Origin of an Increased Serum Alkaline Phosphatase in Chicks.* (28381)

H. R. WILSON† AND F. H. WILCOX (Introduced by C. S. Shaffner)
Department of Poultry Science, University of Maryland, College Park

Evidence has been presented(1) which showed a positive relationship between serum alkaline phosphatase level and egg production. Selection of birds for high serum alkaline phosphatase level at 6 weeks of age(2) resulted in development of a line which, in the 5th generation, had serum phosphatase values approximately 3 times those of a random bred control line. This high phosphatase line also demonstrated a higher rate of egg production than the random bred line(3). To study the physiological relationship between alkaline phosphatase and egg production it was desirable first to determine the source of the increased serum phosphatase of the high line.

Bodansky(4) has suggested that serum alkaline phosphatase has diverse origin and the major sources are thought to be bone, intestine, liver and kidney. These tissues have high phosphatase activity in both lines (2). Inhibitory(5), electrophoretic(6) and immunochemical(7) studies have demonstrated tissue differences in phosphatase characteristics. In this study various inhibitors were used in an effort to detect similarities or differences in the serum and tissue phosphatases within and between lines.

Methods and materials. Six-week-old male chicks from a White Leghorn random bred control line and a White Leghorn line bred for a high serum alkaline phosphatase were used in all cases. These stocks have been previously described(2,8). The birds were fed *ad libitum* throughout the experiment. Blood was taken by heart puncture, incubated at 37°C for one hour, the serum collected and stored at −15°C. The birds were killed by cervical dislocation. The right lobe of the liver, the duodenal loop, the frontal lobe of the left kidney and the left tibiotarsus were removed immediately and frozen at −15°C.

In a preliminary study pooled sera from each of the lines were analyzed for alkaline phosphatase by the method of Bessy *et al.* (9) in the presence of various inhibitors. When tissues were analyzed, the modification of Nimni(10) was used. Inhibitors were incorporated into the para-nitrophenyl phosphate substrate mixture and amounts (for most inhibitors 0.1, 0.01, and 0.001 M) expressed as concentration in the substrate. Analyses were performed on pooled samples of unfiltered tissue homogenates or appropriate dilutions thereof. All alkaline phosphatase units are given as mM nitrophenol released per liter of serum or kg of tissue per hour.

Results and discussion. Results of the preliminary study indicated that zinc chloride, lithium oxalate, sodium tungstate, cadmium sulfate,‡ arginine, tyrosine, cysteine, glycine, alanine, formaldehyde, glutathione, ethylenediamine tetraacetic acid, iodacetate and sodium tetraborate-decahydrate (sodium borate) considerably inhibited serum phosphatase of both lines. Of these, only sodium borate was clearly selective for the high line serum phosphatase; therefore it was used in the subsequent tissue experiment.

Tissue selectivity can be seen in the inhibitory action of sodium borate (Table I). The duodenal phosphatase of both lines was inhibited to a greater extent than that of other organs. Analysis of variance(11) showed that this difference was significant. Serum phosphatase inhibition followed that of the duodenum very closely in the high line. This might have been expected if a major portion of the phosphatase in the blood was contributed by the duodenum. This serum-duodenum relationship was not apparent in the random bred line, in which the serum phosphatase followed more closely

* Scientific Article No. A1049, Contribution No. 3455 of Maryland Agri. Exp. Station.

† Present address: Dept. of Poultry Science, Univ. of Florida, Gainesville.

‡ Results with cadmium sulfate were confused by the formation of a precipitate.

TABLE I. Residual Tissue Alkaline Phosphatase Activity in Presence of Sodium Borate.

Inhibitor	Conc (M)	Random bred line					High alkaline phosphate line				
		Serum	Bone	Duodenum	Kidney	Liver	Serum	Bone	Duodenum	Kidney	Liver
					Exp 1*						
Sodium borate	.1	14†	36	4	24	21	7	33	3	24	21
	.01	55	101	20	77	51	28	101	17	72	67
	.001	95	140	52	94	88	58	126	58	92	114
	Control	22‡	535	6200	880	560	103	660	4950	911	394
					Exp 2§						
"	.1	10	28	3	25	23	5	28	3	26	21
	.01	46	84	15	75	59	21	80	15	77	59
	.001	68	96	45	92	92	39	96	40	95	87
	Control	50	790	11900	1306	530	192	790	9750	1222	550

* Each value represents duplicate analyses on serum and tissues pooled from 4 birds.
† % of control values.
‡ Control values are in mM nitrophenol/liter or kg/hr.
§ Each value represents duplicate analyses on serum and tissues pooled from 20 birds.

the pattern of liver phosphatase inhibitions.

When the data were combined from all 3 levels of sodium borate (Table II), there was clearly no difference between lines in the reaction of individual tissues, except that serum phosphatase of the high line was inhibited more than that of the random bred line. This suggests that the increased serum phosphatase in the high line is of intestinal origin, since phosphatases from the other 3 tissues would not cause greater inhibition in serum phosphatase. The expected residual activity in serum of the high line was calculated on this basis using the observed residual activity of the intestine of both lines and the serum of the random bred line. The calculated value (29%) was close to the observed value (26%). It thus appears likely that all of the increased phosphatase activity in the high line is of intestinal origin. These findings agree with those of Madsen and Tuba(5) who indicated that a major portion of rat serum alkaline phosphatase originates in the intestine, but differs from those in humans where the major portion appears to be osseous in origin(13).

TABLE II. Mean Tissue Alkaline Phosphatase Activity in Presence of Sodium Borate. (Data of all levels combined from Exp 1 and 2.)

Line	Tissue	Residual alkaline phosphatase activity (% of control)*
Random bred	Serum	48[b]
	Bone	81[d]
	Intestine	23[a]
	Kidney	65[c]
	Liver	56[c]
High Phosphatase	Serum	26[a]
	Bone	77[d]
	Intestine	23[a]
	Kidney	64[c]
	Liver	62[c]

* Means having different exponential letters are significantly different according to Duncan's Multiple Range Test(12).

Summary. Several chemicals were tested and found to be *in vitro* inhibitors of chicken serum alkaline phosphatase. Sodium borate inhibited serum phosphatase more in a line bred for high serum level than in a random bred control line. Subsequent studies with this inhibitor on tissues from the two lines showed that intestinal phosphatase activity was depressed significantly more than that in bone, liver, and kidney. These studies indicate that the major portion, if not all, of the increased serum phosphatase of the high line originates in the intestine.

1. Gutowska, Marie S., Parkhurst, R. T., Parrott, E. M., Verburg, R. M., *Poultry Sci.,* 1943, v22, 195.
2. Wilcox, F. H., *J. Exp. Zool.,* 1963, v152, 195.
3. Wilcox, F. H., Van Vleck, L. D., Shaffner, C. S., *Proc. XII World's Poultry Cong.,* 1962, 19.
4. Bodansky, A., *J. Biol. Chem.,* 1934, v104, 473.
5. Madsen, N. B., Tuba, J., *ibid.,* 1952, v195, 741.
6. Moss, D. W., King, E. J., *Biochem. J.,* 1962, v82, 19.
7. Schlamowitz, M., *J. Biol. Chem.,* 1954, v206, 369.
8. King, S. C., Carson, J. R., Doolittle, D. P.,

World's Poultry Sci. J., 1959, v15, 139.

9. Bessy, O. A., Lowry, O. H., Brock, Mary J., *J. Biol. Chem.*, 1946, v164, 321.

10. Nimni, M. E., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 402.

11. Snedecor, G. W., *Statistical Methods*, 1957, 5th Ed., Iowa State College Press, Ames.

12. Duncan, D. R., *Biometrics*, 1955, v11, 1.

13. Schlamowitz, M., Bodansky, O., *J. Biol. Chem.*, 1959, v234, 1433.

Received March 20, 1963. P.S.E.B.M., 1963, v113.

Corn Sterols and Avian Atherosclerosis.* (28382)

HANS FISHER, HAROLD S. WEISS AND PAUL GRIMINGER
Department of Poultry Science, Rutgers, The State University, New Brunswick, N. J.

Peterson *et al.*(1,2) have reported that relatively high levels of dietary soybean sterols (1.3%) prevented hypercholesterolemia and atherosclerosis in cholesterol-fed chicks, and lowered blood cholesterol in man. They suggested that the soy sterols interfered with cholesterol absorption. Beveridge *et al.*(3) showed that a considerable part of the hypocholesterolemic activity of corn oil was associated with its sterol fraction, and Fisher *et al.*(4) reported that the beneficial effects from feeding unsaturated plant fats to chickens were attributable primarily to their sterol fraction rather than to the unsaturated fatty acid fraction. Reiner *et al.*(5), in reporting on hypercholesterolemic sterols derived from marine algae, suggested that appreciable amounts of sterol might be absorbed and thus act in a manner other than by interference with absorption of cholesterol alone. This view is supported by the finding of Fisher *et al.*(4) that the feeding of a small quantity of corn sterols does not increase cholesterol excretion of chickens.

The present work was undertaken to study the effect of prolonged ingestion of a low level of corn oil sterols (0.05% of diet) in counteracting the atherogenicity of a diet containing 20% whole egg powder. We also checked (a) whether the atherogenic properties of egg powder were due to its fat content, and (b) whether lasting effects might remain in chickens given a brief, early exposure (3 months out of 20) to an atherogenic egg powder diet.

Experimental. Day-old Leghorn cockerels, divided into 6 groups of 30 birds each, were used for this study and maintained throughout a 20-month experimental period in temperature-regulated rooms. For the first 12 weeks, 2 groups were given the control ration supplemented with 20% egg powder, which supplied 0.5% cholesterol. At the end of this time the egg powder diet of one group was replaced with the control ration for the remainder of the experimental period. The other 4 groups were respectively given the following diets throughout the experimental period: (a) a control ration (for composition see Fisher *et al.*(4)), essentially free from cholesterol and containing a small amount of fat derived from soybean meal; (b) control ration supplemented with 2% corn oil and 0.05% corn sterols;† (the former was added to facilitate sterol absorption, should it occur) (c) control ration supplemented with 10% egg oil,‡ which was equivalent to the fat supplied by 20% whole egg powder and (d) control ration supplemented with both 20% whole egg powder and 0.05% corn sterols. The measurements made on the experimental animals by methods previously described(6,7) included blood pressure, blood clotting time, blood and aortic cholesterol, aortic hydroxyproline, and scoring of the

* Paper of the Journal Series, N. J. Agri. Exp. Station, Dept. of Poultry Science. Supported in part by grants-in-aid from N. J. Heart Assn. and U. S. Public Health Service.

† Corn oil sterols were kindly supplied by A. E. Staley Manufacturing Co., Decatur, Ill., through the courtesy of Dr. S. S. Chang.

‡ Egg oil was generously supplied by Henningsen Inc., New York.