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Inadequate Maternal Nutrition and Hydrocephalus in Infant Rats¹

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Richardson and Hogan (1) observed hydrocephalus in infant rats as the result of feeding an inadequate diet to the mother. In this abnormality the head is dome shaped and greatly enlarged. The brain cavity is filled with serum and transmits light readily. In some cases the eyes are abnormally small and muscular incoordination usually develops if the affected rat survives long enough.

In order to demonstrate more conclusively that hydrocephalus is caused by a nutritional deficiency it seemed desirable to produce the abnormality in an unrelated colony of rats. The colony at the Texas Station, which has been maintained for more than 10 years without the introduction of any new strains, was suitable for such a study. A total of 38 females have received Diet A, which is essentially the same in composition as that used by Richardson and Hogan. This diet is composed of casein (acid washed), 25 grams; Cerelose, 57 grams; wood pulp, 3 grams; salts, 5 grams; lard, 10 grams; choline chloride,² 0.1 gram; inositol,² 0.01 gram; p-aminobenzoic acid,² 0.05 gram; vitamin A, 3,000 I.U.; vitamin D, 425 I.U.; α -tocopherol,² 2.5 mg.; Menadione,² 2.5 mg.; thiamine chloride,² 1.0 mg.; riboflavin,² 1.0 mg.; pyridoxine hydrochloride,² 1.0 mg.; calcium pantothenate,² 4.0 mg.; niacin,² 5.0 mg.; and biotin,² 0.02 mg.

Some of the experimental females were from mothers which received a stock diet and others were from mothers which received a synthetic diet, but in every case they received Diet A from 28 days of age until the observations were discontinued. A female was observed until it was evident that she would not produce any additional young.

A total of 10 young have developed typical hydrocephalus. The incidence was 1.5 per cent, or approximately the same as that given in the earlier report. None has occurred in the offspring of females which received a stock diet composed of

natural feedstuffs. The hydrocephalus in 5 of the 10 young was identified at birth. It was identified again in these same young when they were 10 days old by observing the transmission of light through the brain cavity, and finally by autopsy. It was not identified in the other 5 until they were about 10 days old. Twelve additional young appeared to be hydrocephalic at birth, but none of these survived longer than two days, and these early identifications have not been entirely reliable.

Richardson and Hogan observed one case of hydrocephalus in the offspring of a mother which received 5 per cent of dried yeast in the diet. This observation suggested that a small amount of yeast in the diet would furnish very little, if any, of the factor which prevents hydrocephalus, and at the same time it would supply sufficient pteroylglutamic acid for normal reproduction.

Intestinal synthesis of some unrecognized factor might decrease the incidence of hydrocephalus, even though the diet itself was low in this factor. The addition of a sulfonamide to the diet would decrease this intestinal synthesis and thus increase the incidence of the abnormality. In order to test this possibility 12 females were given Diet B, which is the same as Diet A with 2 per cent of dried yeast and 1 per cent of sulfasuxidine substituted for equal amounts of Cerelose. So far, these 12 females have produced 92 young and none has been hydrocephalic. These data are too insufficient to be conclusive, but they indicate that the addition of sulfasuxidine to the diet does not increase the incidence of the abnormality under these conditions.

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Effect of Flavonols on *Clostridium botulinum*

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While conducting experiments on the microbial spoilage of vegetables, the authors found that, although asparagus is readily attacked by many organisms, it is a poor medium for growth of *Clostridium botulinum*. The thought was entertained that the flavonol compound described by Campbell (1) as occurring plentifully in asparagus, and subsequently shown by DeEds and Couch (2) to be rutin, might be responsible. Following the report by Naghski, Copley, and Couch (3) on the suppression of *Staphylococcus aureus* by quercetin, an aglucone derivative of rutin, tests were made to determine the action of rutin, quercetin, and quercitrin² (a rhamnoside of quercetin) on *Cl. botulinum*. Three sets of flasks, each flask containing 15 grams of green peas and 15 ml. of corn steep-casein medium, were inoculated with a mixture of approximately 1,000 detoxified spores of *Cl. botulinum*, Types A and B, per flask. The reaction of the medium was pH 6.60. One flask in each set was left untreated as control. Weighed amounts

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² Thanks are due F. DeEds, Pharmacology Laboratory, and J. F. Couch, Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, for furnishing quercetin, quercitrin, and rutin for use in these experiments.

¹ Authorized for publication as technical article No. 1057, Texas Agricultural Experiment Station.

² Kindly supplied by Merck and Company, Rahway, New Jersey.

ot rutin, quercetin, and quercitrin were added to give concentrations ranging from 60 to 160 p.p.m. The flasks were heated for 5 minutes at 100° C. and incubated anaerobically for 10 days at 23° C. Sterile filtrates were then prepared and tested for toxin by inoculating 0.20-ml. amounts into mice.

Rutin in concentrations up to 1,000 p.p.m. did not prevent growth of *Cl. botulinum* or interfere with toxin production. Quercitrin was effective only in concentrations of about 1,000 p.p.m. The action of quercetin was, however, well marked, amounts of from 80 to 160 p.p.m. in the course of several trials preventing toxicity. In one experiment, using corn steep-casein medium alone, no toxin was demonstrated in a concentration of quercetin of 20 p.p.m. The action is antibacterial, only an occasional cell appearing in smears after incubation. Concentrations of quercetin as high as 1,000 p.p.m. failed, however, to inactivate preformed toxin of *Cl. botulinum* in 72 hours.

The limited action of quercitrin in preventing growth of *Cl. botulinum* in the present experiment may have been due to the presence in the sample of a small amount of quercetin. Also, it is considered possible that in certain samples of asparagus enough quercetin may naturally be present to check growth of *Cl. botulinum*. As lately shown by Naghski, Copley, and Couch (4), the compound is antagonistic to *Brucella abortus* and *Aerobacillus polymyxa* as well as to staphylococci. Whether anaerobic organisms in addition to *Cl. botulinum* are affected is at present uncertain.

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IN THE LABORATORY

A Flower Marker for Plant-breeding Operations

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Wherever controlled cross-pollinations are made on a large scale, any simplification of the method of marking flowers is desirable. String tags, which are often used for this purpose, are tedious to apply, especially to smaller flowers; require pencil or ink marking; and are susceptible to weather damage, which may even obliterate identification markings.

The requirements for this type of marker have been met economically by small pieces of "Twist-Ems"—a product devised for tying plants and for bunching root vegetables, consisting of a wire strand sealed by a waterproof adhesive between two narrow strips of heavy paper. These markers, used here for periods of three to five months, have proved to be quite weather resistant; moreover, they have been so durable that it has been possible to salvage them for satisfactory use in a second season.

When used to tag pollinated tomato flowers, "Twist-Ems" are cut into inch lengths. Each piece is folded at one end, placed on the flower so that the pedicel lies within the fold, and then folded in the same direction at the other end (Fig. 1). The second fold serves to lock the first one and thus prevents loss of the marker. Any identification markings can be protected from weathering by keeping them inside the folds. Although firmly attached, the marker will not prevent further growth; the wire of "Twist-Ems" is so easily bent that it does not constrict the developing pedicel.

Markers intended for various crosses can be distinguished by painting them different colors in fade-proof lacquers. Two or more colors can be applied in various combinations to the

same marker in longitudinal or oblique stripes, thereby increasing greatly the number of different identifiable markers. Various combinations of letters or numbers (dates if desired) can be printed on the strips by means of an improvised rotary rubber stamp. The parents of a particular cross combination

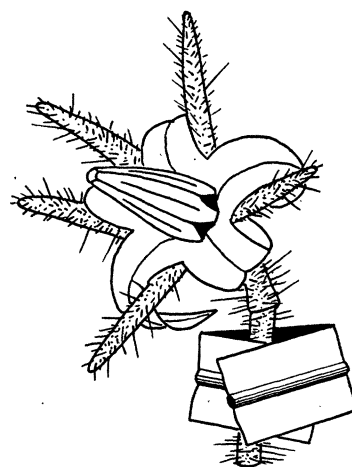


FIG. 1. Diagram of the flower marker placed on the pedicel of a tomato flower.

can then be identified by reference to a key to the color or number combinations.

These markers have been used successfully for tagging flowers of tomatoes, asparagus, and cabbage as well as in marking stems and petioles of particular ages where later identification was needed. Many other uses in biological research might be found where large-scale marking is required.