REFERENCES

1. M. J. FREGLY, I. W. WATERS and J. A. STRAW, *Fedn Proc.* **24**, 255 (1965).
2. D. KUPFER and L. PEETS, *Biochem. Pharmac.* **15**, 573 (1966).
3. W. PEARL and D. KUPFER, *Fedn Proc.* **26**, 683 (1967).
4. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177**, 75 (1949).
5. D. KUPFER and L. L. BRUGGEMAN, *Analyt. Biochem.* **17**, 502 (1966).
6. P. HOCHSTEIN and L. ERNSTER, *Biochem. biophys. Res. Commun.* **12**, 388 (1963).
7. Z. A. PLACER, L. L. CUSHMAN and B. C. JOHNSON, *Analyt. Biochem.* **16**, 359 (1966).
8. A. M. GOTTO, R. M. HUTSON, A. W. MEIKLE and O. TOUSTER, *Biochem. Pharmac.* **14**, 989 (1965).
9. A. H. CONNEY, E. C. MILLER and J. A. MILLER, *Cancer Res.* **16**, 450 (1956).
10. H. V. GELBOIN and N. R. BLACKBURN, *Cancer Res.* **24**, 356 (1964).

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An interaction of disulfiram and ethanol on lipid metabolism

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IT IS well known that the administration of a single large dose of ethanol to fasted rats produces an accumulation of liver triglycerides.[1–2] Among the several factors that have been postulated to contribute to this effect, the availability of free fatty acids (FFA), as substrate for the synthesis of triglycerides seems to be an important one. In fact it has been demonstrated that the fatty acid moieties of triglycerides accumulated in the liver after ethanol administration originate from adipose tissue[3] and that an inhibition of the lipolytic activity of adipose tissue reduces the accumulation of triglycerides in the liver of ethanol treated rats.[4–5]

Disulfiram (TETD or Antabuse (R)) was introduced in 1948 in the treatment of chronic alcoholism because of its unpleasant symptoms produced in connection with ethanol.[6]

Since fatty liver has been observed in the pathogenesis of chronic alcoholism in man[7] it was of theoretical and possible practical interest to study the interaction between ethanol and disulfiram on the accumulation of liver triglycerides in rats. Overnight fasted male Sprague–Dawley rats of the average body weight 170 ± 10 g received 16 ml/kg of 50% v/v ethanol by oral route, or disulfiram 200 mg/kg by i.p. route, or both treatments. Results reported in Table 1 indicate that disulfiram given simultaneously with ethanol was able to prevent the formation of fatty liver.

TABLE 1

Treatment	Time* (hr)	Liver Triglycerides mg/100 g ± S.E.	Plasma FFA μEquiv/l ± S.E.	Adipose tissue FFA μEquiv/ g ± S.E.
Controls	—	217 ± 34	716 ± 96	10·7 ± 0·4
Disulfiram	8	234 ± 18	821 ± 40	—
Ethanol	8	1168 ± 83	621 ± 75	10·3 ± 0·6
Disulfiram + Ethanol	8	264 ± 20†	248 ± 14†	3·5 ± 0·2†
Ethanol	18	1044 ± 77	—	—
Disulfiram + Ethanol	18	312 ± 59†	—	—

* Time = Interval between treatment and sacrifice.

The rats received 16 ml (50% v/v) of ethanol/kg by stomach tube and at the same time Disulfiram i.p. in a dose of 200 mg/kg.

Disulfiram was suspended in saline (40 mg/ml) by means of a few drops of Tween 80.

Triglycerides were determined according to Van Handel and Zilversmit[12] with minor modifications. FFA in plasma and in epididymal adipose tissue were determined according to Trout *et al.*[13]

Number of animals in each group was at least 5.

† = $P < 0.01$ in respect to ethanol.

The effect was still present 18 hr after the treatment. Since FFA are important substrates for the synthesis of triglycerides, determinations of FFA were carried out in plasma and in adipose tissue.

It is clear that at 8 hr the plasma and the adipose tissue FFA are low only in the group treated with disulfiram and ethanol (see Table 1). Other experiments have been performed in order to establish the levels of plasma FFA at different times, before the onset of the fatty liver, in the groups treated with ethanol or with disulfiram + ethanol. Table 2 shows that ethanol itself depresses plasma

TABLE 2

Time hr	PLASMA FFA μEquiv/l. ± S.E.		
	Ethanol	Disulfiram + Ethanol	Disulfiram
0	716 ± 96	716 ± 96	656 ± 32
1	335 ± 49	374 ± 31	618 ± 59
2	295 ± 42	298 ± 6	656 ± 6
4	347 ± 19	270 ± 33*	607 ± 47
6	395 ± 27	293 ± 22*	508 ± 25
7	563 ± 57	319 ± 18*	531 ± 23

Time = interval between treatment and sacrifice.
The rats received ethanol (50% v/v) 16 ml/kg oral, disulfiram 200 mg/kg i.p., or both.
Plasma FFA were determined according to Trout with minor modifications.
* = $P < 0.01$ in respect to ethanol.

FFA but beginning from the 4th hr after the treatment the group receiving disulfiram + ethanol had always lower plasma FFA than the group receiving only ethanol. It may be therefore concluded that the inhibition of fatty liver induced by disulfiram is at least in part related to a decreased availability of plasma FFA. The mechanism by which plasma FFA are reduced is not yet clear. Disulfiram is known to inhibit the synthesis of noradrenaline a powerful lipolytic agent[8] blocking the dopamine-β-hydroxylase.[9-10] The dose of disulfiram used does not presumably decrease the catecholamine in the adipose tissue[11] and it does not affect lipolysis. However it may be possible that in the combination disulfiram-ethanol the disulfiram metabolism is impaired and an higher concentration may be available in the adipose tissue. Other studies are in progress.

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REFERENCES

1. S. MALLOW and J. L. BLOCH, *Am. J. Phys.* **184**, 29 (1956).
2. N. R. DI LUZIO, *Am. J. Phys.* **194**, 453 (1958).
3. M. POGGI and N. R. DI LUZIO, *J. Lipid Res.*, 437 (1964).
4. A. BIZZI and S. GARATTINI, *Progr. Biochem. Pharmac.* **3**, 320 (1967).
5. A. BIZZI, E. VENERONI, A. LIETTI and S. GARATTINI, *Eur. J. Pharmac.* in press (1968).
6. J. HALD and E. JACOBSEN, *Acta Pharmac. Tox. Kbh.* **4**, 305 (1948).
7. C. S. LIEBER, D. P. JONES and J. H. MENDELSON, *Trans Ass. Am. Physns* **76**, 289 (1963).
8. B. B. BRODIE, R. P. MAIKEL, and O. N. HERN, in *Handbook of Physiology. Sect. V- Adipose tissue* (Eds. A. E. RENOLD and G. F. CAHILL JR), p. 583 Am. Physiol. Soc. Washington (1965).
9. J. M. MUSACCHIO, M. GOLDSTEIN, B. ANAGNOSTE, G. POCH and I. J. KOPIN, *J. Pharmac. Exp. Ther.* **152**, 56 (1966).

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10. M. GOLDSTEIN, *Life Sci.* **5**, 175 (1966).
11. P. F. SPANO, L. VARGIU, F. CRABAI, S. CONGIU, R. GESSA and G. L. GESSA, *Boll. Soc. ital. Biol. sper.* **48**, 640 (1967).
12. E. VAN HANDEL and D. B. ZILVERSMIT, *J. Lab. Clin. Med.* **50**, 152 (1957).
13. D. L. TROUT, E. H. ESTES JR. and S. J. FRIEDBERG, *J. Lipid Res.* **1**, 199 (1960).

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Mode of spirolactone action: competitive inhibition of aldosterone binding to kidney mineralocorticoid receptors*

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KAGAWA[1] obtained pharmacological evidence that the spirolactone class of aldosterone antagonists competitively interact with mineralocorticoids for receptor sites in target tissues. However, Lockett and Roberts[2] were unable to demonstrate antagonism of aldosterone-induced sodium retention by two spirolactones in adrenalectomized, hypophysectomized animals. As an alternative explanation, Lockett and Roberts suggested that spirolactones antagonize the renal actions of mineralocorticoids by changing the rate of secretion of growth hormone.

Fanestil and Edelman[3] demonstrated that kidney nuclei contain physiologically specific receptors for mineralocorticoids. This provides a method for testing the mechanism of spirolactone antagonism of aldosterone action. The results confirm Kagawa's inference by indicating that the water-soluble spirolactone, SC-14266,* competitively inhibits aldosterone interaction with renal mineralocorticoid receptors.

METHODS AND RESULTS

Extraction of unmetabolized ^{3}H-aldosterone, determination of protein and DNA, and liquid scintillation counting methods were as previously described.[3] In the first experiment, 22 adrenalectomized rats (purchased from Charles River Breeding Laboratories) were injected sc. with $28{\cdot}6 \times 10^{-11}$, $14{\cdot}3 \times 10^{-11}$ or $7{\cdot}15 \times 10^{-11}$ mole ^{3}H-aldosterone (sp. act., 35 c/m-mole; supplied by New England Nuclear Corp.). One-half of the animals simultaneously received 5×10^{-7} mole SC-14266 in 1 ml saline by a separate sc. injection. Thirty minutes later the animals were sacrificed under ether anesthesia, heparinized blood was collected by cardiac puncture and the kidneys were excised. The purified kidney nuclear fraction was isolated by centrifugation through 2·2 M sucrose as previously described in detail.[3] The amount of unmetabolized ^{3}H-aldosterone per mg DNA in the isolated nuclei was plotted against the plasma concentration of unmetabolized ^{3}H-aldosterone in double reciprocal fashion (Fig. 1). In the absence of spirolactone, the reciprocal of the nuclear aldosterone content was linearly dependent upon the reciprocal of the plasma aldosterone concentration. In the animals which received spirolactone, these relationships were again linear, but the slope of the line was markedly increased with little change in intercept value, effects that are characteristic of competitive inhibition.

In a second experiment, 10 adrenalectomized animals were injected with $2{\cdot}8 \times 10^{-9}$ mole ^{3}H-aldosterone (10 μc). Half of the animals received by separate injection $2{\cdot}8 \times 10^{-5}$ mole SC14266 (this is approximately the same ratio of aldosterone:spirolactone used in the first experiment). Thirty minutes later the kidneys were removed, homogenized and separated into purified nuclear, mitochondrial, microsomal and 100,000 *g* supernatant fractions (see ref. 3 for experimental details).

* The systematic name for SC-14266 is potassium 3-(3-oxo-17 beta-hydroxy-4,6-androstadien-17-alpha-yl) propionate.