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Intestinal Disaccharidase Activities in the Chick

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1. Disaccharidase activities of the small and large intestines of the chick were studied. 2. Homogenates of the small intestine readily hydrolysed maltose, sucrose and palatinose (6-*O*-α-D-glucopyranosyl-D-fructose), hydrolysed lactose slowly and did not hydrolyse trehalose and cellobiose. 3. Within the small intestine the disaccharidases were located mainly in the intestinal wall; the activity in the contents accounted for less than 5% of the total activity. 4. The disaccharidases were non-uniformly distributed along the small intestine, the activities being greatest in the middle section. 5. The disaccharidase activities increased with age between 1 and 43 days. 6. Homogenates of the large intestine and contents readily hydrolysed maltose, sucrose, palatinose and lactose and hydrolysed cellobiose and trehalose slowly. 7. The large-intestinal disaccharidases were located mainly in the contents. 8. Similar K_m and pH optimum values were found for the maltase, sucrase and palatinase activities of the large and small intestines. 9. The lactase activity of the large intestine was markedly affected by diet and had different K_m and pH values from the small intestinal lactase. 10. Low activities of intestinal disaccharidase were found in 12-day-old embryos and marked increases in the intestinal disaccharidases of the developing embryo occurred 2–3 days before hatching.

There is a close correlation between the intestinal disaccharidase activities of an animal and its ability to utilize disaccharides. Numerous reports exist (e.g. Anderson, Messer, Townley & Freeman, 1963; Prader & Auricchio, 1965) of humans showing disaccharide intolerance due to intestinal disaccharidase deficiencies, and disaccharidase assays of intestinal biopsy specimens have become an important diagnostic procedure.

Most reports on intestinal disaccharidases have been confined to mammalian species and have been concerned with such aspects as location of activity within the mucosal cells, distribution along the intestine and development with age.

Very little is known about the intestinal disaccharidases of avian species. Maltase and sucrase activities have been demonstrated in the small intestine of the chick (Plimmer & Rosedale, 1922; Mendel & Mitchell, 1907), but, apart from the work of Laws & Moore (1963) on the maltase activity of acetone-dried powders of the chick small intestine, there is no information on the kinetics, distribution and development of disaccharidases in that organ. The present investigation was undertaken to gain such information and to relate it to the findings with mammalian species.

MATERIALS AND METHODS

Embryos and chicks. Eggs produced by Light Sussex hens mated with Rhode Island Red cocks were incubated for 21 days in a standard commercial incubator. The chicks were housed in groups of 10–15 in electrically heated tier brooders and were given food and water *ad lib.* Male and female chicks were used in all experiments. For most experiments the diet was a practical-type chick mash, though comparative studies were made with chicks receiving a diet of purified ingredients. The compositions of the two diets are given in Table 1. All investigations were performed on material from individual chicks except with embryos younger than 18 days, when it was necessary to pool samples from a number of birds. Between five and ten birds were used in each experiment.

Preparation of homogenates. After being kept overnight without food the chicks were killed by breaking the neck, the abdomen was opened and the entire alimentary tract between the pylorus and the cloaca was removed. The pancreas and any adhering mesentery were cut away. On some occasions the excised tissue was subdivided into sections as follows: (i) the duodenum, extending from the gizzard to the entrances of the pancreatic and the bile ducts; (ii) the jejunum, extending from the entrances of the pancreatic and bile ducts to the yolk stalk; (iii) the ileum, extending from the yolk stalk to the ileo-caecal junction; (iv) the caeca; (v) the colon. The whole intestinal tract or sections thereof was homogenized with 25 ml. of 0·15 M-NaCl

Table 1. *Composition of chick diets*

Practical-type chick mash		Purified ingredient diet	
Substance	Composition (%)	Substance	Composition (%)
Ground maize	35·0	Maize starch	60·25
Ground wheat	30·0	Casein (vitamin-low)	18·00
Miller's offals	8·5	Gelatin	10·00
Fish meal	10·0	Salt mixture*	6·00
Dried skim milk	7·5	L-Cystine	0·30
Dried grass	3·0	Choline chloride	0·15
Dried brewer's yeast	3·0	Inositol	0·10
Limestone flour	1·5	Vitamin supplement†	0·20
Salts (NaCl, 93·4; $MnSO_4,4H_2O$, 6·0; KI, 0·06%)	0·5	Corn oil‡	5·0
Arachis oil (containing 64 i.u. of vitamin D_3 and 680 i.u. of vitamin A/g.)	1·0		

* The salt mixture supplied $CaCO_3$ (1·71 g.), $CaHPO_4,2H_2O$ (1·71 g.), KH_2PO_4 (1·33 g.), NaCl (867 mg.), $MgSO_4,H_2O$ (267 mg.), $FeSO_4,7H_2O$ (67 mg.), $MnSO_4,4H_2O$ (27 mg.), $ZnSO_4,7H_2O$ (13 mg.), KI (3·7 mg.) and $CuSO_4,5H_2O$ (1·6 mg.) in each 100 g. of diet.

† The vitamin supplement supplied thiamin hydrochloride (300 μg.), pyridoxine hydrochloride (400 μg.), riboflavin (600 μg.), calcium pantothenate (1·5 mg.), nicotinic acid (4 mg.), biotin (20 μg.), pteroylmonoglutamic acid (75 μg.) and cyanocobalamin (2 μg.) in each 100 g. of diet.

‡ Containing α-tocopheryl acetate (1 mg.), menaphthone (500 μg.), vitamin A (1700 i.u.) and vitamin D_3 (160 i.u.) in 5 g.

in an MSE homogenizer at top speed for 3 min. The volume of the homogenate was adjusted to 100 ml. with 0·15 M-NaCl and centrifuged at 1000*g* for 5 min. at 4°. The supernatant was filtered through gauze and stored in small quantities at −20°.

Unless otherwise stated the homogenates used included intestinal walls and intestinal contents; although overnight starvation gave an empty small intestine there was still a considerable quantity of material in the large intestine.

Separation of intestinal wall and contents in the living bird. The birds were anaesthetized with ether and a diagonal incision was made in the right side of the abdomen to expose the duodenum. A ligature was placed just distal to the pylorus. The duodenum was cut immediately below the ligature and a glass cannula inserted and tied into position. Another ligature was placed on the proximal side of the ileo-caecal junction and the ileum was severed just above it. A glass cannula, attached to a length of polythene tubing leading into a collecting vessel, was inserted and tied into the cut end of the ileum. The duodenal cannula was connected to a reservoir of 0·15 M-NaCl at 38° and saline (usually about 50 ml.) was allowed to flow through the intestine until all the contents had been removed.

A glass cannula was passed through the cloaca into the rectum and tied into position. The contents of the caeca and colon were washed out through it with several (about four) 5 ml. quantities of warm saline introduced alternately through the tips of the caeca by means of a hypodermic syringe. The bird was then killed with ether and the small intestine, caeca and colon were removed.

Determination of enzymic activities. Disaccharidase activities were determined by the method of Dahlqvist (1964), in which the production of glucose after incubation of substrate and homogenate (suitably diluted with 0·15 M-NaCl) at 37° for 1 hr. was measured with tris–glucose oxidase reagent. Buffers used were 50 mM-sodium maleate, pH 5·6 for lactase, cellobiase and trehalase, pH 5·8 for maltase and pH 6·2 for sucrase and palatinase, all with 28 mM substrate. One unit of disaccharidase activity is defined as that hydrolysing 1 μmole of disaccharide/60 min. at 37°. Two requirements for comparative enzyme studies are: (i) a linear relationship between enzyme concentration and the measured product; (ii) a linear relationship between the length of the incubation period and the measured product. By varying the amount of homogenate and the duration of the incubation it was found that both these requirements were fulfilled by the intestinal disaccharidases of the chick under the above assay conditions until at least 10% of the substrate (initial concentration 28 mM) had been hydrolysed.

Determination of protein. The protein content of the homogenates was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951). Bovine plasma albumin was used to prepare a standard curve.

RESULTS

In preliminary experiments it was found (Table 2) that homogenates of the small intestine from 3-week-old chicks readily hydrolysed maltose, sucrose and palatinose, hydrolysed lactose slowly and did not hydrolyse cellobiose and trehalose. The homogenates of the large intestines of these chicks also hydrolysed maltose, sucrose and palatinose and in addition exhibited a relatively high lactase activity.

Table 2. *Disaccharidase activities in homogenates of the small and large intestines of 3-week-old chicks*

The activities are means ± S.E.M. for seven chicks fed on a chick-mash diet.

	Activity (total units)	
	Small intestine	Large intestine
Maltase	14380 ± 1599	1774 ± 356
Sucrase	1952 ± 322	508 ± 109
Palatinase	452 ± 59	211 ± 51
Lactase	45 ± 7	381 ± 155
Cellobiase	0	39 ± 10
Trehalase	0	16 ± 7

Table 3. *Michaelis constants, K_m, of the chick small-intestinal and large-intestinal disaccharidases*

The K_m values were calculated from the initial disaccharidase activities at low substrate concentrations (below 0·1 M for maltose and lactose and below 0·02 M for sucrose and palatinose) by the graphical method of Lineweaver & Burk (1934) and Dixon (1953).

	K_m (mM)	
	Small intestine	Large intestine
Maltase	3·14	2·8
Sucrase	16·4	11·8
Palatinase	3·41	5·55
Lactase	28·2	7·68

The cellobiase and trehalase activities again were either very low or absent.

Properties of the intestinal disaccharidases. (*a*) Effect of pH. By using different buffer systems disaccharidase activities were measured over the pH range 2·0–9·0 (Fig. 1). The maltase, sucrase and palatinase showed similar pH–activity curves in the small and large intestines, the optimum pH values being 6·2, 6·4 and 6·0 respectively. The lactase, on the other hand, had very different pH–activity curves in the small and large intestines. The former had an optimum at pH 3·6 and the latter at pH 6·4.

(*b*) Effect of substrate concentration. In Fig. 2 the disaccharidase activity ($V_{obs.}$) at various substrate concentrations (0–0·2 M) is expressed as a percentage of the maximum disaccharidase activity ($V_{max.}$). The $V_{max.}$ and the K_m values (Table 3) were calculated from the $V_{obs.}$ at low substrate concentrations (below 0·1 M for maltase and lactase and below 0·02 M for sucrase and palatinase) by the graphical method of Lineweaver & Burk (1934) and Dixon (1953). Substrate concentrations higher than 0·02 M caused marked inhibition of the sucrase and palatinase activities, whereas there was no inhibition of the maltase and lactase activities even at 0·2 M substrate concentration. The K_m values

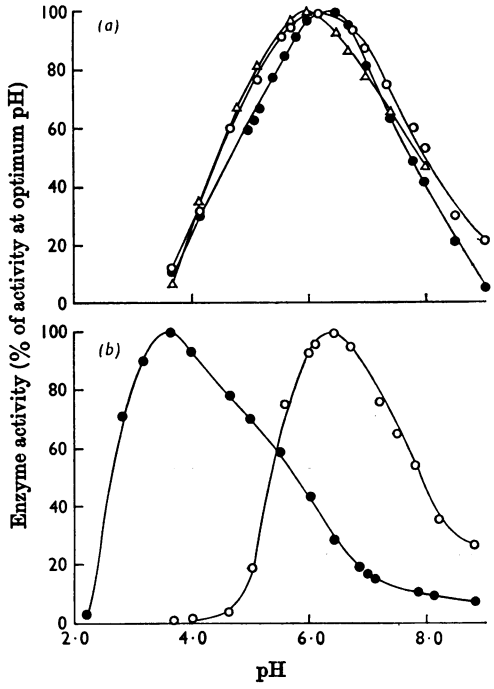

Fig. 1. pH–activity curves for the intestinal disaccharidases of the chick: (*a*) maltase (○), sucrase (●) and palatinase (△) activities of a homogenate of the small intestine; (*b*) lactase activity of homogenates of the small intestine (●) and large intestine (○). The buffers used were: pH 2·2–3·4, 50 mM-glycine–HCl; pH 3·6–5·4, 50 mM-sodium acetate–acetic acid; pH 5·5–7·0, 50 mM-sodium maleate; pH 6·2–8·0, 50 mM-sodium phosphate; pH 7·5–9·0, 25 mM-sodium veronal–HCl.

(Table 3) of the maltase, sucrase and palatinase activities in the small-intestinal homogenates were similar to the K_m values of the corresponding activities in the large-intestinal homogenates. The K_m values for the lactase activity of the two homogenates, however, were different.

Distribution of the disaccharidases between intestinal contents and wall. Since breakdown of the intestinal mucosa begins very soon after death it was necessary to wash out the intestinal tract in the live anaesthetized bird to achieve good separation of the contents from the wall. For assays the contents were homogenized and homogenates of the washed intestine were prepared as described above. Four-week-old birds starved overnight and birds allowed to feed up to the time of experimentation were compared. The results in Table 4 show that in the starved birds 71% of the total maltase, 67% of the total sucrase, 64% of the total palatinase and 17% of the total lactase activity of the whole intestinal tract was located in the small intestine.

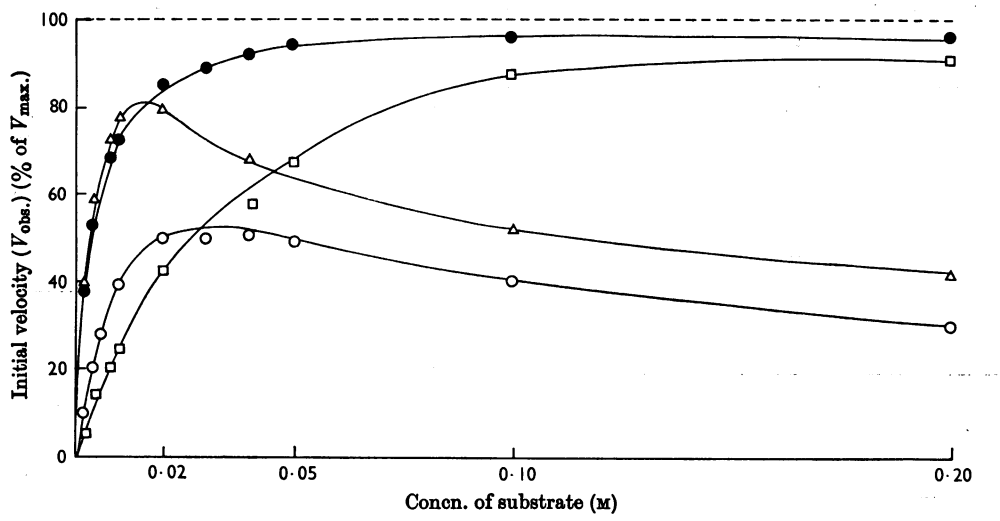

Fig. 2. Effect of substrate concentration on the disaccharidase activities of homogenates of the small intestine of the chick: ●, maltase; ○, sucrase; △, palatinase; □, lactase. The maximum velocities ($V_{max.}$) are shown as broken lines and were calculated as described in the text.

Table 4. *Distribution of disaccharidase activity between the intestinal wall and contents of starved and fed birds*

Five 4-week-old chicks fed on chick-mash diets were used in each group. The contents were obtained from live anaesthetized birds as described in the Materials and Methods section. Total activity equals sum of activities in the small-intestinal wall and contents plus the activities in the large-intestinal wall and contents. The results were analysed statistically by using an analysis of variance with one-way classification and Student's *t* test: NS, $P > 0{\cdot}1$; † $P < 0{\cdot}1$; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

	Activity (total units)			S.E. of	
	Starved birds	Fed birds	Difference	difference	Significance
Maltase					
Total	15497	16353	+856	4856	NS
Small-intestinal contents	409	647	+238	104	†
Small intestinal wall	10694	14822	+4128	4364	NS
Large-intestinal contents	4124	617	−3507	866	**
Large-intestinal wall	270	267	−3	46	NS
Sucrase					
Total	3363	3372	+9	885	NS
Small-intestinal contents	90	170	+80	28	*
Small-intestinal wall	2166	2933	+767	784	NS
Large-intestinal contents	1082	246	−836	216	**
Large-intestinal wall	25	23	−2	4	NS
Palatinase					
Total	884	842	−42	212	NS
Small-intestinal contents	20	73	+53	39	NS
Small-intestinal wall	538	707	+169	181	NS
Large-intestinal contents	320	55	−265	67	**
Large-intestinal wall	6	7	+1	1	NS
Lactase					
Total	513	239	−274	113	*
Small-intestinal contents	0	12	+12	7	NS
Small-intestinal wall	77	80	+3	13	NS
Large-intestinal contents	422	135	−287	117	*
Large-intestinal wall	14	12	−2	2	NS

Table 5. *Distribution of disaccharidase activities along the digestive tract of the chick*

The results are the means ± S.E.M. of the activities for eight 4-week-old chicks.

	Activity (total units)			
	Maltase	Sucrase	Palatinase	Lactase
Duodenum	2672±266	322±52	74±38	15±3
Jejunum	7843±262	1111±226	247±53	19±8
Ileum	4888±450	646±93	161±20	4±2
Caeca	1699±336	506±50	208±47	337±144
Colon	139±28	17±6	4±2	3±2
Pancreas	94±16	9±3	32±7	0

Within the small intestine these activities were concentrated mainly in the intestinal wall, less than 5% being found in the contents. In the large intestine the distribution was reversed; 93–98% of the total activity was shown by the gut contents.

Statistical analysis of the results obtained with starved and fed birds is shown in Table 4.

Distribution of disaccharidases along the digestive tract (Table 5 and Fig. 3). The disaccharidase activities of the pancreas and colon were very low. The maltase, sucrase and palatinase activities were located mainly in the small intestine and were highest in the distal jejunum and proximal ileum. The lactase activity of the small intestine was low and tended to be higher in the middle and proximal parts than in the distal part. The caeca contained relatively large amounts of all four disaccharidases; the lactase activity was considerably greater than in any other part of the intestinal tract, but varied with diet (see below).

Development of disaccharidase activities after hatching. The development of intestinal disaccharidase activity was studied in chicks receiving either chick mash or the diet of purified ingredients (Table 1). In the age range studied (1–43 days) the development of the disaccharidase activities in the small intestine was very similar in the two groups (Fig. 4). In terms of total activity all the enzymes were lowest in 1-day-old chicks and increased with age.

The specific activities (i.e. activities/mg. of protein) of the small-intestinal disaccharidases were also very similar with the two diets because, despite different growth rates (Table 6), the actual weights of the small intestines were very similar on both diets, and the protein content of the homogenates was closely correlated with intestinal weight. The specific activities of the small-intestinal maltase, sucrase and palatinase (Fig. 4) decreased between 1 and 8 days, owing to a rapid increase in the small-intestinal weight without a concomitant increase in total activity. Subsequently the specific activities increased, the rate of increase falling off after 36 days.

In the large intestine there was again no significant difference due to diet in the total maltase, sucrase and palatinase activities. The activities all increased at an approximately linear rate with age. There was, however, a difference in the lactase activity in the large intestine of the chicks on the two diets. The activities were considerably higher in the chicks receiving the mash than in the chicks receiving the diet of purified ingredients (Table 7).

Disaccharidase activities in the developing embryo. Studies were made with intestines from embryos taken daily from 12 days of incubation onwards. Maltase, sucrase, palatinase and lactase activities were demonstrated in all embryos studied (Table 8). The activities in the younger embryos were low and it was necessary to pool samples to obtain measurable activity. The total activity of each of the disaccharidases increased with age up to 18 days at a rate comparable with the rate of increase in the protein content of the homogenates of the whole intestine, so that the specific activities remained fairly constant over this period. From 19 days to hatching the total maltase, sucrase and palatinase, but not lactase, activities increased more rapidly than the protein content.

DISCUSSION

Although the factors controlling the development of intestinal disaccharidases are not known (Herzenberg & Herzenberg, 1959), it appears that the intestinal disaccharidases of mammals develop in a way that allows the utilization of the carbohydrates that the animal is likely to encounter under natural feeding conditions. Thus in most young suckling mammals the β-glycosidase activities (lactase and cellobiase) are high (Doell & Kretchmer, 1962; Rubino, Zimbalatti & Auricchio, 1964; Siddons, 1968) and allow efficient utilization of the high lactose content of the maternal milk. The young sea-lion is unusual among mammals in that it has no intestinal lactase (Kretchmer & Sunshine, 1967). The milk of the sea-lion, however,

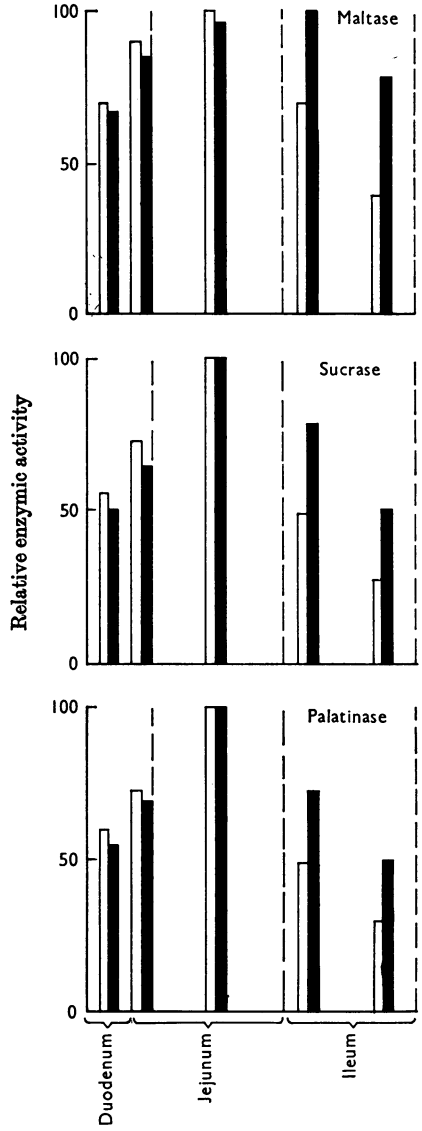

Fig. 3. Disaccharidase activities of homogenates of various sections of the small intestine of the chick. The activities are expressed in terms of activity/cm. of intestine (□) and activity/mg. of protein (■).

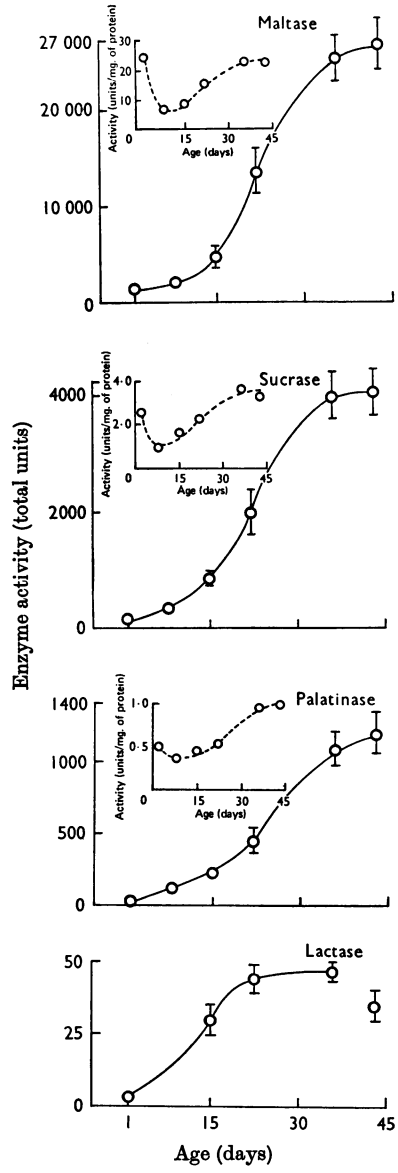

Fig. 4. Change with age in the disaccharidase activities of the small intestine of the chick. Five chicks were used at each age and the results are mean values with their standard errors indicated by vertical bars. The inset diagrams show the change with age in the specific activities of the disaccharidases.

contains no lactose (Pilson & Kelly, 1962). After weaning the lactase activity of the mammalian small intestine decreases and the maltase and sucrase activities increase (Welsh & Walker, 1965; Blair, Yakimets & Tuba, 1963). This change in enzymic activities is consistent with the change in dietary carbohydrate at this point in the mammal's life. However, it should not be construed from this that the diet directly controls disaccharidase activity, since attempts to induce disaccharidase activity by feeding with specific disaccharides have generally been inconclusive (Alvarez & Sas, 1961; Heilskov, 1951*b*; Fischer, 1957; Reddy, Pleasants & Wostmann, 1968).

Table. 6. *Increase with age in the live weight, small-intestine weight and large-intestine weight of chicks receiving a chick-mash diet or a diet of purified ingredients*

The compositions of chick-mash diet (SCM) and the diet of purified ingredients (SCG) are given in Table 1.

	Live wt. (g.)		Small-intestine wt. (g.)		Large-intestine wt. (g.)		Small-intestine wt. / Live wt.	
Age (days)	SCM	SCG	SCM	SCG	SCM	SCG	SCM	SCG
1	41·4	41·4	1·06	1·06	0·46	0·46	0·026	0·026
8	50·6	70·9	4·31	4·79	1·31	1·32	0·085	0·068
15	94·5	132·8	5·84	5·84	1·62	1·06	0·062	0·044
22	175·9	235·3	8·56	8·63	3·17	2·21	0·049	0·037
36	337·1	462·1	11·58	12·70	4·66	3·15	0·031	0·028
43	460·2	497·3	14·31	12·83	5·52	3·48	0·031	0·026

Table 7. *Disaccharidase activities in the large intestine of chicks of various ages*

The compositions of chick-mash diet (SCM) and the diet of purified ingredients (SCG) are given in Table 1. The results are the means of five birds in each group.

	Activity (total units)				
				Lactase	
Age (days)	Maltase SCM	Sucrase SCM	Palatinase SCM	SCM	SCG
1	176	17	0	0	0
8	1099	342	102	208	29
15	1089	318	112	285	62
22	2341	662	237	838	82
36	2838	1339	497	1817	114
43	6463	1900	571	2035	115

The newly hatched chick may be likened to the weaned mammal in that most of the carbohydrate in its diet will be in the form of starch and the lactose content of its diet is likely to be very small. It is not surprising therefore to find high maltase and low lactase activities in the small intestine of the chick. The relatively high sucrase and palatinase activities found would not be expected if it is assumed that intestinal disaccharidase activity is correlated with dietary disaccharides. However, it is possible that these activities may be due to non-specific α-glycosidases. In mammals, chromatographic studies have shown that there are no specific sucrase and palatinase enzymes, but there are several enzymes [five in humans (Auricchio *et al.* 1963), three in pigs (Dahlqvist, 1959)] capable of hydrolysing maltose, some of which are specific for maltose and others that also have sucrase and palatinase activities.

In many respects, e.g. pH optima, K_m values and distribution along the small intestine, the small-intestinal maltase, sucrase and palatinase activities of the chick are very similar to the corresponding mammalian enzymes (Dahlqvist & Thompson, 1964; Blair & Tuba, 1963; Malhotra & Philip, 1964, 1965). A specific trehalase activity that is present in most mammals was not found in the chick.

Another difference between the chick and mammals is in the pH optimum of the small-intestinal lactase activity. In the chick maximum activity was observed at pH 3·6 compared with pH 5–6 for the mammalian lactase activity (Wallenfels & Fischer, 1960; Heilskov, 1951*a*). Studies (Koldovsky & Chytil, 1965; Asp & Dahlqvist, 1968) have shown, however, that there are at least two β-galactosidases in the mammalian small intestine; one has optimum pH 3–4 (i.e. similar to the chick) and is a heterogalactosidase, whereas the other has optimum pH 5–6 and is specific for lactose. Koldovsky *et al.* (1965) found that the non-specific heterogalactosidase is not located in the brush border of the mucosal cells and probably does not participate in digestion.

That hydrolysis of disaccharides in the mammalian small intestine occurred inside the mucosal cells rather than in the lumen was first suggested because the hydrolytic activity of the intestinal contents was too low to account for the observed rate of digestion of disaccharides (Cajori, 1933; Borgström, Dahlqvist, Lundh & Sjövall, 1957). More recently studies *in vivo* and *in vitro* (Dahlqvist & Borgström, 1961; Miller & Crane, 1961) and the use of histochemical techniques (Dahlqvist & Brun, 1962; Jos, Frézal, Rey, Lamy & Wegmann, 1967; Jos, Frézal, Rey & Lamy, 1967) have demonstrated conclusively that the disaccharidases are located in the epithelial cells of the villi and that disaccharides are hydrolysed intracellularly. That this situation may hold for the chick is indicated by the finding of low disaccharidase activity in the small-intestinal contents; feeding increased this activity, but even so the maltase activity, as calculated under the conditions of the assays *in vitro*, was sufficient to hydrolyse less than 50% of the maltose intake (calculated from the starch content of the diet).

Table 8. *Disaccharidase activities in the intestine of the developing chick embryo*

Ten embryos were used in each group. The results are mean values ± S.E.M. The values in parentheses are the activities/mg. of protein.

Age (days)	Activity (total units) Maltase		Sucrase		Palatinase		Lactase	
12	0·9	(0·52)	0·16	(0·09)	0·06	(0·04)	0·12	(0·08)
13	1·4	(0·52)	0·37	(0·14)	0·28	(0·10)	0·18	(0·07)
14	2·6	(0·42)	0·75	(0·16)	0·23	(0·04)	0·20	(0·03)
15	4·2	(0·46)	1·40	(0·16)	0·26	(0·03)	0·38	(0·04)
16	4·7	(0·48)	1·50	(0·16)	0·51	(0·05)	0·33	(0·03)
17	8·6	(0·46)	2·90	(0·15)	0·84	(0·05)	0·84	(0·05)
18	16·9 ± 1·9	(0·86)	3·0 ± 0·13	(0·15)	1·8 ± 0·11	(0·09)	1·08 ± 0·10	(0·05)
19	62·3 ± 4·3	(2·17)	9·7 ± 0·73	(0·34)	3·0 ± 0·26	(0·11)	0·48 ± 0·06	(0·02)
20	160·0 ± 26·2	(4·36)	13·7 ± 1·46	(0·38)	4·2 ± 0·03	(0·12)	1·00 ± 0·14	(0·02)
21	616·5 ± 107·2	(6·10)	66·5 ± 6·49	(0·64)	9·9 ± 1·38	(0·09)	4·10 ± 0·12	(0·04)

An intracellular locus of disaccharide hydrolysis lends added importance to the distribution pattern of the disaccharidases along the small intestine because the location of optimum activity presumably reflects the site of absorption. A non-uniform distribution of disaccharidase activity along the small intestine has been observed in a number of mammals (Dahlqvist, 1961; Malhotra & Philip, 1964, 1965) and in most cases the distribution has been found to be similar to that observed in the chick, the activity being highest in the middle part of the small intestine and decreasing in both proximal and distal directions.

Disaccharidases in the large intestine were present almost exlusively in the contents. Enzymes in caecal contents could arise in a number of ways: (i) secretion by cells lining the caecum; (ii) passage from the small intestine; (iii) production by the caecal flora. The similarity in the chick between the pH optima and K_m values of the small-intestinal and large-intestinal maltase, sucrase and palatinase activities suggests that these enzymes originate in the small intestine and pass into the caecum. The lactase activity, on the other hand, had very different pH optima and K_m values in the small and large intestines, suggesting that caecal lactase is either produced by the caecal wall or is of bacterial origin. Preliminary studies (R. C. Siddons, unpublished work) have shown that caecal lactase activity in germ-free chicks is much lower than in normal chicks and it seems likely that the caecal lactase is predominantly of bacterial origin. In addition the activity of caecal lactase, unlike that of the other disaccharidases, appeared to be directly influenced by the diet; chicks given chick mash had more caecal lactase activity than chicks given the diet of purified ingredients. This may be because the chick mash contained lactose (added as dried skim milk) whereas the 'purified diet' contained no lactose. The lactose probably passes unchanged through the small intestine and its presence in the caecum might encourage the establishment of lactose-fermenting organisms; it might also induce organisms already present to produce lactose-hydrolysing enzymes. The induction of β-galactosidase in *Escherichia coli* by β-galactosides has been known for a long time and is widely used as a system for studying protein synthesis (Lederberg, 1950; Cohn, 1957).

Studies with human foetuses have shown that the small-intestinal disaccharidases are formed long before they are normally required for the digestion of disaccharides. For example, lactase activity has been demonstrated in 2–3-month-old foetuses (Auricchio, Rubino & Mürset, 1965) and the sucrase that is also present in the 2–3-month-old foetus reaches an activity comparable with that of the adult by 35–36 weeks of intrauterine life (Fomina, 1960). Only low disaccharidase activity was found in the intestine of 12-day-old chick embryos. Marked increases in activity occurred only during the last few days of embryonic development. It is during this stage in embryonic development that the small-intestinal villi elongate and increase in number (Pap, 1933; Joos, 1941). Doell & Kretchmer (1962) have shown that β-galactosidase activity is present in the rat at day 18 of gestation and Kammeraad (1942) has shown that this is the period of rapid differentiation of intestinal epithelial cells. Thus it would seem that the development of enzyme activity reflects morphological changes in the developing intestine.

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