TABLE 1. RELEASE AND UPTAKE OF TRIGLYCERIDE BY ISOLATED PERFUSED RAT LIVER

Treatment of donor animal	Release experiments μmoles of triglyceride per g of liver in the perfusate	Uptake experiments Percent of original cpm in the perfusate removed per g of liver
(A) CCl_4 in mineral oil	0·7 ± 0·2 (5)	3·2 ± 1·5 (5)
(B) Mineral oil only	1·4 ± 0·9 (5)	—
(C) No treatment	1·0 ± 0·4 (5)	2·3 ± 1·2 (5)

Figures indicate means ± standard deviations. The figures in parentheses indicate the number of experiments.

Statistical analysis: I. Release experiments
1. A *vs.* B, $t = 4{\cdot}92$, $P = 0{\cdot}001$
2. A *vs.* C, $t = 8{\cdot}35$, $P = 0{\cdot}0001$
3. B *vs.* C, $t = 0{\cdot}89$, $P = 0{\cdot}25$

II. Uptake experiments
1. A *vs.* C, $t = 1{\cdot}08$, $P = 0{\cdot}20$

The inhibition of triglyceride release by the liver occurs in the presence of significantly elevated levels of hepatic triglycerides.[2, 6] Accordingly, the release of triglycerides by the liver is not dependent solely upon the direction of the concentration gradient, but also may be affected by chemical or hormonal effects on various metabolic steps within the hepatic cell. Similar conclusions may be inferred by comparison of rates of outward transport of triglycerides from livers of fed and fasted rats.[3, 4] The livers from the animals fasted for 48 hr, in contrast to those from fed rats, do not release triglycerides into the perfusate, even though the concentration of hepatic triglycerides in the liver from fasted rats is equal to or greater than that in the liver from fed animals.

The mechanism by which CCl_4 induces these changes in hepatic lipid metabolism is unknown. In these experiments, CCl_4 was administered to the animal, not added to the perfusate *in vitro*. The changes ascribed to CCl_4 may be attributable to a direct hepatotoxic effect of this agent and of $CHCl_3$, as suggested by the observations of Brauer *et al.*, on the isolated perfused liver,[7] or may be mediated via adrenergic mechanisms, as indicated by the work of Calvert *et al.* with intact animals.[8,9]

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The depleting effect of vincamin on the cerebral serotonin level

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VINCAMIN is a cristalline alkaloid isolated from the plant *Vinca minor L.* with an unknown structural form. According to investigations hitherto carried out it has an indol skeleton. The compound has a considerable central hypotensive effect.[1] As pointed out in our previous paper, the noradrenalin content of the rat cerebrum and of other tissues is reduced by vincamin pretreatment.[2]

To elucidate further the mechanism of action, the influence of the compound on the cerebral serotonin content was investigated.

Our experiments were carried out on Wistar rats weighing 200–250 g. After intraperitoneal administration of vincamin the test animals were decapitated at different times. After removing the cerebellum, half the cerebral tissue was used for each determination. After homogenization in acetone the cerebral tissue was shaken for half an hour, and afterwards centrifuged; the acetone phase was evaporated in N_2 flow in vacuum at 35 °C. The dissolved lipids were removed by washing with petrol ether. The serotonin content of the extract was determined on a rat fundus band preparation according to Vane.[3] The disturbing effect of the noradrenalin was eliminated by enzymic degradation carried out with poliphenoloxidase.[4] The results obtained in our experiments are shown in the following table expressed in terms of mμg/g wet tissue.

TABLE 1. EFFECT OF VINCAMIN ON SEROTONIN CONTENT OF RAT CEREBRUM

Time, hr	Serotonin mμg/g	Mean	Percent of the control	*P*
0	230, 403, 333, 230, 223, 227, 341, 215, 275 326, 226, 238, 345, 298, 326, 313, 385	286 ± 50	100	
2	262, 285, 290, 233	268 ± 19	94	> 0·5
3	246, 220, 295, 161	231 ± 47	81	>0·2
4	167, 120, 211, 185, 122, 251, 124, 145	166 ± 45	58	≪0·01
6	196, 217, 157, 155	181 ± 27	63	≪0·01
16	254, 213, 318, 283	267 ± 38	94	> 0·5

The time recorded is the interval between intraperitoneal administration of 50 mg/kg vincamin and removal of the brain.

In the table only the effect of 50 mg/kg vincamin is given. For smaller doses no significant effect could be detected.

As may be seen from the table, after vincamin administration a slight decrease in the serotonin level could be observed, a significant difference as compared with the control value appeared only after 4–6 hr. In the 16th hr after administration of vincamin the cerebral serotonin level was normal again. The reducing effect is a considerable one but as compared to that caused by reserpin only of a minor degree, since complete depletion of the serotonin level is caused by a 5 mg/kg dose of the latter within 2 hr. Greater depletion cannot be caused by vincamin, as higher doses have a toxic effect and kill the animals.