*Br. J. Pharmacol.* (1992), **105**, 495–499 

# Alterations in cellular cholesterol metabolism following administration of 6-hydroxydopamine to rabbits

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**1** The role of adrenergic mechanisms in the regulation of cholesterol metabolism was investigated by studying the effects of 6-hydroxydopamine (6-OHDA) on serum cholesterol levels and on the activities of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, acyl coenzyme A : cholesterol-O-acyltransferase (ACAT) in the livers and intestines, and cholesterol 7α-hydroxylase in the livers of male New Zealand White rabbits.

**2** Total serum cholesterol levels were significantly reduced ($P < 0.01$) in 6-OHDA-treated animals. This was reflected in the very low density lipoprotein, low density lipoprotein and high density lipoprotein fractions. The reduction in lipoprotein cholesterol levels reflected reduced cholesterol proportions in the lipoprotein fractions.

**3** The 6-OHDA-treated animals also had significantly lower activities of intestinal ($P < 0.001$) and hepatic ($P < 0.01$) HMGCoA reductase. The specific activities of intestinal ACAT, hepatic ACAT and cholesterol 7α-hydroxylase were comparable in both groups.

**4** In contrast to the results observed *in vivo*, 6-OHDA did not have any *in vitro* effect on cholesterol biosynthesis in cultured human leucocytes.

**5** This latter finding suggests that the effects of 6-OHDA on cellular cholesterol biosynthesis *in vivo* are indirect, possibly resulting from the known toxic effect of this drug in sympathetic nerve terminals, and imply a potential role for the sympathetic nervous system in the regulation of cellular cholesterol biosynthesis *in vivo*.

**Keywords:** 6-Hydroxydopamine; lipoproteins; cholesterol metabolism

## Introduction

The effects of adrenergic mechanisms on triacylglycerol metabolism have been well documented (Fain & Garcia-Sainz, 1983; Smith, 1983) but much less information is available on their role in the regulation of cholesterol metabolism. Studies in animals (Dury, 1957; Shafrir *et al.*, 1960; Barrett, 1966; Kunnihara & Oshima, 1983) have described increases in serum cholesterol following the administration of adrenaline, while an increased very low density lipoprotein (VLDL) cholesterol concentration has been observed following the administration of noradrenaline (O'Donnell *et al.*, 1988). *In vivo* (George & Ramasarma, 1977; Devery *et al.*, 1986) and *in vitro* (Edwards, 1975; Edwards *et al.*, 1979; Devery *et al.*, 1986) studies have also demonstrated an increased activity of hepatic-3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase (E.C.1.1.1.34), the rate-limiting enzyme of cholesterol synthesis, in response to noradrenaline and adrenaline. The large pharmacological doses of catecholamines used in these experiments contrast, however, with the low physiological levels present in serum. Consequently, the precise role of these hormones in the regulation of cholesterol metabolism merits further investigation.

This study evaluates the effects of adrenergic mechanisms on lipoprotein metabolism by studying the effects of 6-hydroxydopamine (6-OHDA), an analogue of noradrenaline, on cholesterol in rabbits. It has been demonstrated that 6-OHDA causes widespread destruction of the terminal endings of sympathetic neurones with a resultant decrease in the noradrenaline content of all sympathetically innervated end organs (Thoenen & Tranzer, 1968). The role of the sympathetic nervous system in the regulation of cholesterol metabolism is further studied by examining in rabbits the effects of 6-hydroxydopamine administration on key enzymes regulating cholesterol metabolism. These key enzymes include HMGCoA reductase, acyl coenzyme A : cholesterol-O-acyltransferase (ACAT) (E.C.2.3.1.26), the enzyme regulating cholesterol esterification and cholesterol 7α-hydroxylase (E.C.1.14.13.7), the key enzyme involved in the catabolism of cholesterol to bile acids.

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## Methods

### *Animals*

Male New Zealand White rabbits weighing 3–4 kg received purina rabbit chow *ad libitum* for two weeks before the start of the study. 6-Hydroxydopamine in 0.15 M saline was then injected intraperitoneally into 6 animals at a single dosage of 10 mg $kg^{-1}$ body weight. The route of administration and the dose chosen were effective as judged by the observation of a marked sympathomimetic response in the 6-OHDA-treated animals within 5 min of injection; 6 control animals were injected intraperitoneally with 0.15 M saline alone. Throughout the study period the animals were housed in individual metabolic cages and food intake was monitored. No significant difference in food intake (g/day) was observed between the two groups. The mean weights of the control and 6-OHDA-treated rabbits at the time they were killed were comparable ($3.32 \pm 0.09$ kg c.v. $3.68 \pm 0.13$ kg respectively). Blood was drawn from both rabbit groups for lipoprotein analysis after a 16 h overnight fast, on the 19th day after the administration of 6-OHDA. The animals were killed on day 21, and the livers and intestines were removed.

### *Experimental*

The livers were chilled, minced and then homogenized in 4 vol. ice-cold 0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM EDTA and 30 mM nicotinamide. The homogenate was centrifuged at 800 *g* for 10 min and the resulting supernatant was further centrifuged at 15,000 *g* for 20 min. This supernatant was then centrifuged at 104,000 *g* for 60 min

**Table 1** Effect of 6-hydroxydopamine (6-OHDA) administration on serum lipoprotein levels in rabbits

| | *Untreated animals* ($n = 6$) | *6-OHDA-treated animals* ($n = 6$) |
|---|---|---|
| *Cholesterol* (mmol $l^{-1}$) | | |
| Total | 0.97 ± 0.17 | 0.54 ± 0.04* |
| VLDL | 0.10 ± 0.01 | 0.02 ± 0.002* |
| LDL | 0.29 ± 0.09 | 0.09 ± 0.01* |
| $HDL_2$ | 0.19 ± 0.05 | 0.14 ± 0.02 |
| $HDL_3$ | 0.36 ± 0.03 | 0.27 ± 0.02* |
| *Triglyceride* (mmol $l^{-1}$) | | |
| Total | 0.44 ± 0.06 | 0.41 ± 0.05 |
| VLDL | 0.14 ± 0.04 | 0.12 ± 0.02 |
| LDL | 0.07 ± 0.004 | 0.06 ± 0.01 |
| $HDL_2$ | 0.04 ± 0.004 | 0.04 ± 0.01 |
| $HDL_3$ | 0.16 ± 0.01 | 0.14 ± 0.02 |

Results are expressed as means ± s.e.mean.
VLDL: very low density lipoprotein; LDL: low density lipoprotein; $HDL_2$ and $HDL_3$: high density lipoprotein—see text for details.
* $P < 0.01$ different from untreated animals.

to obtain the microsomal pellet. The pellet was then suspended in 0.25 vol. 5 mM imidazole/HCl buffer, pH 7.4 containing 0.15 mM NaCl and 5 mM dithiothreitol (DTT).

Mucosal cell fractions were obtained from rabbit intestine by differential scraping of the mucosa as described by Dietschy & Siperstein (1965). Cells were homogenized in 0.1 M potassium phosphate buffer, pH 7.4, containing 1 M EDTA and 30 mM nicotinamide. Due to the relative difficulty in subfractionating intestinal cells compared to liver cells, the activities of HMGCoA reductase and ACAT were measured in the whole homogenate of intestinal cells. The protein content of the liver and intestinal cell preparations was determined by the method of Lowry *et al.* (1951).

### *Enzyme assays*

HMGCoA reductase was determined as described by Mitropoulos & Balasubramaniam (1976) in liver microsomal and intestinal whole homogenate fractions of the rabbit. The incubation mixture contained 0.1 M potassium phosphate buffer pH 7.4, 2.5 mM NADP, 25 mM glucose 6-phosphate, 50 mM glutathione, 30 mM nicotinamide, 5 mM $MgCl_2$ and 5 units glucose 6-phosphate dehydrogenase in a total volume of 0.39 ml to which 0.05 ml of the various enzyme preparations were added. Following a preincubation of 10 min at 37°C, the reaction was initiated by the addition of 90 μM DL-hydroxymethyl [3-$^{14}$C]-glutaryl CoA (specific activity 6 Ci $mol^{-1}$). After incubation for various periods of time (30 min for liver microsomes and 60 min for intestinal cell homogenates, the reaction was terminated by the addition of 0.1 ml 12 N $H_2SO_4$, 0.5 ml mevalonolactone (10 mg $ml^{-1}$) and 10 μl DL-[2-$^3$H]-mevalonic acid (50,000 d.p.m./assay) was added as internal standard. Extraction, separation and quantitation of the product was as described previously (Mitropoulos & Balasubramaniam, 1976).

ACAT activity was determined as outlined by Balasubramaniam *et al.* (1978) in hepatic microsomes and intestinal cell whole homogenates. The incubation mixture contained 0.1 M potassium phosphate buffer, pH 7.4, 2 mM ATP, 4 mM $MgCl_2$, 0.2 mM coenzyme A and human albumin (1.2 mg/assay) in a total volume of 0.16 ml to which 0.02 ml of respective enzyme preparations were added. After a preincubation period of 5 min at 37°C, the reaction was started by the addition of 0.01 mM (1-$^{14}$C]-oleic acid. After 6 min, the reaction was stopped by the addition of two successive 2.0 ml aliquots of chloroform:methanol (2:1 v/v) and 0.8 ml water. [$^3$H]-cholesteryl-oleate 10 μl (20–30,000 d.p.m./assay) was added as internal standard. Quantitation of the product was as described previously (Balasubramaniam *et al.*, 1978).

The activity of cholesterol 7α-hydroxylase was measured in acetone-treated powder preparations of rabbit liver microsomal fractions as described by Shefer *et al.* (1981). The incubation mixture contained 0.1 M potassium phosphate buffer, pH 7.4, 10 mM NADPH and 5 mM $MgCl_2$, in a total volume of 0.5 ml to which 0.05 ml of the respective enzyme preparations were added. After a preincubation time of 5 min, 1.0 mM [4-$^{14}$C]-cholesterol (1.3 nCi $nmol^{-1}$) was added. The reaction was terminated after 20 min by the addition of 14 vol dichloromethane:ethanol (5:1 v/v).

Plasma lipoproteins were separated by a one step density gradient ultracentrifugation technique (Demacker *et al.*, 1983). The very low density lipoprotein (VLDL), low density lipoprotine (LDL), high density lipoprotein ($HDL_2$ and $HDL_3$) fractions correspond to the density intervals $< 1.006$ g $ml^{-1}$, 1.006–1.063 g $ml^{-1}$, 1.063–1.100 g $ml^{-1}$ and 1.100–1.210 g $ml^{-1}$ respectively. Total plasma cholesterol and the cholesterol concentrations in each lipoprotein fraction were assayed by an enzymatic colorimetric technique with Boehringer Mannheim GmbH diagnostic kits (Roschlau *et al.*, 1974) and triglycerides were measured with Biomerieux (Charbonnieres les Bains, France) kits (Takayama *et al.*, 1977).

Lymphocyte-enriched peripheral blood mononuclear cells were isolated from healthy laboratory volunteers' blood by centrifugation on Ficoll/Hypaque gradients as described by Mistry *et al.* (1981). *De novo* cholesterol synthesis was estimated by measurement of [$^{14}$C]-acetate incorporation into cholesterol in these cells after exposure to the mitogen, phytohaemagglutinin (PHA), as described by Owens *et al.* (1990). The effects of 6-OHDA on this process were assessed by culturing the cells in the presence of the drug over the 5 h exposure to [$^{14}$C]-acetate. The drug, dissolved in phosphate-buffered saline, was added (10 μl) to the cultures at two final concentrations, $4 \times 10^{-5}$ M and $4 \times 10^{-4}$ M. Phosphate-buffered saline (10 μl) alone was added to control cultures. Assays were performed in triplicate and results are expressed as nmol acetate incorporated into cholesterol per milligram cell protein.

### *Materials*

3-Hydroxy-3-methyl [3-$^{14}$C]-glutaryl CoA (56.6 mCi $mmol^{-1}$), DL-[2-$^3$H]-mevalonic acid lactone (629 mCi $mmol^{-1}$), [1-$^{14}$C]-oleic acid (57.3 mCi $mmol^{-1}$), [1α, 2α(n)$^3$H]-cholesterol (40 Ci $mmol^{-1}$) and [4-$^{14}$C]-cholesterol (50–60 mCi $mmol^{-1}$) were obtained from the Radiochemical Centre (Amersham, U.K.). β-Nicotinamide adenine dinucleotide phosphate (NADP), β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), glucose 6-phosphate, glucose 6-phosphate dehydrogenase, dithiothreitol (DTT), glutathione, human serum albumin (fatty acid-free), oleoyl chloride, nicotinamide, mevalonic acid lactone, coenzyme A, cholesterol and 6-hydroxydopamine (6-OHDA) hydrochloride were all obtained from Sigma-London Chemical Co. Ltd. (London, U.K.). Phytohaemagglutinin (PHA) was obtained from Wellcome Diagnostics (Oxford, U.K.). Kieselgel 60 was obtained from Merck (Darmstadt, Germany).

### *Statistics*

Statistical analysis of changes in the lipoprotein concentrations between the two groups of animals was performed using the Wilcoxon test (two-tailed) as these data were not normally distributed. The Student's *t* test was used to analyse differences in the activities of HMGCoA reductase, ACAT and cholesterol 7α-hydroxylase between the 6-OHDA-treated and untreated animals.

## Results

Table 1 outlines the effect of 6-OHDA administration on serum triglyceride and cholesterol levels and their distribution throughout the lipoprotein fractions in the animals. The treated animals had a significantly lower total cholesterol

**Table 2** Elemental composition of the lipoprotein fractions of the 6-hydroxydopamine (6-OHDA)-treated and control animals

| | *Control animals* ($n = 6$) | *6-OHDA-treated animals* ($n = 6$) |
|---|---|---|
| VLDL (%) | | |
| Cholesterol | 16.1 ± 3.3 | 6.1 ± 0.6* |
| Triglyceride | 40.4 ± 4.4 | 56.0 ± 5.0 |
| Phospholipid | 18.0 ± 1.6 | 11.8 ± 1.2 |
| Protein | 25·6 ± 1.6 | 26.2 ± 5.1 |
| LDL (%) | | |
| Cholesterol | 21.2 ± 3.3 | 14.2 ± 1.4 |
| Triglyceride | 13.5 ± 1.7 | 14.0 ± 2.4 |
| Phospholipid | 26.2 ± 1.2 | 25.1 ± 1.5 |
| Protein | 39.1 ± 3.2 | 46.8 ± 1.6 |
| $HDL_2$ (%) | | |
| Cholesterol | 15.0 ± 1.5 | 11.8 ± 0.5 |
| Triglyceride | 7.7 ± 1.1 | 8.0 ± 0.9 |
| Phospholipid | 34.4 ± 3.0 | 34.1 ± 1.2 |
| Protein | 42.9 ± 2.1 | 46.1 ± 1.3 |
| $HDL_3$ (%) | | |
| Cholesterol | 8.7 ± 0.7 | 8.0 ± 0.3 |
| Triglyceride | 8.6 ± 0.9 | 9.3 ± 1.0 |
| Phospholipid | 18.4 ± 3.9 | 21.8 ± 0.5 |
| Protein | 64.4 ± 3.3 | 60.9 ± 1.2 |

Results expressed as means ± s.e.mean. For abbreviations, see Table 1.
* $P < 0.01$ different from control animals.

($P < 0.01$), VLDL cholesterol ($P < 0.01$), LDL cholesterol ($P < 0.01$) and $HDL_3$ cholesterol ($P < 0.01$). $HDL_2$ cholesterol levels were similar in both groups. No significant differences were observed in total and lipoprotein triglyceride levels between the two groups.

The elemental composition of the lipoprotein fractions of the treated and control rabbits is demonstrated in Table 2. A significant reduction in the proportions of cholesterol was observed in the VLDL particles of the treated group ($P < 0.01$). Although the percentage cholesterol in the LDL, $HDL_2$ and $HDL_3$ particles was also lower in the treated group, the differences were not significant.

The effect of 6-OHDA on the specific activities of the hepatic and intestinal cholesterol metabolising enzymes in the two animal groups is outlined in Table 3. The specific activities of HMGCoA reductase were significantly lower in the livers ($P < 0.01$) and intestines ($P < 0.001$) of the 6-OHDA-treated animals. Intestinal ACAT, hepatic ACAT and cholesterol 7α-hydroxylase activities were similar in both groups.

In an attempt to ascertain whether 6-OHDA might have a direct influence on cholesterol biosynthesis, the influence of 6-OHDA at two different concentrations on the incorporation of [$^{14}$C]-acetate into cholesterol by cultured human leucocytes is shown in Table 4. There was no significant difference between the amount of [$^{14}$C]-acetate incorporated by cells exposed to either drug concentration and that incorporated by control cells.

**Table 3** The effect of 6-hydroxydopamine (6-OHDA) administration on cholesterol metabolizing enzymes in rabbit liver and intestine

| | *Control animals* ($n = 6$) | *6-OHDA-treated animals* ($n = 6$) |
|---|---|---|
| *HMGCoA reductase* (nmol min$^{-1}$ mg$^{-1}$ protein) | | |
| Hepatic | 0.036 ± 0.005 | 0.016 ± 0.005* |
| Intestinal | 0.024 ± 0.003 | 0.007 ± 0.002** |
| *ACAT* (nmol min$^{-1}$ mg$^{-1}$ protein) | | |
| Hepatic | 2.19 ± 0.19 | 2.03 ± 0.57 |
| Intestinal | 1.62 ± 0.26 | 1.95 ± 0.54 |
| *Cholesterol 7α-hydroxylase* (pmol min$^{-1}$ mg$^{-1}$ protein) | 14.29 ± 1.88 | 16.92 ± 1.2 |

Results expressed as mean ± s.e.mean.
HMGCoA reductase: 3-hydroxy-3-methylglutaryl coenzyme A, ACAT: cholesterol-O-acyltransferase.
* $P < 0.01$ different from control animals.
** $P < 0.001$ different from control animals.

**Table 4** Effect of 6-hydroxydopamine (6-OHDA) on leucocyte cholesterol synthesis

| | *Control cells* | *6-OHDA treated cells* | |
|---|---|---|---|
| 6-OHDA concentration | 0 | $4 \times 10^{-5}$ M | $4 \times 10^{-4}$ M |
| [$^{14}$C]-acetate incorporated (nmol mg$^{-1}$ protein) | 70.7 ± 2.1 | 72.9 ± 2.6 | 68.4 ± 2.0 |

Results are expressed as mean ± s.e.mean.

## Discussion

Given the potential of 6-OHDA administration to bring about a chemical sympathectomy in animals (Thoenen & Tranzer, 1968), it is likely that the 6-OHDA-treated rabbits in this study were noradrenaline-depleted and the metabolic effects observed may be a reflection of lower noradrenaline concentrations in these animals. The observation of a brisk sympathomimetic response (manifested by a profound tachycardia) following the administration of 6-OHDA suggests that the dose chosen was sufficient to cause destruction of sympathetic nerve endings (Stone *et al.*, 1964). Since the time interval necessary for the regeneration of sympathetic neurones following 6-OHDA is much longer than the duration of the present study (Goldman & Jacobowitz, 1971; Kostrzewa & Jacobowitz, 1974), it can be assumed that the 6-OHDA-treated rabbits were at least partially sympathectomized at the time of lipoprotein sampling and at the time they were killed. Thus, the alteration in HMGCoA reductase in the 6-OHDA-treated animals in this study may well be a result of the toxic effect of this drug on the sympathetic nerve fibre rather than due to a direct effect of the drug on hepatic and intestinal cholesterogenesis. This postulate is supported by the lack of any direct effect of 6-OHDA *per se* on cellular cholesterogenesis as measured by [$^{14}$C]-acetate uptake in cultured human leucocytes (Table 4). Mitogen-stimulated cultured human leucocytes have been used previously in this laboratory as a model system for studying cellular cholesterogenesis, using [$^{14}$C]-acetate as precursor (Owens *et al.*, 1990; 1991). The accessibility of these cells, their robustness relative to isolated hepatocytes and their capacity to amplify cholesterogenesis through mitogen stimulation makes them a suitable model system. The sensitivity of [$^{14}$C]-acetate uptake process to the specific inhibitor of HMGCoA reductase, mevinolin, validates the use of this procedure to assess indirectly the enzyme's activity in cells. Other workers (Cuthbert & Lipsky 1980: Cuthbert *et al.*, 1986) have used this tissue as a model for human cell sterol metabolism and for the assessment of LDL receptor status in familial hypercholesterolaemia. Given the marked *in vivo* effect of 6-OHDA administration on both hepatic and intestinal HMGCoA (Table 3), the rate-limiting enzyme in cholesterol biosynthesis from acetate in cells, a chemical sympathectomy-mediated depletion of noradrenaline would seem plausible.

The 6-OHDA-treated rabbits in this study had lower serum cholesterol levels compared with their untreated counterparts. These data are consistent with both the reduced cholesterol levels described in patients receiving the selective α-adrenoceptor antagonist, prazosin (Leren *et al.*, 1981; Rouffy & Jaillard, 1984) and the increased VLDL cholesterol levels observed in New Zealand White rabbits following the administration of noradrenaline (O'Donnell *et al.*, 1988). The results

suggest reduced availability of cholesterol for lipoprotein formation in these 6-OHDA-treated animals. The lipoprotein data confirm this, as the reduced lipoprotein cholesterol levels in the treated rabbits (Table 1) are reflected in reduced cholesterol proportions in virtually all the lipoprotein fractions (Table 2). A reduced availability of cholesterol within hepatocytes might reflect decreased cholesterol catabolism to bile acids. The unchanged activity of cholesterol 7α-hydroxylase in the 6-OHDA-treated animals suggests that cholesterol catabolism was not altered. However, the reduced activity of hepatic HMGCoA reductase in the 6-OHDA-treated animals implies suppressed endogenous cholesterogenesis in the liver, thereby reducing the amount of cholesterol available within the hepatocyte for VLDL formation. A similar reduction in intestinal HMGCoA reductase activity in the 6-OHDA-treated animals may also reduce the amount of cholesterol available for lipoprotein formation in this tissue.

The dramatic fall in hepatic and/or intestinal HMGCoA reductase in the 6-OHDA-treated animals raises the possibility that the effects of 6-OHDA might be mediated by a non-specific destruction of hepatocytes and/or intestinal cells. In such an eventuality, we would also have expected a dramatic reduction in hepatic ACAT, cholesterol 7α-hydroxylase and intestinal ACAT activities. However, the specific activities of these 3 enzymes were not altered in the 6-OHDA-treated animals. Furthermore, the doses of 6-OHDA used in these studies were modest and there is considerable evidence accumulated from many animal species to suggest that under such circumstances 6-OHDA destroys catecholaminergic neurones with a high degree of selectivity (Kostrzewa & Jacobowitz, 1974).

The altered hepatic HMGCoA reductase activity in the 6-OHDA-treated model is consistent with the *in vivo* (George & Ramasarma, 1977; Devery *et al.*, 1986) and *in vitro* (Edwards, 1975; Edwards *et al.*, 1979; Devery & Tomkin, 1986) evidence demonstrating enhanced HMGCoA reductase activity in response to noradrenaline. Since this influence of supraphysiological doses of noradrenaline on hepatic HMGCoA reductase activity in rat isolated hepatocytes is not mediated through the adenylate cyclase system (Edwards, 1975), it is unlikely to represent an $\alpha_2$-adrenoceptor-mediated effect. It is possible that the increased HMGCoA reductase activity in response to noradrenaline is mediated through activation of $\alpha_1$-adrenoceptors or it could represent a direct effect of noradrenaline on the enzyme (George & Ramasarma, 1977). In support of the former possibility, it has been shown that noradrenaline induces DNA synthesis in rat hepatocytes via $\alpha_1$-adrenoceptor stimulation (Cruise *et al.*, 1985). The lack of effect of 6-OHDA administration on ACAT and cholesterol 7α-hydroxylase activity is also consistent with the lack of change in the activity of these enzymes following noradrenaline administration (Devery *et al.*, 1986). Thus, it appears that the sympathetic nervous system regulates cholesterol metabolism within cells by promoting cholesterol synthesis. By contrast, the increased serum cholesterol concentration and increased activity of hepatic HMGCoA reductase observed following vagotomy (Scott & Tomkin, 1985) suggest that the parasympathetic nervous system regulates intracellular cholesterol metabolism by suppressing cholesterol synthesis. However, in addition to regulating the activity of HMGCoA reductase, the parasympathetic nervous system also regulates the activity of hepatic ACAT and cholesterol 7α-hydroxylase (Scott & Tomkin, 1985), whereas the sympathetic nervous system does not appear to have any effect on these enzymes.

In this study, 6-OHDA did not have any effect on serum triglyceride levels. Previous studies in male New Zealand White rabbits have demonstrated that pharmacological doses of noradrenaline increased VLDL triglyceride concentrations (O'Donnell *et al.*, 1988), and there is experimental evidence (Taggart & Carruthers, 1971) suggesting that the rise in free fatty acid and triglyceride concentrations during periods of acute stress in motor racing drivers may be due to the extremely high levels of catecholamines in their serum (levels which are often supraphysiological). Despite the extensive evidence in the literature implicating the importance of adrenergic mechanisms in the regulation of triglyceride metabolism (Fain & Garcia Sainz, 1983), the data in the present study raise the possibility that adrenergic mechanisms do not have a significant influence on triglyceride metabolism under basal conditions and the effects only become manifest when the levels of catecholamines are very high. In support of this view, it has previously been demonstrated by use of graded infusions of noradrenaline, that plasma levels of greater than 1800 pg $ml^{-1}$ (or nearly 10 times the basal level) are required to produce measurable metabolic changes (Silverberg *et al.*, 1978). By contrast, haemodynamic effects are seen with increments in plasma noradrenaline which are as low as 100 pg $ml^{-1}$ (Cryer *et al.*, 1976). Thus, while noradrenaline can function as a neurotransmitter at relatively low mean concentrations (presumably due to high local synaptic levels), it only functions as a hormone in those rare instances where supraphysiological concentrations are attained (Cryer, 1980).

In summary, this study demonstrates reduced serum cholesterol levels in rabbits that were treated with 6-OHDA. This was associated with a reduction in cellular cholesterol biosynthesis. Similar effects on cholesterol synthesis were not observed *in vitro*, suggesting that the alteration in cholesterol metabolism *in vivo* is not a direct effect of this pharmacological agent. Instead, 6-OHDA may mediate its effect via an indirect mechanism, possibly through its well described effect on sympathetic nerve endings; such a hypothesis implicates the sympathetic nervous system in the regulation of cholesterol synthesis *in vivo*. The clinical significance, if any, of this latter observation remains to be determined.

This work was supported by the Adelaide Hospital Research Fund. The secretarial assistance of Ms Ursula O'Halloran is gratefully appreciated.

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*(Received August 16, 1991*
*Revised October 17, 1991*
*Accepted October 31, 1991)*