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Effect of Selenium in Preventing Exudative Diathesis in Chicks. (23307)

E. L. PATTERSON, R. MILSTREY AND E. L. R. STOKSTAD
Research Division, American Cyanamid Co., Pearl River, N. Y.

Schwarz(1) observed that rats fed a diet containing torula yeast as sole source of protein develop liver necrosis when vit. E is omitted from the diet. This liver necrosis could be prevented by vit. E, cystine or a non-fat soluble substance in vit. E-free casein or brewers yeast which he designated Factor III. Scott *et al.*(2) found that exudative diathesis is produced when chicks are fed a vit. E-free diet containing torula yeast. These workers also found that this could be prevented by alpha-tocopherol but not by gamma-tocopherol or an antioxidant such as p-diphenylphenylenediamine. Exudative diathesis developed on this torula yeast diet in the absence of added unsaturated fatty acids and the incidence of this lesion was not affected by the fat content of the diet. We have found that vit. E-free casein and several defatted pork tissues such as pancreas and kidney will also prevent exudative diathesis. Attempts have been made to isolate this factor from casein and kidney. The activity in casein and kidney was insoluble in water and alcohol. The factor in casein could be made soluble and diffusible by hydrolysis with trypsin and with sulfuric acid. Little or no purification was obtained by use of countercurrent extraction with immiscible solvents or by adsorption on activated charcoal or Magnesol. It could be adsorbed on Dowex-50 (sodium form) and eluted wtih 0.2 N sodium hydroxide. It could also be adsorbed on Dowex-1 (chloride form) and eluted with 0.1 N hydrochloric acid. This suggests that the factor as present in acid hydrolyzed kidney is amphoteric. It was also observed that an ash of acid hydrolyzed kidney was inactive, but that an ash of kidney made alkaline with calcium oxide before burning was active. This activity of an alkaline ash and inactivity of an acid ash suggested that the activity of this factor in kidney was due to an element which forms a volatile inorganic acid. Among the elements which form volatile acids are arsenic, selenium and tellurium. Of these 3, selenium is present in the cystine and methionine fraction of wheat from seleniferous soil (3).

Methods. The basal diet used is shown in Table I. This diet, which contained 40% torula yeast and 5% of vit. E-free lard* gave the same results as the one described by Scott *et al.*(2) which contained 60% torula yeast and 5% vit. E-free lard. Day-old White Rock chicks were depleted on the basal diet 6 to 13 days, and then placed on experimental diets. Chicks on the basal diet and on basal diet plus a vit. E supplement had the same growth rate until they were 2 weeks old. The symptoms of exudative diathesis began to appear at the 18th day. The chicks were examined for lesions twice a week. Alcohol

* Purchased from Distillation Products Industries.

extracted casein was prepared by continuous extraction of crude Argentine casein with alcohol at boiling point of the solvent. Pork kidney, liver, lung, spleen and pancreas were defatted with chloroform and then dried in vacuum at 60° for 18 hours. Acid hydrolysis

TABLE I. Composition of Basal Diet.

Torula yeast	40.0 g
Cerelose	40.7
Vit. E-free lard	5.0
Calcium gluconate	5.0
Cellophane	3.0
Bone ash	2.0
Glycine	.5
DL-methionine	.4
L-arginine monohydrochloride	.24
L-cystine	.2
Salts*	2.0
Vitamins†	1.0

* Salt supplements in mg/100 g of diet were dipotassium acid phosphate 600, monopotassium acid phosphate 450, sodium chloride 600, magnesium sulfate trihydrate 250, ferric citrate 50, manganese sulfate monohydrate 40, copper sulfate pentahydrate 2, zinc acetate dihydrate 1.4, aluminum sulfate octadecahydrate 1.6, potassium bromide .8, cobalt acetate tetrahydrate .4, nickel chloride hexahydrate .2, and sodium molybdate dihydrate .05.

† Vitamin supplements in a cerelose diluent in mg/100 g of diet were meso-inositol 100, calcium pantothenate 5, niacin 5, riboflavin 1, thiamine hydrochloride 1, pyridoxine hydrochloride 1, menadione .027, folic acid .2, biotin .002, and vit. A and D, 1000 and 200 units.

of casein and kidney was carried out by digesting 100 g of casein or 500 g of fresh kidney with 1 l of 2 N sulfuric acid for 4 hours at 120°C. The hydrolysate was diluted 10 times and the sulfuric acid precipitated with calcium oxide. The filtrate was concentrated and dried *in vacuo*. Adsorption and elution with ion exchange resins were carried out as follows: Two hundred forty g acid hydrolyzed kidney were dissolved in 2400 ml of 0.1 M sodium bisulfate at pH 2. This was adsorbed on a 4″ x 25″ Dowex-50 (Na^+) column. The column was eluted first with 20 l of 0.2 M sodium bisulfate at pH 2, and second with 26 l of 0.1 M sodium hydroxide. Two hundred forty g of acid hydrolyzed kidney were dissolved in 2400 ml of a solution containing 0.1 M sodium chloride and 0.02 M potassium dibasic phosphate at pH 9. This was percolated through a 4″ x 20″ Dowex-1 (Cl^-) column. This was developed first with 15 l of a solution containing 0.2 N sodium chloride and 0.01 M potassium dibasic phosphate at pH 9, and then with 30 l of 0.2 N hydrochloric acid. Acid and alkaline ashes of kidney acid hydrolysate were prepared by adding 1.5 equivalents of sulfuric acid or calcium oxide to 500 g of kidney hydrolysate in 840 ml of water. The pH of the solutions were 2 and 9.5 respectively for acid and alkaline solutions. The mixtures were dried in an air oven and then ashed for 16 hours at 500°C. Selenium determinations were made by procedure described in Official Methods of Analysis(4).†

Results. The effect of a number of supplements on incidence of exudative diathesis and growth is shown in Table II. It can be seen that 40 units of tocopherol acetate‡ gave complete protection. In other experiments 12 units were adequate but 4 units were not. The results show that 50 or 100 g of casein added at 6 or 8 days gave almost complete protection against exudative diathesis. When the experiment was continued to 28 days more symptoms may have developed, showing that 100 g of casein did not give complete protection over a long period of time. An amino acid mixture simulating casein, fed at 100 g/kg, was inactive. The amino acid mixture supplied the following quantities of amino acid/kg of diet: l-arginine • HCl, 4.7 g; l-histidine • HCl, 3 g; l-lysine • HCl, 8.3 g; dl-tryptophan, 1.8 g; l-phenylalanine, 5 g; l-cystine, 0.4 g; dl-methionine, 3.6 g; dl-serine, 15.3 g; l-threonine, 4 g; l-leucine, 12 g; l-isoleucine, 6.6 g; dl-valine, 14 g; l-glutamic acid, 33 g; l-aspartic acid, 6.3 g; l-glycine, 0.5 g; l-alanine, 5.6 g; l-hydroxyproline, 2 g; l-proline, 8.3 g and l-tyrosine, 6.3 g.

A number of defatted pork tissues were assayed and the results, given in Exp. III of Table II, show that pork kidney and pancreas were the most active, and heart the least. A commercial acid hydrolysate of

† We are indebted to Mr. W. C. Groth for making selenium analyses.

‡ Supplied as Myvamix, commercial preparation made by Distillation Products, Inc. containing 44 units/g of vit. E activity as tocopherol acetate.

TABLE II. Effect of Various Substances on Incidence of Exudative Diathesis in Chicks.

Exp. No. and time of depletion, days		Supplement/kg diet	No. of chicks started	Wt at 23 days and (survivors)	No. of chicks showing exudative diathesis
I	7	None	11	137 (9)	7
		40 units tocopherol acetate	11	195 (11)	0
II	7	None	9	157 (6)	6
		50 g alcohol extr. casein	9	214 (7)	1
		100 g amino acid mix	6	146 (6)	5
		100 g Sheffield acid hydr. casein + 4 g tryptophan	9	175 (9)	2
III*	13	None	12	203 (12)	9
		50 g alcohol extr. casein	12	231 (11)	6
		100 *Idem*	12	242 (12)	4
		30 g defatted hog liver	12	253 (12)	0
		30 heart	12	227 (11)	9
		30 brain	12	235 (12)	2
		30 kidney	12	242 (11)	0
		30 lung	12	250 (12)	0
		30 pancreas	12	238 (12)	0
		30 spleen	12	236 (12)	1
IV	13	None	10	161 (4)	6
		10 g acid hydr. kidney	10	200 (10)	3
		30 *Idem*	10	219 (10)	0
		Acid ash equiv. to 44 g kidney	10	169 (5)	8
		Alkaline ash equiv. to 41 g kidney	10	213 (10)	2
V	13	None	11	190 (6)	11
		15 g acid hydr. kidney	11	241 (11)	0
		Acid ash equiv. to 80 g kidney	11	197 (11)	8
		Alkaline ash equiv. to 80 g kidney	11	235 (11)	0
VI	13	None	11	170 (5)	11
		45 g acid hydr. kidney	11	240 (11)	2
		Acid filtrate and washings of Dowex-50 column equiv. to 40 g kidney hydr.	11	180 (2)	9
		Alkaline eluate of Dowex-50 column equiv. to 40 g of kidney hydr.	11	256 (10)	0
		Alkaline filtrate and washings of Dowex-1 column equiv. to 40 g kidney hydr.	11	200 (6)	10
		Acid eluate of Dowex-1 column equiv. to 40 g of kidney hydr.	11	227 (11)	1
VII	13	None	12	192 (10)	11
		15 g acid hydr. kidney	12	240 (10)	1
		1 ppm selenium as selenite	12	260 (10)	1
		10 *Idem*	12	250 (11)	0
		10 arsenic as equal mixture of arsenate and arsenite	12	217 (9)	10
VIII	13	None	12	164 (8)	10
		10 g acid hydr. kidney	12	223 (8)	5
		20 *Idem*	12	259 (12)	1
		.3 ppm selenium as selenite	12	252 (12)	0
		1 *Idem*	12	245 (12)	0
		3 "	12	257 (12)	0
		3 tellurium as tellurite	12	222 (12)	4

* Experiment ran 22 days instead of 23 days.

casein§ supplemented with tryptophan was somewhat less active than native crude casein. A sulfuric acid hydrolysate of kidney proved the best source of the factor for fractionation work. The results of Exp. IV in Table II showed that 30 g of this were required for complete protection. The factor

§ A sulfuric acid hydrolysate of casein made by Sheffield Co.

in hydrolyzed kidney was adsorbed on Dowex-50 (Na^+) and eluted by dilute sodium hydroxide. Similarly the activity was also adsorbed on and eluted from the anion exchange Dowex-1. The assay results of Exp. VI on these ion exchange fractions show that the active principle is amphoteric.

The alkaline ash of acid hydrolyzed kidney was active but it is apparent that some activity was lost during the ashing process. The acid ash was less active. The alkaline ash contained 1.8 ppm of selenium and the acid ash contained 1 ppm of selenium. The original hydrolyzed kidney contained 3.5 ppm of selenium. Hydrolyzed kidney yielded 37% of alkaline ash, so approximately 20% of the selenium was recovered during the ashing process. Seven % of the selenium was recovered in the acid ash.

The activity of selenium and tellurium is shown in Exp. VII and VIII. Selenium gave complete protection in Exp. VIII at 0.3 and 1 ppm. Tellurium as tellurite was inactive at 3 ppm. Arsenic showed no activity at 10 ppm.

Discussion. It is apparent from these data that selenium can replace vit. E for prevention of exudative diathesis on chicks on a torula yeast diet. It is not possible to state whether the need for selenium can be completely eliminated by vit. E. The torula yeast in the diet contained less than 0.1 ppm of selenium which would contribute less than 0.04 ppm to the basal diet. Addition of vit. E to this diet gave good growth with no evidence of exudative diathesis at 35 days. If selenium is required in the presence of vit. E the requirement would appear to be less than 0.04 ppm.

Selenium is toxic for many species of animals such as the horse, cow, pig, dog and chicken(5). Poley *et al.*(6) reported that the minimum level of selenium which would depress growth rate of chicks was 10 ppm when given in the form of seleniferous grains. These same workers(7) also found that levels in excess of 5 ppm reduced hatchability and that 10 ppm of selenium decreased it to zero.

There is no convincing published evidence that selenium is necessary for animal nutrition. Poley *et al.*(6) stated that chicks receiving diets containing 5 ppm of selenium in seleniferous grains usually grew better than those receiving none or 2 ppm. In view of the fact that 1 ppm of selenium as selenite is adequate and that Poley's diets contained vit. E in the natural grains used, it seems unlikely that the better growth reported with 5 ppm was due to the additional selenium. Pinsent(8) found that 3 to 8 mμg of selenium/ml was necessary for the formation of formic dehydrogenase by *Escherichia coli.* This suggests a role of selenium for certain phases of bacterial metabolism. Very recently, Schwarz and Foltz(9) have found that selenium is protective against dietary necrotic liver degeneration in rats that are deficient in "factor 3."

Summary. (1) Chicks fed a diet containing torula yeast developed exudative diathesis which could be prevented by either vit. E or a non-fat soluble substance in casein and in a number of pork tissues. (2) This factor could be made water soluble by acid hydrolysis. It was adsorbed on both anion and cation exchange resins and thus behaved as an ampholyte. (3) An alkaline ash but not an acid ash of pork kidney was effective in preventing exudative diathesis. (4) Selenium as selenite prevented exudative diathesis at 0.3 ppm. Tellurium as tellurite was ineffective at 3 ppm. Anionic arsenic at 10 ppm was inactive.

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