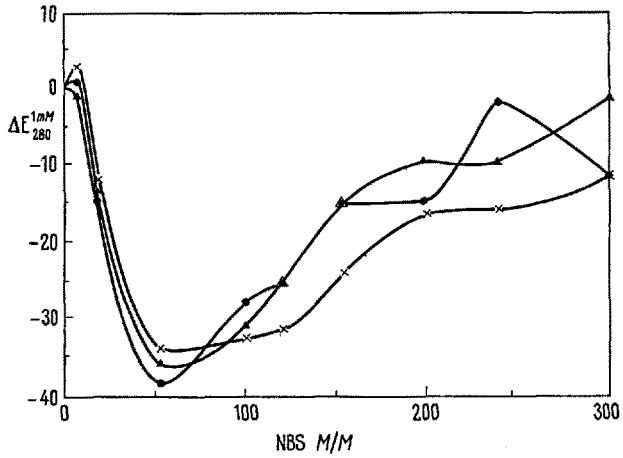

Fig. 2. Change in *mM* absorbancy of creatine phosphokinase treated with increasing amounts of NBS. After 15 min incubation (×). After 160 min incubation (●). After 290 min incubation (▲).

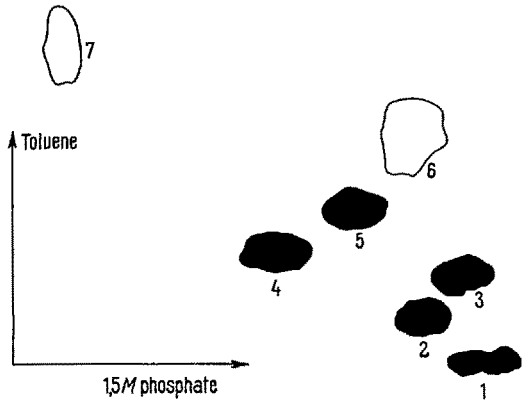

Fig. 3. Paper chromatography of DNP-amino acids from the hydrolysate of intact, dinitrophenylated creatine phosphokinase without NBS treatment (full spots). The additional compounds obtained after NBS reaction are indicated by hollow spots.

	M/*M* of protein		*M*/*M* of protein
(1) glutamic-aspartic	0.044	(5) alanine	0.024
(2) serine	0.022	(6) valine	0.152
(3) threonine	0.096	(7) unknown	0.078
(4) glycine	0.018		

Solvents: Toluene–pyridine–chloroethane and 1.5 phosphate buffer (pH 6.0)[5]. The unknown spot was neither Di-DNP lysine nor Di-DNP tyrosine.

and alanine in quantities, however, so small as to make interpretation of the results difficult (Fig. 3). It might be that 1) protein impurities or absorbed amino acids in the preparation are responsible for these *N*-terminal traces; that 2) certain bonds are somewhat hydrolyzed during dinitrophenylation in alkaline medium or that 3) structure hindrances prevent proper activity by the fluoro-dinitrobenzene onto the end terminals. Two additional spots, valine and an unidentified substance are observed when the protein is exposed to 100 moles of NBS per mole of protein in the 0.1 *M* urea-acetate buffer, pH 4.0 for 1 h (followed by dialysis against 0.5% $NaHCO_3$) before dinitrophenylation, suggesting the existence of a try-val bond in the molecule. (Analysis of the aqueous layer remaining from the ether extraction yielded no additional *N*-residues.) Considered on the mole per mole basis, the quantity of the new *N*-terminal group formed by the action of the NBS is very small. Such bond cleavages, however, have been reported only in yields averaging 20–40% and sometimes even as low as 5–10% or less[6]. Why the number of new *N*-terminals formed does not correspond to the number of tryptophans in the molecule cannot be explained at present. A similar observation has been made with regard to lysozyme where seven residues had been expected[6]. It should also be mentioned that paper electrophoresis at pH 8.6 in barbiturate buffer before and after sulfite exposure[7] of the NBS treated protein did not reveal the presence of any split products.

Zusammenfassung. Behandlung von Kreatinphosphokinase mit überschüssigem *N*-Bromsuccinimid bewirkt eine Umwandlung der vorhandenen peptidartig gebundenen Tryptophanreste, welche sich durch die für diesen Vorgang charakteristische Veränderung des Spektrums anzeigt. Am unbehandelten Enzym liessen sich auch mit einem grossen Überschuss von NBS keine freien endständigen NH_2-Gruppen eindeutig nachweisen.

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The Inhibitory Effect of Duodenal Souring on Shay Ulceration in Rat

SOKOLOV was the first to observe in 1904 that the gastric juice introduced into the dog duodenum intensively reduced the gastric secretion in the Pavlov pouch[1]. The presence of this duodenal inhibitory mechanism was consequently supported by several authors[2–6]. Various investigators produced from the duodenal mucosa the hormonal substances responsible for this effect[7,8]. Enterogastron decreases the gastric secretion also of cats, thus establishing the fact that the substance is not race-specific[9]. The degree of inhibition of experimental ulcers, a method renewed by SHAY[10], and originally described by TALMA[11], is extensively used in the evaluation of duodenal extracts, that is, enterogastron preparates[12]. We were interested in the question of whether duodenal

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souring, induced simultaneously with the SHAY operation, was able to activate a hormone amount sufficient to prevent gastric ulceration.

Methods. 120–150 g rats of our own breed kept on Sós's semi-synthetic diet were used in our experiments[13]. After 48 h of complete fasting—while given water *ad libitum*—the animals were subjected to SHAY's operation under ether anaesthesia and simultaneous intraduodenal HCl injections were given. The animals were decapitated 14 h after the operation. Their gastric content was examined quantitatively with regard to pH and total acidity, and from changes in the gastric mucosa, the ulcer index was determined. The following scheme was used in our calculations:

ulcer and perforation, resp.	of 10 mm diameter	20 points
ulcer	of 5 mm diameter	10 points
ulcer	of 2 mm diameter	5 points
ulcer	of 1 mm diameter	2 points

The points showing the changes in each stomach were added and divided with the number of animals in the group, and thus the value of the ulcer index was obtained.

Results. In the course of our experiments, the animals were divided into two groups. The first group was given simultaneously with the operation a 0.35% HCl solution at body temperature diluted with physiologic saline, and the other group received a 0.9% intraduodenal NaCl solution, 2 ml/100 body weight. The animals were killed in the 15th postoperative hour; their gastric content was subjected to chemical analysis, the results of which are shown in Figure 1.

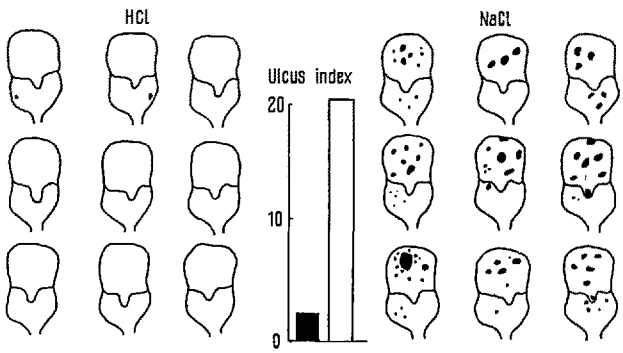

Fig. 1. Scheme of gastric mucosa and ulcer index of SHAY rats treated with intraduodenal HCl and NaCl, resp.

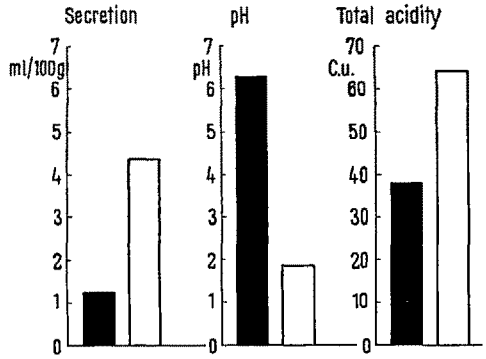

Fig. 2. Amount of gastric secretion, pH and total acidity value (C. u.: clinical unit = ml 0.1*n* NaOH/l gastric juice) in intraduodenally HCl (■) or NaCl (□) treated SHAY rats.

The amount of gastric secretion significantly decreased as compared to the controls; its pH value showed a definite rise and correspondingly the total acidity value dropped after the intraduodenal injection of HCl. The changes detectable on the gastric mucosa are schematically illustrated in Figure 2, and at the same time the value of the ulcer index is shown.

Following intraduodenal HCl injection, only one animal developed a minimal change on the gastric mucosa as can be seen in the Figure whereas serious ulceration was detectable in all of the controls.

It is further on considered noteworthy that, whereas following intraduodenal HCl injection the gastric content was markedly mucous and the surface of the gastric mucosa lubricious, the mucus was completely absent in the controls.

Discussion. SHAY is of the opinion that ulceration after placing a ligature on the pylorus is due to the digestive effect of the sour gastric content gathering because of the increased gastric secretion[10]. According to the French school, the ulcus is said to develop in consequence of the reflectoric vascular spasm elicited by pyloric ligation[14]. We are of the opinion that besides these two reasons the cessation of natural duodenal souring induced by placing a ligature on the pylorus also has a role, and so this ulcer may be considered as an outfall phenomenon of gastrointestinal parahormones. In our former studies, it was demonstrated that after intraduodenal HCl injection a functional capillary dilatation is established in the splanchnic vascular region and, due to electric stimulation of the splanchnic nerve, there is a significantly slighter elevation in blood pressure following duodenal souring than in the previous control period[15,16].

On the basis of all these facts, we presume that in the SHAY rat duodenal souring prevents, through functional capillary dilatation induced in the splanchnic area, the development of reflectoric vascular spasms arising after the ligation of the pylorus. Besides, the protective effect is explained also by the decreased gastric secretion brought about by duodenal souring.

Résumé. L'acidification du duodenum pratiquée parallèlement à l'opération de Shay empêche totalement le développement de l'ulcus chez le rat. Les auteurs expliquent cet effet de la manière suivante: les parahormones activées gênent la sécrétion et la contraction de l'estomac et provoquent dans les vaisseaux splanchniques une vaso-dilatation fonctionelle. De ce fait, la vasoconstriction réflective qui suit la ligature du pylore ne peut pas se réaliser dans les parois de l'estomac.

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