Prevention of Exudative Diathesis in Chicks by Factor 3 and Selenium. (23308)

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Factor 3 is a dietary agent preventing necrotic liver degeneration in the rat(1) and multiple degeneration (heart, liver, kidney and muscle necrosis) in the mouse(2). Recently, Factor 3 has been identified as an organic compound containing selenium, and selenite was found to replace it in the diet (3).* The present study shows that Factor 3 preparations of greatly differing degrees of purity, as well as selenium in organic and inorganic forms, prevent exudative diathesis in the chick when applied at dose levels similar to those effective against liver necrosis in the rat. The data presented here result from collaborative experiments carried out since 1955 at the National Institutes of Health and at Cornell University.

The production of necrotic liver degeneration in the rat, using a vit. E and Factor 3-free, purified ration based on 30% *Torula* yeast as the source of protein, has been described(4). Scott has adapted this diet to the amino acid requirements of the chick and has found that it produces exudative diathesis, inhibition of growth, and death, whereas the liver remains macroscopically normal(5). Exudative diathesis has been noted before to be produced in chicks only by certain types of vit. E-free diets, and not by others(6). The deficiency symptoms in the chick on *Torula* yeast rations were preventable not only by tocopherol but by brewers yeast as well(5). The latter is known to be free from vit. E but it is an effective source of Factor 3(7).

Methods. The Factor 3 preparations were obtained in the course of a project aimed at purification and identification of the active principle from dried, defatted pork kidney powder.† Three fractions of different degrees of concentration were submitted to tests in the chick. Factor 3 activity was determined by prophylactic assay against necrotic liver degeneration using the 30% *Torula* yeast diet and the Fisher strain of rats. Values reported for Factor 3 (Table I) are the result of repeated assays with 10 rats per group. They are expressed in units, 1 unit representing the amount of Factor 3 required daily per rat to afford 50% protection(8). For complete protection against liver necrosis, the Fisher strain of rats requires ca 65 units of Factor 3 in 100 g of diet.

Selenium was tested in 3 different forms: (a) organically bound as seleno-cystathionine; this material was isolated from seleniferous grain by Horn and Breese Jones(9) as an isomorphous compound containing 20.6% selenium,‡ (b) as sodium selenite (Foote Mineral Co.), and (c) as elementary selenium (gray modification). The first 2 materials were supplemented to the rat and chick diets as solutions in water. The elementary selenium was added as a trituration in lactose.

The chick experiments were carried out as follows: At the Nat. Inst. of Health, New Hampshire chicks, 6 or 7 per group, were fed basal diet C34, which is only slightly different from the Cornell diet. It contains *Torula* yeast 60%, cerelose 28.5%, salts 6%(10), vit. E-free lard 4%, glycine 1%, methionine 0.38%, and vitamins(10) except E. At Cornell University, White Plymouth Rock chicks, 17-20 per group, were fed the previously described basal diet A(5) containing *Torula* yeast 58.5%, cerelose 28.3%, salts 4.2%,

* Since various forms of selenium show different biological activities, the term "Factor 3" is used to designate the biologically active, selenium-containing component, or components, present in nutrients and other materials of biological origin. Factor 3 is bound to protein, stable against acid hydrolysis, and water soluble(15).

† Schwarz, K. and others, to be published separately.

‡ The authors wish to thank Dr. M. J. Horn for a generous sample of this material.

TABLE I. Prevention of Exudative Diathesis by Factor 3 and Selenium in Different Forms.

Diet treatment	Dose per 100 g of diet	Units of Factor 3* per 100 g of diet	Nat. Inst. Health			Cornell Univ.		
			Avg wt, 3 wk (g)	No. of chicks	Incidence exudative diathesis	Avg wt, 3 wk (g)	No. of chicks	Incidence exudative diathesis
Basal			164	18†	13	116	17	17
" + d α-Tocopheryl acetate	1.6 mg					164	17	2
" + *Idem*	2.4 "					143	17	1
" + d,l-α-Tocopheryl acetate	10 "		263	6	0			
" + Dried brewers yeast‡	10 g	105	332	6	0	210	17	0
" + Casein, "vit. free"§	10 "		221	6	0			
" + Amino acid mixture‖	10 "	0	148	6	4			
" + Pork kidney powder, dried defatted	2 "	108	242	6	0			
" + *Idem*	5 "	270				225	17	0
" + Factor 3 prep. 3/3722; conc. of α-F3 from pork kidney powder	(1.7 ")¶	106				195	17	11
" + *Idem*	(3.4 ")	212				210	16	4
" + Factor 3 prep. 3/3446A;** conc. of β-F3 from pork kidney powder	(3.2 ")	82	169	6	1			
" + Factor 3 prep. 3/2503; purified α-F3 from pork kidney powder	24 mg	51	244	6	1			
Basal			131	7	6	157	19	14
" + Selenium, as seleno-cystathionine	10 μg	180‡‡	275	4	0	196	17	0
" + " , as sodium selenite	10 "	230††	232	7	0	199	18	0
" + " , elemental	200 "	80‡‡	191	7	2	232	19	0

* Assayed against dietary necrotic liver degeneration in the rat (see *Methods*). † Summary of 3 groups of 6 chicks each. ‡ Brewers dried yeast, composite sample 1956, Brewers Yeast Council, Inc. § General Biochemicals, Inc. ‖ Amino acids used in proportions present in casein. ¶ Mostly NaCl. ** Added to diet at beginning of third week. †† Loc. cit. 3. ‡‡ K. Schwarz and C. M. Foltz, unpublished results.

vit. E-free lard 5%, cellulose 3%, glycine 0.5%, L-arginine HCl 0.24%, DL-methionine 0.3%, and adequate amounts of vitamins except E. In both laboratories chicks were started when one day old. They were housed in electrically heated batteries with wire mesh floors. Food and water were given *ad libitum*. Supplements were added by thorough mixing into the diet at the expense of cerelose.

Results. In Table I a selection of tests carried out at different times at the 2 laboratories is presented. Besides tocopherol, various natural, vit. E-free sources of Factor 3 protected against exudative diathesis. In general, there was good correlation between Factor 3 activity as established in the rat assay, on one hand, and the protective effects obtained in the chick. Dried brewers yeast and vitamin-free casein completely prevented symptoms and promoted good growth when added at 10% levels to the diet. A mixture of amino acids simulating the composition of casein was ineffective. These results are identical to those described for dietary necrotic liver degeneration(10). Fat-extracted pork kidney powder, which is a potent source of Factor 3,† gave excellent growth and prevented exudative diathesis at the 2% and 5% level.

The Factor 3 fractions comprise a relatively crude concentrate of α-Factor 3,§ a concentrate of β-Factor 3 of medium degree of concentration, and a more purified α-Factor 3 fraction which was obtained from pork kidney powder after various steps of purification. These preparations produced degrees of protection in the chick which were predictable from the results obtained in the Factor 3 assay in the rat. The preparation used in the Cornell study (3/3772) reduced exudative diathesis to 25% incidence at the higher level tested. Both preparations tested at Bethesda protected 5 of 6 chicks. One of these gave a good growth response; the other one was added during the third week of the test.

The identity of Factor 3 with the agent preventing exudative diathesis in the chick was established beyond doubt when it became known that purified preparations of Factor 3 contained organic selenium and that sodium selenite would prevent dietary liver degeneration in rats(3). The 3 forms of selenium were tested simultaneously at both Bethesda and Cornell. In the rat, selenocystathionine fully protected against necrotic liver degeneration when supplied at a level equivalent to 4 μg of selenium per 100 g of diet. In the chick, this organic selenium compound completely prevented exudative diathesis at levels equivalent to 10 μg of selenium per 100 g of ration. The same effect was obtained with 10 μg % of selenium in form of sodium selenite. Elemental selenium, on the other hand, was required at higher dose levels in order to prevent liver necrosis in the rat. In the chick assay, 200 μg % protected 5 out of 7 animals in the Nat. Inst. of Health study, and all animals at Cornell. All 3 forms of selenium produced a strong growth promoting effect (Fig. 1). Some inconsistencies in growth rates obtained with different preparations are apparent, but the data on hand are insufficient for conclusive interpretations of the discrepancies.

Discussion. In the past, selenium has received a great deal of attention because of its toxicity. The extensive literature on the subject has been reviewed elsewhere(11)

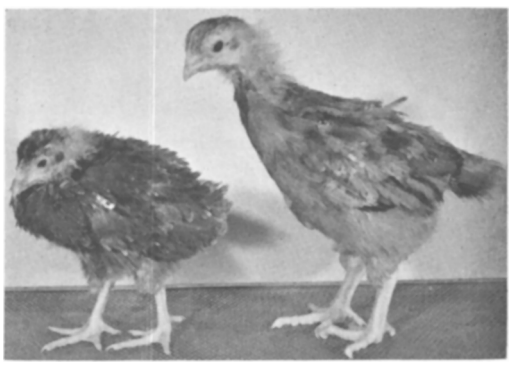

FIG. 1. Effect of selenium on growth and appearance of New Hampshire chicks. Left: Last survivor on unsupplemented basal diet. Right: Chick representing avg of sodium selenite supplemented group.

§ In acid hydrolysates of kidney powder, at least 2 chemically different but related components with Factor 3 activity have been detected. These have been designated α and β Factor 3. (Schwarz, K., Mason, L. H., and Foltz, C. M., to be published).

The viewpoint prevailing until now could be summed up in a sentence by Trelease and Beath, "as far as we know, selenium is toxic but never beneficial to animals or man"(12). The recent findings—that Factor 3 contains selenium in organically bound form, and that it is replaceable by selenite in μg quantities—indicate that *selenium may be a hitherto unrecognized, essential trace element.* It is seen from the present study that in the chick, as in the rat, selenium affords protection against serious and fatal deficiency syndromes at dose levels which constitute only a very small fraction of the minimal toxic dose.

It is evident from our results that a rather close parallelism exists between the rat and the chick with respect to protective effects of Factor 3 and other selenium compounds under dietary conditions which lead to liver necrosis, or to exudative diathesis, respectively. Both diseases are prevented by crude sources of Factor 3, by Factor 3 concentrates, and by selenium in form of seleno-cystathionine and as sodium selenite. Elemental selenium is also effective.

From the activities of crude sources of Factor 3, and also of Factor 3 concentrates, (Table I), it can be calculated that dose levels effective against exudative diathesis in the chick are approximately 2 to 3 times as high as those required by the rat for protection against liver necrosis. These levels appear to be "physiological" *i.e.,* they correspond to the trace amounts of bound selenium (Factor 3) ingested with normal diets. Comparison of the effective doses of selenium with those of vit. E shows that in the chick selenium in form of selenocystathionine or selenite is 500 to 1000 times as active as tocopherol. This ratio is very similar to that in the rat, which is roughly 500:1(3). It is obvious that the presence or absence of selenium in the form of Factor 3, or as an inorganic compound, decisively influences the course of vit. E deficiency diseases. In standard rations used for assaying vit. E in the rat, Factor 3 is amply supplied as an impurity in the vitamin free casein(10). The exact relationship between vit. E and Factor 3 remains to be clarified. Preliminary results indicate that the 2 substances act independently of each other in alternate pathways of intermediary metabolism.†

To our knowledge, a biological function has been previously ascribed to selenium only in one instance: Pinsent found that selenite is required for formation of formic dehydrogenase in *E. coli*(13). Results suggestive of a beneficial effect of selenium in the chick were obtained by Poley and others in their study on selenium toxicity(14). However, their experiments do not permit a conclusive interpretation since they were carried out with seleniferous wheat, and not with pure compounds. Subtoxic levels of the element (200 μg %) produced a positive growth response in 5 out of 6 trials. The amounts used in these studies were much in excess of those found effective in the present investigation.

Summary. It is shown that exudative diathesis in the chick, produced by vit. E-free *Torula* yeast diets, is prevented by crude sources and purified fractions of Factor 3. Selenium, recently identified as the integral part of Factor 3, was highly active. Protection was obtained with 10 μg of selenium per 100 g of diet in form of seleno-cystathionine or sodium selenite; 200 μg of elemental selenium also were effective. All 3 forms of the element stimulated growth.

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Hematocrit Change as Indication of Anaphylactic Shock in the Mouse. (23309)

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Because of their complexity, methods for quantitation of anaphylaxis in large hypersensitive animals(1) are not readily adapted for the mouse. Consequently, the occurrence of anaphylaxis in mice is customarily determined using death as end point. Attempts have been made to estimate sublethal anaphylaxis in mice by observing the appearance of certain gross signs (*i.e.*, cyanosis and convulsions). Because of their subjectivity, these observations are inadequate. Kind(2) found that the rectal temperatures of mice surviving anaphylactic shock were markedly decreased.

In our laboratory we consistently observed that hemoconcentration occurs in mice undergoing anaphylaxis. We observed signs of anaphylaxis similar to those described by Weiser *et al.*(3). Immediately after challenge most of the mice became quiet and huddled in one position. The fur became ruffled and there was mild lacrimation. There soon followed partial paralysis of the limbs and marked cyanosis of the limbs, tail, and snout. Many animals experienced brief but violent convulsions separated by short periods of complete prostration. Nearly all mice which underwent convulsions died within 30 minutes. Nelson *et al.*(4) reported that the hematocrit of pooled mouse blood was markedly increased following fatal anaphylactic shock. The present study was designed to determine whether or not hematocrit change might be a suitable criterion for determination of anaphylaxis in mice.

Methods. Sensitization and challenge. Following randomization, female Swiss-Webster mice (22-27 g) were sensitized with 2 IP injections of 0.25 ml of antigen administered 4 days apart. Twelve days after receipt of the initial sensitizing injection the animals were challenged with an injection in the caudal vein of 0.25 ml of antigen. Two antigen preparations were employed: 1) alum precipitated bovine serum albumin* with *Hemophilus pertussis* vaccine† (APBA + HPV), each 0.25 ml containing 1 mg of bovine serum albumin suspended in 2.5% alum (pH 6.5 to 7.5) with 10^9 to 10^{10} *H. pertussis* vaccine cells; and, 2) the same as above except that *H. pertussis* vaccine was not included. This antigen preparation is referred to as APBA. A single challenge antigen preparation, bovine serum albumin with *H. pertussis* vaccine (BSA + HPV) was used. Each 0.25 ml of this preparation contained 4 mg of bovine albumin and 10^9 to 10^{10} cells *H. pertussis* vaccine. Controls included groups of mice sensitized with one or the other of the 2 sensitizing antigen preparations and challenged with saline, mice injected with saline instead of sensitizing antigen preparation and challenged with BSA + HPV, and groups given saline instead of both sensitization and challenge. *Hematocrit determination*: A modification of the microhematocrit method described by McGovern *et al.*(5) was employed. Four-tenths mm ID microprecipitin capillary

* Bovine serum albumin, 30%, sterile, for use as diluent in Rh testing. Armour Labs.

† Pertussis vaccine (whooping cough bacterin), Wyeth Labs.