IS THE SALIVA OF THE DOG AMYLOLYTICALLY ACTIVE?

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The view that the saliva of certain animals, particularly the carnivora, is devoid of amylolytic power, has been current since the classic investigations of Bidder and Schmidt.[1] With respect to the dog their statement is quite specific.[2] An equally positive and definite conclusion was drawn by Claude Bernard from his experimental observations.[3] Physiological literature since that

[1] Bidder und Schmidt: *Die Verdauungssäfte und der Stoffwechsel*, 1852, p. 14, flg.

[2] Wir haben daher neuerdings nicht nur die reinen Sekrete der betreffenden Drüsen aufgefangen, sondern auch das reine Secret der Mundschleimhaut von Hunden gewonnen. . . . Die so erhaltenen Secrete wurden, jedes für sich, mit Stärkekleister vermischt und einer Temperatur von 40° C. ausgesetzt. In keinem Falle war durch die Trommersche Probe vor 8 Stunden und auch dann nur eine Spur von Zucker nachzuweisen, und wir müssen hiernach auf's Nachdrücklichste wiederholen, dass Keinem der Secrete, durch deren Vermischung die Mundflüssigkeit gebildet wird, allein für sich bei der Umsetzung des Stärkemehls in Zucker irgend eine Fermentwirkung zugeschrieben werden könne. . . . (*loc. cit.*, p. 19 flg.)

[3] "La salive de chien pure et à l'état frais n'agit pas sur l'eau d'amidon; mais elle acquiert cette propriété lorsque, abandonnée à elle-même, elle vient à éprouver une certain degré d'altération. C'est ce que prouve l'expérience suivante. Exp.—Des salives fraîches de chien sous-maxillaire et sublinguale, très gluantes, ont été séparément mises en contact avec de l'eau d'empois d'amidon, et n'ont exercé aucune action pour changer cette substance en sucre. Au bout de deux jours, ces salives ayant été abandonées à elles-mêmes par un temps chaud et orageux, avaient complétement perdu leur viscosité, et alors elles agissaient très énergiquement sur l'eau d'amidon pour le transformer en sucre. De la salive parotidienne fraîche placée dans les mêmes circonstances n'eut pas d'action sur l'eau d'empois d'amidon, et acquit la propriété de la transformer lorsqu'elle eut subi un commencement d'alteration: d'ou il faut conclure qu'à l'état frais, les salives pures ne transforment pas l'eau d'empois d'amidon. (Claude Bernard: *Leçons sur les propriétés physiologiques et les altération pathologiques des liquides de l'organisme*, ii, p. 249, 1859.)

time has repeatedly reiterated these announcements,[1] so that we read in the recent lectures of Starling: "In dogs the mechanical action of saliva is its only one."[2]

The experience in our laboratory has never afforded any occasion to question the accuracy of this statement. It was with some surprise, therefore, that we learned from the paper of Neilson and Terry,[3] entitled "The Adaptation of the Salivary Secretion to Diet," that in all their experiments "the saliva of dogs was found to be active, but varying considerably in its amylolytic power, in different animals." Dogs fed upon a diet consisting principally of bread, with a small amount of meat broth and some ground meat are reported as invariably having a saliva with strong amylolytic powers. The authors claim that both the saliva and gland extracts of dogs fed on a mixed diet containing bread show a much greater amylolytic power than those of street dogs on an unknown diet. An experiment is reported in which a dog was fed on meat for fourteen days, the salivary glands on one side were taken out, and the animal subsequently put on a bread diet for fourteen days before removal of the remaining glands. No sugar was detected in any case within the first hour in the digestion trials with the extracts and starch paste; but on the basis of subsequent observations upon the digestive mixtures the conclusion is drawn that the glands adapt themselves to diet. It is further stated that "probably in all dogs there is an active ptyalin, but it is relatively inert as compared to human saliva."

The importance and interest which attaches to a demonstration of adaptation in digestive secretions need scarcely be emphasized. Earlier alleged evidences of such reactions in the animal organism have lately been subjected to severe criticism,[4] so that analogies in the case of the pancreas and intestine are, at the

[1] They are found, for example, in the text-books of Gamgee, Neumeister, Schäfer, Hammarsten and Abderhalden.

[2] Starling: *Recent Advances in the Physiology of Digestion*, p. 41, 1906. Fermi (*Arch. f. Physiol.*, Supplementband, p. 65, 1901) also failed to find ptyalin in dog's saliva.

[3] Neilson and Terry: *Amer. Journ. of Physiol.*, xv, p. 406, 1906.

[4] Cf. Plimmer: *Journ. of Physiol.*, xxxiv, p. 93, 1906; xxxv, p. 20, 1906; Bierry: *Compt. rend. de la soc. de biol.*, lviii, pp. 700, 701, 1905.

present moment, not well substantiated. Aside from the work of Neilson and Terry we recall only two other recent investigations which attribute amylolytic properties to dog's saliva. Henri and Malloizel have incidentally noted variations in supposed amylolytic activity in the submaxillary saliva of dogs when different stimuli were employed. They remark "La salive sous-maxillaire est toujours très peu active. Dans les cas de l'activité maximum nous avons trouvé au bout de 5 heures, 6 à 7 milligrammes de sucre."[1] When we add that under comparable conditions of experiment lymph formed five times as much sugar and that there was a parallelism between the mucin content of the digestive mixtures and their supposed activity, these experiments have little positive significance. Aside from this inconclusive evidence we have further noted an experiment by Hemmeter[2] in which dog saliva and trypsin solutions were subjected in different and separate portions to various temperatures, and thereafter their amylolytic and proteolytic power tested. Since the investigator was studying a quite different problem and used saliva as a type of amylolytic secretion one must assume that he regards the canine saliva to be active upon starch.[3]

We have made a series of observations upon dogs and cats in order to demonstrate, if possible, the native or acquired amylolytic properties of the saliva. *The experiments have failed to give evidence of any marked or characteristic digestive action upon*

[1] Henri and Malloizel: *Compt. rend. de la soc. de biol.*, liv, p. 331, 1902. Our attention was directed to this paper by Prof. A. J. Carlson.

[2] Hemmeter: *Journ. of the Amer. Med. Assoc.*, Dec. 9, 1905; *Berl. klin. Wochenschr.*, 1905, No. 44a (Ewald Festschrift). In the older literature positive results are recorded by Astaschewsky. Cf. *Jahresber. f. Thierchem.*, vii, 256, 1877.

[3] Professor Hemmeter has informed one of us (M.) that he has observed the inactivity of dog's saliva on boiled starch in some cases. He writes: "I noticed in one dog that when the saliva is collected by a sponge from his mouth and not by catheterization of the duct, that sometimes it is active and sometimes it is not. Even in dogs in which the saliva is obtained by catheterization of the duct and stimulation of the chorda tympani, the saliva is of varying amylolytic power. This is true not only of dogs, but also of human beings . . . some deep seated causes are underlying these discrepancies." Professor Hemmeter adds that he has not investigated the production of sugar in the digestions, but confined his attention to the solution of the starch.

starch paste.[1] Furthermore a careful study of the data submitted by Neilson and Terry renders their positive deductions somewhat less convincing.[2] The reacting solutions as a rule reduced Haines' solution only after comparatively long periods of digestion, and then only slightly. For example, one extract in which the reduction test showed "a trace of sugar on standing" after four hours' digestion with one gram of starch, was ascertained to contain 0.029 gram of sugar (Experiment D); whereas a comparable digestion (Experiment B) with a presumably more active extract is recorded as giving a "heavy reduction at once" after one and one-half hours, while the amount of sugar was estimated at only 0.054 gram from 1.3 grams of starch. In one experiment (B) upon a bread-fed dog[3] the saliva itself was distinctly active. With this exception, the amylase might be regarded as relatively inert, at any rate if we use the observations on the pancreatic amylase of the same animal or the saliva of other species as standards of comparison.

In our experiments upon dogs the saliva was obtained from cannulas in the ducts after stimulation of the chorda tympani, with frequent intervals of rest, unless otherwise stated. The animals were anæsthetized with ether or A. C. E., after administration of a small dose of morphine. The gland extracts were prepared with toluene water and filtered through absorbent cotton. A one per cent paste prepared from best grade arrowroot starch was used in the digestions, which were carried on in the presence of toluene, at 40° C. The progress of amylolysis was noted by applying the iodine test and Fehling's test. Where quantitative estimations were attempted the Allihn gravimetric method was applied, because we regard this procedure as rather more satisfactory than the volumetric processes where such

[1] I am informed by Prof. E. H. Starling that he also has examined both submaxillary and parotid saliva from the dog (after chorda stimulation or pilocarpine injection) a number of times, but has never obtained any amylolytic effect.—L. B. M.

[2] Plimmer also says: "The results obtained by these authors, however, are not very conclusive and further experiments on this point would be of great interest." (*Journ. of Physiol.*, xxxv, p. 21, 1906.)

[3] In the original paper B, p. 409, is labeled "*Meat-fed Dogs*"—evidently a typographical error. The weight of the second submaxillary gland on p. 410 is also apparently printed incorrectly.

small quantities of reducing substances are present. The reducing power of the solutions is expressed in terms of copper. This was necessary because in several of the trials the quantity of maltose could not be calculated from Wein's tables, as the amounts of copper obtained fell below the limits of these tables.

PROTOCOLS.

I. A dog weighing 14 kilos was used. Diet unknown. The saliva was obtained from both submaxillary glands, and portions successively collected were mixed. The final records noted below were made after three to four days digestion at about 40° C.

(*a*) 3.15 p. m. Mixed Saliva .....20 cc.
Starch Paste.....20 "
Sterilized Water and Toluene.... 20 "
60 "
In two hours a quantitative estimation indicated 0.0137 gram Cu in all. Three days later this was practically unchanged: 0.0141 gram Cu in all. After four days the iodine reaction showed a reddish tinge. A slight reduction could be observed with Fehling's solution.

(*b*) 4.05 Saliva .....40 cc.
Starch .....25 "
Water and Toluene...10 "
75 "
The unchanged starch interfered with the filtration of the trace of cuprous oxide precipitated. After four days the solution still gave the blue color with iodine solution and only a slight reduction.

(*c*) 4.15 Saliva ..... 6 cc.
Starch and Toluene . 6 "
12 "
Four days later this gave a reddish blue iodine test and a slight reduction with Fehling's solution.

(*d*) 4.25 A similar trial gave less evidence of digestion after four days.

(*e*) 4.30 5 cc. saliva + toluene. *No starch added.* On the following day this saliva alone yielded 0.0018 gram Cu in an Allihn estimation.

It will be noted that the digestive action, even as indicated by the reduction tests. was minimal at most. In (*a*), for example, despite the large quantity of saliva present, it did not increase, thus suggesting exterior causes for the reaction. The saliva itself, after standing, gave a visible reduction sufficient to account for part of the other reactions.

II. From a dog weighing 15 kilos, saliva was collected alternately from the right and left submaxillary gland, with varying strengths of stimuli (coil distance) and intervals of rest, and it was tested in portions of 5 cc. saliva + 5 cc. starch paste + toluene. Next day the nine tubes all still showed a blue reaction with iodine. Slight reduction tests were obtained, becoming somewhat more distinct in the portions of saliva last collected (after 2 hours).

III. A bitch weighing 9.5 kilos was anæsthetized with ether and A. C. E. and saliva was collected directly by blowing chloroform or ether vapor into the mouth. Digestion trials were made: 4½ cc. saliva + 4½ cc. starch paste (1) with and (2) without addition of toluene. On the following day both tubes gave a blue reaction with iodine solution and a slight reduction. Four days later (1) was clear but still gave a blue reaction with iodine (soluble starch) as well as a marked reduction test; (2) was decomposed.

IV. For quantitative estimations submaxillary saliva was collected by chorda stimulation from the same animal, and digestion trials were arranged as follows:

(1)	(2)
18 cc. Saliva.	18 cc. Saliva.
18 " Starch Paste.	18 " Starch Paste.
18 " Water.	18 " Water.
Toluene.	No Toluene.

Two days later the mixtures still gave a blue reaction with iodine despite the very large quantities of saliva used.

Copper found: (1) 0.0480 gm.; (2) 0.0696 gm.

The toluene was omitted from one of the trials in order to exclude any possible retarding action of the antiseptic on the enzyme. The slightly increased yield of copper in (2) may equally well be ascribed to the action of microörganisms. At any rate no serious inhibition is attributable to the toluene used in our experiments.

V. The dog weighed 12 kilos. Cannulas were introduced into the ducts of both submaxillary and sublingual glands. The periods of stimulation and rest were continued over three hours; the submaxillary and parotid glands were excised, minced and

extracted with toluene water and filtered through absorbent cotton.

Successive portions of saliva, etc., were mixed, *as soon as collected*, with sterilized water, starch paste and toluene. They were examined 24 hours later.

		Iodine Test.	Reduction Test.
(1)	5 cc. Submaxillary Saliva 20 " Starch Paste 5 " Water and Toluene	blue	18 mgm. Cu
(2)	10 " Submaxillary Saliva 10 " Starch Paste Toluene	blue	28 mgm. Cu
(3)	5 " *Boiled* Submaxillary Saliva 20 " Starch Paste 5 " Water and Toluene	blue	none
(4)	1 " Sublingual Saliva 10 " Starch Paste 5 " Water and Toluene	blue	faint
(5)	10 " Submaxillary Saliva 10 " Starch Paste Toluene	purple	positive
(6)	15 " Parotid Extract 20 " Starch Paste Toluene	red	heavy
(7)	Ditto, with Parotid Extract *boiled*,	blue	none
(8)	35 cc. Submaxillary Extract 40 " Starch Paste Toluene	colorless	heavy
(9)	Ditto with submaxillary extract *boiled*,	blue	none

(10) A further experiment was tried with 7 cc. of submaxillary saliva which was *allowed to stand two hours* under cover before being mixed with 5 cc. starch paste and toluene. This mixture gave *no color with iodine* and a *heavy reduction test* on the following day.

(11) A mixture of 4.6 cc. submaxillary saliva + 10 cc. starch paste + toluene was frequently tested. After 1½ hour it began to give a faint reduction test which did not increase in the next 20 hours.

These trials indicate the nearest approach to active digestion which we have recorded.

VI. *Adaptation Experiment.* A dog weighing 14.5 kilos was kept upon a diet of bread, with a little milk and without any meat, during 4 to 6 weeks. Successive portions of submaxillary saliva were examined alone, or with equal quantities of starch paste, with and without toluene.

	(1) Saliva 20 cc. Starch Paste. Toluene.	(2) Saliva 20 cc. Starch Paste. No Toluene.	(3) Saliva 10 cc. Toluene.	(4) Saliva 20 cc. Starch Paste. Toluene.
Reduction test, next day..	.0117 gm. Cu	faint	0.001 gm. Cu	faint
Iodine test, after 2 days		blue		blue
" " " 9 "		decomposed		blue
Reduction test, after 9 days		none		positive

There is no evidence here, in our opinion, that any adaptation has taken place.

VII. *Adaptation Experiment.* A bitch weighing 15 kilos had for months been kept upon a diet of meat, *cracker meal* and lard, which was regarded as very favorable for the development of an adaptation of the secretion. Saliva was collected from both submaxillary glands with varying rates of flow and intensity of stimulation (coil distance). The different portions, usually about 5 cc., were mixed with equal volumes of starch paste + toluene. After one day at 37° C. all still gave a blue iodine test together with reduction tests on standing, which we can best describe as varying from slight to distinct, without any apparent sequence. Thus the most distinct reduction test was given by a specimen obtained from the left gland with weak stimulation, while three specimens secreted under comparable conditions from the right gland showed almost no cuprous deposit.

VIII. The submaxillary saliva of a large dog was collected directly from a duct by chorda stimulation. To 10 cc. portions of starch paste an equal volume of (1) boiled and (2) unboiled saliva was added in two flasks, with toluene. Next day both mixtures still gave a blue color with iodine solution, 5 cc. gave a slight reduction in each with Fehling's solution. The deposited cuprous oxide was, if anything, larger in quantity in (2).

IX. *Experiments with Cats.* The saliva of the cat is generally regarded as devoid of amylolytic properties.[1] We have collected mixed saliva from this animal directly from the mouth by the use of ether vapor stimulation. It fails to clear starch paste or give evidence of amylolytic power. The possible influence of diet has not been considered.

We agree with Plimmer in assuming that adaptation is perhaps more readily established in connection with functions present or dormant than in the case of activities of which there are no direct indications in the species.

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[1] Cf. Cannon and Day: *Amer. Journ. of Physiol.*, ix, p. 396, 1903. Other references of interest in this connection are given in the introductory part of their paper.

The extracts from our protocols have been recorded here in somewhat greater detail than they might otherwise deserve, in order to enable the impartial observer to draw his own conclusions. Our interpretation of the observations has already been indicated. The almost uniform failure of dog's saliva, collected under variable conditions from the mouth cavity or directly from the ducts, to convert starch paste completely to or beyond the dextrin stage under the most favorable conditions of digestion seems to speak against any distinctive amylolytic activity.[1] The more nearly positive results indicated in Experiment V could not be obtained again. The experiments with the gland extracts are not so significant when we recall the wide distribution of amylases in small amounts throughout many tissues and in the blood and lymph.[2] Furthermore the demonstration of a *slight* reducing power must be interpreted with considerable caution. The saliva itself was sometimes slightly effective in this direction; and the presence of sugar in the digestions is an uncertain inference when the quantitative data approach the lower limits of accuracy. This is emphasized by the failure of the slight reducing power at times observed to increase adequately with the period of digestion. While these experiments give no evidence of a specific adaptation of the salivary glands effected by diet, the possibility of a more successful result under still other conditions cannot be denied.

[1] If one accepts the idea of a specificity of amylases as advocated by Ascoli and Bonfanti (*Zeitschr. f. physiol. Chem.*, xliii, p. 156, 1904), it might be objected that the test-starch selected was unsatisfactory. It is, of course, also possible, though unlikely, that the canine saliva contains enzymes which act upon *soluble* starch and dextrins, without converting starch paste.

[2] Cf. for example, Bainbridge and Beddard: *Bio-chemical Journal*, ii, p. 91, 1907.