hypothetical inhibitor of lipoprotein lipase in man [22], may influence the activity of this enzyme in TCDD or streptozotocin treated rats.

The raised HDL cholesterol levels after TCDD are similar to those detected after chlorinated hydrocarbons [6], TCDD being, however, considerably more potent and with a longer duration of activity. The presently accepted physiological role for HDL in man, i.e. removal of tissue cholesterol and transport to biliary elimination sites [23] is, as yet, unproven in the rat. The similarities of the apoprotein changes with those occurring in streptozotocin diabetic rats suggest, however, that HDL cholesterol levels may be increased following non-dietary treatments which enhance liver and protein biosynthesis. In these apparently divergent conditions, i.e. diabetes and TCDD hypercholesterolemia, HDL are probably a newly synthesized reservoir of excess lipids or of apoproteins with as yet unknown functional properties.

In summary, TCDD increases HDL cholesterol levels in rats and modifies apoprotein composition, in particular increasing the CIII-3/CIII-0 protein ratio.

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Center E. Grossi Paoletti, Chemotherapy and Pharmacognosy Chairs, University of Milano, 20129 Milano, Italy

ANDREA POLI
GUIDO FRANCESCHINI
LINA PUGLISI
CESARE R. SIRTORI

REFERENCES

1. L. Jirasek, J. Kalensky, K. Kubek, J. Pazderova and E. Lukas, *Cs. Dermat.* **49**, 145 (1974).
2. R. M. Oliver, *Br. J. ind. Med.* **32**, 49 (1975).
3. A. P. Poland, D. Smith, G. Metter and P. Possick, *Archs envir. Hlth* **22**, 316 (1971).
4. N. E. Jensen and A. E. Walker, *Proc. R. Soc. Med.* **65**, 687 (1972).
5. W. B. Kannel, *Ann. intern. Med.* **74**, 1 (1971).
6. T. T. Ishikawa, S. McNeely, R. M. Steiner, C. J. Glueck, M. Mellies, P. S. Gartside and C. McMillin, *Metabolism* **27**, 89 (1978).
7. L. A. Carlson and B. Kolmodin-Hedman, *Acta med. scand.* **192**, 29 (1972).
8. G. J. Miller and N. E. Miller, *Lancet* **1**, 16 (1975).
9. G. R. Warnick and J. J. Albers, *J. Lipid Res.* **19**, 65 (1978).
10. R. J. Havel, H. A. Eder and J. H. Bragdon, *J. clin. Invest.* **34**, 1345 (1955).
11. L. I. Gidez, J. B. Swaney and S. Murnane, *J. Lipid Res.* **18**, 59 (1977).
12. J. L. Witztum and G. Schonfeld, *Diabetes* **27**, 1215 (1978).
13. B. A. Schewets, J. M. Norris, G. L. Sparschu, V. K. Rowe, P. J. Gehring, J. L. Emerson and C. G. Gerbig, *Envir. Hlth Perspects.* **5**, 87 (1973).
14. P. W. Albro, J. T. Corbett, M. Harris and L. D. Lawson, *Chem.-biol. Interact.* **23**, 315 (1978).
15. N. E. Miller and P. J. Nestel, *Clin. Sci. molec. Med.* **45**, 257 (1973).
16. R. Pelkonen, R. Fogelholm and E. A. Nikkila, *Br. Med. J.* **4**, 85 (1975).
17. K. A. Narayan, *Atherosclerosis* **13**, 205 (1971).
18. E. A. Nikkila, M. Kaste, C. Ehnholm and J. Viikari, *Br. Med. J.* **2**, 99 (1978).
19. C. B. Blum, R. I. Levy, S. Eisenberg, M. Hall, R. H. Goebel and M. Berman, *J. clin. Invest.* **60**, 795 (1977).
20. H. Baron, P. S. Roheim and H. A. Eder, *J. clin. Invest.* **57**, 714 (1976).
21. R. L. Hamilton, M. C. Williams, C. J. Fielding and R. J. Havel, *J. clin. Invest.* **58**, 667 (1976).
22. W. V. Brown and M. L. Baginsky, *Biochem. biophys. Res. Commun.* **46**, 375 (1972).
23. Y. Stein, M. C. Glangeaud, M. Fainaru and O. Stein, *Biochim. biophys. Acta* **380**, 106 (1975).

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Effect of oxamniquine on *Schistosoma mansoni:* some biological and biochemical observations*

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Ornithine-δ-transaminase [E.C.2.6.1.13] (OTA) figures importantly in the biosynthesis of the amino acid proline in mammals [1, 2] and has also been reported to occur in the blood fluke *Schistosoma mansoni* [3]. Senft [4] noted that free proline concentrates in the nervous system and the germination areas of the adult schistosomes.

Oxamniquine (UK-4271) is a fairly new schistosomicide [5] and there are as yet no published data regarding its mode of action at the molecular level. Consequently it was deemed interesting to test the effect of this drug on the OTA activity in *S. mansoni* both *in vitro* and *in vivo*. The *in vitro* conditions for evaluating the action of oxamniquine on OTA from *S. mansoni* were the following: adult worms grown in ICR mice were removed live by perfusion and subsequently maintained in Petri dishes in M199 + 10 per cent inactivated fetal calf serum in the presence of 20 mM HEPES, 200 U/ml penicillin G and 200 μg/ml streptomycin. After adding varying amounts of oxamniquine to the Petri dishes, these were placed for 0, 3, 6, 9, 12, 18 or 24 hr in a 10% CO_2 incubator at 37°, and following the incubation the worms from each dish were lyophilized. In the *in vivo* experiments, groups of 22 mice with 7-weeks-old schistosome infections were injected i.m. with a single dose of 200 μg/kg oxamniquine. During the next 11 days, two mice per day were killed by cervical dislocation and the worms in the liver and intestine of each mouse counted separately, then pooled and lyophilized. The OTA enzyme assay was performed on homogenates from the lyophilized

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worm material by the method of Strecker [2], as modified by Ertel and Isseroff [6].

In the *in vitro* studies, all worms were still alive 24 hr after the addition to the maintenance medium of 0.2 mg/ml oxamniquine. This is unlike the findings of Foster and Cheetham [5] who reported 44 per cent dead male worms and 21 per cent dead females with the same dosage. Similarly to Chavasse *et al.* [7], the present authors found hyperactivity of the worms at the stated concentration of oxamniquine. Furthermore, none of the worms adhered to the bottom of the plastic Petri dishes whereas untreated (control) worms did. Preliminary trials failed to show any inhibitory effect of the oxamniquine at this concentration on the OTA activity. At 0.4 mg oxamniquine/ml, however, there was 50 per cent mortality of male schistosomes and 10 per cent mortality of female schistosomes after 24 hr of incubation. All the worms, in fact, already exhibited sluggish movement and an evidently damaged tegument 6 hr after introduction of the drug into the maintanance medium. An oxamniquine concentration of 0.6 mg/ml was fully schistosomicidal after 12 hr. Therefore a concentration of 0.4 mg/ml was used for the enzymatic studies *in vitro*. At this concentration oxamniquine, after 6–18 hr of incubation, caused a 27–30 per cent decrease in the schistosome OTA activity compared to that in the control (untreated) worms.

As for the enzymatic studies *in vivo*, it can be seen from Fig. 1 that the OTA activity of the worms diminished steadily during the 11 days post drug administration to the mouse host, so that on the 11th day a decrease of about 87 per cent in the enzymatic activity was registered compared to that on day 0. It can also be seen from the figure that the percentages of male and female worms shifted to the liver as a result of the drug were almost identical from the start. Foster and Cheetham [5] found a greater female shift during the first 4 days post drug administration but the dose of oxamniquine per mouse used by them was 50 mg/kg, whereas in the present investigation 200 mg/kg were employed. On day 4, most of the worms found in the liver were paired, active and contained abundant caecal hematin, but from the 6th day on, the worms were encountered separate, the hematin content progressively diminished and movement considerably slowed down. Death of worms was noted from the 8th day on. Thus on the basis of the described criteria, namely, OTA activity, hepatic shift, hematin content, motility and viability of the drug-treated worms, it is clear that a single administration of oxamniquine produced a steady decrease in the schistosome OTA activity along with a constantly increasing detrimental effect on the adult worms. These results certainly do not prove nor imply that oxamniquine acts as a schistosomicide by inhibiting OTA activity. However, a decrease of this activity could conceivably interfere with the metabolism of *S. mansoni* either by diminishing the proline production of the worm or by leading to the accumulation of excess ornithine in it. According to Senft [8], proline appears to be synthesized in *S. mansoni* from arginine as the final step in the sequence of: arginine→ornithine→Δ′-pyrroline→proline. Arginine, in turn, plays a role in the urea secretion and nitrogen balance of schistosomes. Perhaps, then, the accumulation of excess ornithine in the blood fluke *S. mansoni* might interfere with its nitrogen balance.

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Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel

MIRIAM GOLDBERG
DANIEL GOLD
ELIEZER FLESCHER
JACOB LENGY

REFERENCES

1. A. Meister, *Biochemistry of the Amino Acids*, 2nd Edn, Vol. 2, p. 685. Academic Press, New York (1965).
2. J. H. Strecker, *J. biol. Chem.* **240**, 1225 (1965).

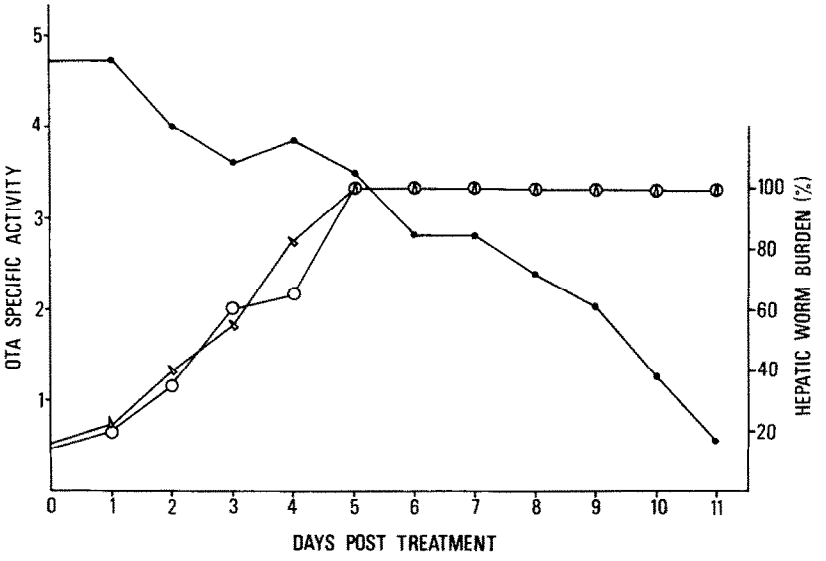

Fig. 1. *Schistosoma mansoni:* OTA specific activity of the adult worms and their proportion in the liver of the host mouse following its treatment by a single dose of oxamniquine (200 mg/kg, administered intramuscularly). —●—, OTA acitivity; —○—, per cent of male worms in liver; —△—, per cent of female worms in liver. Day 0 baseline data obtained on worms removed from untreated mice. Each point represents a mean of 8 separate determinations (or counts).

3. M. Goldberg, E. Flescher and J. Lengy, *Exp. Parasitol.* **47**, 333 (1979).
4. A. W. Senft, *Comp. Biochem. Physiol.* **27**, 251 (1968).
5. R. Foster and B. L. Cheetham, *Trans. R. Soc. Trop. Med.* **67**, 674 (1973).
6. J. Ertel and H. Isseroff, *Rice Univ. Stud.* **62**, 79 (1976).
7. C. J. Chavasse, M. C. Brown and D. R. Bell, *Ann. trop. Med. Parasitol.* **72**, 293 (1978).
8. A. W. Senft, *Ann. N.Y. Acad. Sci.* **113**, 272 (1963).

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Release of noradrenaline from cat cerebral arteries by different drugs and potassium

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The existence of receptors for 5-hydroxytryptamine (5-HT, serotonin) which mediate the vasoconstrictor effects of this amine in the cerebral blood vessels, has been demonstrated [1–3]. Apart from this direct effect on the tryptaminergic receptors, it has been observed that 5-HT has the ability to release noradrenaline from several tissues [4–9], and this indirect effect contributes to the overall actions of 5-HT. Furthermore, Fozard and Mwaluko [6] have demonstrated on the isolated rabbit heart that this noradrenaline release is calcium-dependent, suggesting that it is an exocytotic-like process. However, Starke and Witzell [9] have reported that the greater effects of serotonin on the perivascular adrenergic nerve endings of the rabbit pulmonary artery seem to be tyramine-like.

The aim of the present investigation was to study in a comparative manner the mechanism of noradrenaline release evoked by serotonin from cat pial arteries with that induced by tyramine, ionophore X537A and potassium (K^+) whose patterns or mechanisms of adrenergic neurotransmitter release have been extensively studied in peripheral tissues. Thus, tyramine is taken up by the adrenergic nerve terminals and causes stoichiometric displacement and release of noradrenaline [10] by a non-exocytotic mechanism [11, 12]. Ionophore X537A is a drug which increases the permeability of membranes to calcium and other divalent and monovalent ions [13] causing secretion of catecholamines apparently by a non-exocytotic process [14–16]. Finally K^+ releases noradrenaline from adrenergic fibres by a process similar to the physiological efflux of adrenergic neurotransmitter, i.e. by exocytosis [17, 18].

Cats of either sex ranging in weight from 1.5 to 3 kg were anesthetized with 35 mg/kg of sodium pentobarbital administered intraperitoneally and killed by bleeding. The brain was carefully removed and the circle of Willis arteries with their ramifications were dissected and placed in a Petri dish which contained ice-cold Kreb–bicarbonate solution (KB). In this medium the blood was removed and the arteries (10–20 mg) pooled. Afterwards the arteries were placed on a cylindrical nylon net and put into a beaker containing 4 ml of oxygenated KB. After a 15 min equilibration period at 37°, the tissues were then exposed to (±) (^{3}H)-noradrenaline (^{3}H-NA, 2×10^{-7}M, specific activity 10.7 Ci/mmole) for 30 min and thoroughly washed with fresh KB solution at 10 min intervals during a 100 min period.

To estimate the spontaneous tritium release, the arteries were successively immersed in 5 vials containing 2 ml of fresh KB solution for 3 min periods. The drug-evoked release was analyzed by transferring the tissue to another 4 vials, each one containing 2 ml of KB with the appropriate concentration of the drugs studied (140 mM K^+; 10^{-5}M for all other drugs); finally the arteries were again exposed to fresh KB in another 5 vials in order to recover the basal level of tritium efflux. Total radioactivity present in the media was analyzed by adding 0.5 ml of each sample to 10 ml of Bray's solution [19] and it was measured in a Nuclear Chicago liquid scintillation counter, model ISOCAP 300, using the external standard method to correct for quenching. The results are expressed as cpm/mg of wet tissue.

The composition of the normal KB was: NaCl, 119 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4.7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM; glucose, 11.1 mM and disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), 0.03 mM. This solution was equilibrated with 95% O_2 and 5% CO_2 and the final pH was 7.4–7.5. In some experiments, 80 min after the incubation period, the arteries were washed with KB without calcium to the end of the experiment.

The following drugs were used: 5-hydroxytryptamine creatinine sulfate and tyramine hydrochloride (Sigma); potassium chloride (Merck); (±) (^{3}H)-noradrenaline hydrochloride (Radiochemical Centre, Amersham), and ionophore X537A (Ro2-2985, Lasalocid, Hoffman-La Roche).

Serotonin and tyramine were prepared as stock solutions in physiological saline containing 0.01% (w/v) ascorbic acid and kept frozen (−20°). Stock solutions of ^{3}H-NA were made in 0.01 N HCl and stored at 4°. X537A was dissolved in ethanol and stored at −20°; the final concentration of ethanol in the KB solution was always 0.1%.

High-K^+ solution (140 mM) was prepared by adding KCl saturated solution to KB to the adequate amount. In three experiments, the effects of and equivalent hyperosmolar Krebs solution (made up with sucrose, 252 mM) on ^{3}H-efflux were tested.

Results were expressed as means ± S.E. of the means. Deviations from means were statistically analyzed by the Student's *t*-test. A probability value of less than 5 per cent was considered significant.

The spontaneous tritium efflux from pial arteries preloaded with ^{3}H-NA showed a rapid initial decay followed by a moderate loss, which practically levelled off after 90 min of washout.

Figure 1 shows the pattern of ^{3}H-release evoked by serotonin. Addition of 5-HT (10^{-5}M) to the incubation medium produced a rise of the release of radioactivity which reached the peak 6 min later, followed by a slow decrease.

Calcium-deprivation greatly inhibited the secretory effects of 5-HT. If the background release is subtracted from the drug-evoked release, the net efflux of tritium in the absence of calcium is significantly lower than in the presence of this cation ($P < 0.01$).

Tyramine (10^{-5}M) considerably increase the release of radioactivity from the brain arteries. This release was superior to that produced by the same concentration of 5-