have had experience with one male dog which, even at doses producing polydipsia and polyuria as well as negative nitrogen balance, exhibited no change in circulating eosinophil level. Also the sequence in which the various changes (eosinopenia, negative nitrogen balance, polydipsia and polyuria) usually occur would suggest a differential sensitivity of each of these responses to dose.

Summary. 1. In dogs large, prolonged, oral doses of Meticorten induced polydipsia, polyuria, and negative nitrogen balance. 2. Blood glucose levels and glucose tolerance curves were unaffected by the treatment, and no evidence of albuminuria and glycosuria was obtained.

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Protective Action of Glutamine, Cysteine and Other Substances Against Experimental Lathyrism in the Rat. (22325)

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Although the toxic action of sweet peas, *Lathyrus odoratus*, is known to be due to β-aminopropionitrile (occurring as its γ-glutamyl derivative in the sweet pea)(1,2,3), the specific metabolic disturbance caused in the rat by the ingestion of this substance is still undetermined. It is to be expected that discovery of a specific compound having a protective action against sweet pea toxin will aid in revealing the metabolic site at which the toxin acts. The finding of a protective agent may also be of considerable medical importance because of the possible relationship between odoratism (sweet pea lathyrism) and a number of human diseases of unknown etiology(4,5).

Methods. Male, albino, weanling rats, 24-28 days of age and weighing 48-64 g were used in all experiments. All rats were Sprague-Dawley (Holtzman) strain. Because all young rats receiving substantial amounts of sweet pea meal in their diets rapidly develop lathyrism without exception, screening tests for protective agents can be conducted with a relatively small number of rats. For screening purposes, therefore, supplements were usually fed to 3, but sometimes 2, rats at each level. *Diets.* In a few of the earlier experiments the basal diet contained 50% sweet pea meal* and 25% corn starch and was made up as previously described(6). In most of the screening tests, however, the basal diet contained 30% sweet pea meal and 45% corn starch. Supplements were incorporated by replacing an equivalent amount of starch. All diets contained 10% casein (not vitamin-free) except as noted below. In a few instances supplements were added to Rockland Rat Diet containing 0.1% of synthetic β-aminopropionitrile hydrochloride.† Since high levels of casein exhibit some protective action(8,9, 10) the following device was used with supplements which appeared to have anti-lathyrismic properties. Five per cent of supplement was incorporated into a diet containing 30%

* Sweet peas were generously supplied by Mr. Raymond H. Coulter of Ferry-Morse Seed Co.

† β-aminopropionitrile was synthesized by the method of Buc(7), then converted to hydrochloride and crystallized from 95% alcohol.

sweet peas and 30% casein. Rats receiving this diet were compared to rats receiving unsupplemented diet containing 30% sweet peas and 35% casein. If the supplement had appreciable protective activity, it could be expected to cause improvement above that produced by the increment of casein which it replaced.

Evaluation of Supplements. The animals were weighed weekly or oftener. Observations of muscle tone, activity, etc. were made at frequent intervals. The primary criteria for determining the severity of the advancing lathyrism, however, were evaluations of skeletal changes. These were made by palpation of mandibles, spine, and thorax(8). Levels of supplements which cause decreased growth rate sometimes appear to have protective action due to the fact that, other things being equal, the more slowly growing animal develops skeletal deformities at a slower rate. For this reason it was necessary to differentiate between a more slowly developing lathyrism due to decreased food consumption and a positive protective action. Because assessment of severity of the symptoms was subjective, and because there is some individual variation in the rate of progress of the disease, only those supplements which caused an unquestioned improvement definitely outside the symptom complex bracketed by control animals were considered to exert any protective action. Supplements which appeared to be beneficial, but caused an improvement which could conceivably lie within extremes of individual variation, were fed to more rats at the same level as before and at higher levels. Supplements which were found toxic at the levels fed (severe growth inhibition or death) were re-tested at lower levels.

Results. Partial protection shown by high levels of *protein* (casein and gelatin) have already been reported(8). Casein hydrolysate (enzymatic) fed at a 30% level (with 10% casein) showed some protective activity, although it proved to be less protective than casein itself. Lactalbumin was almost equal to casein in protective activity. Zein exerted no protective action. Zein at 30% (with 10% casein), in spite of its amino acid deficiencies, had no growth-inhibiting effect; its effects on growth and on development of lathyrism could not be distinguished from that of starch.

When 5% *L-cysteine-HCl* was incorporated into the basal diet, some inhibition of growth resulted. A 4% level permitted growth comparable to that obtained with the unsupplemented basal diet. Delayed development especially of mandibular exostoses could readily be distinguished by palpation for the first 2 or 3 weeks after weanling rats were placed on diet. This protective action was temporary, however, for at 3 to 4 weeks cysteine-fed rats were no better off than controls, *i.e.*, cysteine did not decrease eventual severity of the skeletal changes but only delayed their onset. At 2.5% cysteine-HCl gave only very slight, barely perceptible, protection. When L-monosodium glutamate (10%), L-monosodium glutamate plus glycine (10% + 5%), L-leucine (10%), L-lysine-HCl (10%), glucosamine-HCl (5%), or pyridoxine-HCl (0.5%) were added to the basal diet containing 4 or 5% of L-cysteine-HCl, no protection over that due to cysteine was obtained. A diet containing 5% cysteine-HCl plus 30% casein gave somewhat better protection than a diet containing 35% casein without added cysteine. The high level of casein in this diet overcame the growth-depressant effect of cysteine. A level of 5% of cysteine-HCl gave significant protection also to rats receiving 0.1% of β-aminopropionitrile-HCl in Rockland Rat Diet, although the growth-depressant effect of the cysteine was here again in evidence. Addition of 5% *L-glutamine* to basal sweet pea diet also caused delay in onset of skeletal changes. In contrast to the growth-depressant effect of cysteine, glutamine had a growth-stimulating effect. The protective action of glutamine was of longer duration than that of cysteine. Five weeks after beginning of experiment, although skeletal deformities by this time were marked, rats receiving glutamine were not only larger than control rats, but their fur was sleeker, they had better muscle tone and were more active. A 10% level of glutamine gave no more protection than did a 5% level. A diet containing 5% glutamine plus 30% casein seemed to give slightly better protection than

a diet containing 35% casein without added glutamine, however the beneficial effect of glutamine in this diet was not clear-cut. Glutamine (5%) added to Rockland Rat Diet containing 0.1% β-aminopropionitrile delayed skeletal changes during the first 2 weeks of the feeding trial. After 3 weeks on this diet, however, there was no detectable difference between these rats and rats receiving the same diet without added glutamine. Rats receiving Rockland diet containing the aminopropionitrile grew better than rats receiving sweet pea diet. Glutamine had no growth-stimulating effect in the Rockland diet.

Addition of 5% L-cysteine-HCl plus 5% L-glutamine to basal sweet pea diet had growth-stimulating effect, but protection against skeletal changes was no greater than with either of these supplements alone. Pyridoxine-HCl seemed to vitiate the beneficial effects of glutamine; rats receiving 5% L-glutamine plus 0.5% pyridoxine did no better than rats receiving the unsupplemented basal ration.

The following *amino acids* were not protective: L-lysine-HCl (10%), L-leucine (10%), L-cystine (5%), L-monosodium glutamate (up to 20%), L-glutamic acid (10%), L-ammonium glutamate (10%), L-histidine-HCl (5%-growth inhibition), DL-aspartic acid (10%—some growth inhibition), L-asparagine (10%), β-alanine (5%), DL-methionine (2%; 10%—toxic), glycine (5%), and DL-Serine (4%; 10%—toxic). The partially protective action of lysine reported by others (11) was not confirmed in this study.

The following *carbohydrate derivatives* were found to have no protective action: glucosamine hydrochloride (5%; 15%—toxic), glucuronic acid lactone (5%), chondroitin sulfate (5%), glucosamine-HCl plus chondroitin sulfate (5% + 5%), and sodium glucuronate (10%). Since there was some question as to whether or not glucosamine-HCl exerted slight protective action at the 5% level, and because cysteine was later found to be somewhat protective, glucosamine-HCl and L-cystine HCl (5% + 5%) were fed together. Glucosamine did not provide any protection above that due to cysteine.

None of the following *vitamins* gave any protection: pyridoxine-HCl (0.5%), Ca pantothenate (various levels up to 5%), L-ascorbic acid (50 mg/100 g), Ca pantothenate plus L-ascorbic acid (20 mg + 50 mg/100 g). Liver powder (1:20, GBI) at 4% level plus added pantothenate and ascorbic acid was ineffective.

The following *miscellaneous supplements* gave no protection: monoethanolamine-HCl (up to 10%—levels of 5% and above caused severe growth inhibition), taurine (5%), sodium acetate (5%), sodium propionate (5%), sodium pyruvate (1.5%), betaine (1%), brewers yeast (15%; 30%—toxic), ribose nucleic acid (10%), sodium glycollate (2%), sodium thioglycollate (up to 1%—growth inhibition), sodium thiocyanate (up to 0.5%), ammonium chloride (4%), prostigmine bromide‡ (up to 3.5 mg/100 g), glutathione (100 mg of monosodium salt/day by subcutaneous injection), and cortisone acetate (0.5 mg/day by subcutaneous injection). An alcoholic extract of casein was prepared by continuously extracting casein with 95% ethyl alcohol for 8 hours. When this extract was added in amounts equivalent to 100 g of casein per 100 g of basal diet (modified to contain 30% casein) no additional protection was obtained.

Discussion. The metabolic site at which sweet pea toxin acts is still obscure. That the metabolism of sulfur is in some way involved seems likely. Protein synthesis itself does not seem to be disturbed, since the animals grow relatively well until the terminal phase of the disease is approached and since no toxic lesions can be demonstrated in liver, kidney or other organs(4,13).

Glutamine also appears to be involved in the metabolic defect. Because glutamate does not seem to be able to substitute for glutamine, it might be inferred that the presence of the amide group of glutamine is critical. However, the interference is not merely one of γ-amidation of glutamate, because methionine sulfoxide, which is known to interfere with γ-amidation of glutamic acid, does not give

‡ Prostigmine bromide was generously supplied by Dr. M. J. Schiffrin, Hoffmann-LaRoche, Inc. Prostigmine methylsulfate has been reported to be effective in treatment of human lathyrism(12).

rise to a syndrome comparable to odoratism even when administered to rats in large doses (rats fed 1% methionine sulfoxide grew normally).§ A clue to the problem may lie in recent publications of Boström, Rodén, and Vestermark(14,15) who reported that glutamine, but not glutamic acid, appears to act as an accelerator of chondroitin sulfate synthesis and probably plays a part in biosynthesis of mammalian mucopolysaccharides. In this connection, it has been pointed out repeatedly that sweet pea lathyrism in the rat seems specifically to involve mesodermal tissues and that the metabolic defect appears to be localized in the ground substance, which is rich in mucopolysaccharides (*cf.*, for example, 4 and 16).

Conclusions. Of a wide variety of supplements tested for their protective action against development of skeletal deformities in rats fed sweet peas or β-aminopropionitrile, none gave complete protection. Only L-cysteine-HCl, L-glutamine, and proteins were partially protective. Of the proteins tried, casein, gelatin, lactalbumin and casein hydrolysate gave some protection; zein did not. The protective action of glutamine may be related to its probable role in chondroitin sulfate and mucopolysaccharide synthesis.

Note added in press. Experiments completed since this paper was submitted for publication indicate that the protection afforded by L-cysteine-HCl is prolonged if the diet is made up fresh weekly. Under these conditions the protection against development of skeletal changes was greater with cysteine than with glutamine. The decreased protective activity of L-cysteine in an aging diet is probably due to a gradual loss of cysteine because of the lability of its sulfhydryl group.

§ Unpublished data.

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Relation of Brain Stem to Renal Electrolyte Excretion.* (22326)

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Experimental evidence that a cerebral lesion might affect formation of urine was first presented by Claude Bernard. He produced polyuria without glycosuria in the experimental animal by puncturing the median eminence of the floor of the fourth ventricle

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