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PHOSPHATASE ACTIVITIES OF CHICKEN LIVER AND DUODENUM: CHARACTERISTICS, LEVELS DURING DEVELOPMENT, AND HYDROCORTISONE-INDUCED CHANGES

CORNELIUS F. STRITTMATTER

Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, N.C. 27103 (U.S.A.)

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SUMMARY

1. Changes in specific activity levels of phosphatases were studied in homogenates and cell fractions of liver and duodenum from chick embryos and chicks at various stages of development. The phosphatases studied included the activities with glucose-6-phosphate, fructose-1,6-diphosphate, inorganic pyrophosphate, or ATP as substrate, all at pH 6.5 in the presence of 5 mM Mg^{2+}, and activity with *p*-nitrophenyl phosphate as substrate at pH 5.0, 6.5, and 9.5 in the presence of 5 mM Mg^{2+} or 5 mM Zn^{2+} or without added metal. In liver, there were increases about the time of hatching in the largely particulate phosphatase activities with ATP and with *p*-nitrophenyl phosphate in the presence of Mg^{2+}, but decreases in the primarily soluble activity with *p*-nitrophenyl phosphate at pH 6.5 in the presence of Zn^{2+} and in the activities with Glc-6-*P* or Fru-1,6-P_2 as substrate. In duodenum there was a marked and persistent increase about the time of hatching in activity with Glc-6-*P*, in addition to a dramatic increase of phosphatase activity with *p*-nitrophenyl phosphate or β-glycerophosphate at pH 9.5 which began shortly before hatching and reached a transient peak shortly after hatching. The duodenum also showed moderate increases in activities with Fru-1,6-P_2, PP_i and ATP, in the largely particulate activity with *p*-nitrophenyl phosphate at pH 5 or 6.5 in the presence of Mg^{2+}, and in a largely soluble activity with *p*-nitrophenyl phosphate at pH 5 in the presence of Zn^{2+}. No marked changes in intracellular distribution of activities between particulate and soluble state were observed during the developmental period studied.

2. The phosphatase activities of the chick tissues showed varied patterns of stimulation by or requirement for Mg^{2+}, usually similar to the patterns known for analogous mammalian enzymes, and activities with several substrates were substantially inhibited by 5 mM Zn^{2+}.

3. Administration of hydrocortisone to developing 13-day embryos gave rise in 18-day embryo duodenum to marked increases above normal 18-day levels of phosphatase activity with *p*-nitrophenyl phosphate and β-glycerophosphate at pH 9.5 and with Glc-6-*P* at pH 6.5, and to modest increases of the other activities studied. The changes are consistent with a general acceleration of normal duodenal develop-

ment by hydrocortisone. In the liver, hydrocortisone administration led to substantial decreases in activity with Glc-6-*P*, Fru-1,6-P_2 and *p*-nitrophenyl phosphate, but no appreciable changes in the other activities studied.

INTRODUCTION

Many studies have been made of the marked increase during embryonic development of alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) activity in the intestinal mucosa in the chicken[1], where the rise begins shortly before hatching, and in the mouse[2], where increases occur shortly before birth and again shortly before weaning. Premature evocation of these increases by administration of glucocorticoids has been documented in both the developing chick embryo[3] and the mouse[4], and the developing mouse duodenum has been extensively used for studies of the normal and induced changes of alkaline phosphatase levels[4-7]. Studies have also been made of developmental changes in levels of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) in chick liver[8,9] and rat liver[10,11], of acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) and inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) in rat liver[12] and of both Mg^{2+}- and Zn^{2+}-stimulated acid phosphatase activities with *p*-nitrophenyl phosphate as substrate in chick liver[13].

The present study was undertaken as a coordinated investigation of developmental changes in a spectrum of phosphatase activities in the liver and duodenum of chick embryos and chicks. The duodenum was chosen as an organ that undergoes major functional differentiation about the time of hatching, and the liver as one that is already functional in the early embryo, but does undergo significant alterations of metabolic roles in later development. The effects of hydrocortisone administration on developmental patterns for various phosphatases were studied in both organs. Because Mg^{2+} and Zn^{2+} have varied stimulatory and/or inhibitory effects on different mammalian and microbial phosphatases, the effects of these ions on the avian phosphatase activities were also examined.

METHODS

Animal development and injections

Fertilized white Leghorn chicken eggs were incubated at 38 °C and 65% humidity. Embryo and chick ages refer to full days of incubation or days after hatching. Only embryos of the developmental stage[14] appropriate to the period of incubation were used for tissue preparations. Chicks were provided with water and Purina Chick Startena *ad libitum*. For the hormone studies on embryos, a single injection of 0.25 mg hydrocortisone sodium succinate in 0.05 ml sterile isotonic saline was made below the egg shell membrane, usually after 13 days of incubation, and the hole sealed with collodion. Control embryonated eggs were injected with an equal volume of sterile isotonic saline.

Tissue preparations

Embryos and chicks were killed by decapitation. Tissues were quickly excised

and trimmed free of connective tissue, rinsed with cold 250 mM sucrose–1 mM EDTA (pH 7.0) (sucrose–EDTA medium), blotted and weighed. Entire livers were used. The duodenum, the portion of the intestine from the gizzard to the bile duct entrance, was cut into several segments and gently washed inside and out with the sucrose–EDTA medium prior to blotting and weighing. Tissue from different animals was pooled to give 1–2 g samples from embryos or young chicks and 3–5 g samples from older chicks. All subsequent steps were carried out at 0–2 °C. The tissue samples were homogenized in 5 vol. of cold sucrose–EDTA medium in a motor-driven glass homogenizer with teflon pestle at 200–500 rev./min; the number of strokes was adjusted to give about 90% breakage of cells, except for connective tissue, as estimated by microscopic examination. Homogenates were strained through gauze, and were made up with sucrose–EDTA medium to a final concentration of 100 mg wet wt of tissue per ml.

For tissue fractionations, the homogenate (H) was centrifuged at 700 × *g* for 10 min to give a "nuclear pellet." The pellet was suspended in sucrose–EDTA medium and recentrifuged, then suspended in 10 mM Tris–maleate buffer (pH 7.0)–1 mM EDTA (Tris–maleate–EDTA medium) to give the nuclear fraction (N). The combined supernatant solution from the two centrifugations was centrifuged at 100 000 × *g* for 60 min to give a particulate fraction (P), which was resuspended in Tris–maleate–EDTA medium, and a supernatant fraction (S), which was made up with Tris–maleate–EDTA medium to the original volume of homogenate used. Samples of fraction S were taken to 75% saturation with $(NH_4)_2SO_4$ by addition of saturated $(NH_4)_2SO_4$ solution; after standing 15 min, the mixture was centrifuged at 20 000 × *g* for 10 min, and the precipitate was suspended in Tris–maleate–EDTA medium to give the soluble-precipitated fraction (SP). Homogenates and fractions routinely were frozen immediately after preparation and were stored at −15 °C until assayed, usually after 1 day. The activities of the frozen-and-thawed preparations were equal to or greater than the activities seen with fresh preparations.

Assays

Phosphatase activities of homogenates or fractions were assayed by determination of inorganic phosphate released from substrates during incubation at 37 °C for 15 min in 50 mM acetate buffer (pH 5.0), in 50 mM Tris–maleate (pH 6.5), or in 100 mM 2-amino-2-methyl-1-propanol (pH 9.5). Substrates were added in 10 mM final concentration, except for use of 20 mM Glc-6-*P* and 3 mM PP_i. The incubation mixtures routinely contained 5 mM $MgCl_2$ or 5 mM $ZnSO_4$, except for use of 3 mM metal when PP_i was the substrate, and contained 0.1 mM EDTA. The reaction was initiated by addition of enzyme to the pre-warmed incubation mixture in a final volume of 1.0 ml, and the reaction terminated by addition of 0.5 ml cold 10% trichloroacetic acid. After centrifugation of the cooled mixture, samples of the supernatant fluid were assayed for inorganic phosphate by the procedure of Lowry and Lopez[15].

Protein content of tissue preparations was determined by the micromethod of Lowry *et al.*[16], using bovine serum albumin as a standard.

Chemicals

Bovine serum albumin, 2-amino-2-methyl-1-propanol and the sodium salts of

Glc-6-*P*, Fru-1,6-P_2, *p*-nitrophenyl phosphate, phenyl phosphate, ATP and β-glycerophosphate (reagent grade) were obtained from Sigma Chemical Company. Hydrocortisone sodium succinate was obtained from the Upjohn Company. Other chemicals were of reagent or certified grade and were obtained from Fisher Chemical Company.

RESULTS

Phosphatase activities of 4-day chick liver and effects of metal ions

Initial experiments with 4-day chicks were carried out to identify appropriate tissues, phosphatase activities, and assay conditions for the projected study of developmental changes in phosphatases. Table I includes the most pertinent activities in chick liver homogenate and subcellular fractions. The enzymes assayed with added Mg^{2+} showed maximum activities with the 5 mM Mg^{2+} routinely employed, except for the activity with PP_i as substrate, where 3 mM Mg^{2+} and 3 mM PP_i were used to give maximal activity. From the data in Table I, it may be calculated that 80–100% of the various activities in the homogenate were recovered in the sum of the nuclear, particulate and soluble fractions. Further, the activities concentrated in the soluble fraction (S) could be recovered almost completely in the $(NH_4)_2SO_4$-precipitated fraction (SP) derived from the soluble fraction.

With pNPP as substrate, the activity of the particulate (P), soluble (S) and SP fractions at either pH 5.0 or 6.5 were only slightly stimulated by Mg^{2+}, and the activities with or without added Mg^{2+} were concentrated in the particulate fraction. Stimulation of *p*-nitrophenyl phosphate hydrolysis by added Zn^{2+} was localized almost entirely in the original soluble fraction (S), and was more marked in the SP preparation derived from the soluble fraction. The different effects of Zn^{2+} at pH 5.0 and pH 6.5 on the activity of homogenates with *p*-nitrophenyl phosphate as substrate resulted from the presence in the soluble fraction of a Zn^{2+}-stimulated enzyme(s), which was more active at pH 6.5 than at pH 5.0, and the presence in the particulate fraction of enzymes which were inhibited by Zn^{2+}.

The effects of 5 mM Mg^{2+} and 5 mM Zn^{2+} on other phosphatase activities were varied (data not tabulated). The activity of the particulate (P) fraction with Glc-6-*P* as substrate was equally high with or without added Mg^{2+}, and the activity in the presence or absence of Mg^{2+} was 95% inhibited by Zn^{2+}. The activity of the particulate (P) fraction on ATP was reduced 95% by omission of added Mg^{2+}, and reduced 30% by substitution of Zn^{2+} for Mg^{2+}. The activities of the SP fraction on Fru-1,6-P_2 or PP_i were reduced 90% by omission of added Mg^{2+} or by substitution of Zn^{2+} for Mg^{2+}, and the activities with added Mg^{2+} were inhibited 80–90% by the simultaneous addition of 5 mM Mg^{2+}. The low activity shown for liver homogenate at pH 9.5 with *p*-nitrophenyl phosphate and added Mg^{2+} was reduced about 20% by omission of added Mg^{2+} and was reduced 50% by substitution of Zn^{2+} for Mg^{2+}.

Phosphatase activities of 4-day chick duodenum and effects of metal ions

As is also shown in Table I, chick duodenum contained substantial levels of the major phosphatase activities seen in liver, and the intracellular distribution of the duodenal phosphatase activities between particulate (P) and soluble (S) fractions was similar to those seen for the corresponding activities in liver. The soluble Zn^{2+}-stimulated activity with *p*-nitrophenyl phosphate at pH 5.0 and 6.5 was obscured in

TABLE I

LEVELS AND INTRACELLULAR DISTRIBUTION OF PHOSPHATASE ACTIVITIES IN 4-DAY CHICK LIVER AND SMALL INTESTINE

Homogenates and nuclear (N), particulate (P), soluble (S) and soluble-precipitated (SP) fractions of 4-day chick tissues were prepared and assayed for phosphatase activities and protein content as described under Methods. Where indicated, 5 mM Mg^{2+} or 5 mM Zn^{2+} was added. The data tabulated are the mean values for 3 typical fractionations; the range of values was within ±10% of the mean except in those values indicated by an asterisk. Specific activities are expressed as nmoles P_i released/min per mg protein in the fraction assayed and protein content as mg protein/g original tissue. pNPP = *p*-nitrophenyl phosphate.

Tissue	*Fraction*	*Specific activity of phosphatases with designated substrate, metal and pH*											*Protein content (mg/g tissue)*
		Glc-6-P Mg^{2+} *pH 6.5*	*Fru-1,6-P_2* Mg^{2+} *pH 6.5*	*PP_i* Mg^{2+} *pH 6.5*	*ATP* Mg^{2+} *pH 6.5*	*pNPP* — *pH 6.5*	*pNPP* Mg^{2+} *pH 6.5*	*pNPP* Zn^{2+} *pH 6.5*	*pNPP* — *pH 5.0*	*pNPP* Mg^{2+} *pH 5.0*	*pNPP* Zn^{2+} *pH 5.0*	*pNPP* Mg^{2+} *pH 9.5*	
Liver	Homogenate	64.5	70.5	619	545	50.2	59.2	109	56.5	71.0	54	14.0	186
	N	58.5	32.7*	256	419	40.6	50.9	36	37.2	40.0	14*	12.6*	22
	P	100.0	11.8	161	1010	66.2	78.5	15	87.5	99.0	14*	15.1	93
	S	0	159.8	1670	85	27.2	28.8	267	30.7	44.1	139	11.9*	59
	SP	—	152.0	1660	55	16.1	23.2	311	33.0	37.5	197	10.7	56
Duodenum	Homogenate	17.3	16.7	733	219	33.3	48.7	40	51.4	60.0	48	367.0	150
	N	12.3	6.2*	247	164	20.5	34.8	24	31.5	43.1	10*	185.0	39
	P	29.0	12.7	159	429	42.9	74.5	29	58.7	74.5	20	603.0	63
	S	0	37.4	2250	25*	22.5	40.0	83	35.3	40.0	132	75.0	40
	SP	—	40.1	2850	20*	20.6	35.3	115	29.7	35.3	271	59.0*	34

assays of the homogenate at both pH values because Zn^{2+} markedly inhibited the activity with *p*-nitrophenyl phosphate in the particulate fraction (P). The Zn^{2+}-stimulated activity with *p*-nitrophenyl phosphate in the duodenum, unlike that in the liver, was higher at pH 5.0 than at pH 6.5.

The effects of 5 mM Mg^{2+} and 5 mM Zn^{2+} on phosphatase activities of the duodenal SP fraction with Fru-1,6-P_2 or PP_i as substrate and of the duodenal particulate (P) fraction with ATP as substrate were very similar to those noted above for the analogous activities in liver fractions (data not tabulated). However, unlike the liver enzyme, the activity of duodenal particulate (P) fraction on Glc-6-*P* was reduced 35% by omission of added Mg^{2+}; addition of Zn^{2+} in the presence or absence of Mg^{2+} resulted in activities similar to that seen without added metal. The high activity of the particulate (P) fraction at pH 9.5 with *p*-nitrophenyl phosphate and added Mg^{2+} was reduced 90% by omission of Mg^{2+}, was reduced 80% by substitution of 5 mM Zn^{2+} for Mg^{2+}, and was reduced 20% by addition of Zn^{2+} *plus* Mg^{2+}.

Changes in wet weight and protein concentration

As indicated in Table II, the total wet weight of the chicken liver and duodenum increased many-fold during the period from 12-day embryo to 12-day chick, but

TABLE II

CHANGES IN WEIGHT AND PROTEIN CONTENT OF LIVER AND DUODENUM DURING DEVELOPMENT OF CHICK

Total wet weight of original tissues was obtained and mg protein per g original tissue was determined on tissue homogenates as described under Methods. Each value tabulated represents the mean and standard error of the mean from assays of 3 to 6 preparations, except for 19-day embryo and 2-day chick liver values, where each value is the average of results from two preparations.

Age	*Liver*		*Duodenum*	
	Wet weight (g)	*Protein (mg/g)*	*Wet weight (g)*	*Protein (mg/g)*
12-day embryo	0.068 ± 0.002	165 ± 6	0.005 ± 0.0004	100 ± 9
15-day embryo	0.149 ± 0.005	181 ± 8	0.020 ± 0.003	115 ± 4
18-day embryo	0.435 ± 0.010	187 ± 3	0.050 ± 0.002	124 ± 3
19-day embryo	0.450	183	0.061 ± 0.004	120 ± 5
20-day embryo	0.452 ± 0.010	190 ± 7	0.086 ± 0.005	122 ± 6
6-hour chick	—	—	0.170 ± 0.014	129 ± 9
1-day chick	0.756 ± 0.043	194 ± 10	0.233 ± 0.018	144 ± 9
2-day chick	0.98	190	0.315 ± 0.030	148 ± 6
3-day chick	1.23 ± 0.07	185 ± 5	0.540 ± 0.064	147 ± 2
4-day chick	1.45 ± 0.08	183 ± 5	0.689 ± 0.024	152 ± 4
5-day chick	1.66 ± 0.10	190 ± 7	0.860 ± 0.092	155 ± 5
8-day chick	2.15 ± 0.11	195 ± 8	1.13 ± 0.062	165 ± 3
12-day chick	3.22 ± 0.10	192 ± 8	1.52 ± 0.090	163 ± 7

the protein concentration per g tissue increased only about 15% in the liver and 60% in the duodenum. The general patterns of developmental changes in enzyme levels would therefore not be substantially different if specific activities were expressed as units per g wet weight rather than per mg protein.

Developmental changes of phosphatase levels in liver and duodenum

Homogenates rather than homogenate fractions were used for studies on changes of phosphatase levels during development to avoid problems arising from possible changes in enzyme stability, ease of homogenization or intracellular distribution during development. However, preliminary studies on phosphatases in liver and duodenum from 18-day embryos (data not tabulated) showed substantially the same intracellular distributions and effects of metal ions as were described above for the phosphatase activities in 4-day chick liver and duodenum.

Table III shows that the levels of various phosphatase activities in liver change to a moderate degree and in different patterns, including both overall increases and decreases, during the developmental period from 12-day chick embryo to 12-day chick.

TABLE III

CHANGES IN PHOSPHATASE ACTIVITY LEVELS IN LIVER DURING DEVELOPMENT OF CHICK

Homogenates prepared from livers of chick embryos and chicks of various ages were assayed as described under Methods for phosphatase activities with the substrates indicated, at the pH indicated, and in the presence of added 5 mM $MgCl_2$ or $ZnSO_4$. Specific activities of phosphatases are expressed as nmoles P_i released/min per mg protein. Each value tabulated represents the mean and standard error of the mean of results from 3 to 6 preparations, except for the 19-day embryo and 2-day chick data, where each value is the average of results from two preparations. pNPP = *p*-nitrophenyl phosphate.

Age	*Specific activity of phosphatases*						
	Glc-6-P Mg^{2+} *pH 6.5*	*Fru-1,6-P_2* Mg^{2+} *pH 6.5*	*PP_i* Mg^{2+} *pH 6.5*	*ATP* Mg^{2+} *pH 6.5*	*pNPP* Mg^{2+} *pH 6.5*	*pNPP* Zn^{2+} *pH 6.5*	*pNPP* Mg^{2+} *pH 5.0*
12-day embryo	105 ± 4	129 ± 6	725 ± 21	375 ± 24	45 ± 2	160 ± 5	53 ± 2
15-day embryo	101 ± 4	126 ± 6	640 ± 12	380 ± 21	50 ± 2	149 ± 4	57 ± 3
18-day embryo	98 ± 3	128 ± 4	600 ± 10	370 ± 12	53 ± 1	142 ± 3	60 ± 2
19-day embryo	102	115	590	425	55	135	65
20-day embryo	115 ± 6	108 ± 4	570 ± 13	510 ± 26	56 ± 2	135 ± 3	65 ± 3
1-day chick	111 ± 4	89 ± 5	585 ± 14	540 ± 24	54 ± 2	132 ± 4	63 ± 1
2-day chick	112	86	605	555	56	131	63
3-day chick	87 ± 5	80 ± 5	620 ± 11	555 ± 25	57 ± 3	121 ± 3	64 ± 3
4-day chick	60 ± 3	71 ± 3	620 ± 10	550 ± 12	60 ± 2	110 ± 4	68 ± 3
5-day chick	63 ± 4	61 ± 4	600 ± 14	535 ± 16	62 ± 3	97 ± 3	72 ± 3
8-day chick	79 ± 4	54 ± 4	610 ± 12	520 ± 19	68 ± 3	93 ± 3	77 ± 3
12-day chick	103 ± 4	59 ± 3	630 ± 14	530 ± 24	76 ± 3	91 ± 3	87 ± 4

As is shown in Table IV, most of the duodenal phosphatase activities showed substantial increases about the time of hatching. These increases include a marked rise of activity with Glc-6-*P* from a negligible level in the 18-day embryo to a high level in the 2-day chick that persists through the next 10 days, and dramatic increases of activities at pH 9.5 with *p*-nitrophenyl phosphate or *β*-glycerophosphate which decrease substantially from the peak level in the 2-day chick. Comparison of duodenum fractions from 18-day embryo and 4-day chicks (not tabulated) showed that the increase of homogenate activity with *p*-nitrophenyl phosphate in the presence of Zn^{2+} reflected increases of both Zn^{2+}-stimulated activity in the soluble fraction and of Zn^{2+}-inhibited activity in the particulate fraction.

Effects of hydrocortisone on developmental changes

Some effects of hydrocortisone administration to 13-day embryos on phos-

TABLE IV

CHANGES IN PHOSPHATASE ACTIVITY LEVELS IN DUODENUM DURING DEVELOPMENT OF CHICK

Homogenates prepared from duodenums of chick embryos and chicks of various ages were assayed as described under Methods for phosphatase activities with the substrates indicated, at the pH indicated and in the presence of added 5 mM $MgCl_2$ or $ZnSO_4$. Specific activities of phosphatases are expressed as nmoles P_i released/min per mg protein. Each value tabulated represents the mean and standard error of the mean of results from 3 to 6 preparations.

Age	*Specific activity of phosphatases*							
	Glc-6-P Mg^{2+} *pH 6.5*	*Fru-1,6-P_2* Mg^{2+} *pH 6.5*	*PP_i* Mg^{2+} *pH 6.5*	*ATP* Mg^{2+} *pH 6.5*	*pNPP* Mg^{2+} *pH 5.0*	*pNPP* Zn^{2+} *pH 5.0*	*pNPP* Mg^{2+} *pH 9.5*	*β-Glycerophosphate* Mg^{2+} *pH 9.5*
12-day embryo	0.1	4.0 ± 0.8	440 ± 55	77 ± 10	27 ± 2	14 ± 2	7.2 ± 1.0	3.0 ± 0.7
15-day embryo	0.1	4.1 ± 0.8	435 ± 40	70 ± 8	26 ± 2	14 ± 1	10.4 ± 0.9	4.2 ± 0.6
18-day embryo	0.2 ± 0.1	4.0 ± 0.7	390 ± 27	73 ± 7	25 ± 1	14 ± 2	15.0 ± 1.5	5.8 ± 0.6
19-day embryo	1.0 ± 0.3	4.1 ± 0.6	410 ± 25	73 ± 6	32 ± 3	17 ± 2	27.4 ± 4.0	10.0 ± 1.5
20-day embryo	2.2 ± 0.3	7.4 ± 0.6	435 ± 25	93 ± 7	39 ± 3	19 ± 2	79.5 ± 8.2	24.6 ± 2.5
6-hour chick	7.1 ± 1.0	12.3 ± 1.5	705 ± 38	181 ± 9	46 ± 3	32 ± 3	309 ± 25.0	96.0 ± 8.2
1-day chick	16.7 ± 1.2	27.8 ± 2.0	710 ± 30	241 ± 17	58 ± 2	41 ± 3	1025 ± 40.0	265.0 ± 15.0
2-day chick	18.0 ± 0.8	27.6 ± 1.2	725 ± 27	256 ± 14	56 ± 2	45 ± 2	1080 ± 43.0	285.0 ± 16.0
3-day chick	17.2 ± 0.9	21.1 ± 1.5	735 ± 21	225 ± 13	59 ± 2	46 ± 1	415 ± 22.0	106.0 ± 5.0
4-day chick	16.1 ± 0.4	15.8 ± 0.8	770 ± 14	211 ± 10	57 ± 1	48 ± 1	355 ± 10.0	99.4 ± 2.0
5-day chick	15.9 ± 0.8	14.2 ± 1.1	745 ± 19	208 ± 13	55 ± 3	48 ± 2	268 ± 16.0	77.4 ± 2.6
8-day chick	16.3 ± 1.2	13.5 ± 1.2	695 ± 31	168 ± 13	54 ± 2	47 ± 2	266 ± 8.0	70.0 ± 3.7
12-day chick	17.1 ± 1.2	16.0 ± 1.2	700 ± 20	164 ± 11	53 ± 3	47 ± 2	260 ± 9.0	69.4 ± 3.1

phatase levels in liver and duodenum of 18-day chick embryos are summarized in Table V. Preliminary studies established that these times of hormone administration and tissue harvest resulted in significant changes of enzyme activities and that the embryos were still grossly normal in development and appearance at harvest but were smaller than control embryos. The livers and duodenums from 18-day hormone-treated embryos were normal in appearance, but their wet weights were lower than those from control embryos (Table V). Hormone-treated embryos harvested at 19 or 20 days of age showed greater changes from normal in phosphatase levels, but an increasing mortality; 60% of the hormone-injected chicks survived to 18 days but less than 10% survived to hatching.

The specific activity levels and protein content of homogenates from livers and duodenums of control embryos injected with saline (Table V) were very similar to those shown for untreated embryos in Tables III and IV. Comparison of specific activities in preparations from duodenum of 18-day hormone-injected embryos *vs* 18-day saline-injected controls in Table V shows that all of the phosphatase activities assayed were higher than normal in preparations from hormone-injected embryos, especially the activities with *p*-nitrophenyl phosphate at pH 9.5 and with Glc-6-*P*. The slight increase seen for specific activity with *p*-nitrophenyl phosphate at pH 5.0 with added Zn^{2+} was more clearly seen in assays (not tabulated) on the SP fraction in which this activity is concentrated: the specific activity was 84 in the duodenal SP fraction from 18-day saline controls, and 220 in the SP fraction from 18-day hormone-treated embryos. As may be seen by comparison with the activities in saline-injected 20-day embryos and 6-h chicks (Table V), the increases in the duodenum of 18-day hormone-injected embryos were, for most of the enzymes, similar to the increases which normally occurred between the 18-day embryo stage and the time of hatching.

Compared to liver from control saline-injected embryos, the livers from 18-day hormone-injected embryos showed substantially decreased activities at pH 6.5 with Glc-6-*P*, with Fru-1,6-P_2 and with *p*-nitrophenyl phosphate in the presence of Zn^{2+}, but only minor changes from normal in the other activities examined (Table V). The decreases from normal in the liver of 18-day hormone-injected embryos did not closely parallel the developmental changes normally seen between 18-day and 20-day embryo liver (Table V), but the major decreases occurred in those activities which normally decrease substantially in the first week after hatching (Table III).

DISCUSSION

The high levels of various phosphatase activities found in embryonic chick liver and the lack of marked increases in these activities about the time of hatching are consistent with the extensive functioning of the liver in the avian embryo long before hatching. The transient decreases of glucose-6-phosphatase activity and fructose-1,6-diphosphatase (fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) activity reported here may reflect a decrease of gluconeogenic activity in chick liver in response to the renewed availability of carbohydrate in the diet after hatching The patterns of change during development reported here include data consistent with previous observations on developmental changes in avian liver of the levels of glucose-6-phosphatase[8,9], ATPase (ATP phosphohydrolase, EC 3.6.1.3)[17] and acid *p*-nitrophenyl phosphatases[13].

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TABLE V

EFFECT OF HYDROCORTISONE INJECTIONS OF 13-DAY CHICK EMBRYOS ON DEVELOPMENT OF PHOSPHATASE ACTIVITIES IN DUODENUM AND LIVER

Homogenates of duodenum and liver from 18-day embryos that had been injected with 0.25 mg hydrocortisone at 13 days and from control embryos and chicks of various stages that had been injected with saline at 13 days were assayed for phosphatase activities and protein content. Details of procedures are given under Methods. Specific activities of phosphatases are expressed as nmoles P_i released/min per mg protein. Each value tabulated is the mean of results from 3 experiments; the range of values was within ±10% of the mean except for values marked.*

Tissue	*Specific activity of phosphatases*									*Wet weight of organ*	*Protein content*
	Glc-6-P Mg^{2+} *pH 6.5*	*Fru-1,6-P_2* Mg^{2+} *pH 6.5*	*PP_i* Mg^{2+} *pH 6.5*	*ATP* Mg^{2+} *pH 6.5*	*pNPP* Mg^{2+} *pH 6.5*	*pNPP* Zn^{2+} *pH 6.5*	*pNPP* Mg^{2+} *pH 5.0*	*pNPP* Zn^{2+} *pH 5.0*	*pNPP* Mg^{2+} *pH 9.5*	*(g)*	*(mg/g)*
18-day embryo duodenum, saline	0.2*	4.7	380	71	24	—	24	15	15	0.051	122
18-day embryo duodenum, hormone	7.8	7.0	530	115	38	—	33	25	132	0.042	145
20-day embryo duodenum, saline	2.8	6.2	450	99	26	—	35	21	82	0.091	122
6-h chick duodenum, saline	8.5	10.5	740	192	41	—	48	34	315	0.159	133
18-day embryo liver, saline	101	131	610	375	55	147	61	48	11*	0.440	183
18-day embryo liver, hormone	34	62	635	400	49	103	54	42	12	0.335	143
20-day embryo liver, saline	111	92	595	545	57	127	64	47	11	0.475	187

There have been no previous extensive studies on developmental changes of the duodenal phosphatase activities reported here, except for alkaline phosphatase, and there apparently were no previous observations on an intestinal enzyme analogous to the soluble, Zn^{2+}-stimulated *p*-nitrophenyl phosphatase, active at acid pH. Most of the duodenal phosphatase activities studied here reached a peak of activity in the 2-day chick, the time of maximum activity previously reported for chick duodenal alkaline phosphatase[1,3]. However, the percent of increase differed greatly for the various activities, and the activities showed different patterns of change after this stage of development (Table IV). The change in ratio of activity at pH 9.5 with *p*-nitrophenyl phosphate *vs* that with β-glycerophosphate in the duodenum during development (Table IV) is similar to but different in time course from the previously reported change in the ratio of alkaline phosphatase activity with phenylphosphate *vs* β-glycerophosphate as substrate in the duodenum of the developing chick embryo[18]. In part the different patterns of increase seen for various phosphatases may reflect preferential proliferation of cell types in which particular enzymes are concentrated, but the large increases probably reflect primarily an increase in the amount of activity associated with individual cells. Histochemical studies with both chick[1,3] and mouse[4] indicate that alkaline phosphatase activity increases markedly in the individual duodenal mucosa cells as these cells migrate to the apex of the villus.

The effects of Mg^{2+} and Zn^{2+} on a given phosphatase activity usually were similar for analogous preparations from chick liver and duodenum, and the effects generally resemble effects noted with mammalian enzymes. However, Mg^{2+} stimulated the chick duodenum glucose-6-phosphatase but not the corresponding liver enzyme. Mammalian liver glucose-6-phosphatase is not stimulated by Mg^{2+} but purified preparations of the enzyme contain a small amount of magnesium[19]. The marked activation by Mg^{2+} of the activities with PP_i, ATP or Fru-1,6-P_2 resembles the requirement for Mg^{2+} or similar divalent ion of analogous enzymes in mammalian liver[20–22]. The activation by Mg^{2+} of *p*-nitrophenyl phosphatase activity at alkaline pH in both chick liver and duodenum resembles the marked stimulation reported for chick duodenum alkaline phosphatase[23], and purified mammalian intestine alkaline phosphatase has almost absolute requirement for Mg^{2+} or similar metal ion[24]. In contrast, the *p*-nitrophenyl phosphatase activities at acid and neutral pH are only slightly or not at all stimulated by Mg^{2+} in either chick tissues (Table I) or mammalian liver[25,26].

Zinc atoms appear to be firmly bound and essential components of alkaline phosphatase preparations from *Escherichia coli*[27], chick duodenum[23] and mammalian tissues[28–30]; these preparations can be inactivated by dialysis against chelating agents and often can be reactivated by addition of Zn^{2+}. Zinc has also been implicated in the function of several phosphatases that are active at neutral pH[31–33]. However, as reported here for avian phosphatases and by other workers[24,34,35] for phosphatases from mammalian tissues, 1–5 mM Zn^{2+} is inhibitory to some enzymes that are not activated by Zn^{2+} or that require or are stimulated by low levels of Zn^{2+}. It is therefore of interest that chick liver and duodenum contain soluble *p*-nitrophenyl phosphatases, active at neutral or acid pH, that are markedly stimulated by 5 mM Zn^{2+}, and we report elsewhere[36] further studies to characterize these enzymes.

Previous studies indicated that hydrocortisone administration to chick embryos produced an increase of alkaline phosphatase activity in the intestine but that acid phosphatase activity was not increased significantly[1,3]. We have observed substan-

tial increases in a number of other phosphatase activities in 18-day chick embryo duodenum as a result of hydrocortisone administration to 13-day embryos (Table V). These increases do not necessarily indicate specific effects on particular enzymes, and are consistent with a general acceleration of essentially normal development of the intestine following glucocorticoid administration[1,3]. However, some degree of abnormality in development following hormone administration is suggested by abnormalities in organ and embryo size and by the high mortality, which were seen both in the earlier studies[1,3] and in our experiments.

The major decreases in glucose-6-phosphatase, fructose-diphosphatase and Zn^{2+}-stimulated *p*-nitrophenyl phosphatase activities of 18-day chick embryo liver found after hydrocortisone treatment may reflect secondary responses to other metabolic changes evoked by the hormone, in addition to a possible acceleration of normal developmental changes, particularly since these decreases were greater than the decreases that normally occurred in these activities over the entire developmental period up to the 12-day chick (Tables III and V). These hormonal effects on development appeared to be distinct from the transient increase in hepatic glucose-6-phosphatase activity seen in chicks or in 18- and 19-day chick embryos, 12 to 18 h after injection of hydrocortisone[37].

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