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ADAPTATION OF CANINE PANCREATIC ENZYMES TO DIET COMPOSITION

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SUMMARY

1. Lipase, protease and amylase activity in canine pancreatic juice were determined during the feeding of basal, high fat, high protein and high carbohydrate diets for 3-week periods.

2. The level of lipase in pancreatic juice was significantly augmented by increasing the dietary intakes of fat ($P < 0{\cdot}05$) and carbohydrate ($P < 0{\cdot}05$) but was not affected by increasing the protein intake.

3. Protease activity was highly augmented by increasing the intake of protein ($P < 0{\cdot}01$), was less strongly augmented by increasing carbohydrate ($P < 0{\cdot}10$), but was not affected by the level of dietary fat ingested.

4. Enzyme activities in canine pancreatic juice were modified by the nature of the diet on which the animals were maintained and the response for a given enzyme may be influenced by more than one dietary constituent.

INTRODUCTION

Pavlov reported that the enzymes secreted by the pancreas can be modified by the composition of the diet (Knox & Greengard, 1965). At that time, however, enzyme catalysis was an unknown and the means for adequately estimating enzyme activity were not established. Later, Grossman, Greengard & Ivy (1942–43) observed increased amylase and trypsin activity in rat pancreatic tissue after feeding high starch and high protein diets, but a high fat diet did not change lipase activity. Using more precise enzyme assay techniques, Reboud, Ben Abdeljlil & Desnuelle (1962) enlarged upon the early studies of Grossman *et al.* (1942–43). They

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reported that amylase and chymotrypsinogen in rat pancreatic tissue extracts followed closely the starch and casein content of the diet, respectively, but that lipase was unaffected by diet. For the rat, it has been reported that enzyme distributions in pancreatic juice are similar to those in pancreatic tissue extracts (Ben Abdeljlil & Desnuelle, 1964). Later evidence indicates that amylase and trypsin levels in tissue extracts vary independently in the rat, amylase being related to the level of dietary carbohydrate and trypsin to dietary protein (Howard & Yudkin, 1963).

Little information is available concerning the effect of diet composition on pancreatic enzyme distribution in other species. In the dog, pancreatic juice can be collected from conscious, essentially normal animals, thereby permitting the measurement of enzyme levels in a given animal fed different diets. Our interest in the present study was centred on the individual effects of fat, protein and carbohydrate intake on lipase and protease levels in pancreatic juice of the dog.

METHODS

Animals. Three adult dogs of mixed breed and of either sex ranging in weight from 15 to 25 kg were used for the intermittent collection of pancreatic juice. The animals were maintained in temperature controlled quarters (23° C) and were allowed to run free in a compound (6 × 6 ft.) except when individually caged during feeding. A gastric cannula was fitted in the ventral wall of the stomach to allow drainage of gastric juice. An intestinal cannula was positioned to permit the collection of uncontaminated pancreatic juice according to the method outlined by Thomas (1950). The accessory pancreatic duct was not ligated and at least 6 months elapsed between surgery and experimentation.

Collection of pancreatic juice. The animals were suspended in a canvas sling during the collection of pancreatic juice; they passively accepted this physical restriction after several preliminary trials. Before making the test feedings and collections, the gastric and intestinal cannulae were opened, the accumulated contents drained (10 min) and the pancreatic duct intubated. During the collection, the gastric cannula remained open to permit continuous drainage of gastric contents. Pancreatic secretions were collected in a graduated cylinder surrounded with ice.

A 10–20 g portion of each animal's diet was orally presented to induce a psychic stimulation of pancreatic secretion. In each instance a marked increase in flow rate occurred in 1–2 min. In a separate experiment with the same animals a 15 min basal secretory rate of about 0·5 ml. was observed which increased ten to twelvefold when a test stimulus of basal diet was presented. Collection of pancreatic juice in the present experiment began about 4–5 min after the test food was given and continued until about 1 ml. was obtained. The criterion used to discontinue collection was a volume of pancreatic juice greater than 0·8 ml. but less than 2·5 ml. The mean stimulated flow rate observed during the entire experiment was 0·3 ml./min and the mean volume obtained at each collection was 1·4 ml. Samples were collected from each animal at intervals of 2–3 days as outlined in the experimental design, and excessive loss of pancreatic juice was prevented by collecting a minimum sample. It has been reported that pancreatic enzymes are secreted in a parallel fashion over short time periods (Babkin, 1950) and at the time of test the animals had not eaten

for 24–30 hr. It was presumed, therefore, that the pancreas was charged with zymogen granules and that a representative sample was obtained. The order of collection was varied so that any change in secretion due to time after last feeding was evenly distributed between all animals. The samples of pancreatic juice were stored at −20° C until assay.

Protein determination. The protein content of pancreatic juice was determined using the biuret reaction (Gornall, Bardawill & David, 1949) with bovine serum albumin as the standard.

Lipase assay. Lipase activity was determined by titrating, with 0·0597 N sodium hydroxide, the caprylic acid released from a tricaprylin substrate at pH 8. For the assay an automatic titrating pH-stat Radiometer was used in conjunction with a continuously stirred incubation chamber held at 38° C. The amount of sodium hydroxide added to maintain pH 8 was recorded as a linear cumulative graph. The substrate was prepared according to the procedure outlined by Marchis-Mouren (1959). The reactive mixture contained 6 ml. substrate plus 1 ml. diluted pancreatic juice which was added when the substrate had equilibrated to 38° C. Pancreatic juice was diluted with 0·01 M Tris buffer (pH 8) and assayed at protein concentrations of 75, 50 and 25 μg. The enzyme activity at these protein concentrations produced a linear curve which passed through the origin. Lipase assays were conducted within 6 hr after collection of the pancreatic juice. A lipase unit was defined as the amount of enzyme which liberated 1 μ-equiv acid/min.

Protease assay. Before the protease assay, the proteolytic enzymes were activated using an enterokinase preparation (Nutritional Biochemicals Inc.). A 10 ml. volumetric flask containing 1 mg pancreatic juice protein and 25 mg enterokinase, made to volume with 0·1 M phosphate buffer (pH 7·6), was incubated for 18 hr at 5° C. In a preliminary study, maximum protease activity occurred at an enterokinase concentration of 20–25 mg pancreatic juice protein.

Protease activity was determined according to the method of Anson (1938), except that the incubation temperature was 30° C and the substrate was prepared from crystallized bovine haemoglobin (Nutritional Biochemicals Inc.). Activated pancreatic juice was diluted with 0·1 M phosphate buffer (pH 7·6) to a protein concentration of 100, 50 and 25 μg/ml. and was assayed for activity at each dilution in duplicate. The μ-equiv tyrosine released was proportional to protein concentration. A protease unit was defined as the amount of enzyme which liberates 1 μ-equiv tyrosine during 10 min of incubation.

Diets. Four diets were used. A commercial diet (Speak, supplied by General Mills Corporation, composition: fat 6 %, protein 19 %, carbohydrate 40 %, fibre 2·5 %, ash 7·5 %, water 25 %) served as the basal. The other three were prepared by diluting the basal with cottonseed oil, casein or a mixture of carbohydrates which contained 50 % starch, 25 % glucose, and 25 % sucrose. The diets were prepared before each treatment period with each animal's daily allotment packaged separately and stored at 5° C until fed. The food was mixed with water before feeding to increase palatability. Water was available *ad libitum* and the dogs were fed once daily at the rate of 20 g diet/kg body wt. The amounts of the diluents added per 100 g basal diet and the actual daily intakes of fat, protein and carbohydrates are shown in Table 1.

Experimental design. The order in which the three animals received the diets is shown in Table 2. Each of the diets was fed to a given dog for a 3-week interval (i.e. duration of treatment period). An individual treatment period began on a Sunday, and no collections of pancreatic juice were made during the first week. In the second and third weeks a single collection was obtained from each dog on Monday, Wednesday and Friday. The last treatment period was incorporated into the design to check critically whether or not dietary effects had stabilized in a 3-week period.

Statistical analyses. Standard analysis of variance procedures could not be used to evaluate the patterns of response to the diets of different fat, protein and carbohydrate contents with respect to pancreatic enzyme activity. First, the design (Table 2) was 'non-orthogonal' in that: (i) only three of the four diets were compared in a given period, (ii) although each dog received all four diets, one was received twice and that diet differed between dogs. Secondly, the increase in intake of one of the variables was accompanied by changes of the other two (Table 1). Because of these complications a basic least squares procedure was invoked (Harvey, 1966; Steel & Torrie, 1960).

TABLE 1. Daily intakes of fat, protein and carbohydrate

Diet	Daily intake from diet (g/kg body wt.) Fat	Pro-tein	Carbo-hydrate	Fibre	Ash	Water	g diluent/100 g basal diet
Basal	1·2	3·8	8·0	0·5	1·5	5·0	
High fat	4·0	3·2	6·8	0·4	1·3	4·3	17·5 (Cotton-seed oil)
High protein	0·8	8·0	6·0	0·4	1·1	3·7	35·0 (Casein)
High carbo-hydrate	0·4	1·2	16·0	0·2	0·5	1·6	200·0 (Carbo-hydrate)

TABLE 2. The experimental design

Period*	Dog A	Dog B	Dog C
1	Basal	Protein	Fat
2	Protein	Fat	Carbohydrate
3	Carbohydrate	Basal	Protein
4	Fat	Carbohydrate	Basal
5	Fat	Carbohydrate	Basal

* Duration of each period was 3 weeks starting on a Sunday. Collections were made on Monday, Wednesday and Friday of the last 2 weeks.

Parameters were fitted to account for the differences between dogs, periods, collection days within periods, and the interactions among these factors. This was done because with the design used, these variations are not a part of experimental error. The daily intakes (g/kg body wt.) of fat, protein and carbohydrate were introduced as independent variables. This device assessed the dietary effects in terms of regression coefficients representing the change of enzyme activity per unit increase of a dietary factor. To test whether or not responses to shifts in diets had stabilized, parameters were also fitted for the interaction of diet with collected days within periods.

RESULTS

The specific activities for lipase and protease on the four diets, adjusted for dog and period effects, are shown in Figs. 1 and 2, respectively. These Figures provide comparison of the four diets *per se*, but, because the fat, protein and carbohydrate levels all varied from one diet to another, they

do not show the relations between enzyme activities and the individual dietary factors. These relations are measured by the regression coefficients given in Table 3.

Analyses of variance of the specific activities are presented in Table 4. These analyses reflect the significance of the effects of fat, protein and carbohydrate indicated in Table 3. They also provide information on the

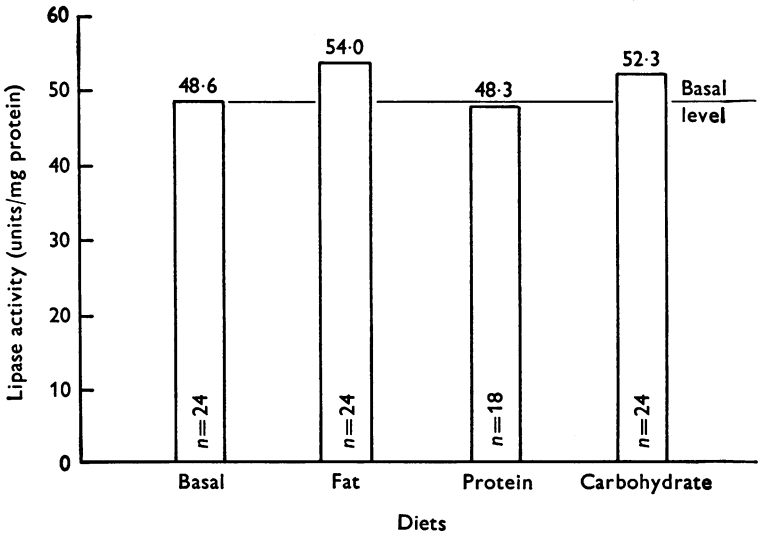

Fig. 1. Effect of diet composition on mean specific activity of pancreatic lipase.

TABLE 3. Relations of specific activities of pancreatic enzymes (units/mg protein) to intakes (g/kg body wt). of fat, protein and carbohydrate

Dietary constituent	Change in activity per unit increase in intake of constituent	
	Lipase	Protease
Fat	2·46*	0·02
Protein	0·62	1·30†
Carbohydrate	0·91*	0·47‡

* Significant at $P < 0.05$; † Significant at $P < 0.01$; ‡ Significant at $P < 0.10$.

stabilization of dietary effects, on the variability between dogs, periods, collection days and their interactions, and on the magnitudes of experimental errors.

Lipase responses. The specific activity of lipase in pancreatic juice increased significantly ($P < 0.05$) as the intake of fat in the diet increased

(Tables 3, 4). The increase was 2·46 units for each g increase in fat intake/kg body wt. over the range, 0·4–4·0 g/kg. The intake of protein had no significant effect on the specific activity of pancreatic lipase (Tables 3, 4) even though the protein intake varied from 1·3 to 8·0 g/kg. Lipase activity was significantly augmented ($P < 0·05$) by an increased intake of carbohydrate (Tables 3, 4); the amount was 0·91 unit for each g carbohydrate/kg body wt. over the range, 6–16 g/kg. The very small F-ratios for the interactions of days with the intakes of fat, protein and carbohydrate indicate that responses had stabilized.

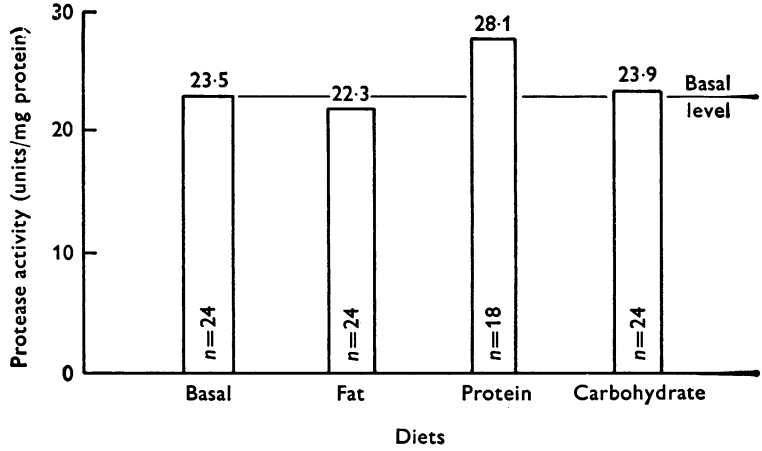

Fig. 2. Effect of diet composition on mean specific activity of pancreatic protease.

Protease responses. The specific activity of protease was not affected by dietary fat level. It was significantly affected, however, by the protein level, increasing 1·30 units for each g protein intake/kg body wt. Increasing the level of carbohydrate intake increased the protease activity somewhat, but the effect was not clearly significant ($P < 0·10$). The very small F-ratios for the interactions of days with the intakes of dietary constituents (Table 4) indicate stabilization of responses.

Results pertinent to experimental technique. The variations between dogs, periods or both were substantially larger than some or all responses to the dietary factors for both enzymes (Table 4). Between day, day by dog and day by period effects in the case of lipase, were significant as were day by period effects for protease. Further, the F-ratios were usually greater than one for these three effects.

Error (*a*) (Table 4) measures the degree to which the period to period trends would have varied between dogs had no treatments been imposed. Error (*b*) (Table 4) measures the degree to which the trend from one collec-

tion day to another in a given period would have varied between dogs had no treatments been imposed. If the day to day fluctuations on a given dog were independent, then error (*a*) would be about the same size as error (*b*). For lipase and protease error (*a*) was much smaller than error (*b*). Apparently, the day to day fluctuations on a given dog were compensatory so that the means for the periods (averages of the six collections) varied much less than might be expected from the day to day fluctuations.

TABLE 4. Analysis of variance for specific activities of lipase and protease

		Lipase		Protease	
Variance source	Degrees of freedom	Mean square	*F*-ratio	Mean square	*F*-ratio
Between dogs	2	227·53	14·27*	55·33	12·15*
Between periods	4	55·98	3·51	966·01	212·05†
Fat intake	1	234·22	14·69*	0·02	0·00
Protein intake	1	23·29	1·46	102·65	22·53†
Carbohydrate intake	1	111·09	6·97*	29·33	6·44‡
Error (*a*)	5	15·94	—	4·56	—
Between days	5	62·32	1·31	24·92	1·46
Days × dogs	10	27·02	0·57	27·07	1·58
Days × periods	20	63·02	1·33	48·55	2·84*
Days × fat intake	5	18·30	0·38	4·40	0·26
Days × protein intake	5	26·62	0·56	5·37	0·31
Days × carbohydrate intake	5	22·51	0·47	3·63	0·21
Error (*b*)	25	47·54	—	17·09	—
General mean		51·0		24·19	
Coefficients of variation (%):					
For a period mean		3·2		3·6	
For a collection day		13·5		17·1	

* Significant at $P < 0·05$, † Significant at $P < 0·01$, ‡ Significant at $P < 0·10$.

The experimental errors, when expressed as coefficients of variation (Table 4), were well within the upper range for biological work. In fact, those for period means, error (*a*) for lipase and protease were very small (3–4 %).

These results relevant to experimental technique will be discussed subsequently.

DISCUSSION

The pancreas is a major source of digestive enzymes, and an animal's diet is subject to varying levels of intake of fat, carbohydrate and protein. The results obtained in the present study show that the distribution of pancreatic enzymes changes in response to the amounts of the various

components of the diet on which a dog is maintained. Early workers (Babkin, 1950) reported that the pancreatic enzymes in the dog are secreted in a parallel fashion over short time periods. Later workers (Guth, Komarov, Shay & Style, 1956) observed, however, that feeding various diets to a dog over a period of 4 days resulted in a non-parallel secretion of enzymes, but they could not relate this fluctuation to dietary composition in a meaningful way. In the rat, it has been shown that pancreatic enzyme adaptation to the diet does occur over a period of several days (Reboud *et al.* 1962).

Pancreatic lipase activity was found to be directly related to the intake of fat and carbohydrate, but not to protein, in the present experiment. This is in contrast to the results reported for the rat (Reboud *et al.* 1962), in which the lipase (of tissue homogenates and samples of pancreatic juice) was unaffected by a high fat or a carbohydrate régime, but was depressed by high protein. Thus a species difference in the adaptability of pancreatic lipase is indicated. An increase in lipase activity associated with the level of dietary carbohydrate has not been reported previously. Glucose, the major product of carbohydrate digestion, has been implicated as an inducer for pancreatic amylase in the rat (Ben Abdeljlil & Desnuelle, 1964). Why it increased lipase activity in the dog is puzzling, however, and needs further study.

Increasing the protein intake of the dogs significantly enhanced protease activity in the pancreatic juice. An increase in carbohydrate appeared to increase protease activity moderately, but the level of fat intake was without effect. The assay used for protease activity was not specific for individual proteolytic enzymes. Thus, it is not known which of the proteolytic enzymes were involved. It has been reported for rats that both chymotrypsinogen and trypsinogen were increased in pancreatic tissue homogenates when rats were fed a high protein diet as compared to a high starch diet (Ben Abdeljlil & Desnuelle, 1964). Further work is necessary to substantiate the apparent carbohydrate response on protease activity here observed.

The data indicate that a difference may exist between the rat and dog in capacity to modulate pancreatic enzyme levels to changes in diet composition. It has been reported that species differences exist in the relative distribution of pancreatic enzymes. For example, bovine pancreatic juice contains little amylase or lipase and is predominantly composed of proteolytic enzymes. In contrast, the dog, rat, pig and man have substantially greater amounts of lipase and amylase (Neurath, 1961). This suggests that the degree to which the amount and the nature of the pancreatic secretions can be affected by changes in diet is related to the dietary habits of the species.

Comments on technique. The magnitudes of variability between dogs, periods and days and of their interaction demonstrate the efficacy of using experimental designs of the change-over type for the kind of work reported here. This efficacy is reflected in the small coefficients of error variation obtained. These designs utilize an animal as its own control, a feature common to much physiological work. They also subject the different animals to the treatments in different sequences in such a way that any general time trends in behaviour (period and day effects and their interactions) do not confound the comparison of treatments. This is a distinct advantage. If improper change-over patterns are used, time trends which might exist (inherent in the animals or due to environment, shifts in techniques, reagent changes, etc.) are confounded with treatment effects. In this event treatment effects and time trends cannot be distinguished even by complex statistical methods. Extensive series of change-over designs, some of which should be very useful for physiological work, are available (Patterson & Lucas, 1962).

The small coefficients of variation observed indicate that precise comparisons of treatments can be observed in work of this sort if proper change-over designs are used and properly analysed. The compensatory day to day fluctuations observed for lipase and protease could be attributed to either technique or physiologic phenomena and the authors have not been able to explain them in terms of technique.

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