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Short Communications

Factor 3 and 6-Selenoctic Acid

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Factor 3 was recognized as a nutritional agent *per se* in 1951 by Schwarz [1]. It protects against dietary liver necrosis in the rat and the pig [2], multiple necrotic degeneration (heart, liver, kidney, and muscle necrosis) in the mouse [3], as well as against exudative diathesis in the chick [4,5] and the turkey. Factor 3 has been shown to be an organic compound of high biological potency which contains the element selenium as an essential constituent [6]. Recently G. Bergson described the synthesis of 6-selenoctic acid, the selenium analogue of 6-thioctic acid; the author stated that "the hypothesis may be advanced that Factor 3 is identical with or related to 6-selenotic acid" [7]. This assumption can be eliminated for the following reasons:

(a) The possible involvement of thioctic acid or of compounds with similar physicochemical properties was excluded in 1951, in the course of extensive unpublished fractionation studies on Factor 3 carried out at Bethesda. Various procedures which separated thioctic acid from Factor 3 were applied to prepurified Factor 3 fractions from enzymatic casein digests and other sources. For instance, continuous extractions at a low pH with solvents such as benzene removed thioctic acid readily, but failed to remove the Factor 3-activity from the aqueous phase *.

(b) Following the discovery of selenium as an integral part of the Factor 3 molecule, a large variety of organic selenium compounds has been tested for Factor 3 activity, using the quantitative assay against dietary necrotic liver degeneration in the rat. An appreciable degree of chemical specificity was detected [8,9]. Several types of selenium compounds are inactive, while others prevent liver necrosis. The majority of the compounds tested, including selenate, selenite, and also the selenium analogues of cystine, cystathionine and methionine, was found to afford 50 % protection at dose levels which supply 2—3 μg of selenium per 100 g of diet (ED_{50} = 2—3 μg % Se). A purified preparation of α-Factor 3 from pork kidney powder was more potent than any other compound investigated. The fraction was accurately analyzed for selenium by radio activation analysis; its ED_{50} was established to be 0.7 μg % Se.

Animal assays for Factor 3 potency, at Bethesda, with 6-selenoctic acid from Uppsala have resulted in a value of 3.8 μg % Se for 50 % protection (Table 1) * §. Comparison of this ED_{50} value to that for α-Factor 3 clearly indicates that the latter is different from 6-selenoctic acid. The potency of the thioctic acid analogue is only 1/5 of that of the α-Factor 3 preparation, and somewhat less than that of selenite, selenocystine, etc. At present, the structure of the organic part of the Factor 3 molecule remains to be established.

Experimental. Synthesis and properties of 6-selenoctic acid have been described in this journal [7]. For the animal tests, the substance was dissolved in ethanol and added to the basal diet. Details of the standardized procedure

* Schwarz, K. *Unpublished results.*

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§ A test with 6-selenoctic acid in chicks on an exudative diathesis producing diet (30 % *Torula* yeast, 28 % purified soy protein, 4% vitamin E free lard), carried out by J. G. Bieri, showed that 15 μg % of selenium in form of this compound protected all animals against the disease, while 7 out of 8 controls succumbed to the deficiency.

Table 1. Factor 3-activity of 6-selenoctic acid

Amount in diet		No. of animals	No. of dead animals *	Protection %	ED_{50}
Compound I μg %	Selenium μg %				μg Se/100 g diet
0	0	30	27		
3.8	2	20	17	16	
5.7	3	10	6	43	3.8
7.6	4	10	5	57	
11.4	6	10	2	83	
19	10	10	0	100	

* The experiments were terminated on the 30th day.

of the Factor 3 assay using a vitamin E and Factor 3-free 30 % *Torula* yeast ration [10], and weanling, inbred Fischer 344 rats [11] have been specified elsewhere. For evaluation of the results, the average velocity of death from liver necrosis (V_{st}) was calculated from individual survival times [12]. The effects were expressed in "% protection", *i.e.*, in per cent reduction of the velocity as compared to that of the control groups (% protection = 100 — 100 × V_{st} exp./V_{st} contr.). Weighted averages were used, and the ED_{50} was computed from those results which fell on the ascending part of the dose response curve (between 15 and 85 % protection). The assay method, set up twice weekly with 10—15 groups of 10 rats each, yields very consistent ED_{50}'s for selenite, Factor 3 and other compounds tested.

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Preparation of Monoperphthalic Acid

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The method for preparing monoperphthalic acid by oxidising phthalic anhydride with an alkaline solution of hydrogen peroxide, first suggested by Böhme [1], gives according to *Organic Syntheses* [2] normally 65—70 % yield. We found, however, this reaction to be somewhat capricious, sometimes giving only 40—50 % yield. By slightly modifying the procedure we succeeded in eliminating the capriciousness, and at the same time the yield was raised to 90—95 %.

The essential problem is to keep the solution sufficiently cool during the reaction. Operating the same charge as indicated in *Organic Syntheses* we cooled the solution in a carbon dioxide-acetone mixture and maintained a temperature below —5° during the whole reaction. At this temperature a crystal-layer will cover the walls of the reaction vessel, thus preventing the transmission of the heat of reaction to the cooling mixture. It is, therefore, necessary constantly to scrape down the crystals from the walls, which may easily be effected when using a beaker as reaction vessel instead of a flask. Using this procedure no foaming is observed and practically no phthalic acid was formed.

After the addition of sulphuric acid the temperature is allowed to rise to 0° and enough water is added to dissolve the inorganic material. The solution is extracted 5 times with ether, the ether-solution washed 3 times with ammoniumsulphate solution and dried over magnesiumsulphate.

The yield, ranging from 90 to 95 %, was estimated iodometrically as indicated in *Organic Syntheses*.

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