The Lipase and Esterase Activities of the Pancreas and Small Intestine of the Chick

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Apart from the work of Hewitt & Schelkopf (1955), little in the nature of a general study of the digestive enzymes of the chick has appeared since the papers of Shaw (1912-13) and Plimmer & Rosedale (1922). The findings presented in these three papers are somewhat limited since the techniques employed were only qualitative or, at the most, semi-quantitative. Some of the enzymes concerned with the digestive processes in the chick were therefore investigated.

The work here reported is concerned with a comparison of the activities of pancreatic and intestinal lipase and esterase in chicks of different ages. During this investigation it became clear that lipase activity (as defined by Desnuelle, 1961) with an emulsified triglyceride as substrate was confined almost entirely to the pancreatic extracts, whereas esterase activity with a water-soluble substrate was confined almost entirely to extracts of the small intestine. This partition of the enzymes between pancreas and small intestine allowed certain properties of the two enzymes to be studied without previous attempts at purification.

EXPERIMENTAL

Measurement of lipase and esterase activity of the pancreas and small intestine of chicks of different ages

Female chicks (1 day old) of a broiler strain were obtained from a local breeder. On arrival at the Laboratory, before they had any access to food, 24 of them were killed. The remainder were given a commercial chick starter diet and drinking water *ad lib*. Groups of 12, 6 and 6 chicks chosen at random were killed at 10, 20 and 30 days of age respectively. So that each bird should be in a similar post-absorptive state the food trays were removed 12 hr. before the birds were killed. Immediately after death the pancreas and small intestine were removed from each bird as rapidly as possible and the contents of the intestine quantitatively removed by washing with water. The small intestines of the 10-, 20- and 30-day-old chicks were divided into three sections: the duodenum (from the gizzard to the pancreatic and bile ducts), the upper and lower small intestine (obtained by dividing the intestine from the pancreatic and bile ducts to the ileocaecal junction into two sections of equal length). The small intestines of the 1-day-old chicks

were not subdivided and were treated subsequently as a whole. With minimum delay all tissues were transferred to a deep-freeze cabinet, where they were stored for further use at -20° .

Before acetone-dried powders of these tissues were prepared certain of them were bulked, owing to the limited amount of material available from the younger birds. For the 1-day-old chicks four samples of each tissue (pancreas and whole small intestine) were obtained by randomly pooling the tissues from groups of six birds. For the 10-day-old chicks four samples of each tissue (pancreas, duodenum, upper and lower small intestine) were obtained by pooling the tissues obtained from groups of three birds. The tissues from the 20- and 30-day-old chicks were all treated individually.

Acetone-dried powders were prepared by a method similar to that of Morton (1955). The frozen tissues were chopped into small sections and homogenized with 10 vol. of acetone at 0° either in a Potter-Elvehjem glass homogenizer or, according to the quantity of tissue available, a stainless-steel Waring Blender. The homogenates were centrifuged and the solid residues washed and re-centrifuged with three successive portions of ice-cold acetone. They were then dried under reduced pressure and passed through a sieve (B.S. size 50) to remove any remaining connective tissue. The acetone-dried powders were weighed and stored in a desiccator at 0° .

Enzyme solutions were prepared by extracting known weights of the acetone-dried powders with 0.15M-sodium chloride (Morton, 1955) and portions of the extracts were taken for enzyme assay. All enzyme assays were performed at pH 7.0 in the sodium acetate buffer described by Boissonnas (1948), since despite the liberation of acid during incubation the pH of the mixture remains under these conditions relatively constant (Boissonnas, 1948).

In view of the accumulating evidence that water-soluble substrates are unsuitable for the measurement of lipase activity and the concept that there are two distinct groups of hydrolytic enzymes, one acting exclusively on emulsified esters and the other on esters in true solution (Desnuelle, 1961), the activities of the pancreatic and small-intestinal extracts were each determined with a water-soluble substrate and an emulsified triglyceride. The water-soluble ester used was Tween 20 (polyoxyethylene sorbitan monolaurate) obtained from Honeywell and Stein Ltd., London; it was purified by the method of Bier (1955). The substrate consisted of a 20% (v/v) solution of the purified Tween 20 in 0.2M-sodium acetate adjusted to pH 7.0. So that enzyme extracts could be assayed for activity against an emulsified triglyceride, a 5% (w/v) emulsion of triolein was

prepared as described by Sarda & Desnuelle (1958) in a 5% (w/v) gum acacia solution that also contained sodium acetate (0.2M) and was adjusted to pH 7.0.

Enzyme assays were carried out at 37° with 2 ml. of enzyme extract and 5 ml. of buffered substrate (pH 7.0) by the technique of Boissonnas (1948). Acid liberated during the reaction was titrated with 0.01M-sodium hydroxide to pH 8.2. With Tween 20 as substrate the end point of the titration was determined visually with phenol red as indicator, but with the emulsified triglyceride as substrate the end point of the titration was determined electrometrically. After correction for blanks, determined with boiled enzyme extracts, enzyme activity was expressed in terms of μ equiv. of acid liberated/30 min./mg. of acetone-dried powder. To check the reproducibility of substrate preparation, extracts of acetone-dried powder of the pancreas and small intestine of 30-day-old chicks were included with each series of enzyme assays. This technique of extraction of the acetone-dried powders and method of assay for enzyme activity gave highly reproducible results. For example, four weighed samples of acetone-dried powders of the pancreas and of the whole small intestine were extracted. The mean activities (μ equiv. of acid liberated/30 min./mg.), standard errors of the means and coefficients of variation were, for the pancreas with triolein, 21.6, ± 0.23 and 3.04% respectively, and, for small intestine with Tween 20, 9.42, ± 0.088 and 2.65% respectively.

That inactivation of the pancreatic lipase and intestinal esterase did not occur to any appreciable extent during the preparation of the acetone-dried powders is indicated by the following experiment. The whole small intestine of a 30-day-old chick was divided longitudinally into two portions of approximately equal weight. From one of the portions an acetone-dried powder was prepared as quantitatively as possible and was extracted with 0.15M-sodium chloride. The other portion of fresh intestine was homogenized with 0.15M-sodium chloride. The hydrolytic activity with Tween 20 as substrate was determined in portions of the filtered homogenate and extract of acetone-dried powder. The activity, calculated as μ equiv. of acid liberated/30 min./g. of fresh tissue, determined with the fresh homogenate was 750 and with the acetone-dried powder 713. In a similar experiment with the pancreas of the same bird the hydrolytic activities with triolein as substrate, again calculated as μ equiv. of acid liberated/30 min./g. of fresh tissue, were 3774 with the fresh homogenate and 3550 with the acetone-dried powder.

Comparison of the properties of the pancreatic lipase and intestinal esterase of the chick

To study certain properties of the two enzymes, sufficient quantities of acetone-dried powders were prepared from the tissues of the pancreas and the whole small intestine of a large number of 30-day-old female chicks. The diet of the birds, treatment of tissues and preparation and extraction of the acetone-dried powders were as described above.

To determine the pH optimum for intestinal esterase a series of unbuffered 20% (v/v) solutions of Tween 20 were prepared with pH values varying from 6.0 to 10.0. Mixtures of 5ml. of intestinal extracts (equivalent to 20 mg. of acetone-dried powder) and 20 ml. of substrate were incubated at 37° for 10 min., during which time the pH of the

reaction mixture was maintained constant at the initial value by the continuous electrometric titration (with 0.01M-sodium hydroxide) of the acid liberated during the reaction. The pH for optimum activity of pancreatic lipase was determined similarly with 5 ml. of pancreatic extract (equivalent to 5 mg. of acetone-dried powder) and 20 ml. of an unbuffered 5% (w/v) emulsion of triolein in 5% (w/v) gum acacia solution as substrate. For both, the results were expressed as μ -equiv. of sodium hydroxide required/10 min.

In the experiments designed to investigate the effect of substrate concentration on the hydrolytic activity of the pancreatic and intestinal extracts, triacetin and methyl butyrate, both of which exist in true solution at low concentrations in aqueous medium, were chosen as substrates. Solutions or emulsions of these substrates at concentrations varying from 0.045 to 1.15M for methyl butyrate and from 0.076 to 2.51M for triacetin were prepared in a 5% (w/v) solution of gum acacia containing 0.2M-sodium acetate at pH 7.0. Enzyme activity was measured at pH 7.0 and 37° by the Boissonnas (1948) procedure as described above, and results were again expressed as μ equiv. of acid liberated/30 min./mg. of acetone-dried powder.

The same techniques of preparation of substrate and enzyme assay and method of expressing results were used to compare the activity at pH 7.0 and 37° of both enzyme extracts towards a variety of tri-, di- and mono-glycerides, cholesterol esters and lecithin.

RESULTS

Enzyme activities of the pancreas and small intestine of the chick at various ages. Results were submitted to an analysis of variance and the multiple-range test of Duncan (1955), the 1% level of significance being accepted as indicative of a real biological difference. The standard error of the mean of each group was calculated from the 'within-age group' mean square and the number of observations in the group.

Table 1 shows that the hydrolytic activity of the chick pancreatic extracts with triolein did not differ very markedly during the 30 days after hatching, although the activity at 10 days was significantly higher ($P < 0.01$) than at 30 days. In each age group the pancreatic extracts exhibited a much smaller activity with the water-soluble Tween 20 than with the emulsified triolein. The activity with Tween 20 in the 1-day-old chick was about twice that ($P < 0.01$) at 10, 20 or 30 days of age.

To compare the hydrolytic activity of the whole small intestine of the 1-day-old chicks with triolein and Tween 20 with the corresponding values for the older birds, the activities of the whole small intestine of the 10-, 20- and 30-day-old chicks were calculated from the weights and the activities determined on the separate sections of the intestine, i.e. the duodenum, and upper and lower small intestine. These results are given in Table 2,

from which it is evident that, in contrast with the pancreas, the hydrolytic activity of the intestine with Tween 20 was much greater than with triolein. Although there were no significant differences in the hydrolytic activities with triolein in chicks of different ages, there was a highly significant difference ($P < 0.001$) between the activity with Tween 20 in the 1-day-old chicks and the chicks of other age groups. Between 1 and 10 days of age the hydrolytic activity with Tween 20 of the whole small intestine of the chick increased approximately ninefold and then remained relatively constant up to 30 days of age.

The hydrolytic activities with triolein and Tween 20 of the duodenum, upper and lower small intestine of chicks 10, 20 and 30 days of age are given in Table 3. The difference in activity with the two substrates was very marked in each section of the intestine, but there were no significant differences with either substrate between the various age groups. However, it appeared that there was a difference in hydrolytic activity with triolein and Tween 20 between different sections of the intestine. To investigate this further the values

given in Table 3 for each section of the intestine and for each substrate were pooled over the three age groups. These pooled results are given in Table 4 and it is evident that the hydrolytic activity of the upper intestine with triolein was significantly higher ($P < 0.01$) than that of the duodenum and lower small intestine, whereas the hydrolytic activity of the duodenum with Tween 20 was significantly higher ($P < 0.001$) than that of the upper and lower small intestine.

Properties of the pancreatic and intestinal enzymes. The pH-activity curves for the pancreatic extracts with triolein as substrate and for the intestinal extracts with Tween 20 as substrate are shown in Fig. 1. Under the conditions of the experiment and with these substrates the pH for optimum hydrolytic activity of the pancreatic extracts was approximately 8.70 whereas that of the intestinal extracts was approximately 8.30. Even at pH values as high as 10.0 both extracts still possessed more than half the activity exhibited at the optimum pH.

The effects of increasing concentrations of methyl butyrate and triacetin on the hydrolytic

Table 1. *Hydrolytic activity with triolein and Tween 20 of pancreatic extracts obtained from chicks of various ages*

Mean values are given with the corresponding standard errors and the activities are expressed as μ equiv. of acid liberated/mg. of acetone-dried powder/30 min. Figures in parentheses refer to the number of acetone-dried powders analysed for each age group. Significance of differences was calculated by the multiple-range test (Duncan, 1955): the mean values differ significantly ($P < 0.01$) except when they share a common underlining.

Age of chicks (days) ...	10	20	1	30
Activity with triolein	24.1 (4)	21.7 (6)	21.3 (4)	18.7 (6)
		S.E.M. (4) ± 1.04 } 16 D.F. S.E.M. (6) ± 0.846 }		
Age of chicks (days) ...	1	10	20	30
Activity with Tween 20	0.950 (4)	0.578 (4)	0.528 (6)	0.518 (6)
		S.E.M. (4) ± 0.0762 } 16 D.F. S.E.M. (6) ± 0.0624 }		

Table 2. *Hydrolytic activity with triolein and Tween 20 of whole intestinal extracts obtained from chicks of various ages*

Mean values and the number of observations per group are given with the corresponding standard errors. Activities are expressed and significance of differences was calculated and indicated as in Table 1.

Age of chicks (days) ...	1	10	20	30
Activity with triolein	0.070 (4)	0.285 (4)	0.217 (6)	0.182 (6)
		S.E.M. (4) ± 0.0592 } 16 D.F. S.E.M. (6) ± 0.0490 }		
Age of chicks (days) ...	20	10	30	1
Activity with Tween 20	7.40 (6)	6.73 (4)	6.62 (6)	0.820 (4)
		S.E.M. (4) ± 0.571 } 16 D.F. S.E.M. (6) ± 0.466 }		

Table 3. *Hydrolytic activity with triolein and Tween 20 of duodenal, upper and lower small-intestinal extracts obtained from chicks of various ages*

Mean values, number of observations, standard error of the means and significance of differences are expressed as in Table 1.

Age of chicks (days)	...	10	20	30
Activity with triolein				
Duodenum		0.288 (4)	0.0917 (6)	0.100 (6)
Upper small intestine		0.370 (4)	0.447 (6)	0.285 (6)
Lower small intestine		0.040 (4)	0.0283 (6)	0.153 (6)
<div style="display: flex; justify-content: flex-end; align-items: center;"> <div style="margin-right: 10px;"> S.E.M. (4) ± 0.0938 S.E.M. (6) ± 0.0762 </div> 39 D.F. </div>				
Activity with Tween 20				
Duodenum		8.23 (4)	9.06 (6)	7.34 (6)
Upper small intestine		5.07 (4)	6.85 (6)	6.70 (6)
Lower small intestine		7.70 (4)	5.44 (6)	6.03 (6)
<div style="display: flex; justify-content: flex-end; align-items: center;"> <div style="margin-right: 10px;"> S.E.M. (4) ± 0.690 S.E.M. (6) ± 0.563 </div> 39 D.F. </div>				

Table 4. *Difference in hydrolytic activity with triolein and Tween 20 of duodenal, upper and lower small-intestinal extracts*

Mean values, obtained by pooling results for the different age groups, are given with the corresponding standard errors. Activities, number of observations and significance of differences are expressed as in Table 1.

	Upper intestine	Duodenum	Lower intestine
Activity with triolein	0.367 (16)	0.144 (16)	0.0781 (16)
	S.E.M. (16) ± 0.0469 , 39 D.F.		
	Duodenum	Upper intestine	Lower intestine
Activity with Tween 20	8.21 (16)	6.35 (16)	6.22 (16)
	S.E.M. (16) ± 0.345 , 39 D.F.

Fig. 1. Influence of pH on the hydrolytic activity of the pancreatic extracts with triolein (○) and of the intestinal extracts with Tween 20 (●).

activity of the pancreatic and intestinal extracts with these two substrates are shown in Figs. 2 and 3. Under the conditions of the experiment the saturation concentrations in true solution of

methyl butyrate and triacetin were 0.19 and 0.33M respectively. Above these concentrations a second phase, i.e. emulsified substrate, appeared. With both methyl butyrate and triacetin the activity of the pancreatic extracts increased somewhat with increase in substrate concentration in true solution but with the appearance of emulsified substrate there was a pronounced enhancement of enzyme activity. In marked contrast, the curves relating hydrolytic activity of the intestinal extracts to substrate concentration showed that increasing concentration of methyl butyrate and triacetin in true solution gave rise to considerable increase in enzyme activity. With the appearance of emulsified substrate there was no great enhancement of intestinal enzyme activity although there was in fact a slight inflexion of the curves at the saturation concentrations of both substrates.

The activities of the pancreatic and intestinal extracts with various triglycerides and Tween 20 are given in Table 5. Under the experimental conditions triacetin and Tween 20 were present in true solution only, whereas the remaining glycerides were present as emulsions. From the results

Fig. 2. Effect of concentration of methyl butyrate on the hydrolytic activity of the pancreatic (●) and intestinal (○) extracts.

Fig. 3. Effect of concentration of triacetin on the hydrolytic activity of the pancreatic (●) and intestinal (○) extracts.

presented in Figs. 2 and 3 it is not surprising that the activities of the intestinal extracts were greater than those of the pancreatic extracts with triacetin and Tween 20 as substrate but that the reverse obtained with tributyrin, tricaproin, trilaurin, triolein and tristearin. With the emulsified glycerides the substrate specificity of the intestinal extracts, although at a much lower activity, was very similar to that of the pancreatic extracts.

The hydrolytic activities of the pancreatic and small-intestinal extracts with a variety of substrates are given in Table 6. The three glycerol acetates at the concentration tested were again present in true solution, tri- and di-acetin being hydrolysed more readily by the intestinal extract. The finding that the intestinal extract was without action on monoacetin was nevertheless unexpected. The other substrates listed in Table 5 were present

Table 5. *Hydrolytic activities with various triglycerides and Tween 20 of the pancreatic and intestinal extracts*

Activities are expressed as μ equiv. of acid liberated/mg. of acetone-dried powder/30 min.

Substrate	Enzyme activity	
	Pancreatic extract	Intestinal extract
Triacetin	0.6	2.0
Tributyrin	117.0	4.0
Tricaprylin	87.3	2.1
Trilaurin	36.1	0.8
Triolein	24.4	0.1
Tristearin	5.6	0.1
Tween 20	0.5	5.0

Table 6. *Hydrolytic activities with a variety of substrates of pancreatic and small-intestinal extracts of 30-day-old chicks*

Activities are expressed as in Table 1.

Substrate	Enzyme activity	
	Pancreatic extract	Intestinal extract
Monoacetin	0.0	0.0
Diacetin	0.0	0.4
Triacetin	0.5	1.8
Mono-olein	10.8	0.1
Triolein	25.5	0.1
Monostearin	2.6	0.3
Tristearin	5.8	0.1
Lecithin	3.4	0.0
Cholesterol acetate	0.1	0.0
Cholesterol oleate	0.3	0.0

as emulsions and were hydrolysed more readily by the pancreatic extracts. The activities of the pancreatic extracts with triolein and tristearin as substrates were about twice as great as with the corresponding monoglycerides. Conversely monostearin was hydrolysed more readily than tristearin by the intestinal extracts. Unlike the pancreatic extracts, the intestinal extracts appeared to have no action on lecithin and the two cholesterol esters.

DISCUSSION

In marked contrast with the lipolytic activity of the pancreas, the enzyme activity of the small intestine with Tween 20 was low in the newly hatched bird but increased markedly during the next 10 days. Richardson, Berkowitz & Moog (1955) showed that the concentration of a 'non-specific esterase' in the duodenum of the chick, determined with β -naphthyl acetate as substrate, increased in older birds above the value at hatching.

The fact that both the pancreas and small intestine of the chick possess hydrolytic activity

with triolein and Tween 20 raises the possibility of there being one pancreatic enzyme with high and low substrate affinity for triolein and Tween 20 respectively and one small intestinal enzyme with low and high substrate affinity for triolein and Tween 20 respectively. Such a possibility seems unlikely since the hydrolytic activity of the small intestine with triolein did not increase, as did the activity with Tween 20, during the first 10 days after hatching. Also the distribution of lipolytic and esterolytic activities was different in the various sections of the small intestine (Table 4). It seems more likely therefore that at least two distinct enzymes were present in both tissues. This concept would certainly agree with work on other species reported by Sarda & Desnuelle (1958), DiNella, Meng & Park (1960), Stansfield (1960) and Wills (1961).

The pH optima for hydrolytic activity of the pancreatic and small-intestinal extracts with triolein and Tween 20 respectively agree very well with those given by Stansfield (1960) for the corresponding tissues of the pig with the same substrates. Although the pH optima of both enzymes are above 8.0, they have been assayed at pH 7.0. This is nearer to the physiological pH at which they normally operate since the pH of the small-intestinal contents of the chick rarely exceeds 7.0 (Sturkie, 1954).

The results of the experiments on the effect of increasing concentrations of methyl butyrate and triacetin on the enzyme activity of the chick pancreatic extracts (Figs. 2 and 3) are almost identical with those obtained for pig pancreatic lipase by Sarda & Desnuelle (1958). Similar activity-concentration curves have been given by Schonheyder & Volqvartz (1945) for a lipase preparation with tricaproin as substrate. According to Desnuelle (1961) the term lipase should be restricted to hydrolytic enzymes that have little or no action on esters present in true aqueous solution but are dependent on the presence of an interface for activity; the term esterase should be applied to enzymes whose activities are confined to the hydrolytic cleavage of the ester bonds of water-soluble substrates. Sarda & Desnuelle (1958) showed that after extensive purification pig pancreatic lipase exhibited no activity whatsoever with methyl butyrate in aqueous solution. The weak but definite activity of the chick pancreatic extracts with methyl butyrate and triacetin (Figs. 2 and 3) at low concentrations may indicate the presence of two enzymes, the lipase being of considerably greater quantitative significance than the esterase.

The relationships between the activity of the chick intestinal extracts and increasing concentrations of methyl butyrate and triacetin (Figs. 2 and 3) show a general similarity to those between

the activity of horse liver esterase and increasing concentrations of these substrates reported by Sarda & Desnuelle (1958). However, whereas Sarda & Desnuelle (1958) found that maximum activity of horse liver esterase was attained well before the saturation point of methyl butyrate and that enzyme activity did not increase with the appearance of a second phase, the activity of the chick intestinal extracts continued to increase somewhat beyond the saturation concentration of the substrate. This finding, together with the evidence of a slight discontinuity in both curves at the point of appearance of the interface, presumably indicates again that the extracts contained both a lipase and an esterase, but that in contrast with the pancreas the esterase was the enzyme of major importance. With tributyrin as substrate Aldridge (1954) observed an increase in the hydrolytic activity of extracts of acetone-dried powders of rat pancreas when the substrate was emulsified. No such increase occurred in similar experiments with extracts of acetone-dried powder of rat intestinal mucosa.

The experiments on the activity of the pancreatic extracts with various emulsified triglycerides (Table 5) indicate an order of substrate specificity very similar to that reported for purified pig pancreatic lipase by Desnuelle (1961) and Wills (1961), and for extracts of rat pancreas acetone-dried powders by Aldridge (1954). As was to be expected, the activity of the intestinal extracts with the emulsified triglycerides was very low. Even so they had the same relative substrate specificity as the pancreas extracts, which yet again suggests the presence in the intestinal extracts of small concentrations of a true lipase.

The activity of the chick pancreas extracts (Table 5) with tri- and mono-stearin and tri- and mono-olein supports the general contention of Desnuelle (1961) and Wills (1961) that pancreatic lipase hydrolyses tri-, di- and mono-glyceride with increasing difficulty. The activity of the intestinal extracts with tri- and mono-stearin and tri- and mono-olein was again low but whereas there was no activity with monoacetin, slight and appreciable activity was observed with diacetin and triacetin respectively. The fact that the glycerol acetates were all water-soluble at the concentration used indicates that the intestinal esterase is not lacking in specificity even with water-soluble esters.

The ability of the chick pancreatic extracts to hydrolyse lecithin is consistent with the various reports of phospholipase activity of the pancreas in other species (Shapiro, 1952; Hanahan, 1952). Although there was apparently no phospholipase activity in the chick intestinal extracts (Table 5), Epstein & Shapiro (1957) have shown phospholipase B to be present in rat intestinal mucosa.

As has been reported for the rat (Swell, Boiter, Field & Treadwell, 1955), the pancreas of the chick contains a cholesterol esterase but the small intestine does not.

As yet the important question of the identity of the natural substrate and physiological function of the very active esterase of the chick small intestine cannot be answered. Certainly, water-soluble carboxylic acid esters cannot amount to more than a trace in the normal diet of the bird. This intestinal esterase might be identical to the 'non-specific esterase' of the chick duodenum described by Richardson *et al.* (1955), who suggested from histochemical evidence that it is an intracellular or non-secreted enzyme that participates in some undetermined fashion in the movement of molecules across the intestinal epithelium.

SUMMARY

1. In chicks from 1 to 30 days of age the lipase activity of the pancreas remained fairly constant, but the esterase activity of the small intestine increased markedly from 1 to 10 days of age and then remained constant.

2. Of the three sections of the small intestine examined, lipase activity was greatest in the upper small intestine, whereas esterase activity was greatest in the duodenum.

3. Although lipase activity was almost entirely confined to the pancreas, and esterase activity to the small intestine, evidence is given that both enzymes are present in both tissues.

4. The optimum pH for chick pancreatic lipase with triolein as substrate was 8.7, whereas that for intestinal esterase with Tween 20 as substrate was 8.3.

5. The hydrolytic activity of the pancreas with increasing concentrations of methyl butyrate and triacetin increased markedly at the saturation concentration with the appearance of emulsified substrate. The hydrolytic activity of the small intestine with methyl butyrate and triacetin was high when both substrates were present in true solution only, and increased only slightly with the appearance of emulsified substrate.

6. Of a number of emulsified triglycerides investigated the hydrolytic activity of the pancreatic

and small-intestinal lipase was highest with tributyrin, but decreased with increasing chain length of the constituent fatty acid. Tristearin and triolein were hydrolysed more readily by the pancreatic lipase than were the corresponding monoglycerides.

7. Cholesterol esters and lecithin were hydrolysed by the pancreas, but not by the small intestine of the chick.

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