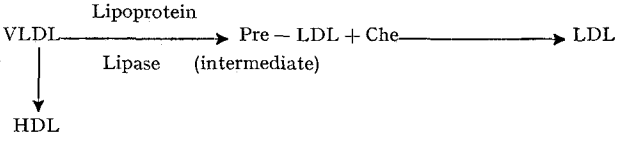
serum. The separated fractions of pre-beta, beta, and alpha-lipoproteins were cut out of the gel, dispersed in 10 ml of P.C.S. and counted. The radioactivity was calculated as counts per min in the lipoproteins contained in 0.1 ml of serum.

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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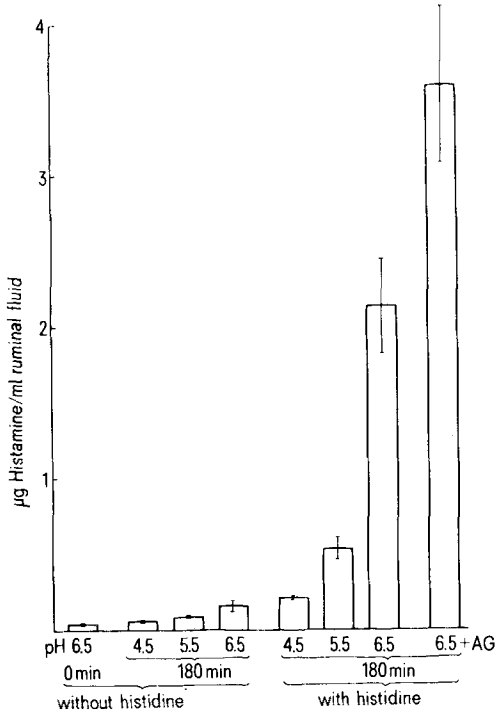
dition of a concentrated $NaHCO_3$ solution from a tightly fitting syringe. Histidine-HCl 4.7 mM as substrate and bis-(aminoguanidine-)sulfate 0.1 mM as inhibitor of the histaminase (diaminoxydase E.C. 1.4.3.6.) were added to the incubation mixture when required. The fluid was extracted as follows: pH was adjusted to 2 by adding HCl and solids removed by filtration through cheese cloth; 40 ml of filtrate was saturated with NaCl and Na_2CO_3 at 65 °C and shaken for 20 min with 100 ml n-butanol. The butanol phase was collected after centrifugation and this step repeated once. To the pooled butanol extracts, 1 part 0.1 n-HCl and 3.75 parts of heptanol were added in a separating funnel. After shaking, the aqueous phase was cooled in ice and evaporated under vacuum by slowly raising the temperature to 50 °C. The dry extracts were dissolved in Tyrode solution and assayed on the guinea-pig ileum, benadryl serving to distinguish histamine effects from other activities (which were negligible). Histamine-HCl served as standard substance. All results are given as free histamine-base per ml of original ruminal fluid. Acetylhistamine was determined by hydrolyzing the dry extract in 1 ml water and 9 ml concentrated HCl for 1 h at 120 °C in a sealed glass vial. On average recovery of histamine from water, salt solution, salt solution with grass powder or complete incubation mixture was 93.7% ± 2.3 (SEM) without significant deviation from the mean in any of the fluids. The results are shown in the table and the figure. The histamine content of ruminal fluid immediately after removal from the forestomach was 0.037 ± 0.0045 µg/ml (10 extractions from 7 specimens). Incubation for 3 h at normal pH (6.5) increased this value to 0.157 ± 0.013 µg/ml original ruminal fluid (7 extractions from 4 specimens) which is statistically significant ($p = 0.038$). The average rate of net formation during 3 h was 6 nmoles/ml min. As seen in the table, the rate was less at lower pH values. Adding 4.5 mM histidine to the incubation mixture increased the rate of histamine formation by a factor of about 20 (7 experiments, $p < 0.001$). At this high rate of formation, the same positive correlation with pH as seen without added histidine was observed. Addition of 0.1 mM aminoguanidine, known to be a comparatively specific inhibitor for diaminoxydase increased the rate of net histamine formation from 116 nmoles/ml min to 180 nmoles/ml min ($p = 0.024$). In 8 experiments with 4.7 mM histidine in the incubation mixture, the extracts were subjected to acid hydrolysis. This treatment did not increase the response of the guinea-pig ileum to the extract, which demonstrates that acetylhistamine formation in the ruminal fluid is negligible. In summary, the results show that ruminal fluid is able to decarboxylate histidine and that net histamine production is the result of formation and simultaneous destruction, probably by enzymatic deamination of histamine. Under normal feeding conditions, histidine is obviously limiting and free histamine concentration is of the order of 10^{-8} to 10^{-7} g/ml. Net histamine formation strongly decreases with pH values falling below the normal pH range of ruminal content.

2 conclusions may be drawn from these findings: 1. If reports on increased apparent histamine production in ruminal acidosis are correct, the explanation cannot be a low pH optimum of the decarboxylase or a shift away from the pH optimum of the deaminase. Possible reasons might be that low pH selects bacteria rich in histidine decarboxylase, or that it impairs histamine absorption by the well-known effect of pH on the distribution of weak bases across cellular membranes. 2. Since ruminal smooth muscle is remarkably insensitive towards histamine (effective concentration for half maximal response 1.2×10^{-5} g/ml for contraction in ruminal and approximately 3×10^{-6} g/ml for increase in frequency of reticular muscle; Wicki, unpublished) normal histamine content of the rumen will not influence ruminal motility. Only at histamine concentrations exceeding normal values by a factor of 100 might effects be expected. Such concentrations are only likely to occur when protein breakdown is unimpaired but protein resynthesis markedly reduced, i.e. conditions leading to high concentrations of free histidine.

Average rate of histamine formation at 39 °C under different incubation conditions expressed as nmoles per ml original ruminal fluid per min

	pH	Histamine (nmoles/min ml)
Without added histidine	4.5	0.92
	5.5	2.3
	6.5	6.06
With 4.7 mM histidine	4.5	8.7
	5.5	25.1
	6.5	116.3
With 4.7 mM histidine and 0.1 mM aminoguanidine	6.5	180.4

Incubation time 180 min.



Concentration of histamine base at end of incubation time (180 min) calculated per ml original ruminal fluid (mean ± SEM). Histidine, when added, was at a concentration of 4.7 mM in the incubation mixture. *AG*, 0.1 mM aminoguanidine in the incubation mixture. Column at far left: concentration in vivo.