Fibrinolysis, etc. Lysis of the fibrin clots in A and B (Table I, A) occurred overnight, as did T_2 (Table I, B) and T_3 (Table II). This confirms the fibrinolytic actions of natural plasma protease. Fibrinolysis was absent, even after 7 days at 37°C, in the enzyme-free controls and in the presence of S.B.I., while it was delayed for 3-4 days by the cited amounts of T.I. Thrombinolysis requires further study, but did not occur with the proteases used in the present study, except trypsin (Table I, T_5). This could be a matter of enzyme strength, however.*

The absence of significant prothrombinolysis (*cf.*[9]), due to choice of very weak enzyme preparations, is apparently the key to the successful demonstration of these important "thromboplastic" effects.

Summary. The natural protease *(tryptase)* in several plasma protein fractions resembles a weak pancreatic trypsin in its fibrinogenolytic, fibrinolytic, and "thromboplastic" effects. These actions are inhibited by crystalline trypsin-inhibitors from pancreas and soybean. The clear demonstration of thromboplastic action in these quantitatively-controlled tests is direct proof of participation of plasma tryptase in the blood-clotting system.

* More recent tests show that a sufficiently potent tryptase is thrombinolytic.

9 Seegers, W. H., and Loomis, E. C., *Science*, 1946, **104**, 461.

15776

Chronic Oral Toxicity of Alpha-Naphthyl Thiourea.*

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Richter[1] proposed the use of alpha-naphthyl thiourea (usually abbreviated ANTU) as a specific poison for the control of Norway rats because of its high toxicity to rats and its relatively low toxicity to all other species tested. Its emetic property protected dogs in most cases. Although Richter showed that animals became tolerant to acute doses of ANTU, the experiments of McClosky and Smith[2] indicated a cumulative action with successive doses to rabbits and cats. In experiments conducted for short periods of time the chronic effects of ANTU paralleled closely those obtained with thiourea and 2-thiouracil.[1] Because of Richter's report of tolerance to ANTU the present study was undertaken to determine the effects of small amounts of ANTU ingested during the lifetime of the rat.

Experimental. Groups of 18 male weanling rats (21 days) from our colony of Osborne-Mendel strain were started on each of 7 diets containing respectively 0, 50, 100, 200, 400, 600 and 800 ppm ANTU. Ground commercial rat biscuits with 1% added cod liver oil served as the basic diet. The ANTU was mixed with the basic diet by means of a rotary batch mixer. Litter mates were selected and assigned to the various groups according to a randomized design of experiment (balanced incomplete blocks).[3] All

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1 Richter, C. P., *J. A. M. A.*, 1945, **129**, 927.

2 McClosky, W. T., and Smith, M. I., *Pub. Health Rep.*, 1945, **60**, 1101.

3 Fisher, R. A., and Yates, F., *Statistical Tables for Biological, Agricultural, and Medical Research*, Oliver and Boyd, Edinburgh, 1938.

TABLE I.
Mean Gain in Weight of Male Rats Fed Diets Containing ANTU.

Time in mo.	Dosage of ANTU, ppm	No. of animals	Mean gain in wt, g	Standard error of mean, g
3	0	18	324.5	± 8.3
	50	17	315.1	± 7.3
	100	18	262.0	± 9.0*
	200	18	202.9	± 7.7*
	400	17	123.2	± 8.2*
	600	18	105.1	± 7.9*
	800	16	101.8	± 8.1*
12	0	12	498.1	±16.0
	50	16	505.8	± 7.2
	100	15	457.7	±22.9
	200	15	416.3	±15.2†
	400	16	260.6	±28.8*
	600	15	248.5	±11.4*
	800	12	240.3	±14.3*

* $p < .001$
† $p < .01$

animals were kept in individual cages in a room with controlled temperature and humidity and were given free access to their respective diets and water. Body weights and food consumption were determined at weekly intervals. Surviving animals were sacrificed at the end of the 2-year period.

Results. Effects on Growth and Mortality. Growth curves of the experimental groups, with the exception of that for the group on 50 ppm ANTU, deviated markedly from that of the controls. The data on the gains in weight during the fast-growing period of the first 12 weeks (Table I) show that the animals on concentrations of 100 ppm, or more, grew significantly ($p = < .001$) less than those on the control diet. When the gains in weight of the experimental and control animals during the first year were analyzed (Table I), the difference between the animals on 100 ppm ANTU and the controls was not significant ($p = .17$). This fact led to a further analysis of the growth data for the last 26 weeks of the first year. During this period all groups of rats on 100 ppm, or more, ANTU grew slower than the control group; however, the differences in growth between the groups on 100, 200 and 400 ppm ANTU and the controls were not significant. The growth rate of the rats on these concentrations of ANTU had increased so that the value for the retardation of growth had changed from highly significant at 3 months to nonsignificant during the plateau period. This apparent decrease in toxicity undoubtedly was due in part to the development of a tolerance;[1] however, as we have shown in a similar experiment with 2,2-bis (*p*-chlorophenyl)-1,1,1-trichloroethane (DDT),[4] it was due in part to the lesser daily intake of ANTU per kg of body weight as the animals increased in size. The reduction in dosage per kg of body weight was produced by the rapid increase in weight of the rats during the fast-growing period without a corresponding increase in food intake. A dislike for the food containing ANTU was evident in the rats on dosages from 400 to 800 ppm. For the first 3 or 4 weeks on the experimental diet these rats ate less per kg of body weight than control rats; later their food intake gradually became normal. After the first 3 months rats on dosage levels of 600 and 800 ppm were consuming daily more ANTU than the acute lethal dose of nontolerant rats of the same age.

All the deaths, except 4 in the group of rats on 800 ppm ANTU, which occurred during the first year of the experiment were caused either by respiratory infections or by middle ear disease. The 4 deaths in the group on 800 ppm appeared to be a direct

[4] Fitzhugh, O. G., and Nelson, A. A., *J. Pharm. Exp. Therap.*, 1947, **89**, 18.

effect of the ANTU; the animals were small and showed other signs of ANTU poisoning. At 18 months, although fewer animals were living in each group on 200 ppm, or more, ANTU than in the control group, no group had a death rate significantly different from that of the control. At the termination of the experiment the differences appeared to have a definite break at 200 ppm; however, because of the small number of animals the differences at 200 and 800 ppm ANTU were not significant. When the 4 groups from 200 to 800 ppm ANTU were compared jointly with the 3 groups which had an apparent normal death rate, namely, the groups on 50 and 100 ppm ANTU and the control, the difference in mortality rate was found to be highly significant.

Pathology. Autopsy was done on nearly all the 126 rats, and from 83 of them hematoxylin-eosin stained paraffin sections of various tissues were made for microscopic study. Lung, heart, liver, spleen, pancreas, stomach, small intestine, colon, kidney, adrenal, testis and thyroid were sectioned routinely. In addition, sections of the following structures were obtained from the number of animals given: Parathyroid, 39; bones and muscles of knee joint region, 31; skin, 11; lymph nodes, 10; deformed leg and/or foot, 6; eyelids, 6; urinary bladder, 3; brain, 2. Perls' reaction for ferric iron was used on a number of sections to check the nature of certain pigments.

Externally, the rats were stunted and showed spectacle eyes, thinning and coarsening of the hair with increased prominence of the guard hairs, and deformities of the legs and feet (Table II). Internally, there were no characteristic changes, but some nonspecific ones were seen, in addition to those resulting from inanition. Slight or moderate relative or absolute enlargement of the spleen was noted 14 times, most frequently (6 instances) at 200 ppm. Dark semifluid material in the stomach or dark spots on the glandular stomach mucosa were noted 28 times, which together with 4 additional instances of focal hemorrhagic necrosis discovered microscopically make up the 32 listed

TABLE II.
Incidence of the More Frequent Pathological Findings.

Dosage ANTU ppm	No. of animals	Gross findings					No. examined microscopically	Microscopic findings			
		Enucleation	Spectacle eye	Hair loss	Leg deformity	Focal stomach hemorrhages		Hyaline liver	Calcified casts in kidney	Thyroid hyperplasia	Splenic hyperplasia
800	18	13	14	9	2	9	15	5	10	9	7
600	18	8	13	8	3	8	13	5	7	7	8
400	18	7	15	13	3	9	12	2	2	4	8
200	18	5	12	2	0	3	12	3	1	2	7
100	18	1	2	0	0	1	10	0	0	1	2
50	18	1	0	0	0	1	15	1	1	1	5
0	18	1	0	0	0	1	6	0	0	0	0

in Table II. In 7 animals, 5 of which were in the 800 and 600 ppm groups, the thyroid showed questionable or slight generalized enlargement.

The hair changes were most noticeable on the nose and on the rear half of the body and were related in degree to the dosage of ANTU. The cessation of hair growth was present in all rats surviving for the first year on 400 to 800 ppm ANTU and in 2 rats on 200 ppm. Spectacle eyes occurred in all rats on dosages from 200 to 800 ppm ANTU. Changes around the eyes were noticed first in animals on 800 ppm ANTU after about 6 months on the experimental diet, and later they occurred in the animals on lower dosage levels. The 3 instances of spectacle eyes in rats on 100 ppm ANTU appeared after 80 weeks. Sections of skin and eyelids showed mainly atrophy of the various structures. The epithelium was reduced in thickness. Hairs and sebaceous glands were reduced both in number and size. Chronic inflammatory cellular exudate was present in some of the eyelid sections, but not in the skin sections.

Hyperplasia of the thyroid was present microscopically (as judged from increased height of the epithelium and decreased amount of colloid) in about half the animals on 800 and 600 ppm ANTU, and in decreasing frequency at lower dosage levels, as shown in Table II. All the 24 instances of thyroid hyperplasia were of slight degree, except for 3 of slight-moderate and 1 of moderate degree. In a few instances there were papillary ingrowths and heapings of the follicular epithelium which might indicate that the present slight degree of hyperplasia had been somewhat greater at some time in the past.

Microscopic hyperplasia of the splenic pulp (Table II) was of moderate degree in 13 instances and slight in the remaining 24, with the moderate degree more frequent at the higher than at the lower dosage levels. It is probable that parallelism of splenic hyperplasia with dosage of ANTU is not more marked because of counteraction of hyperplasia at the higher dosage levels by inanition, which tends to cause hypoplasia (atrophy) of the spleen. In 24 instances, all at 200 ppm or above, a relative increase in the percentage of myeloid cells in the splenic pulp was noted; these spleens included atrophic as well as hyperplastic ones.

Hyaline change in the cytoplasm of the centrolobular hepatic cells (Table II) was noted in 16 instances, all graded as slight or minimal in degree except for 2 of moderate and 1 of slight-moderate degree. The lesion consisted of increased oxyphilia and slight to moderate hyalinization of the cytoplasm of the centrolobular hepatic cells, with a minimal increase in cell size. It resembled the lesser degrees of the typical lesion in the rat liver caused by the feeding of DDT,[5] but did not show the peripheral segregation of large basophilic cytoplasmic granules and the distinct increase in cell size present in many of the DDT rats. Otherwise, we have not previously noted this lesion. Two rats showed focal necrosis of the liver, an incidence to be expected even in untreated animals. There was no vacuolation of the hepatic cells to indicate hydropic or fatty degeneration.

A slight or even moderate decrease in the thickness of the adrenal cortex was seen in most animals at the higher dosage levels, and less frequently with decreasing dosage level through the 200 ppm group; it was not distinct below that level. There did not appear to be a decrease in the number of cortical cells; the cells were simply smaller than usual, having lost most of the foaminess of the cytoplasm normally present in greater or lesser degree. Fat stains were not done. This change has not been noted previously in simple chronic inanition. The adrenal medulla was not altered.

Calcified tubular casts were found in the renal medulla in very small to moderate numbers (numerous in 2 instances at 800 ppm) and their incidence was proportional to dosage, as shown in the table. They first appeared at 35 weeks. They caused little or no renal tubular destruction. The kidneys otherwise showed nothing of note.

[5] Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. G., Smith, R. B., Jr., and Calvery, H. O., *Pub. Health Rep.*, **1944, 59, 1009.**

Foci of superficial terminal hemorrhagic necrosis of the glandular mucosa of the stomach are rather frequently seen in rats found dead, especially after a short period of treatment (*i.e.*, with a highly toxic agent). However, the incidence (Table II) in ANTU rats at the higher dosage levels was greater than usual, and the lesions were found in sacrificed animals as well as in those found dead. A sharp drop in incidence below 400 ppm will be noted. The lesion was generally not massive.

Bone changes (upper half of tibia and lower half of femur), apart from the peculiar deformities noted below, consisted in an increase in the number of trabeculae of spongy bone, an increase to a lesser extent in their thickness, and irregularity and slight thinning of the cortical bone. Sometimes a small portion of the spongy bone had the appearance (increased oxyphilia and decreased density) of being newly formed. In some animals small to moderate amounts of fibrous tissue surrounded some of the spongy bone and encroached on the marrow. Few osteoblasts or osteoclasts were seen, suggesting a slow tempo or chronic nature of these changes.

The changes in the 31 bone marrows sectioned were complicated by the associated ones in the bone, but generally speaking slight myeloid hyperplasia was frequent at the higher dosage levels, less frequent at 200 ppm, and absent below that level. On the whole the marrow in the epiphyses was not as hyperplastic as that in the shafts. The remarks about inanition in connection with the spleen also apply here. The ultimate cause of the hyperplasias in the spleen and bone marrow is uncertain.

Deformities of the legs and feet were noted after the 40th week in 8 animals receiving 400 or more ppm ANTU. There were contractures of the extremities, so that the affected animals had the appearance of walking on their heels and elbows, combined with twisting of the feet or legs; sometimes the paws would face backwards. Chronic granulomas of the feet were more severe and the incidence was distinctly higher in treated animals than usually have occurred in similar experiments with other substances.

Structures unchanged from their appearance in the controls by the feeding of ANTU were lung, heart, lymph nodes, proventriculus, small intestine, colon, and parathyroid. Pancreas and testis differed only in showing nonspecific changes from inanition. Pigmentation in spleen and adrenal was as generally seen in adult rats. The incidence of middle ear infection, chronic pneumonia, and various spontaneous tumors was not increased.

Discussion. Both the hair changes and the spectacle eyes generally are considered to be caused by nutritional deficiencies. Richter[1] has reported that feeding cystine prevents the cessation of hair growth. Circumocular alopecia, or the "spectacle eye condition," has been reported to occur in biotin deficiency[6] and in inositol deficiency;[7] however, this condition often is associated with dermatitis elsewhere and is not necessarily a sign of a single deficiency of a member of the vitamin B complex.[8] Many of the rats on the higher dosages of ANTU showed inanition to a marked degree, and therefore complex nutritional deficiencies were probably present.

Destruction of bone and joint tissues in the feet and lower legs by suppurative inflammation and the reactive processes consequent thereto appeared to be the underlying cause of the deformities of the legs and feet. The subsequent contractures and twisting are accounted for in the same way that they occur in human cases unless they are prevented. The chronic granulomas of the feet, which are occasionally seen in our rats, presumably resulted from the trauma of the cage wires. It seems reasonable to assume that the various nutritional, endocrine and osseous upsets produced by feeding ANTU caused the tissues of the feet to be a more favorable location than they are usually for

6 Nielsen, E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 349.

7 Paveek, P. L., and Baum, H. M., *Science*, 1940, **92**, 502.

8 Sullivan, M., and Nicholls, J., *Arch. Der. and Syph.*, 1942, **45**, 917.

bacterial growth. Except where involved by adjacent inflammatory processes, the muscles showed surprisingly little change. The lack of muscle changes rules out to some extent a vitamin E deficiency.

Summary. When ANTU was fed chronically to rats for 2 years in concentrations of 50 to 800 ppm in the diet, the concentrations from 100 to 800 ppm were toxic. Externally, the rats were stunted and showed spectacle eyes, thinning and coarsening of the hair and deformities of the legs and feet. Histopathological changes consisted of hyperplasia of the thyroid, hyperplasia of the splenic pulp, hyaline changes in the cytoplasm of the centrolobular hepatic cells, a slight to moderate decrease in the thickness of the adrenal cortex, calcified tubular casts in the renal medulla, slight myeloid hyperplasia of the bone marrow, and slight modifications in bone structure. The degree of injury in each case was related to the concentration of the ANTU. The concentrations of 600 and 800 ppm ANTU produced a marked tolerance.

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Separation of Two Mutually Antagonistic Chromatophorotropins from the Tritocerebral Commissure of *Crago*.

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From the time of Koller's original contention that he had discovered in the rostral region of the thorax of the shrimp, *Crago*, a new endocrine source, producing a principle which blackened white-adapted specimens,[1] repeated attempts to confirm this conclusion have been generally unsuccessful at the hands of other investigators. More recently it has been shown that the circumoesophageal connective region,[2] or more specifically the tritocerebral commissure, of *Crago*[3] possesses very potent chromatophorotropic activity. Extracts of the commissures in sea-water, injected into eyestalkless specimens produce within 5 minutes striking lightening of the body and simultaneously blackening of the tail-fin (telson and uropods). It has been postulated upon the basis of such observations as the following, that the tritocerebral commissure of *Crago* possesses 2 mutually antagonistic principles for general body-coloration: (1) extracts of commissures from different individuals show variation in their relative influences in body-lightening and tail-fin-darkening, at times even darkening the body instead of lightening it,[3] (2) stimulation of the stubs of the eyestalks in eyestalkless animals results in body-lightening and tail-fin-darkening when the stimulus is strong, and, overall blackening if the stimulus is weak,[4] (3) comparative studies of crustacean nervous systems show some to possess only body-lightening activity (*e.g.* the crab, *Uca*), others predominantly body- and tail-fin-darkening activity (*e.g.* the lobster, *Homarus*).[5]

A clear proof, however, that the observed results of the action of extracts of *Crago* tritocerebral commissures are due to 2 hormones (1) a body-blackening hormone, and (2) an antagonistic body-lightening one obviously requires the chemical fractionation of the *Crago* commissure into 2 portions, each possessing only one of these 2 actions.

1 Koller, G., *Z. vergl. Physiol.*, 1928, **8**, 601.

2 Brown, F. A., Jr., and Ederstrom, H. E., *J. Exp. Zool.*, 1940, **85**, 53.

3 Brown, F. A., Jr., *Physiol. Zool.*, 1946, **19**, 215.

4 Brown, F. A., Jr., and Wulff, V. J., *J. Cell. Comp. Physiol.*, 1941, **18**, 339.

5 Brown, F. A., Jr., and Saigh, L. M., *Biol. Bull.*, 1946, **91**, 170.