Activity of NDV-envelope proteins

Material	Hemagglutination		Neuraminidase (μg NANA)	H
	Chick ετ	Guinea-pig ετ		
NDV	1280	320	21	42
NDV + ether	0	0	0	0
NDV + arcton	640	160	8	42
NDV + tween + ether	1280	320	25	—
NDV + triton × 100	1280	320	23	—
NDV + nonidet	1280	320	22	—
NDV + 0.125% SDS	640	320	0	—
NDV + 0.250% SDS	640	320	0	—
NDV + 0.750% SDS	0	0	0	—
NDV + 0.750% SDS dial.[a]	0	0	0	—
NDV + SDC	0	0	0	—
NDV + SDC dial.[a]	0	0	0	—

[a] The dialysis was performed for 48 h against 0.85% NaCl.

by the method of AMINOFF[8]. The percent of the hemolysis of the chick erythrocytes caused from the virus, which expressed the hemolytic activity (H), was estimated according to NUMAZAKI et al.[9].

Results. The results in the Table show that the activities of HA, N-ase, and H were lost after the extraction of viral lipids with ether. Arcton does not completely extract the lipids, and partial activity remained. Disruption of the virus with triton, tween 80, and nonidet did not inhibit the HA and N-ase, but SDS and SDC completely destroyed these activities.

Discussion. It can be concluded that the phospholipids-protein linkages are very important for the biological properties of NDV-envelope proteins, since the solubilization of the lipids with tween 80 and triton does not influence the phospholipid-protein linkages[10] but the same solubilization with SDS and SDC completely breaks these linkages, causing conformational changes[1,11,12]. According to SCHEID and CHOPPIN[6], HA and N-ase reside on a single polypeptide molecule. It can be suggested that the active center of the viral enzyme occupies the part of the molecule which is bound to phospholipids of viral envelope. Hydrophilic end of the molecule is necessary for hemagglutination, which occurs only when the protein molecules aggregate in the presence of phospholipids.

Summary. Treatment of NDV with anionic detergents or lipid solvents destroys the activities of hemagglutinin and neuraminidase. After disruption of the virus with non-ionic detergents, the activities of envelope proteins remain unchanged. It is suggested that the phospholipids are very important for the biological activity of NDV-envelope proteins.

LILIA WASSILEWA and D. NACHKOV

Institute of Infectious and Parasitic Diseases, Boul. P. Slavejkov 15, Sofia 6 (Bulgaria), 8 July 1975.

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Is the Basal Activity of Rat Stomach Histidine Decarboxylase Affected by Antrectomy?

The activity of histidine decarboxylase in the oxyntic mucosa of the rat stomach is low in the fasted state and high following feeding or treatment with gastrin or insulin[1,2]. With the exception of gastrin itself, all agents or treatments that activate the enzyme do so by releasing gastrin[3]. After removal of the bulk of endogenous gastrin through antrectomy, feeding and treatment with insulin or other gastrin-releasing agents failed to activate the enzyme[3–10]. Pentagastrin, however, still raised the enzyme activity[3,4]. Thus, gastrin seems to be the physiological mediator of the enzyme activation[3,11]. Contradictory results are on record as to the effect of antrectomy on the enzyme activity in the fasted rat. JOHNSON et al.[11,12] reported that basal enzyme activity was abolished, while ROSENGREN and SVENSSON[13], SVENSSON[14] and LUNDELL[15] found the activity to be significantly reduced by antrectomy. By contrast, we have never observed any effect of antrectomy on the enzyme activity in the fasted state[3–10]. In our studies, all rats were fasted for 48 h before the basal enzyme activity was determined. Considerably shorter fasting periods have been used by others[13–15]. So far the influence of the time of fasting on the enzyme activity has not been studied systematically. The present study was undertaken to determine the rate and extent of decline of the enzyme activity in unoperated and antrectomized rats after withdrawal of food. At the same time, the serum gastrin concentration was measured. 125 adult male Wistar rats weighing 150–200 g were used. 23 rats had been antrectomized 4–6 weeks before the actual experiments, as previously described[4]. The animals were either fed ad libitum or fasted for a defined period of time (not exceeding 48 h). During fasting, the rats were kept single in cages with wire mesh bottoms in

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Effect of food deprivation on serum gastrin concentration and gastric histidine decarboxylase activity[a]

	Serum gastrin concentration (pg eqv SHG/ml)		Histidine decarboxylase activity (pmoles CO_2/mg/h)	
Unoperated rats				
Fed ad libitum	144 ± 14	(15)[d]	56.2 ± 9.8	(15)[d]
Fasted 6 h	123 ± 16	(8)[d]	38.9 ± 6.7	(14)[d]
8 h	98 ± 5	(5)[d]	29.4 ± 4.1	(5)[d]
10 h	91 ± 16	(5)[c]	28.6 ± 5.1	(5)[c]
12 h	74 ± 10	(14)[d]	18.2 ± 3.0	(9)[c]
18 h	62 ± 8	(8)[c]	13.9 ± 3.0	(9)[b]
24 h	43 ± 8	(16)	10.8 ± 1.4	(18)[c]
36 h	34 ± 6	(8)	6.8 ± 1.1	(10)
48 h	27 ± 5	(12)	5.8 ± 0.5	(4)
Antrectomized rats				
Fed ad libitum	42 ± 6	(8)	6.6 ± 0.9	(8)
Fasted 24 h	—		7.3 ± 0.6	(4)
48 h	35 ± 7	(4)	6.6 ± 1.1	(11)

[a] Mean ± SEM (n). Significance of difference compared to the 48 hour values is given by [b] $0.05 > p > 0.01$; [c] $0.01 > p > 0.001$ and [d] $0.001 > p$; Student's t-test.

order to prevent coprophagia. They had free access to water. Upon sacrifice, the abdomen was opened under diethyl ether anesthesia and blood was drawn from the aorta. Serum was lyophilized and kept at −25 °C until analysis. Gastrin was determined by radioimmunoassay, using antibodies raised in rabbits against synthetic human gastrin I. The technique, its accuracy and reliability on rat serum has been described elsewhere[3]. The oxyntic mucosa was scraped off the stomach wall and homogenized in 0.1 M phosphate buffer, pH 7.0, to a final concentration of 100 mg (wet weight) per ml. After incubation of 0.5 ml of the homogenate with 1-^{14}C-L-histidine (4×10^{-4} M; 1.3 mCi/mM) in the presence of pyridoxal-5-phosphate (10^{-5} M) and reduced glutathione (5×10^{-4} M) at 37 °C under nitrogen for 1 h (final reaction volume 0.53 ml), the $^{14}CO_2$ produced during the reaction was collected and measured by liquid scintillation counting. The results were corrected for non-enzymatic decarboxylation by incubating boiled samples. Enzyme activities are expressed as pmoles $^{14}CO_2$ produced per mg mucosa per h[3].

The results are summarized in the Table. In the unoperated, freely fed rats, the serum gastrin concentration was high. It decreased after withdrawal of food, to reach a minimum at between 24 and 48 h of fasting. In the antrectomized, freely fed rats, the serum gastrin concentration was low and was not further lowered by fasting for 48 h. Following food deprivation, the gastric histidine decarboxylase activity in normal rats was markedly but slowly reduced. After 24 h of fasting, the basal enzyme activity was not yet established. After 36–48 h, the enzyme activity appeared to be maximally reduced. In freely fed antrectomized animals, the histidine decarboxylase activity was low and it was not further reduced by fasting. It is evident from the results that a fasting period of 18 h, as employed by ROSENGREN and SVENSSON[13], SVENSSON[14] and LUNDELL[15], is too short to establish a basal level of histidine decarboxylase activity. Any difference in enzyme activity between unoperated and radically antrectomized rats fasted for less than 36–48 h is therefore probably due to incomplete fasting.

The results of the present investigation emphasize the importance of adequate fasting in attempts to determine basal gastric histidine decarboxylase activity[16].

Summary. The serum gastrin concentration and the gastric histidine decarboxylase activity are high in freely fed, unoperated rats but low in antrectomized rats. Following food deprivation the serum gastrin level and the enzyme activity are reduced simultaneously in the unoperated rats. After fasting for 36–48 h – but not before – the enzyme activity drops to the same low levels as in antrectomized rats.

R. HÅKANSON, G. LIEDBERG and J. F. REHFELD

Departments of Pharmacology and Surgery, University of Lund, Sölvegatan 10, S-221 85 Lund (Sweden) and Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen (Denmark), 28 May 1975.

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Invertase in Cell-Free Culture Fluids of *Streptococcus mutans* Strain SL-1

Intracellular invertases have been purified and characterized from several strains of the cariogenic organism *Streptococcus mutans*[1–4]. FUKUI[5] has recently reported that invertase found extracellularly for *S. mutans* strain HS-6 has properties that are distinct from those of the intracellular invertases. The current investigation was directed at the purification and characterization of invertase from cell-free culture fluids of *S. mutans* strain SL-1.

Materials and methods. Cultures were grown anaerobically 10–14 h in a chemically-defined medium (Table). All subsequent procedures were performed at 3–7 °C unless otherwise stated. Extracellular protein was precipitated from the cell-free culture solutions, drawn at intervals during organism growth, by addition of ammonium sulfate to 80% saturation, followed by overnight settling. After centrifugation at 13,000 × g, the protein fraction was taken up in 0.020 M potassium phosphate at pH 6.7 (150 ml per l of original culture) and dialyzed against this buffer for 40 h, against distilled water for 4–5 h and lyophilized.

Extracellular protein preparations were dissolved in 0.025 M potassium phosphate buffer (pH 6.7) containing 0.04% NaN_3, and were chromatographed on a Sephadex G-100 column (2.5 × 40 cm) which had been equilibrated and washed in the same buffer. Eluted fractions with

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