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Distribution of Se^{75} in Serum Proteins of Chicks and Influence of Selenium on Albumin Recovery.* (28723)

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Time-distribution studies on dog serum proteins(1) have demonstrated that albumin is the immediate acceptor of injected Se^{75}. After some time, however, the selenium is released to or bound by other serum proteins, chiefly alpha-2 and beta-1 globulins, indicating that albumin may act in the transport of selenium. Similar studies have not been conducted with chicks, although it has been shown(2) that Se^{75} retention in various tissues, including blood, is markedly influenced by the level of selenium in the diet. A high incidence of exudative diathesis(3), which is accompanied by serum protein changes, characterized by lowered A/G ratios(4) is produced by feeding chicks a torula yeast-glucose type diet. Vitamin E or selenium will prevent this condition(5). In studying serum protein changes in chicks recovering spontaneously from exudative diathesis, Bieri and Pollard(6) indicated that total protein concentrations were usually elevated, while the A/G ratios varied from normal to very low. Thus, it appeared that during recovery, globulin synthesis proceeded faster than albumin synthesis.

It is shown here that serum albumin is not the immediate acceptor of Se^{75} in chicks, and that oral administration of selenium appears markedly to influence albumin synthesis (or turnover) in Vit. E and selenium depleted chicks.

Methods. Two groups of New Hampshire $\times$ Delaware cross-bred male chicks were reared in battery brooder compartments for 3 weeks. One group was fed a basal diet composed principally of torula yeast and glucose monohydrate, similar to that used by Scott *et al*(3). The second group was fed the same diet, supplemented with selenium (1 mg/kg) as selenious acid. At 3 weeks, 2 chicks were randomly selected from each group and moved to small wire-floored cages

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TABLE I. Effect of Time After Dosing on Distribution of Se^{75} in Chick Serum Proteins.

Diet supplement	Hr after dosing	Activity of whole serum (cpm)	Radioactivity of serum proteins (% of total)				
			Albumin	Globulins			
				Alpha-1	Alpha-2	Alpha-3	Beta & gamma
None	2	312	6	6	18	16	49
	4	347	7	6	14	15	49
	8	335	4	3	15	16	57
	12	228	3	3	19	20	50
	24	183	0	2	12	16	67
	48	187	1	2	13	10	70
	96	119	0	0	9	11	77
	144	69	0	0	0	15	78
	192	58	0	0	7	10	74
Selenium (1 mg/kg)	2	383	11	3	17	20	44
	4	370	17	11	14	15	29
	8	338	8	3	16	16	51
	12	158	6	4	17	18	51
	24	70	0	0	16	16	61
	48	38	5	5	16	8	63
	96	14	0	0	0	14	86
	144	24	0	4	8	12	62
	192	3	0	0	0	0	100

in an isolation room equipped with space heaters. The chicks were continued on their previous diet regimes. After 24 hours each chick was given an oral, 0.5 μl dose of $H_2Se^{75}O_3$ in neutral solution containing approximately 10 μc of radioselenium and 0.0528 mg of selenium. The dose was administered directly into the crop by means of a polyethylene tube attached to a syringe.

At 0, 2, 4, 8, 12, 24, 48, 96, 144 and 192 hours after dosing, blood (0.5 ml) was obtained, *via* heart puncture, from each chick and allowed to clot for 4 hours and then centrifuged. Electrophoretic analyses were performed in a Spinco model R, Durrum-type paper electrophoresis apparatus, according to the directions of the manufacturer.† The Spinco Analytrol calibrated recording densitometer and automatic integrator was used to evaluate relative amounts of protein in the stained material which had been separated electrophoretically. The various serum protein fractions plus background strips were cut from the electrophoresis paper and placed in 1¼ × 12½ cm test tubes. The tubes were placed in a well detector with a 2″ thallium activated sodium iodide crystal and 30 min counts were obtained. The counts from various protein fractions plus background strips represented total serum protein Se^{75} activity.

Strips were also prepared using a 1:1 mixture, either of water and $H_2Se^{75}O_3$ (0.3 μc/ml) or serum from untreated chicks and $H_2Se^{75}O_3$. The latter mixture was allowed to stand 3 hours prior to application. Following application of 0.01 ml of the mixtures, one-half of the strips were dried, rolled up tightly and placed in test tubes for counting. The remainder of the strips were subjected to electrophoretic procedures, with all the appropriate rinses, prior to drying.

Results and discussion. Table I shows the distribution of Se^{75} in the various serum protein fractions as influenced by time. Grouping of the beta and gamma fractions was necessary since clear separation of these proteins was not always attained. At 2 hours, nearly 50% of the Se^{75} activity was found in the beta and gamma globulin fractions with only minor activity in the albumin or alpha-1 globulin fractions. From 2-24 hours, 30-40% of the activity was found in the alpha-2 and alpha-3 globulin fractions, with approximately equal amounts in each. Afterwards, the beta and gamma values increased gradually with a concomitant decrease in other fractions. Alpha-3 globulin was a possible exception; it retained 10-15% of the Se^{75} ac-

† Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.

tivity. The results indicate that beta and gamma serum proteins in the chick accept as well as retain selenium, which contrasts to the dog, where albumin appeared to act as a selenium carrier(1).

Reduced retention of Se^{75} in birds fed selenium supplemented diets confirms previous observations from this laboratory(2). Slightly higher initial Se^{75} activity of albumin in the selenium supplemented birds is likely attributable to the marked reduction in serum albumin in Vit. E and selenium depleted birds (Fig. 1). When Se^{75} was mixed with water or serum from untreated birds, 100 and 98%, respectively, of the activity was lost during the electrophoretic processes. Thus, it appears that selenium is firmly bound to serum proteins only *in vivo*.

Oral administration of Se^{75} had a marked effect on A/G ratios in depleted chicks (Table II). Changes were already apparent at 48 hours and by 96 hours the values were approaching the initial values for selenium supplemented chicks. Electrophoretic patterns

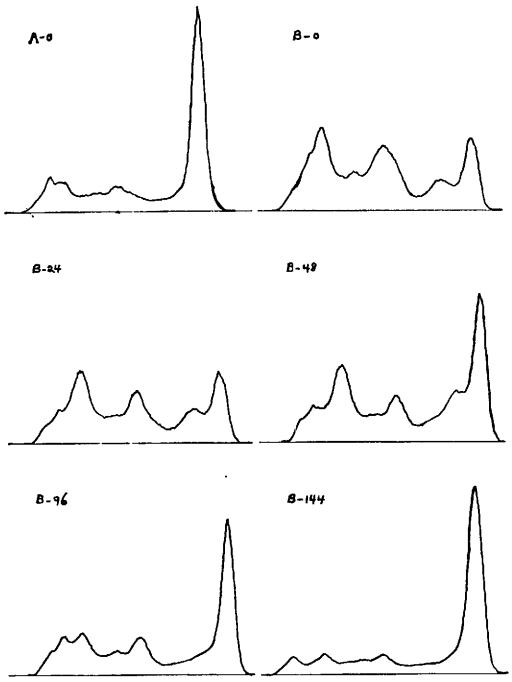

FIG. 1. Influence of Se^{75} on serum proteins. Chick A received a selenium supplemented diet and chick B a selenium deficient diet. Numbers after the letters refer to hr after administration of the dose when blood sample was taken.

TABLE II. Effect of $H_2Se^{75}O_3$ Administration on Serum Albumin/Globulin Ratios in Chicks Fed Diets With and Without Selenium Supplementation.

	Albumin/globulin ratios*			
Hr after admin	Basal diet		Basal + Se	
	Chick 1	Chick 2	Chick 1	Chick 2
0	.24	.50	1.24	1.40
24	.29	.30	1.00	1.26
48	.53	.59	1.14	1.24
96	.89	.91	1.48	1.51
144	.82	1.72	1.73	2.16
192	.95	1.57	1.50	1.72

* Ratios represent an avg of 4 determinations per chick.

(Fig. 1) show that the marked increase in A/G ratios was due to both an increase in the albumin fraction and a reduction in the globulins. This is in direct contrast to results obtained by Bieri and Pollard(6) with chicks spontaneously recovering from exudative diathesis. The dramatic changes in the serum protein pattern in depleted chicks receiving a single dose of Se^{75} together with an increase in A/G ratios on the administration of Se^{75} to chicks supplemented with selenium (Table II) shows that selenium either played a role in synthesis of serum albumin or in the rate of its turnover.

Summary. Se^{75} was orally administered to 3-week-old chicks fed either selenium deficient or supplemented diets. Distribution of Se^{75} in serum proteins with time and its effect on serum protein pattern were determined electrophoretically. Selenium in the diet had no effect on the distribution of Se^{75} in the protein fraction, but markedly decreased its retention. After 2 hours the major portion of the dose was found in the beta and gamma fraction and the activity in this fraction increased with time. Marked increases in A/G ratios in depleted chicks were observed within 96 hours after administration of Se^{75}. Albumin was increased and globulin fractions were decreased. Increases in A/G ratios, somewhat smaller in magnitude, were also noted in chicks receiving a diet supplemented with selenium.

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Serologic Properties of Bentonite Particles Coated with Microbial Polysaccharides. (28724)

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The basic procedures for detection and measurement of antibody *in vitro* are relatively few. Some were developed before the turn of the century. Although in recent years variations have been devised for studying the interaction of antigens and antibodies, there is a need for easily prepared, sensitive and dependable serologic reagents for measurement of antibody. The technique of fixing antigen to the surface of normal or of tanned erythrocytes, which came into general use about 10 years ago, has provided a practical and convenient means for measurement of antibodies(1) but despite its utility the coated red cell technique has not proved to be ideal. Disadvantages of the method are: the complex antigenic reactivity native to the red cell surface; lack of uniformity of red cells, and problems related to their lability; and finally, for reasons which are not clear, a number of antigens are not taken up by such cells.

To devise more appropriate surfaces for antigen-antibody reactions various particulates have been investigated including collodion(2), latex(3), barium sulphate(4), bismuth tannate(5), etc. Bozicevich and his coworkers had shown that bentonite (aluminum silicate) particles could be used to advantage for this purpose(6,7,8,9). During studies with various purified polysaccharide antigens fixed on bentonite, certain advantages of these coated particles as serologic reagents became evident. This communication concerns the findings of a systematic exploration of the factors influencing the preparation and performance of polysaccharide-coated bentonite particles.

Materials. Bentonite (Volclay, American Colloid Co., Chicago, Ill.) is a mineral clay, mined in Wyoming, consisting principally of aluminum silicate. The preparation by differential centrifugation of a stock bentonite suspension with appropriate range of particle size has been described(7). The polysaccharides employed as antigens, their distinguishing features, source and reference to their method of preparation are listed in Table I. The immune sera had been prepared previously by immunization of man, rabbit, horse and mouse in connection with other studies. The schedule of injections varied widely for the different situations, but represented intensive immunizations; in some instances the immunizing preparation was the intact bacterium from which the polysaccharide was derived.

Methods and results. The methods employed were those described by Bozicevich and coworkers(7,8,9) with some modifications. Ten ml of stock bentonite is sedimented, the pellet brought up to 1 ml with distilled water, the desired quantity of antigen in 1 ml of saline is added, and the two are mixed and allowed to stand at room temperature for 1 hour. One ml of 1% crystallized bovine serum albumen (BSA) is added, the mixture left at room temperature for an additional 15 minutes, after which 15 ml distilled water and 1 ml of a 0.1% solution of methylene blue are added and the antigen-bentonite particles are washed twice