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*).

JOSEPH J. LALICH

Department of Pathology, Medical School, University of Wisconsin, Madison

References and Notes

1. W. Dasler, *Science* 120, 307 (1954).
2. S. Wawzonek *et al.*, *ibid.* 121, 63 (1955); T. E. Bachhuber *et al.*, *Proc. Soc. Exptl. Biol. Med.* 89, 294 (1955).
3. J. T. Garbutt *et al.*, *Federation Proc.*, in press.
4. S. H. Lipton, J. J. Lalich, F. M. Strong, *J. Am. Chem. Soc.*, in press.
5. Ralston, Purina Co., St. Louis, Mo.
6. β-Aminopropionitrile fumarate was kindly supplied by Abbott Laboratories, North Chicago, Ill. Ethylamine, ethanolamine, and propionitrile were purchased from K & K Laboratories, Long Island, N.Y. Cyanoacetic acid and α-cyanoacetamide were purchased from Eastman Organic Chemicals, Rochester, N.Y.
7. J. J. Lalich, *A. M. A. Arch. Pathol.* 61, 520 (1956).
8. D. Richter, *Biochem. J.* 31, 2022 (1937).
9. ———, *ibid.* 32, 1763 (1938); R. L. Hartles and R. T. Williams, *ibid.* 43, 296 (1948).
10. H. Blaschko, *Pharmacol. Revs.* 4, 415 (1952).
11. This work was supported by funds from the U.S. Public Health Serivce [grant No. A 538 (C8)].

14 February 1958

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.

DOUGLAS E. SMITH

Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois

References and Notes

1. G. P. Fulton, F. I. Maynard, J. F. Riley, G. B. West, *Physiol. Revs.* 37, 221 (1957).
2. This work was performed under the auspices of the U.S. Atomic Energy Commission.
3. H. U. Zollinger, *Experientia* 6, 384 (1950); I. Mota, W. T. Beraldo, L. C. U. Junqueira, *Proc. Soc. Exptl. Biol. Med.* 83, 455 (1953).
4. Compound 48/80, a polymer of N-methyl-homoanisylamine and formaldehyde, was supplied through the courtesy of Dr. Edwin J. de Beer of the Wellcome Research Laboratories.
5. D. E. Smith, *Abstr. of Communications, 20th Intern. Physiol. Congr., Brussels* (1956), p. 835; D. E. Smith, *Am. J. Physiol.* 193, 573 (1958).
6. A description of the details of the late events following treatment with the other test substances is in preparation.
7. E. Wehrle and R. Amann, *Klin. Wochschr.* 23, 624 (1956).

14 February 1958