SPECIAL ARTICLES

HISTAMINE RELEASE FROM BLOOD CELLS IN ANAPHYLAXIS IN VITRO[1]

IT is a well-established fact that in anaphylaxis tissues from some species of animals release a substance with all the known biological and, as far as examined, chemical properties of histamine. This substance, subsequently referred to as histamine, is released in anaphylactic shock *in vitro*[2] and *in vivo*,[3] and many investigators have shown that anaphylactic reactions such as the smooth muscle contraction accompanied by the histamine release take place in isolated organs without the presence of blood. Such observations necessarily led to a revision of the earlier assumption that the blood is the site of antigen-antibody union and formation of an "anaphylatoxin" (histamine), and to-day it is accepted that these reactions take place in the cells. The shift of emphasis from the body fluids to tissues as the site of antigen-antibody union and subsequent histamine release has perhaps detracted from the possibility that blood—as a suspension of cells—may also release histamine in anaphylactic shock. The following is a brief report on investigations in this direction.

Rabbits were sensitized by several intravenous injections of egg albumin (Merck). From 10 to 30 days after the last injection, blood was taken by heart puncture, heparinized, and divided into two samples. To one sample egg albumin, which had previously been dissolved in a small amount of Locke's solution, was added, so as to make it 1:1000 egg albumin in blood. The other sample served as an untreated control. Both samples were incubated at 37° C. for 10 minutes. The plasmas were next separated by rapid centrifugation, filtered, and their histamine extracted according to Code's modification of Barsoum and Gaddum's method.[4] (At the beginning of the extraction process, a corresponding amount of egg albumin was added to the control sample in order to exclude the possibility that this substance by itself might be the source of any increased histamine content in the "shock" blood.) The assay was carried out on the isolated atropinized guinea pig ileum. In all instances in which "sensitized" blood had thus undergone "shock" *in vitro*, its plasma contained a higher histamine level than the control samples which had not been incubated with antigen. The differences ranged from 100 to 600 per cent. in rabbits, and were also very distinct but somewhat smaller in a few experiments on sensitized dogs and guinea pigs. No differences in plasma histamine were found when blood from unsensitized animals was incubated with egg albumin and compared with untreated samples, or when cell-free plasmas from sensitized animals were incubated with antigen and compared with controls.

From these studies, it may be concluded that blood cells from sensitized animals release histamine into the plasma when they are in contact with the antigen. Which cells are concerned with this shock reaction is the subject of investigations which are in progress. In the animal species examined, the quantities of histamine thus set free by blood in shock *in vivo*, as estimated from the *in vitro* experiments are of an order sufficient to be physiologically active and would be large enough to play a definite role in anaphylactic shock.

The results so far obtained also suggest the possibility of following anaphylaxis in one and the same animal. This would not involve disturbing the sensitization of the animal as when shock *in vivo* is produced, or sacrificing the animal as is necessary for the Schultz-Dale experiment on isolated smooth musculature. An extension of these studies to cases of human allergy is planned.

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[1] Aided by a grant from the John and Mary R. Markle Foundation.

[2] R. Bartosch, W. Feldberg and E. Nagel, *Pflügers Arch.*, 230:129, 674, 1932.

[3] C. F. Code, *Amer. Jour. Phys.*, 127: 78, 1939.

[4] C. F. Code, *Jour. Phys.*, 89: 257, 1937.

POTASSIUM DEFICIENCY IN AMMONIUM- AND NITRATE-FED TOMATO PLANTS

SYMPTOMS of potassium deficiency in tomato plants grown with nitrate nitrogen have been observed by numerous investigators and are generally known.[1] Substituting ammonium nitrogen for nitrate nitrogen produces entirely different symptoms.

Four groups of seedling tomato plants were established in white quartz sand and were supplied with the following solutions: (1) complete nutrients with nitrate nitrogen; (2) complete nutrients with ammonium nitrogen; (3) lacking potassium with nitrate nitrogen; (4) lacking potassium with ammonium nitrogen. The solutions were supplied to the plants at pH values previously recommended.[2]

The plants supplied with the two complete nutrient treatments made a luxuriant growth for the duration of the experiment. After three weeks the plants supplied with a minus potassium-nitrate solution exhibited early deficiency symptoms[1] (stunted growth, foliage containing abundant starch, pin-point necrotic areas appearing on the curled margins of the older leaves).

The plants supplied with a minus potassium-ammonium nitrogen solution exhibited symptoms on the seventh day, but very different from the nitrate

[1] M. E. Wall, *Soil Science*, 47: 143–161, 1939.

[2] V. A. Tiedjens, *Plant Physiology*, 9: 31–57, 1934.

plants. The foliage was darker green. The plants were stunted and contained considerable starch. This starch, however, rapidly disappeared after four days. The leaves wilted and died in twenty-four hours after the appearance of the first symptoms without changing color, or exhibiting any of the symptoms associated with the nitrate-supplied plants.

Data from the analysis of the four groups of plants showing the ammonium nitrogen in the leaves and stems are graphically shown in Fig. 1.

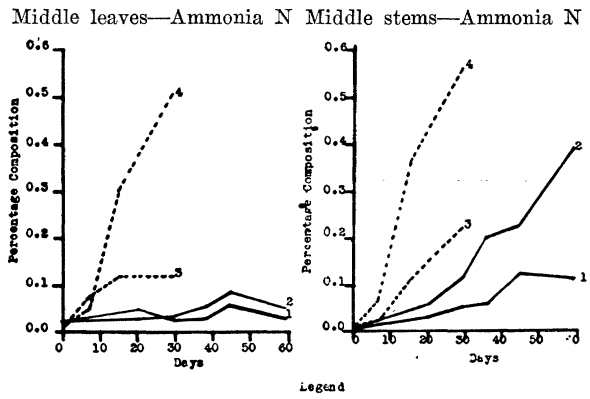

FIG. 1. Nitrogenous fractions of Rutgers tomato plants receiving nitrate and ammonium nitrogen and plus-potassium and minus-potassium nutrient solutions.

The comparatively high concentration of ammonium nitrogen in those plants supplied with ammonium, but no potassium, apparently was responsible for the rapid deterioration and collapse of the leaf tissue. Carbohydrates likewise decreased very rapidly as ammonium increased in the foliage. However, the cause of the injury must be attributed to the lack of potassium in preventing ammonium from being converted to amino and protein nitrogen. The chemical reactions involved in the metabolic cycles of potassium-deficient plants supplied with nitrate or ammonium nitrogen differ only in the fact that the ammonium plants have at hand a large supply of readily assimilated nitrogen. The nitrate plants, on the other hand, must first form ammonia through the reduction of nitrates. These facts account for the rapid completion of the cycle of chemical reactions in the potassium deficient plants supplied with ammonium, requiring less than two weeks to bring them about, while it required three to eight weeks for similar processes to take place in the plants supplied with nitrate. This is probably responsible for the two different types of deficiency symptoms observed.

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NEW JERSEY AGRICULTURAL EXPERIMENT STATION

POLYPLOIDY IN SOYBEAN, PEA, WHEAT AND RICE, INDUCED BY COLCHICINE TREATMENT

WHEN day-old seedlings of soybean, pea and wheat (*Mei-Yü* variety) and 2-day-old seedlings of paddy rice (*Mar-Tze-Tao* variety) were soaked in 0.05–0.1 per cent. solutions of colchicine for 24 or 48 hours under ordinary conditions of temperature and light, the plants that developed were found to be tetraploid, as shown by microscopic examination of their root-tips. Compared with normal plants, those from treated seedlings generally had thicker and rougher leaves, larger cells, larger nuclei and larger stomata. In plants grown from seedlings that had received the 24-hour treatment both shoots and roots were notably more sturdy than those of the controls. When soybean seeds were placed in 0.05 per cent. colchicine solution and allowed to germinate there, the resulting plants showed these same peculiarities. When seedlings of soybean and pea were immersed in 0.05 per cent. colchicine solution for 24 hours in darkness, their leaves soon died but new leaves were formed after a week or two. When a film of lanoline containing 1 per cent. of colchicine was applied to shoot tips of soybean and pea seedlings, or when 4–6 drops of 0.05 per cent. colchicine solution was applied in the same way, treatment resulted in shortening of internodes and curling of leaves.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODIFIED KENDALL TUBE FOR PURIFYING NITROGEN[1]

KENDALL[2] has described a method of purifying nitrogen in a glass tube containing a double roll of copper gauze, heated by radiation from a nichrome coil at the center of the tube. Oxygen is removed by direct combination with the heated copper gauze, which slowly becomes tarnished. The tarnished gauze is restored to bright copper by slow flushing of the tube with hydrogen, and can be so used interminably.

In our studies of bacterial enzymes, we have used

[1] This work has been aided by the Graduate Medical Research Fund of the University of Minnesota.
[2] E. C. Kendall, SCIENCE, 73: 394, 1931.