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HYPOCHOLESTEROLEMIC AND HYPERTRIGLYCEROLEMIC EFFECTS OF CHRONIC CYANIDE INTOXICATION IN RABBITS.

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ABSTRACT

The effect of chronic cyanide toxicity on liver and serum lipoprotein profiles was investigated in New Zealand White rabbits using a combination of gravimetric and colorimetric procedures. Two groups of three-month-old rabbits (6 per group) were fed either pure growers mash or growers mash containing 400ppm inorganic cyanide for 42 days. Initial and weekly estimation of serum total cholesterol, HDL cholesterol and triacylglycerols were carried out on both groups. Same parameters were estimated in liver on termination of feeding.

Results showed that the cyanide treatment led to significant decreases in serum total cholesterol and serum HDL cholesterol while serum triacylglycerols were significantly increased ($p<0.05$). However, while the cyanide group had significantly raised liver triacylglycerols ($p<0.05$), liver total cholesterol and HDL cholesterol were significantly decreased relative to controls ($p<0.05$). These results suggest that chronic cyanide exposure exerts hypocholesterolemic and hypertriacylglycerolemic influences in rabbits probably due to impairment of hepatic lipoprotein metabolism.

KeyWords: Cyanide toxicity;hypocholesterolemia, hypertriacylglycerolemia, rabbits.

INTRODUCTION

Cyanide is a very potent respiratory poison in all aerobic organisms. As a specific inhibitor of cytochrome oxidase [Smith & McFeters, 1994; Jones et al 1984], it blocks mitochondrial electron transport, oxygen utilization and oxidative phosphorylation [Greer & Jo, 1995; Borron & Baud, 1996]. Extensive reports in the literature have associated chronic cyanide toxicity with the pathogenesis of several syndromes including goiter [Cliff et al, 1986], tropical ataxic neuropathy [Osuntokun, 1981], spastic paraparesis [Howlett et al 1990] and tobacco smoke-mediated visual impairment [Wilson et al, 1966]. Humans may be exposed to cyanide through dietary sources such as cassava [Cooke & Coursey, 1981;Hantson et al, 1999]; beans [Montgomery, 1969; Okolie & Ugochukwu, 1989] and cereals [Panasiuk & Bills, 1988]; and environmental routes such as tobacco smoke as well as smoke arising from incinerators [Borron & Baud, 1996]. The varied and diverse sources of cyanide hazard to humans underscore the need for investigation of its toxic metabolic effects. Studies have demonstrated that chronic cyanide exposure causes extensive degenerative lesions in liver and kidney [Okolie & Osagie, 1999] as well as in lungs of experimental rabbits [Okolie & Osagie, 2000]. The liver is one of the major sites of cholesterol biosynthesis. It has also been reported that cyanide inhibits glucose- 6-phosphate dehydrogenase [Okolie & Osagie, 1998]. Glucose- 6-phosphate dehydrogenase is a key enzyme of the oxidative phase of the pentose phosphate pathway that is needed to generate NADPH required for reductive biosynthesis of cholesterol and fatty acids. Elevated serum cholesterol is a well-known risk factor for the pathogenesis of cardiovascular artery disease [Kannel, 1983]. Since cyanide interferes with NADPH production and exerts deleterious influence on liver tissue, it was considered necessary to investigate the effect of chronic cyanide exposure on serum and liver lipoprotein profiles, using the rabbit as an experimental animal model.

MATERIALS AND METHODS

Experimental design

Twelve New Zealand white rabbits of about 3 months old were purchased from a private breeder in Benin City. The animals were maintained in clean metal hutches and acclimatized to growers mash [product of Bendel Feed & Flour Mills, BFFM Ltd, Ewu, Nigeria] for 3 weeks. Subsequently they were weighed and divided randomly into 2 groups [6 per group]. Members of each group were housed singly in clean, disinfected hutches. One group was fed growers mash containing 400ppm inorganic cyanide [sodium cyanide] while the other group (control) received growers mash only. Prior to feeding, each feed type was mixed with distilled

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water in the ratio of 10:1 (w:v) to minimize feed dust and increase acceptability. Clean drinking water was liberally provided. The animals were fed once daily at the rate of 70g mash/rabbit, while stale feed remnants were weighed and discarded. Blood samples were drawn weekly for estimation of serum total cholesterol, HDL cholesterol and triacylglycerols. The blood was drawn through the rabbit ear veins using sterile disposable 21-guage syringes. Feeding was terminated after 42 days. The animals were weighed and subsequently sacrificed by cervical dislocation. Liver sections were rapidly dissected out, while blood samples for serum analysis were drawn. The blood samples were allowed to clot in plastic, disposable centrifuge tubes, and the yellowish serum samples were recovered by centrifugation. Both liver and serum samples were stored refrigerated and analyzed within 48 hours.

Estimation of serum and liver lipoproteins:
Analysis of total cholesterol, HDL cholesterol and triacylglycerols were carried out directly on serum samples as well as on liver lipid extract. Lipid extraction from liver was done by extraction with 2:1 (v/v) chloroform: methanol [Folch, et al, 1957]. The clear chloroform layer was recovered and gently dried at 40°C by warming in a water bath. The dry residue was taken up in 5ml chloroform. Total cholesterol was assayed colorimetrically by the sulphuric acid-mediated dehydration and oxidation of cholesterol in the presence of ferric chloride to form a purple coloured complex, which absorbs strongly at 560nm [Zlatkis et al, 1977]. HDL cholesterol was estimated colorimetrically as for total cholesterol, but after prior precipitation of VLDL and LDL fractions using sodium phosphotungstate according to the method of Lopez-Virella et al [1977). Serum and liver triacylglycerols were determined colorimetrically according to the method of Gottfried & Rosenberg, [1977] using the Hantzsch condensation reaction. In this process, formaldehyde liberated from glycerol oxidation condenses with ammonium ion and aetylacetone to form a golden-yellow coloured complex, which absorbs strongly at 425nm.

Estimation of serum thiocyanate:
Serum thiocyanate was measured after prior deproteination in a colormetric reaction with Sorbo reagent [Bowler, 1994].

RESULTS

Values of mean feed consumption, weight gains and serum thiocyanate for both groups are depicted in table 1. While weight gains were comparable for the cyanide-treated groups and controls, mean feed intake and serum thiocyanate values were statistically higher [$p<0.05$] in the cyanide-toxified groups relative to controls.

Table 1: Feed intake, weight changes, feed efficiency and serum thiocyanate levels of rabbits in both test and control groups.

	Test (Growers mash + NaCN)	Control
Feed intake (g/rabbit/day)	61.7 ± 2.5^a	49.2 ± 1.7^b
Weight gain (g/rabbit)	244 ± 7^a	213 ± 3^a
Feed efficiency (weight gain/g of feed)	4.0	4.3
Serum thiocyanate [umole/100ml serum]	48.9 ± 2.8^a	8.3 ± 1.1^b

* Values are mean ± S.E.M; n = 6
* For each parameter, values bearing different superscripts across differ significantly ($p<0.05$).

Table 2: Liver total cholesterol, HDL cholesterol and triacylglycerols in the two groups at the termination of feeding.

	Test	Control
Total cholesterol (mg/g fresh wt.)	257 ± 17^a	450 ± 19^b
HDL cholesterol (" " ")	33 ± 5^a	57 ± 8^b
Triacyglycerols (" " ")	130 ± 11^a	83 ± 15^b

* Values are mean ± S.D. (n = 6). For each parameter, values having different superscripts across differ significantly ($p<0.05$).

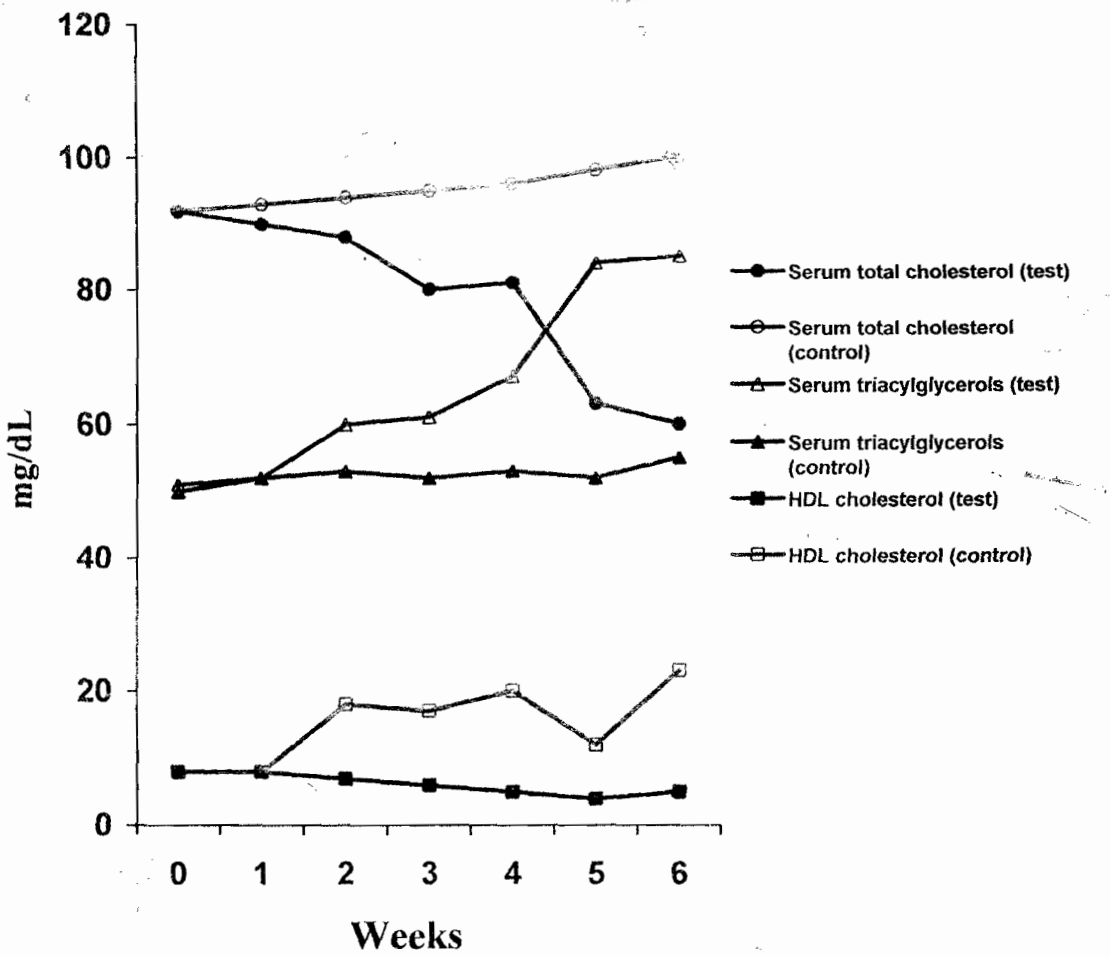

Fig.1.Time-course profiles of serum total cholesterol, serum triacylglycerols and serum HDL cholesterol. [Values are means ± SD: n = 6]

Fig.1 depicts time-course profiles of serum total cholesterol, HDL cholesterol and triglycerides for the cyanide-treated rabbits and controls during the 42-day feeding experiment. Although the initial values of all the parameters were comparable for both groups, the cyanide administration led to progressive and significant ($p<0.05$) reductions in serum total cholesterol and HDL cholesterol while serum triacylglycerols were significantly increased ($p<0.05$).
Table 2 shows liver total cholesterol, HDL cholesterol and triacylglycerols for both groups at the termination of the feeding experiment. While HDL cholesterol and total cholesterol were significantly reduced in the cyanide-fed rabbits, liver triacylglycerols were significantly increased relative to control values [$p<0.05$].

DISCUSSION

The hypocholesterolemic and hypertriacylglycerolemic effects observed in this study can be reasonably ascribed to the toxic effects of cyanide. Similar results have been described by previous workers using cassava-based feed formulations: cassava feeds have been shown to exert significant hypocholesterolemic responses when exogenous cholesterol is administered to rats [Adamson et al, 1983]; as well as rabbits [Adamson & Mbajiorgu, 1985]. Onongbu and Emole [1978] have earlier demonstrated plasma cholesterol-lowering effect of gari in rats, although the decreases were not statistically significant. Gari is a processed form of cassava, a cyanophoric plant containing the cyanogenic glycosides linamarin and lotaustralin [Nartey, 1978]. Although the cyanide content of the gari used in that study was not indicated, it is generally known that gari contains less than 40ppm cyanide [Maduagwu, 1979]. This is well within the innocuous limits of cyanide in foods [Bolhius, 1954]. Since the level of cyanide used in the present study was 400ppm, the significant hypocholesterolemia obtained is not surprising. It has been reported that chronic cyanide poisoning causes degenerative hepatic necrosis in juvenile rainbow trouts, leading to cellular disorganization and loss of the characteristic chord-like pattern of

the hepatocytes [Dixon & Leduc, 1981]. More recently it was demonstrated that cyanide exerts hepatotoxic effects in rabbits, as shown by histological evidence of degenerative hepatic necrosis and congestion [Okolie & Osagie, 1999]. These cyanide-induced pathological changes may compromise some of the biochemical functions of the liver, including cholesterol and lipoprotein biosynthesis, and thus precipitate hypocholesterolemia such as was seen in this study. It can be reasonably argued that since cholesterol arises from exogenous (diet) and biosynthetic sources only, the hypocholesterolemia observed in this study, must be (to a large extent) a consequence of a cyanide-induced interference with the hepatic biosynthetic pathway of cholesterol. This is more so since exogenous cholesterol was not administered to the rabbits. Recently it was reported that cyanide inhibits the activity of glucose- 6- phosphate dehydrogenase [Okolie & Osagie 1998]. This inhibition would result in diminished levels of NADPH, a vital coenzyme required not only by the rate-limiting step of cholesterol biosynthesis (i.e.HMG CoA reductase), but also in the formation of the highly reactive squalene epoxide needed for conversion of squalene to lanosterol. Consequently, the hypocholesterolemia seen in this study can be attributed to cyanide toxicity. The liver is the site of synthesis of plasma lipoproteins. The significant decreases in HDL cholesterol in liver and blood of the cyanide-treated rabbits may be a consequence of impairment in the capacity of the liver to carry out this function. Cyanide-induced hepatic degeneration may also be responsible for the significant increase in triacylglycerols, due possibly to inability of the liver to export them. This finding is consistent with the reports of previous studies, which have demonstrated that cyanide administration to rabbits caused significant lipid accumulation in liver [Padmaja & Panikkar, 1989].

Although the cyanide- fed rabbits consumed significantly more feed than controls, there were no significant differences in body eight gain between the two groups. This is consistent with the finding that cyanide retards muscle development [Ibebunjo et al, 1992]. At moderate doses, cyanide is usually detoxified to thiocyanate by the liver enzyme rhodanese [Cerletti, 1986]. The significantly higher output of serum thiocyanate confirms that indeed the animals were exposed to cyanide intoxication.

In conclusion, cyanide administration significantly lowers serum cholesterol in rabbits, probably due to an impairment of *de novo* hepatic cholesterol biosynthesis. However, the concomitant lowering of HDL fractions is considered undesirable since studies have unequivocally established an inverse relationship between HDL cholesterol and incidence of cardiovascular disease.

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