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THE SPLITTING OF HYALURONIC ACID BY GASTRIC JUICE AND SALIVA OF DOGS

By

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Hyaluronic acid, HyA, is a common constituent of soft tissue of mesenchymal origin. HyA is hydrolyzed, i.a. by hyaluronidase, Hyase, which has a softening action on connective tissue (5). Thus one might assume the occurrence of some HyA-splitting factor(s) in digestive fluids of carnivores. A study (2) on gastric, intestinal, and vesical walls of dog seems to confirm this assumption. In the following investigation the *gastric juice* from dogs was studied with regard to its splitting action on isolated HyA. Some complementary experiments with *saliva* of dogs are also described.

MATERIAL AND METHODS

Gastric juice from 18 dogs of different breeds and varying ages, 8 months to 12 years, was studied. 16 dogs were known to have no digestive disturbances, whereas 1 dog (no. 12) suffered from chronic gastritis and 1 dog (no. 11) refused to eat raw meat.

The gastric juice was obtained as follows: The dogs were kept without food for about 18 hours. The secretion of gastric juice was stimulated by giving a 7 per cent ethanol-water solution through a ventricular tube at a dosage of 10 ml/kg bodyweight. After about 15 minutes samples of gastric juice were withdrawn by suction. Toluene was added as a preservative.

The *saliva* from dogs nos. 16—18 was obtained immediately after withdrawal of the gastric juice by the following procedure: The dogs were injected subcutaneously with pilocarpine — about 0.2 mg/kg bodyweight — to stimulate secretion of saliva. The saliva was collected in a basin held under the mouth of the dog. As above toluene was added.

The *HyA* was prepared from human umbilical cord as a sodium salt (AB. Analyskemikalier, Stockholm). The salt had a nitrogen content of 3.3 per cent, was ninhydrin negative, and contained less than 0.02 per cent sulphur.

The following buffer solutions were used:

1. Glycine — Hydrochloric acid pH 3.4
2. Citrate — Phosphate „ 2.6
3. „ „ „ 3.0
4. „ „ „ 3.4
5. „ „ „ 4.0
6. „ „ „ 4.5
7. „ „ „ 5.0
8. „ „ „ 5.5

They were prepared as described by *Colowick* and *Kaplan* (4). All buffers contained 0.15 M NaCl/1.

The determinations of the HyA-splitting activity were performed in an Ostwald viscometer at 37.0 ± 0.1° C. Three ml of a

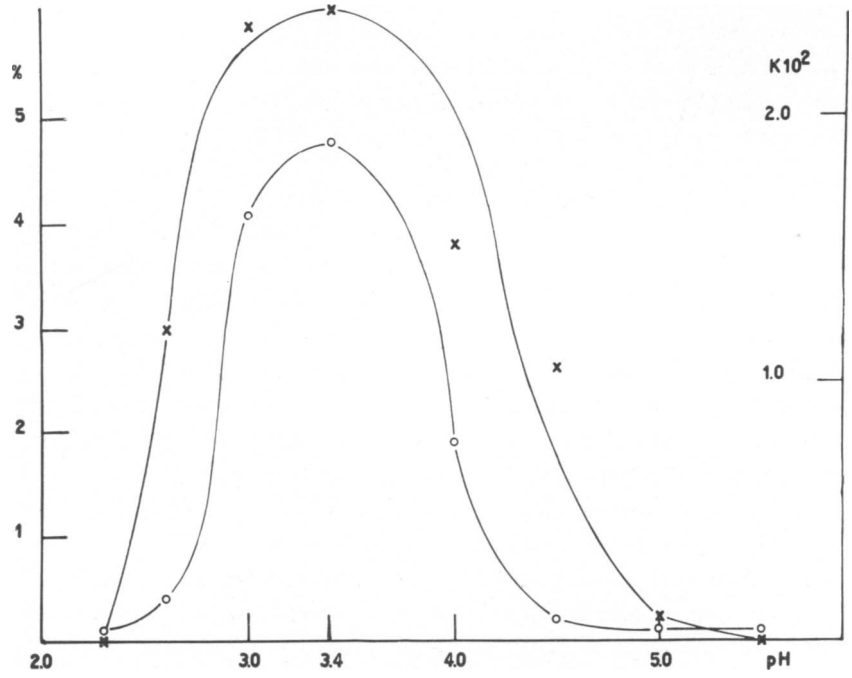

Fig. 1. pH-optimum of gastric juice from dog.
Symbols: -o-o-o- per cent splitting.
-x-x-x- k × 10^2-values.

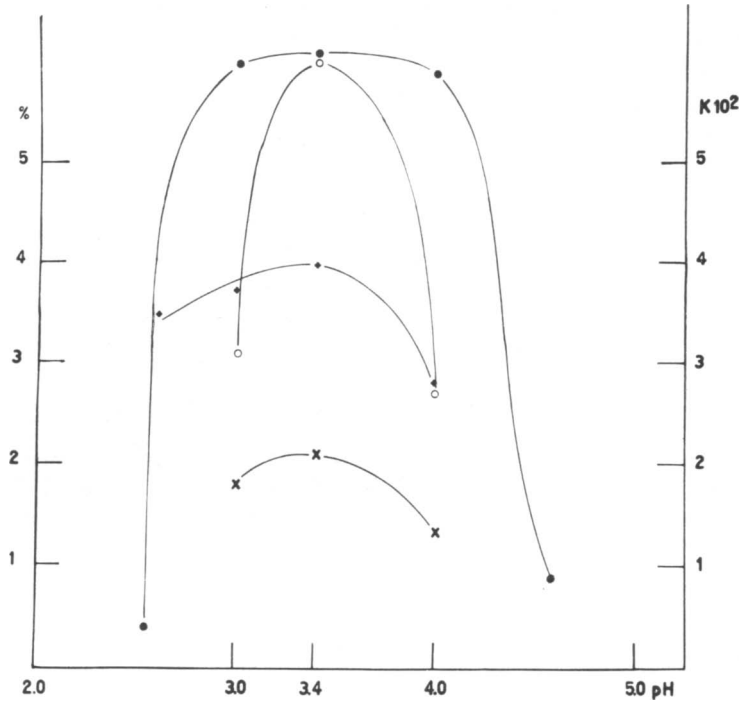

Fig. 2. pH-optimum of gastric juice and saliva from dog.
Symbols: -o-o-o- gastric juice, per cent splitting.
-x-x-x- gastric juice, k × 10^2-values.
-●-●-●- saliva, per cent splitting.
-+-+-+- saliva, k × 10^2-values.

0.3 per cent HyA in buffer 1 and 2—8 resp. was pipetted into the viscometer and 2 ml of a mixture of equal volumes filtered gastric juice (saliva) and buffer 1 (and 2—8) was added with simultaneous insufflation of air. The flow time was recorded immediately after mixing and then every fifth minute. As an expression for the velocity of the reaction the value of k for the equation: log flow time (minutes) = — k × reaction time (minutes), was determined (8).

Controls were run with HyA-solution and gastric juice (saliva) heated at 100° C for 30 minutes.

pH was checked before and after each determination.

The *pH-optimum* of the activity was determined in a few samples by use of buffers 2—8. The values of k obtained were compared with per cent splitting as measured by determination

of reducing groups in the viscometer solutions after 2 hours' reaction time.

The reducing groups were expressed as mg glucose according to the method of Somogyi (13), i.e.: To 4 ml of the viscometric mixture 2 ml saturated $Ba(OH)_2$ and 2 ml 0.45 per cent $ZnSO_4$ were added. The pH of this mixture was adjusted by adding 1-M NaOH within a volume of 1 ml until the phenolphthalein turned pink. After mixing and centrifugation 5 ml of the supernatant was used for the determination of reducing groups. The results are expressed as mg glucose $\times$ 100/mg HyA in the original sample compared with the controls, and are called per cent splitting.

The *HyA-splitting* activity of the different gastric juice and saliva samples was expressed by the velocity constant, k, using buffer 1 as a "physiologic" buffer. In some cases buffer 4 was used for comparison.

RESULTS

The *pH-optimum* of the activity for gastric juice, dog no. 1, is shown in Fig. 1. The $k \times 10^2$-values of the controls were $= 0.00$.

Fig. 1 thus indicates optimal activity at about pH 3.4

Fig. 2 shows the pH activity relation for gastric juice compared with saliva, dog no 17.

The figure shows a fairly good agreement with regard to pH-optimum for saliva and gastric juice, i.e. about pH 3.4. Yet the optimum seems broader for saliva.

The *k-values* — expressions for the HyA-splitting activity — from the viscometric determinations at pH 3.4 $\pm$ 0.1, buffer 1 and 4, are collected in table 1.

The results of the gastric juice experiments thus show wide differences in activity. The absence of activity in some cases, viz. nos. 2, 3, 4, 15 is remarkable.

The few experiments with saliva, viz. dogs nos. 16—18, show rather high k-values. In these cases the gastric juice also shows pronounced activity.

DISCUSSION

The result of *Cadilio* and *Li Voti* (2) showing Hyase activity in the gastric wall of dog seem to be confirmed by the results obtained with gastric juice from dog in this study. However, it

Table 1.

Dog no.	*Gastric juice*		*Saliva*	
	Buffer 1 $k \times 10^2$	Buffer 4 $k \times 10^2$	Buffer 1 $k \times 10^2$	Buffer 4 $k \times 10^2$
1	1.88[2])	2.42[1])		
2	0.00			
3	0.00			
4	0.00			
5	0.13			
6	0.05			
7	0.21			
8	1.63			
9	3.33			
10	1.28			
11[3])	2.87			
12[3])	0.09			
13	0.71			
14	1.04			
15	0.00			
16	2.11		6.65	
17		2.16		4.07
18	1.10		5.21	
Controls	0.00	0.00	0.00	0.00

[1]) immediate determination.
[2]) determination after 2 days. Sample stored at + 3° C.
[3]) gastric disturbances.

is remarkable that in some cases, viz. dogs nos. 2, 3, 4, 15 no activity was found at pH 3.4. It is of interest that dogs nos. 16—18, with gastric juice HyA-splitting activity, also show such activity of saliva. It is not unlikely that the HyA-splitting activity of gastric juice and saliva are of the same origin. The similar pH-optimum of the two in the samples studied may be in agreement with such an assumption. *Bussard et al.* (1) showed the presence of Hyase in saliva of dogs, and because of their great experimental series they were able to show also negative findings in some dogs. The works by *Lisanti* (7), *Chauncey et al.* (3), and *Rovelstad et al.* (12) describe Hyase activity in human saliva and also present results from human saliva without or almost without HyA-splitting activity. Several articles (3, 10, 11, 12) treat the Hyase activity of human saliva in connection with the occurrence of shown or supposed HyA-splitting microorganisms in the mouth.

This study which shows the presence of HyA-splitting acti-

vity in gastric juice and saliva of dogs does not resolve the question whether the activity is endogenous or exogenous in nature or both. The pH-optimum about pH 3.4 in the samples studied suggest a major activity of the HyA-splitting factor(s) in the stomach where it (they) could be of importance for the digestion of meat. If the activity is due to microorganisms, this could indicate that carnivores like herbivores (omnivores) have symbiotic microorganisms in the alimentary tract taking part in the digestion of food.

The optimal HyA-splitting activity found at about pH 3.4 in the few samples studied is of some interest since the experiments by *Bussard* (1) and *Rovelstad et al.* (12) were performed at pH 4.7 and 6.6. If the fairly rapid HyA-splitting activtiy of gastric juice and saliva of dogs, which is destroyed by heating is dependent upon a Hyase, it is interesting that most cited Hyase pH optima (6) are less acid than 3.4. Among the examples cited in the literature are pneumococcal and leech head enzyme pH 5.0—7.00 and 5.4 as determined by increase in reducing groups, and testicular-, Clostridium welchii-, Streptococcus haemolyticus- and Staphylococcus aureus- Hyase pH 7.0, 6.0, 5.5 and 6.6 as measured by viscometric methods. However, comparisons between reported pH-optima should be evaluated judiciously, if the systems used are not quite similar. With this in mind, the acid optimum of about 3.4 for the HyA-splitting activity of gastric juice and saliva of dogs could be due to another kind of Hyase than those cited.

It may be mentioned that also non-enzymic, pH-independent, breakdown of HyA caused by different substances is known (9). If such, heat labile substances are present in the samples not checked for pH-dependence, they could have contributed to the found splitting of HyA.

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SUMMARY

The splitting action on hyaluronic acid by gastric juice (18 cases) and saliva (3 cases) from dogs was studied. The determinations were performed viscometrically with pure hyaluronic acid as substrate. The pH-optimum of the activity was in a few cases determined to pH 3.4 and the activity of the samples were preliminary studied at that pH. It was found that gastric juice in 14 cases out of 18 and saliva in the 3 cases studied showed a splitting action on HyA.

ZUSAMMENFASSUNG

Die Spaltung der Hyaluronsäure durch den Magensaft und Speichel von Hunden.

Die Spaltwirkung von Magensaft (18 Fälle) und Speichel (3 Fälle) vom Hund wurde auf Hyaluronsäure untersucht. Die Bestimmungen wurden viskometrisch mit reiner Hyaluronsäure als Substrat ausgeführt.Optimale Aktivität wurde in einigen Fällen bei 3.4 gefunden. Die Aktivität der Proben wurde versuchsweise bei diesem pH-Wert studiert. Es wurde gezeigt, dass 14 Magensaftproben von 18 und die 3 Speichelproben eine spaltende Wirkung auf Hyaluronsäure ausübten.

SAMMANFATTNING

Spjälkning av hyaluronsyra av magsaft och saliv från hund.

Den spjälkande verkan på hyaluronsyra av magsaft (18 fall) och saliv (3 fall) från hund har undersökts. Bestämningarna gjordes viskometriskt med ren hyaluronsyra som substrat. Aktivitetens pH-optimum fastställdes i några få fall till omkring pH 3.4 och aktiviteten hos proven bestämdes försöksvis vid detta pH. Det visade sig att 14 av 18 magsaftprov och samtliga 3 undersökta salivprov spjälkade hyaluronsyra.

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