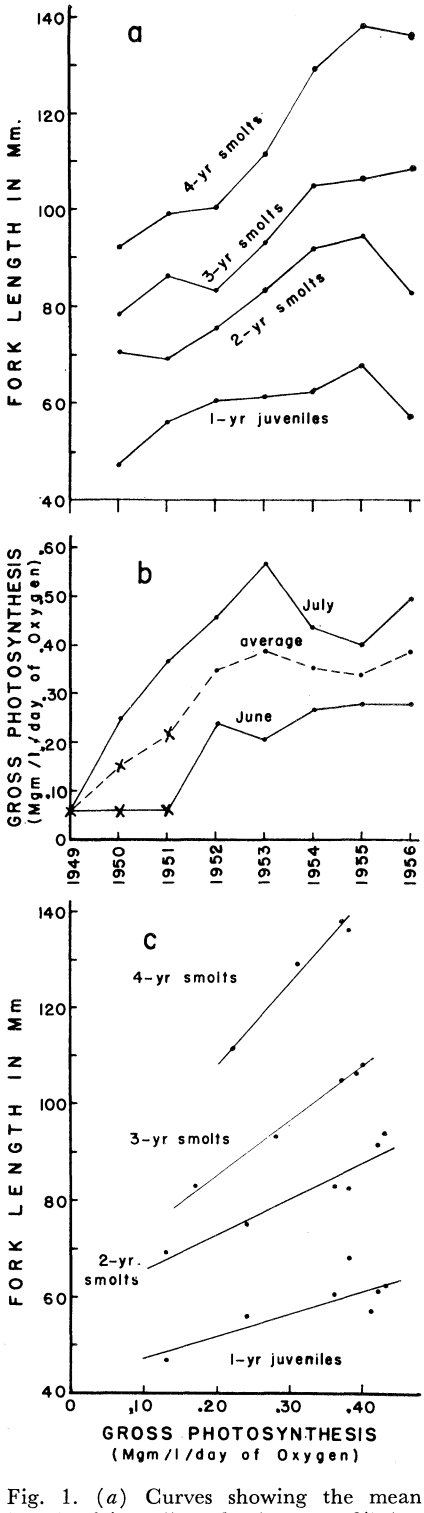


Fig. 1. (*a*) Curves showing the mean length of juvenile red salmon on 27 August of their first growing season and of red salmon smolts migrating to sea in the beginning of their 2nd, 3rd, and 4th year of life for the years 1950–56. (*b*) Curves showing the mean rate of gross photosynthesis during the years 1949–56 for the 40-day periods following the June and July fertilization. Also presented is a curve of the average of the two periods. Points on the curves marked by x's denote the values are estimated or partly estimated. (*c*) Scatter diagrams showing the relation between gross photosynthesis and fork length for each age group of fish. Regression lines are drawn by inspection.

in nearby unfertilized Karluk Lake. Because of the long life cycle of red salmon, data are not yet sufficient to demonstrate whether fertilization has increased the fresh-water survival.

The rate of growth of fish is very sensitive to influence by the food supply. Although plankton and bottom fauna have been sampled regularly, the time-consuming censuses have not yet been completed. However, data are available on the primary productivity as measured by the rate of photosynthesis of the phytoplankton (Fig. 1*b*). These measurements were made by the method originally described by Gaarder and Gran (*2*).

No actual determinations of rate of photosynthesis were made in 1949, but a few determinations were made prior to the July fertilizations of 1950 and 1951, years when the lake was not fertilized in June. On the basis of measurements made during those periods (*1*), it is believed the mean rate of oxygen production would not have exceeded 0.12 mg/liter per day and may well have been about 0.06; the latter figure is plotted as the rate during 1949 and during June of 1950 and 1951. Following the 1951 season the same amount of fertilizer was used as before, but it was applied during two periods, June and mid-July.

A cursory comparison of the curves of seasonal rate of photosynthesis and size of fish reveals a certain correspondence between them (Fig. 1*a* and *b*). To show the relation more clearly, diagrams were made (Fig. 1*c*). It was thought that three periods in time would be of importance in affecting the population size and growth of the new crop of insect larvae hatching in early summer and which would be fed upon by the young juvenile red salmon that had hatched earlier that spring. The period following July fertilization of the year prior was considered important to the survival of the brood stock of insect larvae which was to produce the new generation to be utilized by the fish. Periods following both the June and July fertilizations would influence the growth and survival of the newly hatched larvae. Thus, in Fig. 1*c* the length the first-year juvenile salmon attained each year is plotted against the mean rate of photosynthesis after the June and July fertilizations of that year and the period following the July fertilization of the preceding year. All three periods were weighted equally in establishing the mean. In a somewhat similar manner, smolt size was plotted against the mean rate of photosynthesis over those periods mostly responsible for the development of insect larvae upon which the fish feed during their lake residence.

It might be supposed, since so many steps exist between the original synthesis of food materials by the phytoplankton and growth of fish, and since fish are affected by so many environmental factors in addition to food supply, that a significant correlation would not exist. Nevertheless, the growth of smolts showed a very close relation with the rate of photosynthesis (Fig. 1*c*). The relationship with juveniles at the end of their first growing season is weaker. However, the figures indicate a much closer relation between fish growth and primary photosynthetic productivity than might have been expected a priori (*3*).

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18 March 1958

## Role of Cyanoacetic Acid in Production of Lathyrism in Rats by β-Aminopropionitrile

β-Aminopropionitrile (BAPN) is the toxic factor in *Lathyrus odoratus* meal which produces lathyrism in young rats (*1, 2*). The mechanism by which BAPN exerts such profound effects on mesodermal tissue is not known. Metabolic studies have been performed with BAPN in order to gain some knowledge concerning its toxicity. During these investigations, an acidic metabolite of BAPN was discovered in phenol extracts of rat urine. This metabolite has been isolated from the urinary phenols and crystallized (*3*). The chemical structure of the crystalline derivative has proved to be cyanoacetic acid (*4*). Following an injection of $C^{14}$ cyano-labeled BAPN into rats, 80 to 90 percent of the radioactive material is excreted within 20 hours. Approximately 40 percent of the activity is in unchanged BAPN, and 25 to 30 percent can be recovered in cyanoacetic

Table 1. Changes observed in rats following the feeding of aliphatic amines or nitriles.

| Assay | Chemical ingested | No. of rats | Wt. gain (g) | Gross alterations |
|---|---|---|---|---|
| 1 | None | 3 (0) | 2.9 | None |
| 2 | $HOOCCH_2C{\equiv}N$ | 4 (0) | 2.8 | None |
| 3 | $NH_2COCH_2C{\equiv}N$ | 4 (0) | 2.8 | None |
| 4 | None | 6 (0) | 2.6 | None |
| 5 | $CH_3CH_2NH_2$ | 6 (0) | 2.6 | None |
| 6 | $HOCH_2CH_2NH_2$ | 6 (0) | 2.5 | None |
| 7 | $CH_3CH_2C{\equiv}N$ | 6 (0) | 2.5 | None |
| 8 | $NH_2CH_2CH_2C{\equiv}N$ | 6 (4)* | 1.8 | † |

* Rats died during period of feeding.
† Gross alterations: Femur, fibrous proliferation, 6; Vertebra, kyphoscoliosis, 2; Aorta, ruptured, 3.

acid. The oxidation of BAPN to cyanoacetic acid in the rat is therefore considerable. Before the role of cyanoacetic acid in the development of lathyrism can be appraised, however, it is necessary to establish whether this compound exerts any influence on mesodermal tissue.

Young (41 to 45 g) female Sprague-Dawley rats were used. Rats in assays 1, 2, and 3 were allowed to eat rat pellets (*5*) ad libitum. Test rats received 200 mg of cyanoacetic acid or cyanoacetamide per 100 ml of drinking water each day for 7 weeks. In other assays the rats were fed a 0.3-percent concentration of the following: ethylamine, ethanolamine, propionitrile and BAPN fumarate (*6*) in a semisynthetic diet (*7*) for 7 weeks. In each assay the rats were housed in an open-bottom mesh cage. Autopsies were performed, and the organs were examined for gross changes. The alterations observed in these assays are shown in Table 1.

Neither cyanoacetic acid nor cyanoacetamide in concentrations at which BAPN produces lathyrism showed any evidence of toxicity (assays 2 and 3). Minor alterations of chemical structure in BAPN as represented by the organic amine or nitrile fed in assays 5, 6, and 7 also resulted in loss of toxicity. The incidence of skeletal deformities and aortic rupture in rats fed BAPN (assay 8) was comparable to that of previous observations (*7*).

Present studies show that cyanoacetic acid, cyanoacetamide, and propionitrile resemble other organic nitriles which do not exert any influence on mesodermal tissue (*2*). The fact that cyanoacetic acid does not affect mesodermal tissue when fed suggests that oxidation of the amine in BAPN to a carboxyl is a mechanism of detoxication. Ethylamine and ethanolamine also failed to produce lathyrism. Skeletal deformities observed in lathyrism, therefore, are not due solely to an excess of an aliphatic amine in the diet. Amines of the general type $R \cdot CH_2NH_2$ are oxidized by amine oxidase to aldehydes (*8*) and may eventually be converted to acids (*9*). Blaschko has suggested that amine oxidase might function in detoxication of some toxic amines (*10*). β-Aminopropionitrile is a toxic amine which fosters in some manner the development of skeletal deformities, herniation, and aortic rupture in young rats. The recovery of cyanoacetic acid from urine of rats adminstered BAPN suggests that amine oxidase is involved in detoxication of BAPN. Since cyanoacetic acid does not produce skeletal deformities, tissue changes observed in lathyrism are probably caused by either BAPN or cyanoacetaldehyde (*11*).

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## Dynamics of Release of Histamine from Tissue Mast Cell

It is generally acknowledged that the tissue mast cell contains histamine and heparin (*1*). This report (*2*) is concerned with a series of cytological changes that we have observed in living mast cells treated with histamine liberators. Our findings extend those previously reported (*3*) in living mast cells treated similarly. Microscopic observations and cinephotomicrographic recordings were made of the mast cells of the transilluminated mesentery of the intact, anesthetized Sprague-Dawley rat. Bright-field illumination and magnifications of 400 to 900 were employed. The experiments consisted of supplanting the oxygenated Tyrode's solution normally bathing the preparation with Tyrode's solution containing one of the following test substances: Compound 48/80 (*4*), 1:100,000; stilbamidine, 1:80 to 1:8000; protamine sulfate, 1:5000 to 1:100,000; or toluidine blue, 1:5000 to 1:200,000. All of these compounds bring about the release of histamine from the mast cell (*5*).

Prior to treatment the mast cells of the mesentery are round or spindle-shaped and densely packed with dark granules. Shortly after the introduction of any of the above test solutions there occurs a marked change in the refractile properties of the granules: they suddenly lose their dark appearance and become almost invisible. First one granule and then another reacts until all have become involved and the cell is barely discernible. Correlated with these events is a gradual swelling of the cell to about 1⅓ times its original diameter.

After toluidine-blue treatment, the nucleus of the mast cell takes on a blue color when about 50 to 80 percent of the granules have lost their dark appearance. When most or all of the granules are scarcely visible, metachromatic staining of the faded granules begins. At first a few granules stain purple, then more and more, until apparently all are so stained. As the staining of the granules proceeds, the mast cell shrinks toward its normal size. At this stage, those living cells stained with toluidine blue resemble closely mast cells in mesenteries fixed in alcohol and then stained with toluidine blue (*6*).

I consider the present findings to be indicative of significant chemical changes in the mast cell. The changes in the refractile properties of the granules are interpreted to be a manifestation of the freeing of some material from binding either within or on the surface of the granule where it is osmotically inactive. Once free, the material is osmotically active; water enters the cell and swelling results. It seems likely that the material liberated is histamine which is freed from its known binding with heparin (*7*).

The cytological changes noted here are common to a variety of treatments that cause release of histamine from the mast cell, and the time course of the changes is the same as that for histamine release resulting from such treatments (*5*). I suggest that histamine is freed from its binding with heparin because the histamine liberators have a stronger affinity for heparin than does histamine. The sequence of changes in the experiments with toluidine blue is consistent with such an interpretation; toluidine blue does not stain the granules until binding sites are made available on molecules of heparin. When the molecules of toluidine blue bind heparin, they become osmotically inactive and the cell loses water and becomes smaller in size. The movement of histamine out of the cell at this time also contributes to the loss of water and shrinking of the cell. According to the present interpretation, heparin is not lost from the mast cell treated with histamine liberators.

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