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Serum Lipoprotein Patterns in Rats and the Effects of Some Pollutants

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Serum tissue lipids were analysed to determine the suspected role of dietary oils in combination with some chemicals on induced hyperlipidaemia in rats. Mustard and rapeseed oils were observed to increase triglyceride levels of tissues as well as blood. Trace elements like Vanadium and pollutants like DDT were found to have an effect on these alterations while cobalt was seen to decrease triglyceride level in the heart.

Key Words: Lipoprotein, Pollutants, Dietary oils, DDT

Introduction

The effect of dietary oils on lipid metabolism has been studied by a number of workers (Gopalan et al. 1974, Beare-Rogers 1976, Hulan et al. 1977, Bhatia et al. 1979). Various edible oils like mustard oil and rapeseed oil are widely used in Bengal. The consumption of these oils could be associated with hyperlipidaemias, especially as the incidence of coronary and cerebral atherosclerosis appears to be significantly high in this region. Mustard oil and rapeseed oil, due to the presence of high contents of erucic acid, increases different lipid fractions of heart tissues of rats and monkeys (Beare-Rogers et al. 1972, Gopalan et al. 1974, Hulan, et al. 1977, Beare-Rogers 1976, Roy et al. 1979). Feeding of large amount of rapeseed oil to laboratory animals has been reported to cause growth retardation and some changes in the testes, adrenals and liver and early cardiac lipidosis and later myocardial necrosis or fibrosis (Roine et al. 1960, Abdellatif & Vles 1970a, 1970b, Beare-Rogers et al. 1971, 1972, Roquelin 1972, Vogtmann et al. 1973, Bramer et al. 1973, Borg 1975). Gopalan et al. (1974) attributed the myocardial changes in monkeys fed mustard oil to the harmful effects of high amount of erucic acid in the oil.

Hyperlipidaemias caused by diet are

however influenced by the genotype and ancillary factors like exercise, body weight, trace elements and hormones. This paper reports the effects of trace elements and pollutants on hyperlipidaemias induced by dietary oils.

Materials and Methods

Male albino strain of rats *(Rattus norvegicus)* of 6 weeks age, laboratory-bred, were used. Animals were kept in standard aluminium cages with fresh water supply and special diet prepared in the laboratory. Altogether three sets of experiments comprising of 90 rats were carried out. In each set there were 10 rats and half the number (5) were kept only on standard diet containing one of the three oils and the remaining 45 rats were subjected to treatment with either of the three chemicals viz., DDT, $CoCl_2$ 10 mg/kg body weight, or Vanadium pentoxide 3 mg/kg body weight for 1st week, 4 mg/kg body weight during 2nd and 3rd weeks, together with standard diet. All animals received diet and chemicals for 21 days. The standard diet consisted of protein, fat and adequate amount of vitamins and minerals. Drinking water was given *ad lib.* The animals were weighed immediately before they were put on the diet. On completion of 21 days on diet and chemicals, the animals were sacrificed under general anaesthesia after recording final body weight. The liver and heart were immediately removed, weighed and preserved at -20°C.

Electrophoresis of serum lipoprotein

Electrophoresis of heart blood serum was carried out by polyacrylamide gel disc electrophoresis technique following the procedure standardized earlier by the authors (Bhattacharya & Talukder 1978) and the lipoprotein bands were compared and studied. A three gel method was used.

Thin layer chromatography of tissue lipid fractions

The extraction of lipids from heart and liver tissues was done within 15 days of collection, following the procedure of Folch et al. (1957). The solvent containing tissue lipid extract was used separately for the determination of total lipids by biochemical methods and the different lipid fractions by thin layer chromatography.

Determination of total lipid of rat liver and heart tissues

The total lipid content of the tissues was estimated by the method of Frings et al. (1972).

Results

Polyacrylamide disc gel electrophoresis

The serum electrophoretic lipoprotein patterns of rats fed different oils showed normal lipoprotein bands in all the cases. The bands were somewhat more enhanced in β- and pre-β regions in mustard oil and rapeseed oil fed rats as compared to the rats kept on groundnut oil. With the addition of chemicals, the bands were found to be increased in case of $CoCl_2$ treatment. In some the pattern resembled type IIa hyperlipidaemia (Frederickson et al. 1967).

Total lipids and fractionation by thin layer chromatography

Quantitative estimation of heart lipids showed that there was increase in free cholesterol, free fatty acid and triglyceride content and total lipids in the rats fed mustard oil and rapeseed oil as compared to rats fed groundnut oil with no significant increase in phospholipid values (Plates 1.1, 1.2, 1.3).

In heart tissue of rats fed with and either of the three dietary oils, free cholesterol (FC) showed increase as compared to the animals kept on oil diet alone. In rats fed mustard oil and DDT and rapeseed oil and DDT, free fatty acid (FFA) content was found to be increased. Triglyceride (TG) content was found to be lower than the corresponding values of animals fed oil alone. Ester cholesterol showed higher values in the rats fed DDT and groundnut oil or DDT and rapeseed oil. Lower values were found in case of rats fed DDT and mustard oil. Phospholipid content showed slightly higher values in all cases (figures 1–3).

Animals kept on $CoCl_2$ and groundnut oil showed decreased values of all heart lipid fractions except phospholipid when compared to animals fed groundnut oil alone. The heart tissue of rats fed $CoCl_2$ and mustard oil showed decrease in all lipid fractions except FFA and phospholipid compared to the animals fed oil only. The heart tissue of rats fed $CoCl_2$ and rapeseed oil showed decreased lipid values in respect to all the fractions except free cholesterol and ester cholesterol as compared to that of rats fed oil alone.

In the animals kept on V_2O_5 and either of the three oils, all the lipid fractions (except phospholipid in rats fed mustard oil and V_2O_5) decreased considerably as compared to the tissues of rats fed corresponding oils alone (table 1).

Liver lipid values

Liver tissue of rats kept on mustard oil and rapeseed oil showed small but

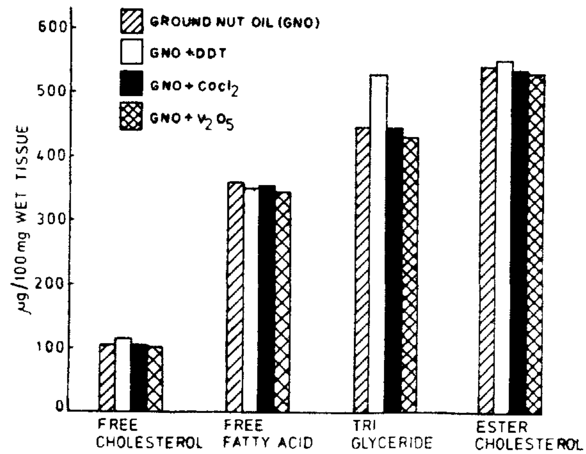

Figure 1 Heart tissue lipid fractions of rats fed groundnut oil and three different chemicals for 21 days

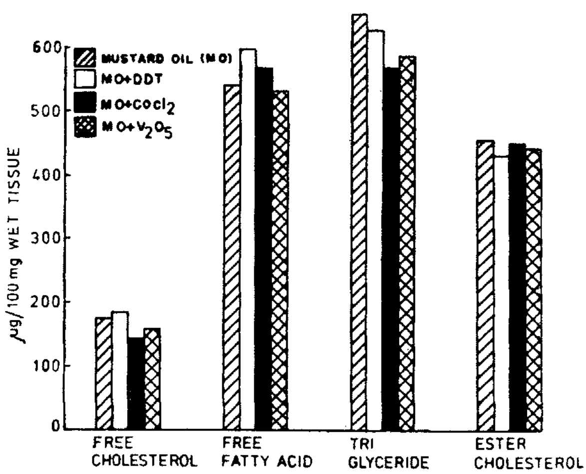

Figure 2 Heart tissue lipid fractions of rats fed mustard oil and three different chemicals for 21 days

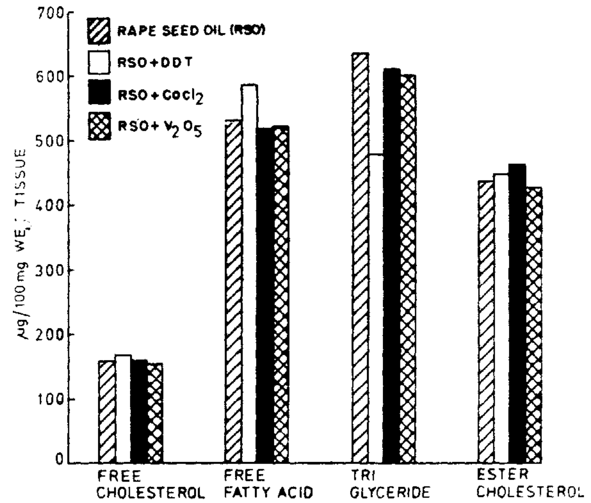

Figure 3 Heart tissue lipid fractions of rats fed rapeseed oil and three different chemicals for 21 days

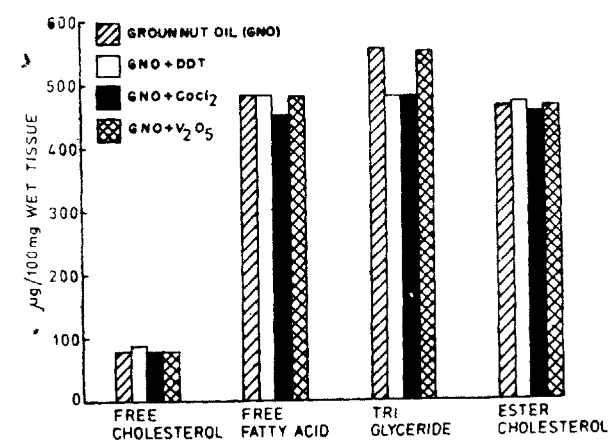

Figure 4 Liver tissue lipid fractions of rats fed groundnut oil and three different chemicals for 21 days

insignificant increase in free cholesterol and ester cholesterol as compared to those fed groundnut oil. Triglyceride content of liver of rats fed mustard oil and rapeseed oil increased significantly as compared to that of rats fed groundnut oil. Increase of phospholipid and total lipid content was found to be insignificant.

Liver tissues of rats fed groundnut oil and DDT showed higher free cholesterol, ester cholesterol, triglyceride and total lipid values than that of rats fed corresponding oil alone. Liver tissues of rats fed mustard oil and DDT, rapeseed oil and DDT showed slightly higher values of free cholesterol, free fatty acid and triglyceride and lower values of phospholipids as compared to tissues of rats fed corresponding oils (figures 4-6).

There was almost no change in lipid fractions of liver tissues of rats fed groundnut oil and $CoCl_2$ from that of rats fed groundnut oil only. Free and ester cholesterol and triglyceride values in tissues of rats fed $CoCl_2$ and mustard oil were somewhat lower as compared to the corresponding liver tissue of rats fed oil alone. Free and ester cholesterol, free fatty acid and triglyceride showed increased values in rats fed $CoCl_2$ and rapeseed oil in comparison to the liver tissues of rats fed rapeseed oil only (figures 4-6).

In liver tissue of animals kept on V_2O_5 and either of the three oils, all the lipid fractions decreased considerably as compared to the corresponding lipid values of liver tissues of rats fed oil alone (table 2).

Discussion

Heart tissue revealed significant increase in free cholesterol, free fatty acid and triglyceride contents in rats fed mustard oil or rapeseed oil as compared to rats fed groundnut oil. In liver, triglyceride values of rats fed mustard and rapeseed oil increased significantly. High erucic acid content of both mustard and rapeseed oil has been said to be one of the factors responsible for increase in these lipid fractions (Beare-Rogers et al. 1972, Borg 1975, Hulan et al. 1977). Hung and Holub (1977) suggested that early accumulation of triglyceride in the hearts of rats fed rapeseed oil arises from a higher rate of synthesis of triglycerides.

Vanadium treatment for 21 days lowered cholesterol, triglyceride, free fatty acid and phospholipid contents in heart and liver tissue as compared to control. Phospholipid in hearts of rats fed mustard oil and Vanadium was slightly higher than rats fed only oil. The effect of Vanadium may be both on the prevention of hepatic cholesterol synthesis as well as due to its oxidative properties (Curran 1954, Brownings 1969). The significant reduction in phospholipids in the liver, along with low free cholesterol, is associated with the hypothesis of Popjak (1946) that free cholesterol controls phospholipids. Snyder and Cornatzar (1958) attributed this decrease in liver phospholipid levels to the oxidative capacity of Vanadium.

Cobalt is found to display action through acyl CoA: 1, 2 diglyceride acyltransferase and a lower activity of microsomal lipase. The fact that all the lipid fractions except ester cholesterol of heart were raised by mustard and rapeseed oil denotes a definite hyperlipidaemic effect, the exact mechanism of which may be worked out after analysis of the different fatty acid fractions.

Chemicals and dietary oils

The effects of hard water and trace elements on lipids have been found to show

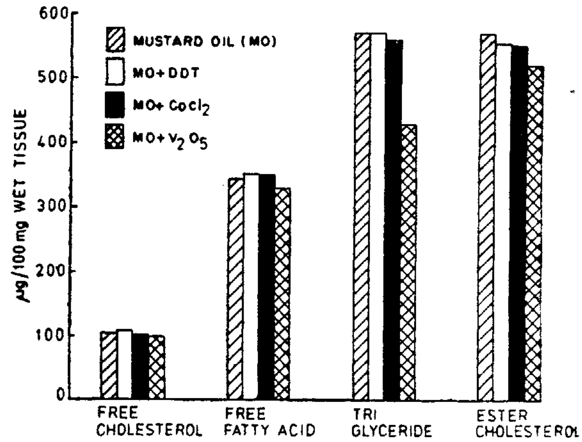

Figure 5 Liver tissue lipid fractions of rats fed mustard oil and three different chemicals for 21 days

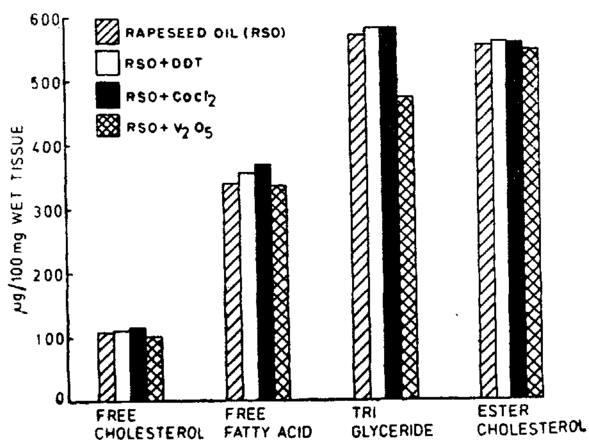

Figure 6 Liver tissue lipid fractions of rats fed rapeseed oil and three different chemicals for 21 days

Table 1 *Effect of dietary oils and pollutants on heart tissue of rats*
(Mean in μg/100 mg wet tissue)

Type of diet	Free cholesterol (FC)	Free fatty acids (FFA)	Trigly-ceride (TG)	Ester cholesterol (EC)	Phospho-lipid (PL)	Total lipid (TL)
N + 10% GNO	78.06	483.69	556.74	466.80	1826.00	3406.94 ±30.88
N + 10% GNO + DDT	89.90	482.97	480.66	471.86	1836.19	3361.59 ±49.26
N + 10% GNO + $CoCl_2$	77.00	448.61	481.46	454.89	1927.53	3404.70 ±42.49
N + 10% GNO + V_2O_5	77.20	480.00	550.40	464.15	1729.18	3303.74* ±61.76
N + 10% MO	176.82	544.31	661.95	459.61	1861.20	3703.50 ±34.35
N + 10% MO + DDT	187.55	598.23	628.35	433.93	1885.87	3733.95 ±36.43
N + 10% MO + $CoCl_2$	171.50	572.17	571.05	456.14	1939.30	3710.30 ±54.16
N + 10% MO + V_2O_5	163.14	535.22	592.87	444.24	1872.96	3608.44* ±27.18
N + 10% RSO	158.97	533.66	637.34	438.61	1855.60	3624.20 ±69.36
N + 10% RSO + DDT	169.55	592.42	484.46	451.47	1894.85	3590.77 ±31.44
N + 10% RSO + $CoCl_2$	160.96	519.18	614.21	467.11	1826.81	3588.28 ±46.28
N + 10% RSO + V_2O_5	155.14	525.11	604.63	432.53	1838.92	3548.97 ±57.81

N = Normal; Values represent mean ± S.D.
**P* < 0.01 (Moderately significant)

Table 2 *Effect of dietary oils and pollutants on liver tissue of rats*
(Mean in μg/100 mg wet tissue)

Type of diet	Free cholesterol (FC)	Free fatty acids (FFA)	Triglyceride (TG)	Ester cholesterol (EC)	Phospholipid (PL)	Total lipid (TL)
N + 10% GNO	105.03	358.91	445.19	539.96	2555.64	3984.72 ±73.95
N + 10% GNO + DDT	114.08	352.59	578.85	574.57	2552.04	4172.13*** ±47.84
N + 10% GNO + $CoCl_2$	105.67	355.87	444.06	534.53	2539.21	3981.16 ±57.02
N + 10% GNO + V_2O_5	102.47	337.49	430.30	528.00	2462.71	3860.97** ±46.59
N + 10% MO	105.72	343.38	569.97	572.06	2610.86	4139.51 ±94.33
N + 10% MO + DDT	111.37	353.74	572.43	566.10	2535.83	4139.51 ±110.00
N + 10% MO + $CoCl_2$	103.31	349.25	560.48	553.04	2634.55	4201.84 ±37.69
N + 10% MO + V_2O_5	100.09	328.53	428.54	523.62	2535.88	3909.29**** ±36.59
N + 10% RSO	107.39	338.66	571.30	554.23	2610.66	4182.25 ±72.91
N + 10% RSO + DDT	110.03	356.35	581.40	558.60	2525.58	4111.09 ±55.86
N + 10% RSO + $CoCl_2$	115.48	368.61	579.15	566.01	2560.47	4189.73 ±63.86
N + 10% RSO + V_2O_5	100.45	336.16	423.24	544.97	2507.19	3912.04**** ±51.83

N=Normal
Values represent mean ± S.D., Degrees of freedom=8, **$P<0.02$ (Significant)
***$P<0.01$ (Moderately significant), ****$P<0.001$ (Highly significant)

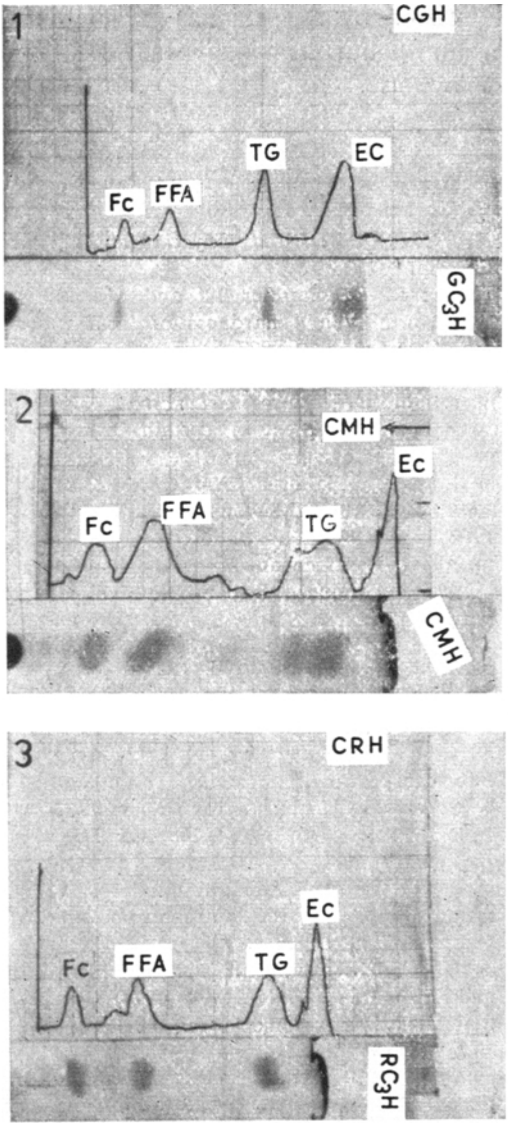

Plate 1

1, heart lipid fractions of rats fed groundnut oil subjected to densitometric measurement after thin-layer chromatography X 1/3 (approx.);

2, heart lipid fractions of rats fed mustard oil subjected to densitometric measurement after thin-layer chromatography X 1/3 (approx.);

3, heart lipid fractions of rats fed rapeseed oil subjected to densitometric measurement after thin-layer chromatography X 1/3 (approx.)

a negative correlation in most cases (Curran 1954, Schroeder 1960, Iacono et al. 1960, Crawford et al. 1968, 1971, Bhattacharya et al. 1969, Harman 1970, Stitt et al. 1973, Nielsen & Ollerich 1973, Seelig & Heggtveit 1974, Hopkins & Mohr 1974). Our study using dietary oils was further extended to work out effect of some chemicals on lipid metabolism by studying their effects on the hyperlipidaemic properties of edible oils. The biochemical mechanism of cobalt toxicity is suggested to be on the TCA cycle (Dingle et al. 1962, Webb 1964, Rona 1971). while studies by Mitala and Goutieri (1971) and Tephly and Hibbeln (1971) suggest its effect on numerous enzymatic systems. In our study $CoCl_2$, in combination with either of the three dietary oils, caused decrease in triglyceride fraction of heart tissue as compared to the animals kept on oils alone. In liver tissue a negligible decreasing tendency in lipid fractions except FFA and phospholipid was evident in rats fed groundnut and mustard oil in combination with $CoCl_2$. The liver of rats fed rapeseed oil and $CoCl_2$, however, had increased values of all lipid fractions except phospholipid.

DDT produced enhancement of some of the cardiac lipids like free cholesterol, free fatty acid and phospholipid; triglyceride was decreased in heart tissue of rats of all the three sets treated with DDT and dietary oils. In liver tissues free cholesterol, free fatty acid and triglyceride values were increased in rats fed mustard and rapeseed oils with DDT as compared to rats fed oil alone. Kelly-Garvert and Legator (1975) observed that DDT was deposited in storage fats in large amounts. DDT also enhances some of the fractions of both hepatic and cardiac tissue lipids of rats when fed with mustard and rapeseed oil as compared to rats fed groundnut oil alone. DDT has been demonstrated to produce increase in several enzyme activities including microsomal NADPH.

The effect of dietary oils could thus be altered by the presence in diet of certain pollutants and pesticides in common use.

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