the Mickle tissue disintegrator and the electron microscope, was described for the separation of the cell wall and the internal protoplasm from the cells of the yeast phase of *Histoplasma capsulatum*. The cell wall contained the antigenic activity associated with complement fixation, protection and toxins. Sensitized animals, on the other hand, showed a delayed type of reaction to either fraction. The protoplasm was more active than the cell wall in the hemolysis of guinea pig erythrocytes.

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## Reproduction in Rats Fed *Lathyrus* Peas or Aminonitriles. (22013)

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Growth disturbances produced by feeding *Lathyrus* peas vary inversely with age and stage of development of the experimental animal(1,2). Profound effects might be expected, therefore, during rapid growth and development of the fetus. Turney, Salmon, and Copeland reported that feeding *Lathyrus hirsutus* peas prevented completion of pregnancy in the rat, but they did not completely describe the nature of the disturbance(3). They were unable to prevent the effect of peas by addition of alpha-tocopherol to the diet(4), although it has since been reported that large doses of that compound give partial protection against lathyrism(5). For these reasons the effect of *Lathyrus odoratus* peas was studied in regard to reproduction. During this investigation, isolation of the active principle of *Lathyrus pusillus* was announced(6). A similar isolation product of *Lathyrus odoratus* was identified as ($\beta$-L-glutamyl-amino) propionitrile(7). The characteristic activity in producing skeletal lesions in rats was also shown by $\beta$-amino-propionitrile(1). This compound also profoundly influenced development of larval frogs(2). Aminoacetonitrile was later found to be more toxic to weanling rats than any compound previously tested(8). A number of aminonitrile compounds were consequently included in this study.

*Methods.* Albino rats of Sprague-Dawley strain were used predominantly. The few hooded rats of Long-Evans strain used gave identical results. The basic diet was ground (Rockland) rat pellets. *Lathyrus odoratus* seeds were finely ground, mixed with this diet in various proportions, refrigerated at 5°C, and fed *ad libitum*. Diets containing syn-

thetic nitrile compounds were stored at –40°F. Feedings were given once daily, and food remaining after 24 hours discarded. All animals were kept on wire-mesh floors. The 50% pea diet produced skeletal deformities and fatal aortic rupture comparable to that reported by Ponseti and Baird(9). Females were first mated when 10-12 weeks of age, and discarded after 3 or 4 matings. Matings were determined by vaginal smear. Specimens for histological examination were fixed in 4% formalin and stained with hematoxylin and eosin.

*Results.* Mature rats of both sexes maintained on 50% pea diet remained in good health for several months. Two male rats placed on this regimen at 6 weeks of age developed extensive skeletal deformities within a few weeks, but accomplished numerous fertile matings between 3 and 9 months. When sacrificed at 10 months they were moderately emaciated and almost helplessly crippled, but showed active spermatogenesis, without testicular atrophy. A third male, started on 50% pea diet at 3 months, was moderately crippled but still potent and fertile when sacrificed 10 months later. This male also showed histologically normal testes. The numerous offspring obtained by mating these males with normal females showed no abnormalities.

The 50% pea diet given to nursing female rats produced no evident toxic effect. In one case this diet was given to the dam of 6 young, beginning on the 3rd day after parturition. In 2 instances this diet was begun on the 20th day of pregnancy, and continued until litters were weaned. Large litters were born, of which 3 in one litter and 5 in the other survived the early neo-natal period. The high peri-natal mortality was typical of results obtained when pea diets were given late in pregnancy, and will be discussed in detail later. The nurslings of all 3 litters made normal weight gains, with no physical or x-ray evidence of skeletal deformity at weaning at 22 days. Three males and 3 females given 50% pea diet after weaning quickly developed severe skeletal deformities, and 2 animals of each sex died of ruptured aortic aneurisms within a month. Those given control diet grew to normal maturity.

Mature female rats on 50% pea diet passed through normal estrous cycles, and mated freely. Conception occurred in a high percentage of matings, with bloody vaginal "placental sign" on about the 13th day. Weight gains were comparable to control animals until the 19th or 20th day, but then weight rapidly decreased, and no living young were born. Pregnant females sacrificed on or before 19th day of pregnancy contained living fetuses, but during the succeeding 3 days there was death and rapid decomposition of all unborn young. Passage of dead fetuses or placental tissue was seldom observed, although vaginal bleeding was often seen at the 22nd or 23rd day. Animals killed within a few days after this time showed involuting uteri with numerous placental sites. Those kept on 50% pea meal soon displayed normal estrous cycles culminating in fertile matings. If the control diet was then given, normal gestations resulted.

TABLE I. Effect of 50% Pea Meal.

| No. rats | Days of gestation diet given | Avg No. viable young |
|---|---|---|
| 9 | 1-22 | 0 |
| 4 | 1-16 | 8.5 |
| 6 | 1-17 | .67 |
| 4 | 16-18 | .25 |
| 6 | 19-22 | 0 |
| 7 | 20-22 | 4 |
| 4 | 21-22 | 12 |

The final few days of gestation constituted the critical period for exhibition of the toxic effect of the pea (Table I). Normal litters were born to females on 50% pea diet during the first 16 days of pregnancy, but giving this diet for one more day resulted in death of almost all fetuses. Giving the diet for as short a time as 3 days after the 16th day was also highly toxic for the unborn young.

Diets containing 20% *Lathyrus odoratus* meal were lethal for the rat fetus, if begun by the 17th day of gestation (Table II). The

TABLE II. Percentage of Lathyrus Pea Meal Begun 17th Day of Gestation.

| No. rats | % pea meal | Avg No. viable young |
|---|---|---|
| 9 | 50 | 0 |
| 8 | 20 | .5 |
| 2 | 15 | 2 |
| 3 | 10 | 10 |

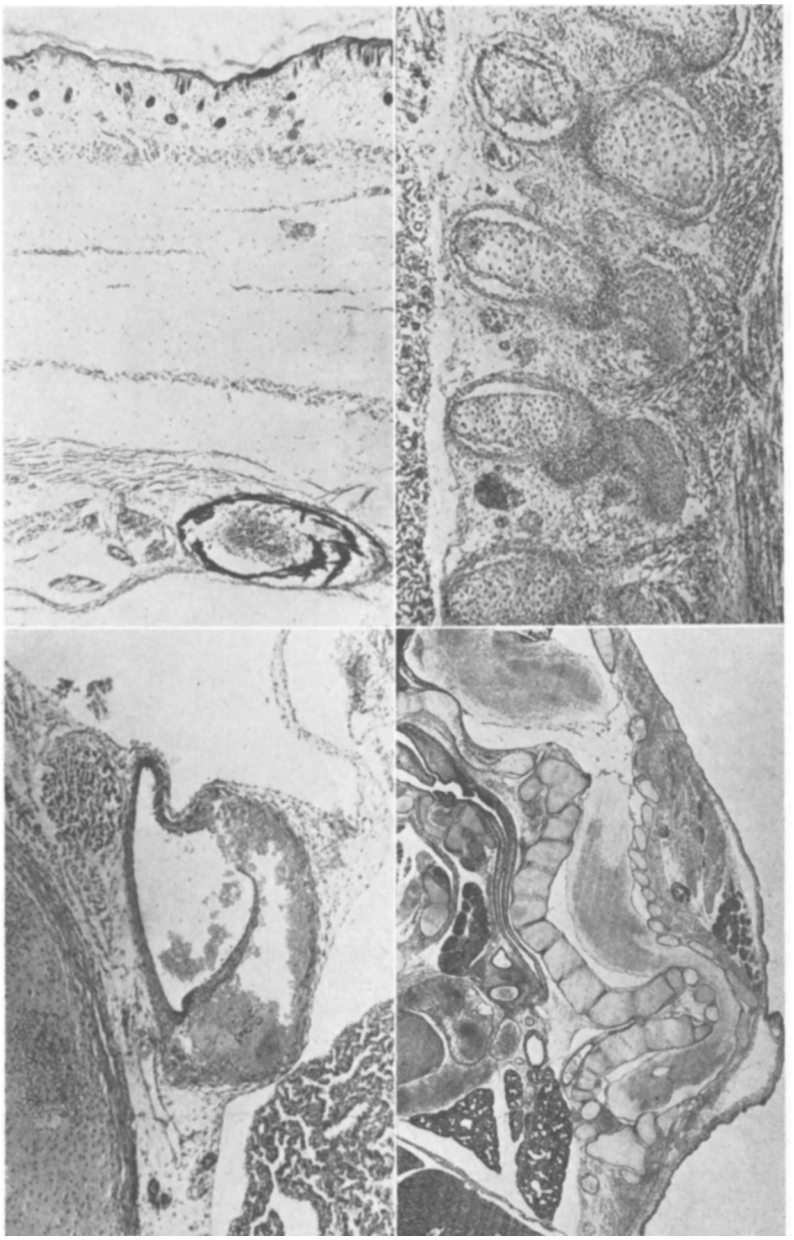

FIG. 1 (top left). Microphotograph, posterior thorax of still-born rat. Includes tissues from skin surface to parietal pleura, with cross section of rib, lower right. There is extreme generalized edema, with paucity of connective tissue and skeletal muscle.

FIG. 2 (top right). Microphotograph, parasagittal section, thoracic spine of 19-day rat fetus, alive at time of sacrifice of dam, who had received 50% pea diet from first day of pregnancy. Note lack of cohesion between cartilaginous spinal structures and perichondrial connective tissue.

FIG. 3 (bottom left). Microphotograph, cross section of dissecting aneurism, thoracic aorta of still-born rat whose dam received 50% pea diet from 17th day of pregnancy. The intima is ruptured and a large mass of blood has forced apart the layers of the media. Complete rupture of the vessel, with massive hemothorax, has not taken place in this case.

FIG. 4 (bottom right). Microphotograph, mid-sagittal section, 19-day rat fetus, litter-mate of fetus shown in Fig. 2. There is acute angulation of the lower thoracic and lumbar spine, with apparent compression of the spinal cord. Diffuse hemorrhage is seen, particularly about great vessels at base of heart. The fetus was alive at time of sacrifice of the dam.

TABLE III. Toxicity of Aminonitriles Begun 17th Day of Gestation.

| Compound | % in diet | No. rats | Avg No. viable young |
|---|---|---|---|
| Aminoacetonitrile* | .0167 | 4 | 0 |
| | .0125 | 10 | 3.1 |
| β-aminopropionitrile | .3 | 3 | 0 |
| | .1 | 9 | 0 |
| | .05 | 6 | 0 |
| | .025 | 9 | 6.2 |
| Bis (β-cyanoethyl) amine | .1 | 3 | 0 |
| | .05 | 2 | 10 |
| Tris (β-cyanoethyl) amine | .3 | 3 | 8 |
| β-dimethylaminopropionitrile | .3 | 2 | 9 |
| β-mercaptoethylamine | .5 | 5 | 3.4 |
| | .25 | 3 | 5.7 |

* Fed as a salt of $H_2SO_4$.

15% level was incompletely lethal, and lesser concentrations apparently harmless. At 20% level many young were born dead, with little evidence of decomposition, while others were feebly alive at birth, but succumbed soon thereafter. Both living and dead were fragile and hydropic in character (Fig. 1). Muscles and connective tissues were poorly developed. There was scanty formation of collagen, and cohesion of various skeletal and connective tissues appeared faulty (Fig. 2). Poor vascular tone, with capillo-venous dilatation and diffuse hemorrhage was commonly seen. Massive hemothorax from aortic rupture was a frequent demonstrable cause of death (Fig. 3). The dissecting aortic aneurisms followed the same pattern seen in weanling rats(1,9). Severe spinal deformities seen in several instances included kypho-scoliosis similar to those typically produced in weanling rats on *Lathyrus* pea diets (Fig. 4).

Several nitrile compounds were toxic for the rat fetus (Table III). Their effect appeared to be qualitatively identical with that of pea diets. Fetal death was readily produced by feeding diets containing β-aminopropionitrile, bis (β-cyanoethyl) amine, or aminoacetonitrile during the latter part of pregnancy. Aminoacetonitrile was the most toxic of these compounds, as shown by Wawzonek and coworkers(8). Lesions produced by these compounds mimicked those produced by pea meal diets, including a high incidence of aortic rupture with hemothorax. As with pea meal diets, little toxicity was shown during the first 16 days of gestation. Thus, normal litters were born to female rats fed a diet containing 0.1% β-aminopropionitrile during the first 16 days of pregnancy, whereas diets with 0.05% of this compound proved uniformly lethal to fetuses when given beginning the 17th day of pregnancy (Table III). It is perhaps significant that the obvious developmental defects produced involved mesodermal tissues, and that development of these lesions occurred during the period of rapid mesodermal growth and differentiation of late fetal life. A possible difference in placental permeability in regard to the toxic compounds might also account for differences in toxicity shown during early and late fetal life.

Two nitrile compounds tested, tris (β-cyanoethyl) amine and β-dimethylaminopropionitrile, showed no toxicity at concentrations used. These results are in accord with previous reports of the detoxifying effect of methylation or acetylation of aminonitrile compounds(10). The final compound listed in Table III, β-mercaptoethylamine, while not an aminonitrile, has produced skeletal growth disturbances typical of lathyrism(11). This compound was found to be relatively innocuous for the pregnant rat and her unborn young, although at comparatively high concentrations many fetuses did die *in utero*. None of these fetuses, however, showed skeletal deformity, ruptured aortae, or other lesions characteristic of lathyrism.

*Summary.* *Lathyrus odoratus* peas and certain aminonitrile compounds chemically related to the active extract of the pea, when added to the diet of pregnant rats, caused death of the fetal rat late in intrauterine life. Death was associated with poor development of the skeleton and other mesodermal tissues, and commonly resulted from rupture of the thoracic aorta at or near the time of birth. No harmful effects on the fetus were demonstrated by feeding these substances to the pregnant female before the 17th day of gestation. *Lathyrus odoratus* peas in 50% dietary concentration did not affect fertility of the adult male or female rat, and males maintained on the diet for as long as 10 months showed no histological evidence of testicular atrophy.

No toxic substance was demonstrated in the milk of nursing females on the pea diet. The feeding procedure employing pregnant female rats proved to be a reasonably accurate and sensitive method for the toxicity assay of aminonitriles. Aminoacetonitrile was the most toxic of the compounds tested.

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## Effect of Diet on Betaine-Homocysteine Transmethylase of Rat Liver.* III. B-Vitamins Other Than Vitamin $B_{12}$. (22014)

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In the rat, a dietary deficiency of vit. $B_{12}$ causes a decrease in the betaine-homocysteine transmethylase activity of the liver(1-4). The addition of a purified "coenzyme" preparation of the transmethylase, to liver homogenates prepared from rats fed a diet deficient in vit. $B_{12}$, has recently been shown to restore the transmethylase activity(4). To ascertain whether this effect is specific for vit. $B_{12}$ the effect of a deficiency of each of the other B-vitamins on the transmethylase activity of rat liver has been investigated.

*Methods.* Albino rats of the Sprague-Dawley strain were used throughout the investigation. The rats were kept in individual cages and were fed *ad libitum*, unless otherwise stated. The complete vitamin mixture used for the various basal diets described below provided in mg per 100 g of ration: thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1; calcium pantothenate, 2; pyridoxine, 0.25; folic acid, 0.02; biotin, 0.01; vit. $B_{12}$, 0.002; inositol, 10; and choline chloride, 150. Each week each rat was given orally 2 drops of halibut liver oil fortified with vit. E and K (5). For the deficient diets the vitamin under study was omitted and in some cases succinyl sulfathiazole ("sulfasuxidine") or the corresponding antivitamin was added to the diet as described in the tables of results. All diets contained 4% of salts 4(6) and 5% of corn oil. The basal diets contained the following percentages of protein, sucrose and supplementary amino acids: Diet I. Casein 25, sucrose 65.8. Diet II. Casein 25, DL-methionine 0.2, sucrose 65.6. Diet III. Casein 12, DL-methionine 0.2, DL-threonine 0.36, sucrose 78.2. Diet IV. Egg white 24, sucrose 66.8, the biotin content of the complete diet was increased to 0.1 mg per 100 g of ration. Diet V. Casein 9, gelatin 12, sucrose 69.8.

At the end of the experimental period the rats were killed by decapitation and were bled, and their livers were removed immediately and chilled in ice. Pooled homogenates

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