crystals to concretion formation is suggestive, but not established. Both entities could be independent consequences of the same phenomenon.

Summary. Crystals and concretions were found in the vaginae of A/Crgl, BALB/cCrgl and C57BL/Crgl mice with persistent estrus produced by injection of estradiol-17β for 5 days starting from day of birth. The concretions appeared at 6-7 months of age. Crystals have also been encountered in vaginal smears of C3H/Crgl and RIII/Crgl mice, which have been in persistent estrus for 2 months. The crystals resemble those of triple phosphates; the concretions consist largely of organic matter (including urate) and calcium phosphate.

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Effect of Selenium on Methionine Formation *in vivo* and *in vitro*.* (27289)

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Hofmeister(1) suggested that salts of selenium and tellurium form methyl compounds in the animal body. He did not mention either the possible methyl source or compounds which serve as methyl donors. du Vigneaud and his school(2) provided the first experimental evidence that transmethylation can occur *in vivo*. They demonstrated that choline, betaine and methionine served as methyl donors. Borsook and Dubnoff(3) have established that choline and betaine can serve as methyl sources in the synthesis of methionine from homocystine, homocysteine and homocysteinethiolactone in tissue homogenates.

Considerable presumptive evidence has been presented in recent years, by use of radioactive selenium, of the biological synthesis of selenium analogues of cysteine(4) methionine and cystine(5,6,7).

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Rosenfeld and Beath(8) reported that in chronic selenosis total nitrogen and sulfur content of all tissues decreased but significant decrease occurred in the liver.

There is no available information on the effect of chronic selenosis on protein metabolism. This report deals with the effect of chronic selenosis on methionine and cystine content of liver and also the effect of inorganic selenium compounds on methionine formation by transmethylation *in vitro*.

Experimental. Adult Sprague-Dawley rats were used in all studies reported here. A group of 10 rats received daily intraperitoneal injection of 2 mg of selenium as sodium selenate. The animals developed chronic selenosis in 4 to 5 weeks. Toxic symptoms were indicated by considerable loss of weight, emaciation or abdominal ascites, anorexia and terminal aphagia. Animals were sacrificed 2 days after development of aphagia. Liver damage was similar to that reported by Rosenfeld and Beath(9). Since emaciation, anorexia and aphagia may influence protein

TABLE I. Methionine and Cystine in Liver in Chronic Selenosis.

Treatment	Nitrogen	Protein	Calculated to 16 g N — Methionine	Cystine
	%			
Normal	3.0 ± .24*	18.8 ± 1.4	3.5 ± .16	1.5 ± .14
Restricted food intake	3.3 ± .16	19.9 ± 1.6	3.3 ± .29	1.0 ± .02
Chronic selenosis	2.2 ± .21	13.8 ± .2	2.5 ± .24	1.27 ± .21

* Standard deviation.

content of the liver, a second group of rats was placed on restricted food intake (3 g per day) for 6 days followed by 2 days starvation. The animals were then sacrificed. The third group of rats was fed *ad libitum* and served as normal control. All animals were fed Purina laboratory chow. At time of sacrifice livers were removed and homogenized in a "Virtis" tissue homogenizer and the protein precipitated with 10% trichloroacetic acid. The precipitated protein was separated by centrifugation.

Duplicate protein samples were hydrolyzed in reflux condenser with 6 N HCl for 24 hours. Concentration of hydrolysate, evaporation and removal of colored pigments were carried out according to Block and Bolling (10). Methionine was determined by Bolling's modification of Sullivan-McCarthy method(10). Cystine was determined by the method of Sullivan and Hess(11). Nitrogen was measured by a micro-Kjeldahl method employing direct nesslerization and checked by standard Kjeldahl distillation procedure.

In vitro methionine synthesis was carried out according to the method of Dubnoff and Borsook(12). Sodium selenate and sodium selenite were dissolved in buffer solution and added in amounts as indicated in the results. Since commercial sodium selenate may contain large amounts of selenite, a test for selenite was carried out to insure the purity of the reagent. DL-Homocysteine, DL-homocystine, betaine · HCl and choline · HCl were purchased from a commercial source. The purity of amino acids used was checked on paper chromatograms. All compounds used gave a single spot with 2-dimensional chromatography and reproducible R_f values. Results of methionine formation by transmethylation are the averages of at least 6 determinations in the absence or presence of varying amounts of selenate and selenite.

Results. Table I presents the experimental data on the livers of the 3 groups of animals. As our previous results indicated there was a decrease in protein content in this tissue(8). This decrease was not due to low food intake and the 2-day aphagia before death inasmuch as animals which received restricted amount of food showed no drop in protein content of liver. Methionine and cystine was calculated to 16% nitrogen. Such adjustment of values to the same percentage of nitrogen, while purely arbitrary, possesses advantages when comparisons are made from one group to the other. The decrease in methionine in chronic selenosis was highly significant; that in cystine was not so striking. It is generally recognized that there is loss of cystine during acid hydrolysis. However, the values reported in these experiments are comparable inasmuch as hydrolysis, in all cases, was carried out under the same experimental conditions.

In chronic selenosis there is considerable liver necrosis(9). The work of Dent(13) indicated that development of acute hepatic necrosis was not related to changes in concentration of methionine and cystine in rat liver. Shaffer and Critchfield(14) analyzed the liver of rats on sulfur-poor diet after several days carbon tetrachloride inhalation; they found no changes in N/S ratio.

Inasmuch as secondary factors, such as restricted food intake, starvation and hepatic necrosis, which accompany chronic selenosis were not responsible for decreased methionine in liver, the possibility that selenium interfered with methionine formation was investigated. Table II gives the results of methionine formation by liver homogenate from homocystine and betaine in presence of selenate and selenite. Both compounds inhibited methionine formation, but selenite always appeared to be more inhibitory than selenate.

The addition of 0.1 mg of selenite to the system depressed methionine formation over 50.0% while selenate showed about 12.0% inhibition. Although selenate is not reduced *in vitro* as readily as selenite, *in vivo* a number of enzymatic systems may be involved before selenate is reduced to selenite. Rosenfeld and Beath(15) examined this possibility and found that in tissue preparations selenate was converted to selenite, volatile and elemental selenium. Autoclaved tissues could not carry out this conversion reaction indicating that these changes were enzymatic in nature. The differential inhibition of methylation of homocystine by betaine in presence of selenate and selenite gives further evidence that selenate is not as active an inhibitor in biological systems as selenite.

TABLE II. Effect of Selenate and Selenite on Methylation of Homocystine by Betaine in Liver Homogenate.

Reaction mixture* Selenium mg	Methionine formed per 100 mg tissue (wet wt) Selenate μg %	Selenite μg %
0	146.3	148.9
.002	144.9	140.4
.01	143.9	133.3
.02	136.3	125.8
.1	128.9	74.2
1.0	120.7	10.0

* Homocystine 25 mg %; betaine 12.5 mg %; 1 ml 1:4 homogenate; selenium, as indicated above; total vol, 4 ml; gas phase, nitrogen; time 3 hr; temp, 38°.

Results of methionine formation by liver homogenate from homocysteine and betaine in presence of selenate and selenite are given in Table III. More methionine was formed from homocysteine than homocystine when homocysteine was the methyl acceptor; however, the extent of inhibition was about the same in both studies. The formation of metallic selenium evidenced by change in color of reaction mixture was observed at termination of the experiment. By testing the various components of reaction mixtures in test tubes, it was found that selenite was reduced by homocysteine to elemental selenium while selenate was not affected. In addition to homocysteine, homocystine, cystine, cysteine, homocysteinethiolactone, GSH and methionine were tested with selenate and selenite and it was found that selenite was reduced to elemental selenium by cysteine, homocysteine, homocysteinethiolactone and GSH. Tsen and Tappel(16) reported catalytic oxidation of cysteine, GSH, Co A and dihydrolipoic acid by selenite. At the end of the methylation experiments both selenate and selenite contained elemental selenium indicating that selenate in presence of tissue homogenate was converted to selenite and elemental selenium. Zalokar(17) reported that when sodium selenite was added to a wild type *Neurospora* culture large quantities of selenium were deposited in the mycelium and a disagreeable odor was produced during this process. No reduction of selenite occurred and no odoriferous product was formed when mycelium was killed by boiling or macerated in a Waring blendor.

TABLE III. Inhibitory Effect of Selenate and Selenite on Methylation of Homocysteine by Betaine.

Reaction mixture* Selenium mg.	Methionine formed† Selenate %	Selenite %
0	100	100
.002	99	92
.01	97	79
.02	92	73
.1	90	68
1.0	85	0

* Homocysteine 25 mg %; betaine 12.5 mg %; other conditions same as in Table II.

† The reaction mixture without selenium taken as 100%.

Experiments were carried out to test the effect of selenate and selenite on methionine formation from homocysteine and homocystine when choline served as methyl donor. Table IV shows the inhibiting effects of sele-

TABLE IV. Selenate and Selenite Inhibition of Methionine Formation *in vitro* by Liver Homogenate.

Reaction mixture* Selenium mg.	Methionine formed† Selenate %	Selenite %
0	100	100
.002	99	94
.01	99	88
.02	95	84
.1	91	73

* Homocysteine 25 mg %; choline 12.5 mg %; other conditions same as in Table II.

† The reaction mixture without selenium taken as 100%.

nite and selenate on homocysteine methylation by choline. Larger yields of methionine were obtained when the methyl acceptor was homocysteine rather than homocystine, and methyl donor betaine, but degree of inhibition by selenate and selenite was the same regardless of methyl donor or methyl acceptor used.

These results present evidence that methionine formation was depressed *in vitro* by selenate and selenite in liver homogenate and this decrease was reflected in the methionine content in liver in chronic selenosis.

Discussion. The factor or factors responsible for nitrogen depletion and decreased protein synthesis in chronic selenosis are not known. Restricted food intake and aphagia which accompanies chronic selenosis do not seem to be contributing factors under present experimental conditions. Our preliminary studies on liver proteins with a tracer dose of radioactive selenium indicated that there were no drastic changes in amino acid composition which would account for the above decrease. The decrease of cystine in chronic selenosis suggested that it may be related to decreased food intake, inasmuch as there was a corresponding decrease of cystine in liver of animals on restricted food intake. The results presented here indicate that selenium depressed methionine formation *in vivo*. *In vitro* experiments confirmed these findings and suggested some explanation for the decrease. Probably 2 factors are responsible for the decrease in methionine: 1. Oxidation of sulfhydryl groups by selenite. 2. The methyl capture by "active" seleno-compounds. There is some evidence to support both mechanisms. These experiments indicate that both processes occur simultaneously *in vitro*. There is some evidence that these reactions occur *in vivo* and they serve as a detoxification mechanism in animal against selenium intoxication. Challenger(18) presents considerable evidence that living mycelium acts on sodium selenite by a succession of methylating and reducing steps to produce dimethyl selenide. Rosenfeld and Eppson (19) reported that addition of choline to diets increased rate of growth and duration of life of rats injected with sodium selenate. Zalokar(17) reported when selenite was added to wild type *Neurospora crassa* cultures selenium was deposited inside the mycelium as red droplets, but no methylated selenium compound was recovered. Elemental selenium in mammalian tissues in chronic selenosis was not detected but lack of recognition does not exclude the possibility of its occurrence. At least we have no evidence that this mechanism of selenium detoxification does not take place *in vivo*.

Summary. The effect of chronic selenosis on methionine and cystine content in liver of experimental animals was studied. The decrease in methionine in chronic selenosis was not associated with restricted food intake and subsequent starvation. In *in vitro* experiments, using rat liver homogenate, sodium selenate and selenite inhibited transmethylation reactions of homocystine and homocysteine by betaine and choline. Selenite was a more active biological inhibitor than selenate. The significance of the results was discussed with reference to the detoxification mechanism in selenium poisoning in animals.

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Effect of Colchicine on Antibody Response in Hamsters. (27290)

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Several substances are known to enhance antibody response. Among them colchicine, which has been reported to enhance antibody response in the rabbit(1,2,3), is of particular interest since the action of the drug on cells has been studied extensively. Most laboratory animals are highly susceptible to the toxic effects of colchicine. The hamster, however, is distinctive in that it can tolerate this drug even in massive dosage(4,5,6). It was therefore determined whether high dosage of colchicine would provide greater adjuvant action in hamsters than in rabbits.

Material and methods. Golden hamsters, weighing 100 ± 2 g, were injected intraperitoneally with an anesthetic (0.1 ml of a 60 mg/ml solution of Nembutal) prior to immunization and to bleeding. A single dose of the antigen, 0.1 ml of 5% rabbit erythrocytes in saline, was administered to each animal *via* the great saphenous vein. Immediately thereafter the hamsters were given, intraperitoneally, colchicine‡ dissolved in distilled water in the amounts indicated in Table I. On the seventh day after administration of the antigen, the animals were bled by cardiac puncture, and the sera separated and stored at −20°C.

Hemagglutination tests were performed by serial 2-fold dilution of sera with 0.9% saline. For the direct hemagglutination tests, 0.2 ml aliquots of 2% rabbit erythrocyte suspensions were added to each reaction mixture containing 0.2 ml of diluted serum. The reaction mixtures were incubated for one hour at 37°C and degree of hemagglutination was determined microscopically. The sera were also tested by the indirect (Coombs) test. As before, 0.2 ml aliquots of 2% rabbit erythrocyte suspensions were added to each reaction mixture containing 0.2 ml of diluted serum. After incubation of the reaction mixtures for one-half hour, 0.1 ml aliquots of rabbit antiserum, prepared against hamster serum, were added to each reaction mixture and then incubated for an additional hour. All sera were heat-inactivated (one-half hour at 56°C) before use.

Results. The toxic syndrome caused by high dose (300 to 500 mg/kg body wt.) of

TABLE I. Antibody Response of Hamsters to Rabbit Erythrocytes Following Administration of Colchicine.

Colchicine dosage mg/kg	Toxicity Dead/ total	Hemagglutination titer (reciprocal) Direct Avg	Direct Range	Indirect Avg	Indirect Range
		Non-immunized controls			
30	0/5	<2	<2	<2	<2
		Immunized Animals			
None	2/10	20	4–32	1392	128–4096
.9	1/5	88	16–256	2816	1024–4096
1.7	1/5	64	64	1664	512–4096
5.1	0/5	70	32–128	563	256–1024
25	0/5	70	32–128	1178	256–4096
50	1/5	96	64–128	576	256–1024
100	1/5	46	8–128	299	128–512
300	4/7*	5	4–8	75	32–128
500	7/9	<2	<2	2	<2–4

* LD_{50} for hamsters, determined separately in 16 animals, was 300 mg/kg (7 of 16 survived for 7 days).

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‡ Colchicine, USP, was obtained from S. B. Penick & Co., New York City.