DNA-phosphorus (DNA-P) was measured after different times of incubation[11]. The release of acid soluble material as a function of time from depurinated DNA is found to be considerably higher than from native DNA. Depurinated DNA incubated without enzyme shows some degradation with time. This could be a tris catalyzed reaction since amine buffers are known to catalyze the degradation of depurinated DNA[12]. Similar results were obtained when the enzyme extracted from purified nuclei was used. The degradation of single stranded DNA containing no apurinic sites was also found to be considerably lower as compared with that of depurinated DNA. In order to demonstrate that the cleavage of depurinated DNA was mostly directed towards apurinic sites, the experiment shown in figure 2 was done. After treatment of depurinated DNA with enzyme for indicated period of time, the reaction mixture was further treated with alkali. This would result in the cleavage of all apurinic sites left undegraded by the enzyme. Addition of NaOH after 90 min of incubation with enzyme did not increase further the acid-soluble material already produced enzymatically. This indicates that the sites of action of alkali were already acted upon by the enzyme. It is, therefore, concluded that the enzyme and alkali cleaved the depurinated DNA at or near apurinic sites.

Depurinated DNA has been shown to be an intermediate in the degradation in vitro of alkylated DNA[13]. Endonucleases specific for alkylated DNA have been reported in B. subtilis[14] and E. coli[15]. DNA was alkylated by dimethyl sulphate, which is a weak carcinogen. Depurinated DNA was obtained by mild heating of alkylated DNA[8], which resulted in the appearance of apurinic sites as shown by alkaline hydrolysis of such DNA. As shown in figure 3, the production of maximum acid-soluble material from alkylated and depurinated DNA is significantly higher than that from native DNA. Alkylated DNA alone in the presence of alkali did not show any degradation. The procedure of heating DNA at pH 3.5 for preparation of depurinated DNA results in the complete denaturation of DNA at 52 °C[2]. In order to determine the native or denatured states of alkylated DNA and depurinated DNA obtained from it, hydroxylapatite chromatography of such DNA was done. The results are shown in figure 4. Whereas part of the molecules of alkylated DNA retain at least partial double strandedness under our conditions, the depurinated DNA is completely denatured. The denaturation of alkylated DNA is a time-dependent phenomenon since it was observed that after 24 h at room temperature this DNA becomes completely denatured.

The apurinic site specific nuclease reported here and by other authors in different systems may be the first enzyme required for the repair of apurinic sites in DNA. This assumes that the mechanism of repair of depurinated DNA is similar to that of UV-irradiated thymine dimer containing DNA. Indeed, using E. coli apurinic site nuclease, Verly et al.[16] have recently demonstrated in vitro repair of apurinic sites in DNA. The nuclease activity on alkylated DNA observed by us raises an interesting question. The enzymatic degradation of alkylated DNA may involve depurination as an intermediate step similar to non-enzymatic degradation of alkylated DNA. A single enzyme may, therefore, be required for both the steps. Conversely 2 separate enzymes may be involved as has been suggested in the case of E. coli endonuclease II[17]. However, the question can only be answered after the enzyme has been purified.

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Serum cholinesterase: Function in lipoprotein metabolism

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Summary. Human serum beta-lipoproteins, isolated by percipitation with heparin-calcium mixture, showed cholinesterase activity. The enzyme activity was almost proportional to the lipoprotein concentration. Rats, treated with neostigmine, a cholinesterase inhibitor, showed a significant decrease in serum beta-lipoprotein and in the incorporation of H^3-lysine into the lipoprotein compared to untreated controls. The decreased incorporation of H^3-lysine into beta-lipoprotein was associated with increased labelling of alpha-lipoprotein. There was no significant difference in the labelling of pre-beta-lipoprotein. We propose that LDL is formed from VLDL in the presence of cholinesterase.

The biological role of serum cholinesterase (ChE) is unknown. However, a relationship between ChE and serum beta-lipoprotein (BLP) has been demonstrated. It has been shown[3] that human BLP released ChE upon ultrasonication and that BLP and ChE recombine upon standing. We demonstrated[4] that ChE inhibitors like physostigmine can destabilize BLP. When BLP was treated with phospholipase D, ChE was released and at the same time, the lipoprotein showed altered electrophoretic mobility[5]. This suggests that the site of attachment of ChE to the lipoprotein molecule is at the phosphorylcholine part of lecithin. It is obvious that a close structural relationship exists between phosphorylcholine and acetylcholine, a natural substrate for ChE.

We next observed[5,6] that when hyper-pre-beta lipoproteinemia was induced in rabbits by treating them with E. coli endotoxin, there was a marked increase in the ChE activity. This activity decreased with the conversion of pre-beta into beta-lipoprotein. This suggests that BLP can bind ChE.

In nephrotic syndrome, an unexplained hypercholesterolemia and hyper-beta-lipoproteinemia has long been re-

Table 1. Serum beta-lipoprotein concentration, H^3-lysine incorporation into precipitated beta-lipoprotein and lipoprotein fractions, cholinesterase activity in total serum

Animals	Beta-lipoprotein (mg percent)	H^3-lysine incorporation into beta-lipoprotein precipitate		H^3-lysine incorporated in lipoprotein fractions		ChE activity
		CPM	PB	B	α	
Control (8)	746 ± 105*	548 ± 31	339 ± 59	594 ± 81	628 ± 92	16.1 ± 1.8
Test (8)	237 ± 21	133 ± 18	420 ± 32	147 ± 32	908 ± 91	8.4 ± 6.7
p	< 0.001	< 0.001	< 0.05	< 0.05	< 0.05	< 0.01

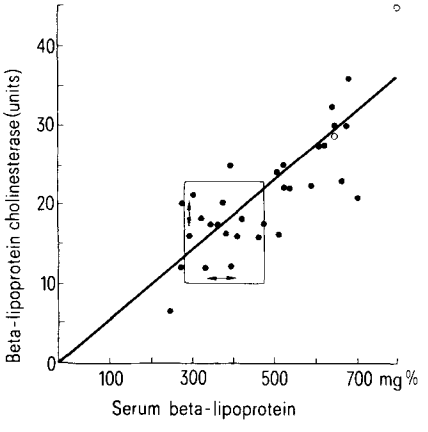
* Standard error of the mean. PB, pre-beta; B, beta; α, alpha.

cognized. These patients have high ChE activity[7]. Our studies[8] in children with nephrotic syndrome in the acute and convalescent phases suggest that hyper-beta-lipoproteinemia is the result of increased ChE synthesis. In a patient with parathion poisoning, we observed[9] a marked decrease in BLP and cholesterol associated with the inhibition of ChE. BLP and the serum cholesterol levels increased proportionally with the rise in ChE activity during recovery of the patient.

Clearly, a definite relationship between ChE and lipoprotein metabolism is suggested. The present study was undertaken to provide information on the overall effect of ChE on lipoprotein metabolism.

Table 2. Serum beta-lipoprotein concentration and H^3-lysine incorporation into precipitated beta-lipoprotein, and ChE activity before and after treatment with neostigmine in 5 rats

	Beta-lipoprotein (mg percent)	H^3-lysine incorporation into beta-lipoprotein CPM	ChE activity (units)
Before	575 ± 51	527 ± 38	17.6 ± 2.1
After	274 ± 27	182 ± 42	5.8 ± 1.7
p	< 0.001	< 0.001	< 0.01



Cholinesterase activity in the serum beta-lipoprotein obtained from 33 patients. The enclosed area represents the normal beta-lipoprotein cholinesterase activity (vertical arrow 10–25 units) and beta-lipoprotein concentration (horizontal arrow: 275–475 mg percent). 2 light dots represent the values in 2 nephrotic patients.

Materials and methods. BLP was measured by precipitation with heparin-calcium chloride mixture[10]. The precipitate thus obtained was washed with the same heparin-calcium chloride reagent and the pellet was dissolved in normal saline (0.1 ml) containing 1% Triton X-100. ChE activity was measured in the dissolved precipitate using propionylthiocholine[11] as the substrate. The following modifications were made for this measurement: 50 µl of sample was incubated with 1 ml of color reagent and 0.2 ml of substrate. The reaction was stopped using 0.25 ml of quinidine sulphate. The ChE activity was then calculated from a precalibrated graph with thiocholine. The units of ChE activity for BLP are defined as the number of micromoles of thiocholine formed from BLP contained in 100 µl of serum per h.

For the in vivo experiments, albino rats (Sprague Dawley) weighing between 300 and 350 g were used. The animals were fasted overnight and 15 µCi of H^3 lysine was injected s.c. into each of the rats. The test animals also received 0.35 mg/kg of neostigmine via the same route. At the end of 2 h, the animals were anesthetized using ether, and the blood was collected by heart puncture.

In another series of experiments, after bleeding the H^3-lysine treated rats at the end of 2 h, neostigmine was administered (doses as above) and blood was collected after $^1/_2$ h.

Serum ChE was measured as described aerlier[11]. BLP was measured by the Hycel kit method[10]. Radioactivity in the BLP precipitate was measured by dissolving it in 10 ml of P.C.S.[12]. For measurement of radioactivity in the lipoprotein fractions, lipoproteins were separated using polyacrylamide gel electrophoresis[13] using 100 µl of

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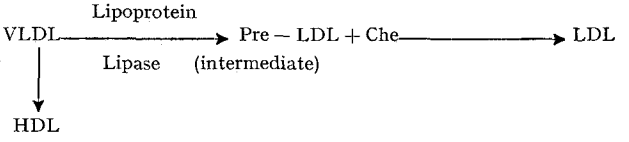
serum. The separated fractions of pre-beta, beta, and alpha-lipoproteins were cut out of the gel, dispersed in 10 ml of P.C.S. and counted. The radioactivity was calculated as counts per min in the lipoproteins contained in 0.1 ml of serum.

Results and discussion. It has been well documented[14,15] that when labeled serum lipoproteins of $d < 1.063$ were separated by either heparin divalent cation precipitation or ultracentrifugation comparable results were obtained, and the precipitated complex contained no other serum proteins. The demonstration of ChE activity in the serum BLP precipitate in this study therefore indicates a structural association between the enzyme and the lipoprotein. The ChE activity is almost proportional to the BLP concentration (figure). Strict linearity could not be obtained because pre-beta-lipoprotein is also precipated with BLP by heparin-calcium chloride mixture.

Since we have already proposed a function for cholinesterase in its interaction with beta-lipoprotein, we decided to investigate the function of ChE in the overall lipoprotein metabolism in the serum of rats treated with a specific ChE inhibitor neostigmine.

It has been suggested[16] that LDL (beta-lipoprotein) is derived from VLDL (pre-beta-lipoprotein). An intermediate lipoprotein, which is unstable, is believe to be formed during this conversion[17]. In the present study, specific inhibition of ChE by neostigmine results in a marked reduction of serum BLP concentration. The labelling pattern of the lipoproteins with H^3-lysine provides evidence of decreased BLP synthesis and of increased formation of alpha-lipoprotein (HDL) (tables 1 and 2). ChE inhibition had no significant effect on pre-beta-lipoprotein. The changes observed appear unrelated to abnormal liver function or lecithin-cholesterol acyl transferase activity, because no increase in serum glutamic pyruvic transaminase activity or decreased cholesterol ester-cholesterol ratio were observed in the treated rats. The dose of neostigmine chosen was to produce about 30–50% inhibition of ChE and is within the human therapeutic range. The treated rats suffered mild short lived twitchings from which all recovered.

The results provided further evidence that ChE has an important function in lipoprotein metabolism. We proposed that ChE influences lipoprotein metabolism as follows:



Recently, decreased levels of alpha-lipoprotein have been implicated in the development of coronary heart disease and atherosclerosis[18,19]. A relationship between hyper-beta lipoproteinemia and atherosclerosis has already been well documented. The present observations suggest the possibility of developing drugs with anti-cholinesterase properties in the treatment of hyper-beta lipoproteinemia and atherosclerosis.

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Histamine formation by ruminal fluid from cattle in vitro

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Summary. Net histamine formation in ruminal fluid is shown to be the result of histidine decarboxylation and histamine deamination. Addition of 4.7 mM histidine increased the rate of net histamine synthesis by a factor of 20 compared to normal. Histamine production sharply decreases at pH values below the physiological range.

Histamine production in the forestomach has been implicated in the pathogenesis of diseased states in ruminants[1,2]. Evaluation of the extent of histamine formation in vivo is complicated by the fact that histamine penetrates the ruminal epithelium[3], and by the possibility that histamine might be synthesized and deaminated by cells belonging to the ruminal wall[4], whereas available studies on in vitro synthesis are controversial[5,6] as to the rate of histamine formation. Therefore reexamining the ability of ruminal fluid (i.e. its microorganisms) to synthesize histamine by decarboxylation of histidine seemed warranted.

Ruminal fluid was obtained through an oesophageal tube from 2 adult cows, 3 h after the last grain feeding (the animals were maintained on 10–12 kg of hay and 1 kg of a concentrated feed, composed of barley, oats and maize). Experiments were always run in duplicates. Avoiding cooling, 80 ml of ruminal fluid were mixed with 20 g of powdered grass and 320 ml of a salt solution containing (mM) $(NH_4)_2SO_4$ 0.63, $CaCl_2$ 1.0, $MgCl_2$ 1.25, KH_2PO_4 5.2, K_2SO_4 9.9, $NaHCO_3$ 40.0, NaCl 78.5[7]. The mixture was incubated at 39 °C under carbon dioxyde in a shaking water-bath. The initial pH was 6.38. Gas escaped through a washbottle maintaining pressure at 3 cm H_2O, and its production was monitored in a gas burette[8]; pH was continuously measured and adjusted by occasional ad-

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