only about one-fiftieth as potent as serotonin in inhibiting [^{3}H]serotonin binding *in vitro*, this compound appears to be more potent as an inhibitor of such binding than any previously reported non-indole compound thought to act as a serotonin receptor agonist. In particular, the compound was more potent than either quipazine or MK-212, two compounds that have been reported to have numerous effects *in vivo* suggested to result from activation of serotonin receptors [8, 9, 15–17]. 1-(*m*-Trifluoromethylphenyl)-piperazine itself has actions consistent with serotonin agonist activity *in vivo* in rats. For instance, it decreases brain serotonin turnover [6] and elevates serum hormones (corticosterone and prolactin) whose secretion has previously been found to be increased by agents that enhance central serotoninergic function [18, 19]. The elevation of serum prolactin by this compound was at one time considered possibly to result from dopamine agonist activity [20], but *in vitro* binding studies (D. T. Wong and L. R. Reid, personal communication) indicated that 1-(*m*-trifluoromethylphenyl)-piperazine had very little ability to inhibit the binding of either [^{3}H]dopamine (IC_{50} 35,000 nM) or [^{3}H]spiperone (IC_{50} 4300 nM) to rat brain membrane receptors. These findings indicate some specificity of this agent for the serotonin receptor.

1-(*m*-Trifluoromethylphenyl)piperazine was a more potent inhibitor of [^{3}H]serotonin binding than of [^{3}H]-D-lysergic acid diethylamide) ([^{3}H]LSD) binding to brain membranes *in vitro*. Its IC_{50} value as an inhibitor of [^{3}H]LSD binding was 300 nM. The same was true for quipazine, another serotonin agonist, whose IC_{50} value as an inhibitor of [^{3}H]LSD binding was 1700 nM. In contrast, the IC_{50} values of serotonin receptor antagonists like metergoline and methysergide were found to be lower with [^{3}H]LSD binding than for [^{3}H]serotonin binding. These differences have been reported previously for serotonin receptor agonists and antagonists [1, 4] and support the idea that 1-(*m*-trifluoromethylphenyl)-piperazine acts on brain serotonin receptors as an agonist rather than an antagonist.

In summary, various 1-phenyl-piperazines and related compounds have been shown to be effective in inhibiting the binding of [^{3}H]serotonin to rat brain membranes *in vitro*. The most effective compound was 1-(*m*-trifluoromethylphenyl)-piperazine. Variation in the piperazine moiety of this compound greatly diminished the ability of this compound to inhibit [^{3}H]serotonin binding. Some variation in the nature of the *meta*-substituent retained activity but compounds with substituents in other positions of the phenyl ring were less active.

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Increased total and high density lipoprotein cholesterol with apoprotein changes resembling streptozotocin diabetes in tetrachlorodibenzodioxin (TCDD) treated rats

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Hyperlipidemia has been frequently mentioned among clinical findings in subjects exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In particular, a 56 per cent incidence of hypercholesterolemia was described [1] in the follow-up of Czechoslovakian factory workers contaminated with TCDD; more recently, a type IIA hypercholesterolemia was diagnosed in three young scientists exposed to TCDD [2]. In other studies, however, a significant prevalence of hyperlipidemia among intoxicated subjects was not detected [3, 4].

These controversial reports, related to a documentedly important prognostic factor for the development of coronary vascular disease [5], suggested that animal studies on this topic be carried out within the special program on long term effects of TCDD in Seveso, Italy. Particular interest was devoted to lipoprotein changes induced by

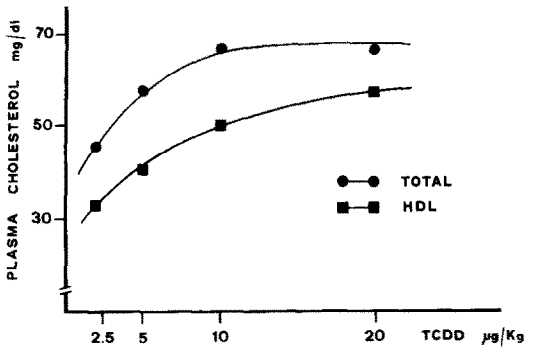

Fig. 1. Effects of single, graded doses of TCDD on plasma total and HDL cholesterol levels. A dose-dependent increase of total and HDL cholesterolemia is evident 21 days after the i.p. administration of the substance.

TCDD, since recent studies have indicated that chlorinated hydrocarbons may increase high density lipoprotein (HDL) cholesterol levels in rats [6], as well as possibly in exposed humans [7]. Elevated HDL cholesterol levels are a hypothetical protective factor against the development of coronary heart disease [8]. The experiments were carried out in the special laboratory on the ICMESA grounds (i.e. of the company where the TCDD accident took place) in Seveso, and were designed to determine lipid and lipoprotein changes after single doses of TCDD. In a first experiment, graded doses of the substance were given to identify the nontoxic dose range and the short term effect. In a successive larger experiment, rats were killed at intervals up to two months after TCDD administration, to assess lipoprotein composition and histological alterations with time. Minimum numbers of animals had to be used in all experiments, to keep environmental contamination, in a heavily polluted area, as low as possible.

Male rats of the Sprague–Dawley strain (Charles River, Calco, Italy) weighing 300–330 g at the beginning of each experiment were used. TCDD (Kor Isotopes, Cambridge, MA) was administered i.p. dissolved in acetone/corn oil (1/9). Controls received the same vehicle. Plasma samples were collected after an overnight fast by free bleeding from the abdominal aorta under light ether anesthesia. Individual plasma cholesterol, triglyceride and HDL cholesterol levels, by heparin–$MnCl_2$ precipitation [9] were determined. Plasma pools collected from two animals were also subjected to preparative ultracentrifugation [10]. Levels of HDL cholesterol were essentially identical when determined by selective precipitation or by preparative ultracentrifuge, as also shown by Ishikawa *et al.* [6]. Isoelectric focusing of ultracentrifugally separated HDL was carried out between pH 3.5 and 10 and 4 and 6 (Ampholine, LKB, Bromma, Sweden) as described by Gidez *et al.* [11], followed by densitometry. Linearity of densitometric response to increasing concentrations of applied lipoproteins was established, as suggested by Witztum and Schonfeld [12]. Lipoproteins were examined by electron microscopy after negative staining with 0.1 M K-phosphotungstate. Liver toxicological tests (AAT, OAT, bilirubin, alkaline phosphatase) were obtained from all animals. Samples from the major parenchymal organs were dissected for light and electron microscopic studies.

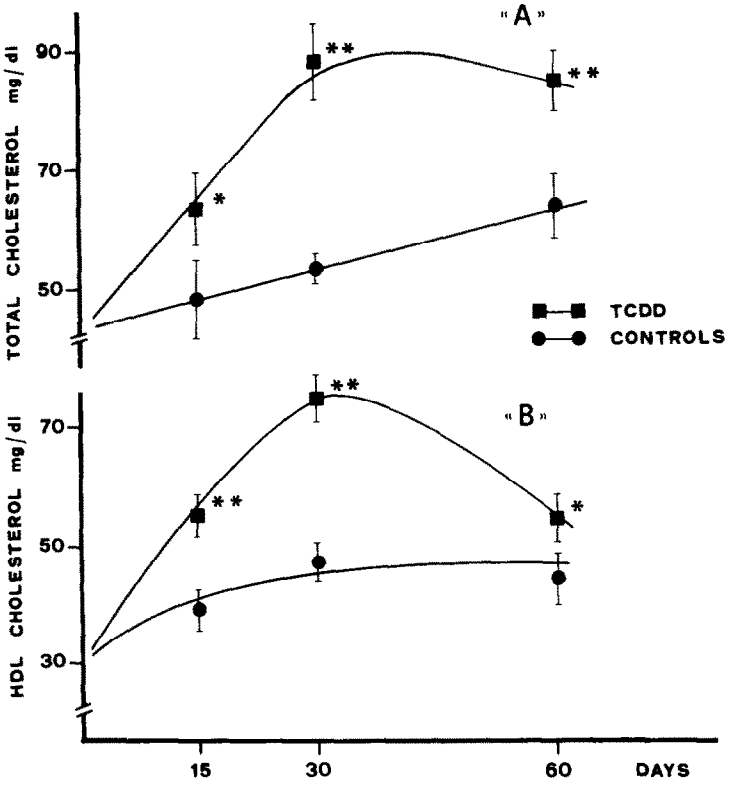

Fig. 2. Time curves of the effects of a single i.p. 20 γ/kg dose of TCDD on plasma total (A) and HDL (B) cholesterol levels. Plasma lipid rise is maximal 30 days after TCDD administration, but is still present after 60 days. Control animals show a slight increase, probably age and growth related. (N = 10, means ± S.D.). *P<0.05; **P<0.01 vs controls.

In a first experiment groups of three rats were administered i.p. graded doses of TCDD, reportedly not lethal [13], i.e. 2.5, 5, 10 and 20 μg/kg. Rats were killed three weeks later for lipid and lipoprotein determinations. Significant hepatomegaly was present in the two groups on the highest doses; no deaths did, however, occur and liver function tests were normal. A progressive dose-dependent elevation of total and HDL cholesterol levels was noted (Fig. 1). A slight excess of HDL cholesterol could also be appreciated with the highest dose (controls having 70 per cent of HDL cholesterol vs 85 per cent for rats treated with 20 μg/kg of TCDD).

The 20 μg/kg dose was therefore selected for the next experiment involving 60 rats. Twelve treated rats and eight controls were sacrificed after 15, 30 and 60 days of treatment. A permanent control group was maintained, since, in the previous report by Ishikawa *et al.* [6], temporal changes of HDL cholesterol levels were appreciable in control animals. Treated rats failed to thrive normally (body weight was, at the start, 308 ± 8 g and 307 ± 5 for control and treated rats, vs 500 ± 17 and 410 ± 13, respectively, after 60 days). A significant increase of liver weight was also detected, particularly at the 15 and 30 day intervals. Steatotic or overtly fibrotic areas in the liver were present in several treated animals. No significant changes in liver toxicity tests were detected and no mortality occurred.

At all three intervals, plasma total cholesterol levels were markedly elevated in TCDD treated rats (Fig. 2A). In contrast, HDL cholesterol levels were significantly higher, both absolutely and as a percentage of total, at the 15 and 30 day intervals in treated rats (Fig. 2B). After 60 days, HDL cholesterol levels were still significantly higher than in controls, but to a lesser extent than at the earlier intervals. No significant changes in plasma triglycerides and in cholesterol distribution in the other lipoprotein fractions (of very low and low density) were detected in treated animals. Electron microscopy of HDL from treated rats did not show marked differences as compared with lipoproteins from controls. The apoprotein pattern of HDL after isoelectric focusing [11] at pH 3.5–10 and 4–6 indicated, particularly after 15 days, less so after 30 days, a decrease of apo CIII-0 and a concomitant increase of apo CIII-3 (Fig. 3) without other significant apoprotein changes.

The experimental findings indicate that a single, acute exposure to TCDD significantly increases cholesterolemia in rats. Animals exposed to a toxic, not lethal dose of TCDD exhibit significantly higher plasma cholesterol levels, as compared to controls, up to the end of a two month experiment. These findings confirm a recent report by Albro *et al.* [14], who also noted increased circulating cholesterol esters in the rat and decreased liver free cholesterol, after oral treatment with sublethal or lethal doses of TCDD. Although the mechanisms of the cholesterol elevation are not clear, it may be noted that treatments with potent inducers of liver metabolizing enzymes, such as phenobarbitone [15] or phenytoin [16], in humans have also been associated with increased cholesterolemia.

The TCDD hypercholesterolemia is accompanied by raised HDL cholesterol levels. This observation is in striking contrast to diet induced hyperlipidemias which, in rats as well as in other animal species, reduce rather than increase HDL levels [17]. Increased HDL cholesterolemia is probably secondary to increased liver protein synthesis, having been detected also after chronic treatment with enzyme inducers, such as phenytoin in humans [18] and chlorinated insecticides in humans [7] and rats [6]. A reduced HDL catabolism, as recently shown after nicotinic acid treatment in man [19], cannot, however, be ruled out.

The apoprotein changes in HDL following TCDD treatment bear a striking resemblance to those occurring in both VLDL and HDL in rats of the same strain with streptozotocin induced diabetes [20]. VLDL apoproteins could not be studied in the present experiment, due to the paucity of available apoproteins in these normotriglyceridemic animals. In both experimental conditions, i.e. streptozotocin diabetes and TCDD hyperalphacholesterolemia, apo CIII-0 is reduced with a compensatory increase of the syalylated CIII-3. These apoprotein changes may be secondary to variations of plasma neuraminidase activity, but more likely reflect an altered rate of synthesis of these same apoproteins. In liver perfusates from rats on a normal diet, in fact, the CIII-3/CIII-0 ratio is about 2/1, whereas in serum it approximates 1/1; a high ratio is, therefore, possibly a marker of newly synthesized lipoprotein particles [12]. Since C peptides of VLDL and HDL belong, in the rat, to a single pool [12], both the hypertriglyceridemia of the diabetic rat and the hypercholesterolemia of the TCDD treated rat, by stimulating the synthesis of the corresponding lipoproteins (VLDL for the diabetic hypertriglyceridemia, HDL for the TCDD hyperalphacholesterolemia), may be responsible for an enlarged C peptide pool, with increased CIII-3/CIII-0 ratio. The increase of CIII-3, a

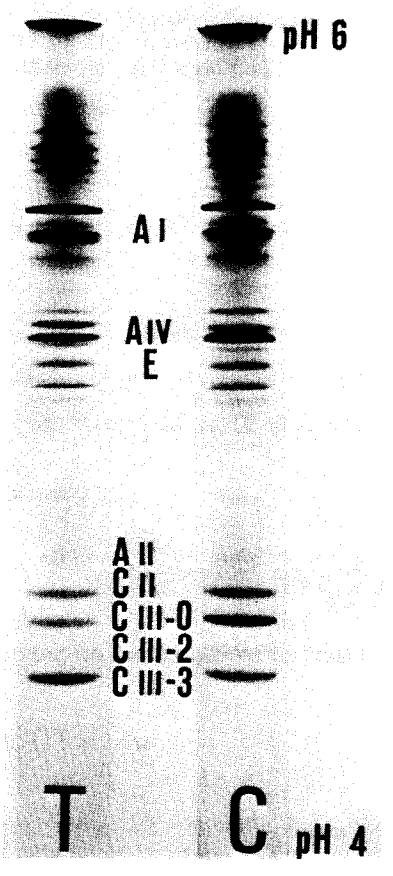

Fig. 3. Isoelectric focusing (pH 4–6) on polyacrylamide gels of HDL apoproteins isolated 15 days after treatment with TCDD (T) and from control animals (C). Apoprotein nomenclature as described by Gidez *et al.* [11]. An altered distribution of CIII apoproteins was observed in treated rats at this interval. By densitometry of CII and CIII apoproteins, percentages of CIII-3 were (N = 10, means ± S.D.): 25.7 ± 1.7 for controls and 36.1 ± 3.0 for the TCDD group ($P < 0.01$) whereas, in the case of CIII-0, densitometric percentages were, respectively, 37.0 ± 3.1 for controls and 27.5 ± 1.6 for the TCDD group ($P < 0.01$). No other significant changes in the distribution of CIII-2, CII and of the other HDL apoproteins were noted.

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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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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