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STUDIES ON DOG SALIVA
IV. THE EFFECT OF CARBOHYDRATE DIET ON THE ACTIVITY OF SOME HYDROLYTIC ENZYMES

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Six mongrel dogs were reared after weaning on a daily diet composed of (1) raw bovine meat (35 %) containing sucrose (65 %), (2) bovine milk with 10 % sucrose, and (3) water. Five control dogs were kept on a similar diet without sucrose. The experiment was carried out for 260 days. No differences between the groups were observed when comparing the rate of growth, eruption pattern of deciduous and permanent teeth and blood sugar concentration. Hydrolases acting on ester and peptide bonds and glycosyl compounds were studied in the supernatant and sediment fraction of centrifuged dog saliva using altogether 41 different substrates. When comparing the values obtained to those of man the following deviations were observed: the sugar diet did not induce the appearance of salivary α-amylase (EC 3.2.1.1.), proline iminopeptidase (EC 3.4.1.4.) nor acid phosphatase (EC 3.1.3.2.) activity. With a few exceptions, the total hydrolytic enzyme activity in the oral cavity seemed to be constant. However, a fourfold increase was noticed in the hydrolysis of 6-bromo-2-naphthyl-α-D-glucoside, possibly representing α-glucosidase (EC 3.2.1.20) activity. The salivary protein concentration increased in the sugar group whereas it decreased in the meat group. No initiation of dental caries was obtained in any of the dogs. These observations were though to support the view that in the oral enzyme spectrum differences due to the diet and interspecies variations do occur.

The effect of diet on plaque formation and the periodontal conditions has been studied earlier in dogs, for example by *Egelberg* (1965 a, b) and *Carlsson & Egelberg* (1965). There are only a few reports available concerning the effect of diet on the oral enzyme spectrum in man or in other species. *Squires* (1953) found that five Kalahari bushmen detained in prison on a carbohydrate diet exhibited a salivary amylase concentration four times as great as similar bushmen who lived on a meat diet in native conditions. *Bates* (1962) found little evidence to support the suggestion that dietary changes might influence the level of amylase in saliva of the rat. Recently, an extensive investigation

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on the effect of dietary sugars on oral enzymes and other parameters has been carried out in man (*Scheinin & Mäkinen*, 1971, 1972; *Paunio, Mäkinen & Scheinin*, 1972; *Mäkinen & Scheinin*, 1971, 1972).

A comparison of the salivary enzyme spectrum between man and dog revealed some interesting differences (*Larmas & Scheinin*, 1971b; 1972), which may have reflected the different behaviour to dental caries between the species. The present study was undertaken partly because the dietary habits formed one of the basic differences and partly because the development of dental caries was thought to be highly influenced by the diet.

MATERIALS AND METHODS

Test animals. Eleven mongrel dogs from three different parents were used. Six of the dogs were born in the institute of parents used in earlier studies (*Larmas & Scheinin*, 1967, 1971 a, b; 1972) and five were obtained at the age of forty days. The dogs were divided into two groups, one group consisting of five dogs (three females, two males), and the other of six dogs (three females and three males). A pilot experiment was made with four other pups.

The body weights were measured weekly during the first one hundred days and bimonthly thereafter until the dogs reached an age of 260 days. All experimental procedures were made without anesthesia. At the age of six months the dogs received a distemper vaccination.

Diets. The dogs were weaned at the age of 45 days. Two experimental groups were formed: a meat group and a carbohydrate group. Five pups of the meat group were reared on a daily meat diet composed of raw bovine meat (100 g), bovine milk (100 g) and water. As a rule the animals received the rations once a day in the morning, except water, which they were allowed to consume freely. After one month the daily amount of meat was increased to 150 g per dog. The carbohydrate diet was composed of raw bovine meat (35 g) and sucrose (65 g). 100 g of this mixture was given to each dog daily during the first month and 150 g after that. Each dog of the carbohydrate group received daily 100 g bovine milk which contained 10 % sucrose (w/v) and water. The carbohydrate group of six dogs received the rations once a day in the morning. As a rule the dogs of this group did not consume all their food until later in the afternoon.

Dental eruption pattern. The eruption of the deciduous teeth of the six pups born in the institute was examined three times a week and a tooth eruption chart for each day was made. Eruption was considered to have

taken place at the time of gingival penetration. The tooth loss was observed in all the dogs studied in connection will the natural exfoliation of the dog teeth.

The eruption of the permanent dentition was studied in all the dogs. Notation was made twice a week when a deciduous tooth was observed to be slightly mobile, loose, or exfoliated, and when a permanent tooth had penetrated the gingiva.

Identification of teeth was made according to the following formula: deciduous, I 3/3, C 1/1, M 3/3; permanent I 3/3, C 1/1, PM 4/4, M 2/3 (*Miller,* 1952).

Collection of saliva. The saliva samples were collected by suction directly from the mouth, without chemical stimulation and anesthesia, by a glass tube and water suction as described earlier (*Larmas & Scheinin,* 1971a). Samples of five ml were collected weekly during the lactation period from six dogs, thereafter three times a week during the first experimental week from all the dogs, after this once a week for one month and thereafter monthly until the end of the experiment. The saliva samples were pretreated in exactly the same way as described earlier (*Larmas & Scheinin,* 1971b).

Enzyme preparation and chemicals. The pretreating of the saliva samples for making the enzyme preparation was the same as described earlier (*Larmas & Scheinin,* 1971 a, b; 1972). All substrates and other chemicals and their sources, unless otherwise stated, were the same as in previous papers (*Mäkinen,* 1966, 1968; *Larmas & Scheinin,* 1971 a, b; 1972). The buffer solutions used were made as described earlier (*Larmas & Scheinin,* 1971a).

Determination of enzyme activity. Determination of the enzyme activity was in principle conducted as described in previous papers (*Mäkinen,* 1966; 1968; *Larmas & Scheinin,* 1971 b, 1972). The enzymes investigated were aminopeptidases (EC 3.4.1.), glycosidases (EC 3.2.) and esterases (EC 3.1.). Different *N*-L-aminoacyl-2-naphthylamines and α- or β-naphthyl esters were used for the assay. Amylase activity with starch as substrate was determined as described earlier (*Larmas & Scheinin,* 1972).

Protein concentration. In this study the protein concentrations were determined using the Folin-Ciocalteu method as described by *Layne* (1965).

Determination of blood sugar. The blood sugar concentration was determined by the method of *Hultman* (1959) at the same time as the body weight was measured.

RESULTS

No differences in body weights between the groups were observed (Fig. 1a). However, the food intake in the sucrose group was seen to decrease occasional-

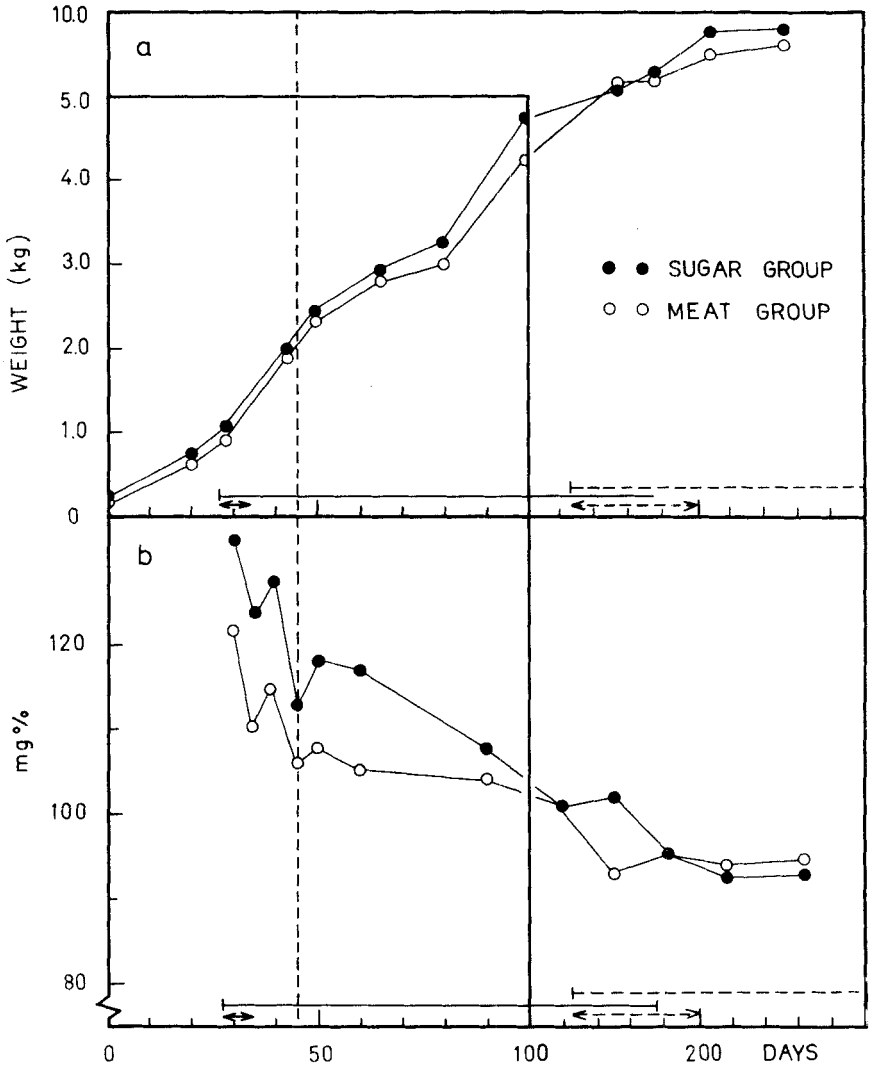

Fig. 1. Average body weights (in kg) and blood sugar concentration (in mg %) of the dogs in the sugar group (solid circles) and in the meat group (open circles). The time of weaning is indicated with the hatched line at 45 days. In the lower part of the figure, the eruption of deciduous teeth is indicated with solid arrows and exfoliation with hatched arrows. The solid horizontal line indicates the time of deciduous dentition and the hatched line indicates the time of permanent dentition.

Table I.

Eruption (T) and Exfoliation (L) of deciduous teeth of the test animals, expressed in days

Teeth		Maxillary		Mandibular	
		Sugar group	Meat group	Sugar group	Meat group
Incisors:	1 (T)	29	29	33	34
	1 (L)	123	125	122	124
	2 (T)[1]	29	29	31	31
	2 (L)	130	133	129	130
	3 (T)[1]	27	28	31	31
	3 (L)	138	135	135	138
Canines:	(T)[1]	27	26	27	27
	(L)	161	165	158	159
Molars:	1 (T)[1]	32	32	32	31
	1 (L)	181	184	158	162
	2 (T)[1]	29	30	31	33
	2 (L)	170	179	181	178
	3 (T)[1]	34	34	33	34
	3 (L)	142	145	179	180

[1] Mean values of six pups born in the Institute.

ly, this period lasting only for a few days. The average weekly food intake was not significantly different in the two diet groups.

Significantly differing results were not observed between the sugar and meat groups, nor between the right and left side of the jaw in the eruption patterns of deciduous and permanent teeth (Tables I and II). Thus the values are mean values of both sides, each figure consisting of the mean of altogether 12 teeth in the sugar and ten in the meat group, except for the values of eruption of deciduous teeth, which are mean values of the six pups born in the institute only.

The blood sugar concentration decreased with increasing age (Fig. 1b). No differences between the two groups were observed.

The protein concentration of saliva increased during the lactation period (Fig. 2). After the diet change (at 45 days age), the protein concentration of saliva sediment decreased in the meat group, and a rapid increase was observed in the carbohydrate group. The protein concentration of the supernatant fluid increased in both groups after ten days following the diet change. In the sucrose group the protein concentration of saliva was higher than in the

Table II.

Eruption of permanent dentition of the test animals, expressed in days

Teeth		Maxillary		Mandibular	
		Sugar group	Meat group	Sugar group	Meat group
Incisors:	1	124	128	122	125
	2	132	136	130	130
	3	138	136	138	140
Canines:		158	163	160	158
Premolars:	1	110	106	132	136
	2	184	182	158	157
	3	171	180	182	181
	4	145	147	184	182
Molars:	1	141	138	134	130
	2	175	168	172	165
	3	—	—	198	187

meat group throughout the experimental period. The range of the individual values was considerable.

The arylaminopeptidase activity of saliva supernatant fluid is shown in Fig. 3. The activities measured at the end of the lactation period are presented in the left part of the figure (Fig. 3a). The most rapidly hydrolyzed *N*-L-aminoacyl-2-naphthylamines at this period were those of L-alanine, L-methionine, L-leucine, DL-phenylalanine, L-glutamic acid (amide bond involving γ-carbon), when enzyme preparations obtained from saliva supernatant fluid were studied. Differences between the groups were small but between the individuals relatively high.

At the end of the experimental period, the most rapid hydrolysis catalyzed by centrifuged saliva was observed with the *N*-L-aminoacyl-2-naphthylamines of DL-phenylalanine, L-leucine, L-glutamic acid, methionine, and L-alanine, in both the sugar and the meat groups (Fig. 3b). As a rule, the rate of the hydrolysis of these substrates was higher in the sugar group than in the meat group. In the meat group the rates of the hydrolysis were somewhat higher during the lactation period than during the consumption of the meat diet.

Fig. 4 shows the results obtained when studying the enzyme activities in dog saliva sediment. After the lactation period (Fig. 4a) the specific activity of the enzymes hydrolyzing various *N*-L-aminoacyl-2-naphthylamines was

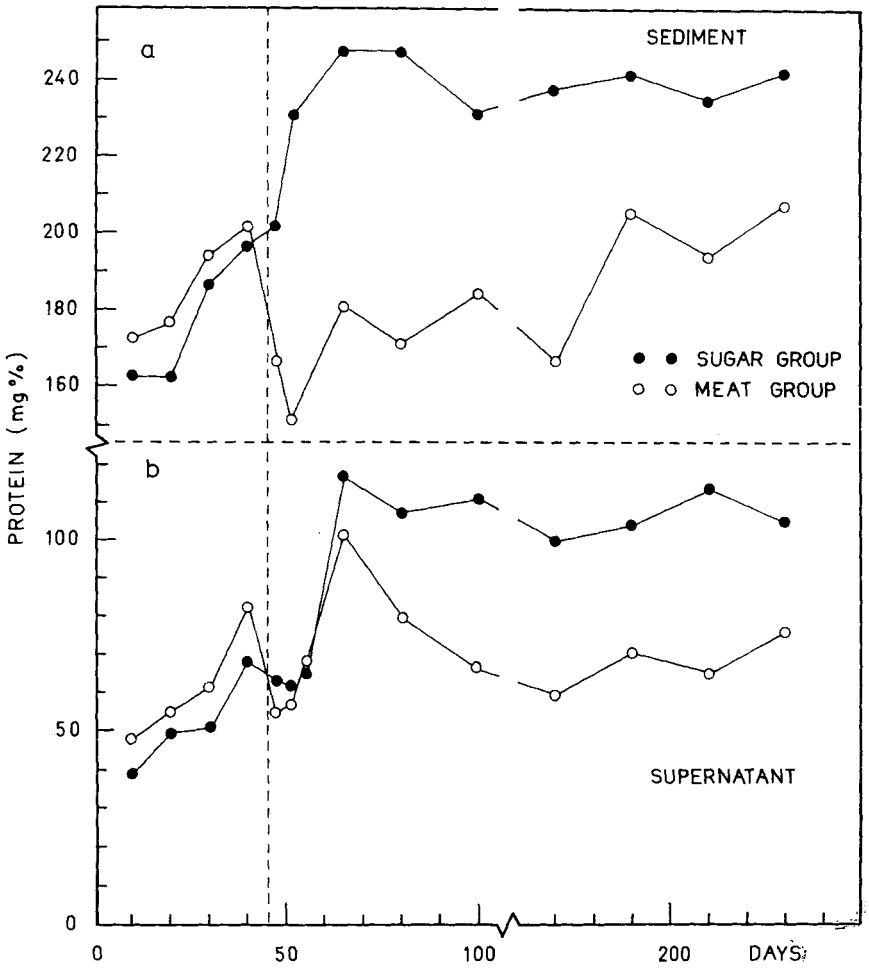

Fig. 2. The average protein concentration of dog saliva sediment (a) and supernatant (b) in mg %. Open circles indicate the meat group and solid circles the sugar group.

increased (Fig. 4 b). This result was the opposite to that obtained with centrifuged saliva. However, the most rapidly hydrolyzed substrates were mainly the same as with the supernatant fraction of saliva. A noticeable increase in the hydrolysis of *N*-L-arginyl, γ-glutamyl, and phenylalanyl was observed in the sugar group.

The rate of hydrolysis of various phosphate esters catalyzed by dog saliva supernatant and its sediment is shown in Fig. 5. During the lactation period enzyme preparations obtained from both centrifuged saliva and its sediment

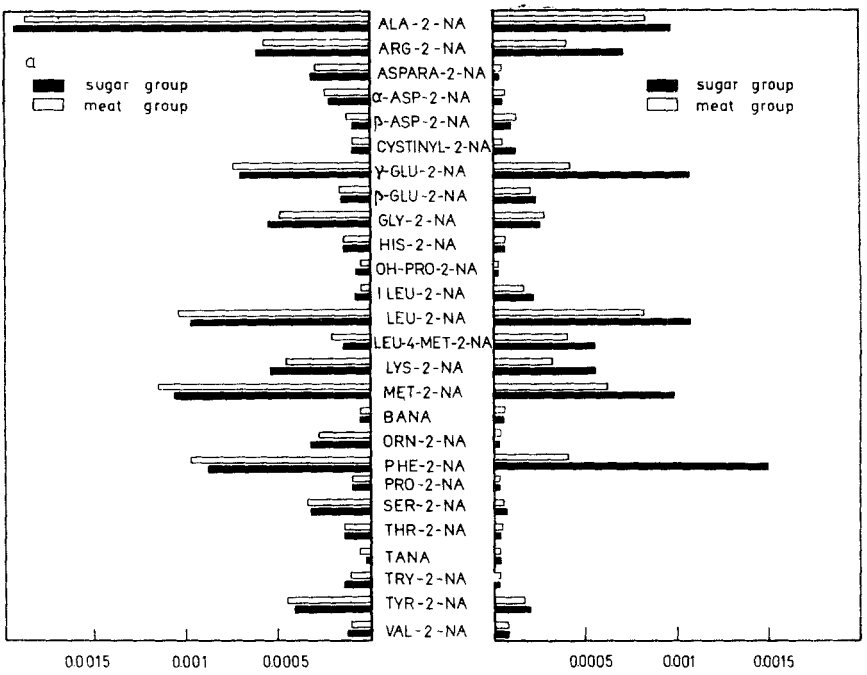

Fig. 3. Hydrolysis of *N*-L-aminoacyl-2-naphthylamines of various amino acids by centrifuged dog saliva at the end of the lactation period (a) and at the termination of the experiment (b) in 0.01 M tris buffer, pH 7.0. The activity is expressed as liberated μmoles of 2-naphthylamine per mg protein and min. The abbreviations are the same as earlier (*Larmas & Scheinin*, 1971 b). Open columns: meat group, black columns: sugar group.

hydrolyzed various phosphate esters at an almost equal rate at pH 4.6, 7.0 and 9.0 (Fig. 5 a, b). On the other hand, at the end of the experimental period, the rate of the hydrolysis at pH 9.0 was increased and at pH 4.6 reduced from the values recorded during the lactation. The sugar diet increased the alkaline phosphatase activity about twofold (Fig. 5 c, d).

Arylsulphatase activity (EC 3.1.6.1.), tested with 6-bromo-2-naphthyl sulphate, did not vary very much during the whole experiment and the activity was localized in the sediment fraction of saliva only (Fig. 6 b, d).

The rate of the hydrolysis of carboxylic acid esters at the end of the experiment (Fig. 6 g, h) was reduced with increasing age of the pups from values determined during the lactation period (Fig. 6 e, f). The decrease was more noticeable in the sugar group than in the meat group. The rate of the hydrolysis of short chain fatty acid esters, catalyzed by enzyme preparations of dog saliva, was the most rapid of all the substrates tested.

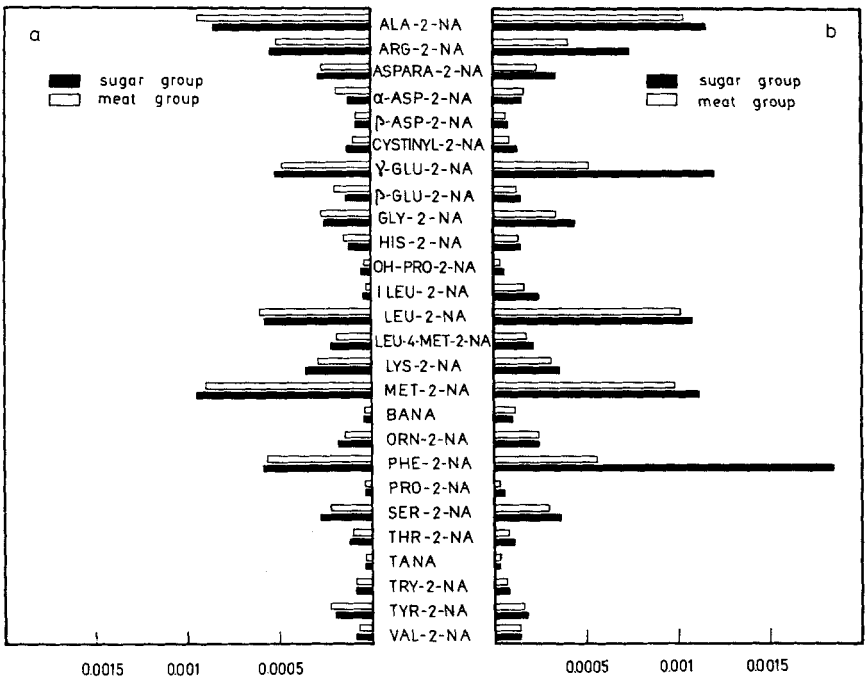

Fig. 4. Hydrolysis of *N*-L-aminoacyl-2-naphthylamines of various amino acids by centrifuged dog saliva sediment at the end of lactation period (a) and at the termination of the experiment (b). The experimental conditions were the same as in Fig. 3.

The rate of the hydrolysis of 6-bromo-2-naphthyl-α-D-glucoside was seen to increase fourfold when catalyzed by enzyme preparations from the sucrose group, compared to the rates obtained in the meat group.

No hydrolysis of soluble starch catalyzed by enzyme preparations obtained from the saliva of both groups could be observed. Thus no amylase-like activity was found.

The rate of hydrolysis of phosphate esters measured at the alkaline side (expressed as liberated naphthol (mg protein × min)) displayed an increasing tendency (Fig. 7 a, b), more easily noticed in the carbohydrate group. On the acidic side, a clearly decreasing tendency in the rate of the hydrolysis could be observed soon after the lactation period, and the hydrolysis of the 1-naphthyl phosphate was markedly reduced with increasing age of the puppies, as seen in Fig. 6 a, b. In the hydrolysis of *N*-L-alanyl-2-naphthylamine no clear difference between the groups can be seen (Fig. 7 c, d), but a marked decreasing tendeucy during the experimental period

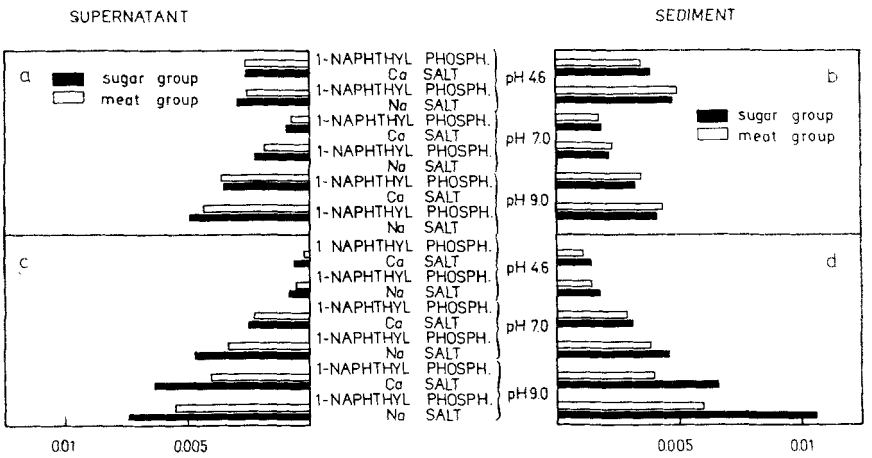

Fig. 5. Hydrolysis of 1-naphthyl phosphate by centrifuged dog saliva supernatant to the right (a, c) and its sediment (b, d) at the end of lactation period to the left (a, b) and at the termination of the experiment (c, d) in 0.01 M tris buffer pH 7.0 or pH 9.0 and in 0.05 M β,β-dimethylglutarate buffer, pH 4.6. The activity is expressed as liberated μmoles of 1-naphthol per mg protein and min. Open columns: meat group, black columns: sugar group.

can be observed. On the other hand, an increase in the rate of the hydrolysis of *N*-L-γ-glutamyl-2-naphthylamine, catalyzed by enzyme preparations of the sucrose group, can be observed when compared to the meat group (Fig. 7 c, d).

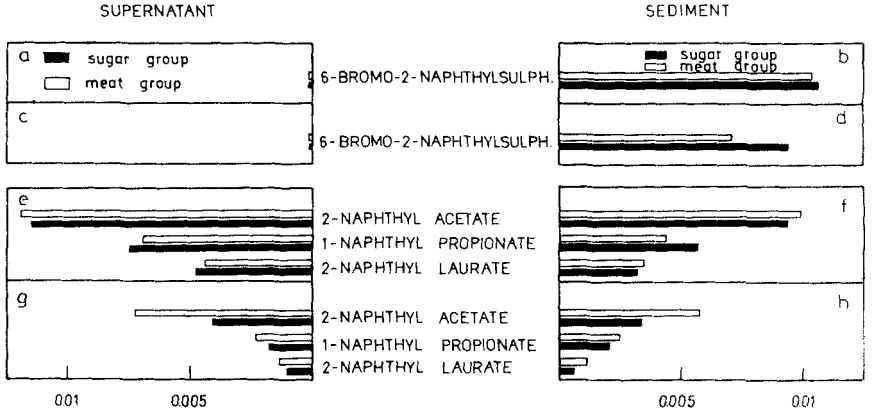

Fig. 6. Hydrolysis of various carboxylic acid and sulphate esters by centrifuged dog saliva supernatant (to the left) and its sediment (to the right) at the end of the lactation period (a, b, and e, f) and at the termination of the experiment (c, d, and g, h) in 0.01 M tris buffer, pH 7.0. The activity is expressed as liberated μmoles 1- or 2-naphthol per mg protein and min. Open columns: meat group, black columns: sugar group.

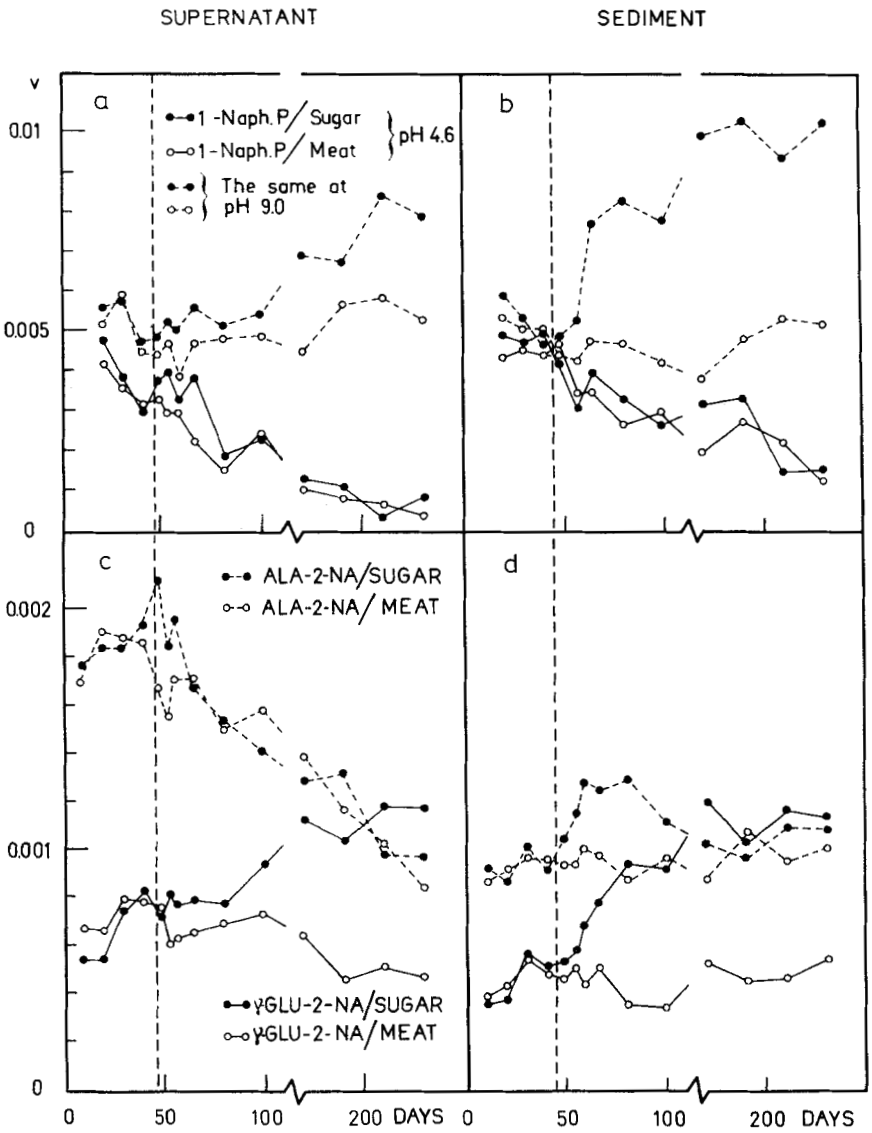

Fig. 7. Hydrolysis of 1-naphthyl phosphate (in 0.01 M tris buffer, pH 9.0) and 0.05 M (β,β-dimethylglutarate buffer, pH 4.6) (a, b) and *N*-L-alanyl- and *N*-L-γ-glutamyl-2-naphthylamine in 0.01 M tris buffer (c. d), pH 7.0 by centrifuged dog saliva supernatant (to the left) and its sediment (to the right) during the whole experiment. Solid circles: sucrose group, open circles: meat group.

Individual differences between the enzyme activity values of the dogs were considerable. No dental caries could be observed in any of the test animals, but in the sugar group slight symptoms of gingivitis appeared within a few days after the change of diet. This developed later to more severe gingivitis and even to parodontitis when terminating the experiment.

DISCUSSION

This series of investigations was planned in order to elucidate some of the inter-species variations in the parameters at the oral cavity between man and dog, who possess a markedly different susceptibility to dental caries. A number of differences could be observed both in the enzyme spectrum (*Larmas & Scheinin*, 1971b, 1972) and in the physico-chemical characteristics (*Larmas & Scheinin*, 1971a) of the saliva between the species.

Since these differences may be due to the different diet between the species, the effect of high dietary carbohydrate concentration on the enzyme spectrum was studied. One of the most interesting results of the present paper was that the sugar diet did not induce the appearance of salivary α-amylase in dog. On the other hand, the hydrolysis of 6-bromo-2-naphthyl-α-D-glucoside was seen to increase fourfold in the sugar group. This induction of an α-glucosidase-like enzyme can be compared to the induction of human salivary invertase on sucrose diet (*Mäkinen & Scheinin*, 1971, 1972). Thus hydrolysis of polysaccharides by dog salivary enzymes seemed to be different from that in man. These differences may partly explain the fact that dog salivary pH never fell below 6.7 during sugar administration (*Larmas & Scheinin*, 1971 a).

The sucrose diet did not induce the formation of enzymes hydrolyzing *N*-L-prolyl-2-naphthylamine, 1-naphthyl phosphate (at acidic side), or 6-bromo-2-naphthyl sulphate in the supernatant fraction of the oral fluid. Thus the difference between man and dog concerning enzymes hydrolyzing these substrates could not be explained as being due to a different diet.

The most noticeable difference during the course of the experimental period was observed in the protein concentration both in dog saliva supernatant and its sediment: after birth the salivary protein concentration increased, more noticeably in the sugar group than in the meat group. On the other hand, the specific enzyme activities exerted a decreasing tendency. Thus the total enzyme activity in the oral cavity seemed to be constant.

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