culturing in its presence; rate of multiplication, vitality, and motility remained the same as in control preparations. *E. histolytica* suspended in solutions of hyaluronidase showed a type of hypermotility, but no changes in vitality or structure. Serial transplantations over 50 generations of *Amoebae* showed that there was enhancement of growth in the presence of hyaluronidase, so that more abundant cultures resulted.

Hyaluronic acid. Hemoflagellates suspended in 0.5% hyaluronic acid showed a steady reduction in motility, particularly of *Leishmania,* but without evidence that the organisms were killed. Serial cultures in the presence of hyaluronic acid showed no effect on the rate of multiplication, vitality, or motility of the organisms over 5 generations. *E. histolytica* exposed directly to hyaluronic acid showed certain structural changes, consisting of hyalinization of the organism and progressive degeneration and rupture, until all the *Amoebae* disappeared. These changes were complete in 15 min with the 0.5% solution of hyaluronic acid and in 70 min with the 0.25% solution; they consisted only of progressive weakening of ameboid activity in 2 hrs with 0.125% solution, while weaker solutions had no effect in 3 hrs. *Amoebae* grown in the presence of 5 mg of hyaluronic acid/5 cc were killed in the 3rd generation; 1 mg/5 cc, in the 5th generation.

Hyaluronidase and hyaluronic acid have indifferent effect upon the hemoflagellates tested. These substances are, however, markedly stimulating and markedly toxic, respectively, to growing cultures of *E. histolytica*; both findings suggest a certain usefulness in culturing *Amoebae* or in attempting to treat amebic infections. While it is not known that *Amoebae* utilize hyaluronidase to invade the host intestine, the observed effect of hyaluronic acid suggests that, if it be used therapeutically, an action both upon the protozoon and upon its power to invade the tissues might be obtained, the latter by inhibition of hyaluronidase possibly secreted by the invader.

References

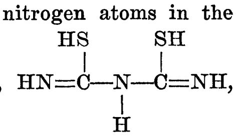
1. DURAN-REYNALS, F. *Bact. Rev.*, 1942, **6**, 197.
2. MEYER, K. *Physiol. Rev.*, 1947, **27**, 335.
3. SENECA, H. T. *Amer. J. trop. Med.*, 1943, **23**, 523.

Responses of Cuttings, Seeds, and Flowers to Dithiobiuret

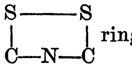
JEAN DUFRENOY and ROBERTSON PRATT

University of California College of Pharmacy, San Francisco, California

Molecular structures that embody reduced forms of sulfur (such as –SH groups) and of nitrogen are of general interest to physiologists, and it is well known that the activity of sulfhydryl groups is influenced by the number and position of neighboring nitrogen atoms in the molecule. Therefore, dithiobiuret,



since it embodies two –SH groups symmetically arranged with respect to three –NH groups, and since it is capable of serving not only as an H donor but as a source of S and of N as well, should be expected to possess some interesting biological properties. The compound is easily oxidized with loss of two hydrogens to form a 5-membered



ring system (*3*). The reaction proceeds rapidly and reversibly over the pH range 0.05–5.2 (*3*). Dithiobiuret operates as a reducing agent at hydrogen-ion concentrations within the biological range also, as is testified by its ability even in low concentrations (0.0005 M) to decolorize rapidly toluylene blue in solutions ranging up to pH values of 7.5 (higher pH values not studied). This dye is but slowly reduced by cysteine (*1*).

This report presents some observations on the response of vine cuttings, germinating seeds, and cut flowers to dithiobiuret,[1] which for convenience will be designated as DTB in the remainder of this report.

Vine cuttings. Cuttings of grape vines (*Vitis treleasi*) with their bases immersed in distilled water normally developed, within 2 weeks, shoots from the upper bud and roots from the lower node. Cuttings similarly placed in solutions containing 20–80 mg of DTB/liter of distilled water developed short roots and stunted shoots from buds, irrespective of the relative position of the bud on the stem. When such cuttings were transferred to distilled water, the roots promptly elongated. Similar tendencies have been observed with cuttings of *Ficus gnaptholocarpa* (*2*).

Cuttings in 0.05 M solution of KH_2PO_4 did not develop any roots in 2 weeks; DTB at 20–80 mg/liter in the phosphate buffer did not invert polarity but had a striking effect in promoting the differentiation of root initials not only from the basal node but all along the basal internodes. Potassium acid phosphate retarded early development of roots and of buds as compared with distilled water, but favored cambial growth at the base of the cutting as well as differentiation of floral organs. Both of the latter effects were enhanced by the presence of DTB. In KH_2PO_4 the increase in diameter was accompanied by longitudinal splitting of the bark and later on by differentiation of root initials, which attained a length of about 10 cm after 3 weeks. Conversely, in KH_2PO_4 + DTB (20 mg/liter) a few root initials differentiated into roots, and these elongated, but slowly, averaging 2.5 cm after 3 weeks. Most of the root initials coalesced into an undifferentiated mass of cells, while the base of each cutting swelled into a tumor-like mass.

Early growth of seedlings under semianaerobic conditions. Seeds of rice (*Oryza sativa* var. Calora) were immersed under 3 cm of a simple nutrient solution (KH_2PO_4, 0.03 M; $Ca(NO_3)_2$, 0.02 M; and $MgSO_4 \cdot 7H_2O$, 0.01 M) or of nutrient plus DTB. Addition of DTB in concentrations less than 10 mg/liter had a depressing effect on germination and on growth of roots and coleoptiles. Concentrations of from 10 to 25 mg/liter were strikingly beneficial, leaves developing to a length of 70 mm in 17

[1] We wish to thank the American Cyanamid Company, New York City, for samples of dithiobiuret; L. Flint, Louisiana State University, for seeds of rye grass; and H. P. Olmo, University of California College of Agriculture, for the vine cuttings.

days. Higher concentrations were less beneficial, so that at 100 mg/liter germination and growth were scarcely better than in the control, where leaves were 25–30 mm in length. Similar results were obtained with seeds of ryegrass (*Lolium multiflorum*), although these seeds benefited from higher concentrations than did those of rice.

Response of cut flowers. The observation that appropriate concentrations of DTB prevented discoloration of the basal section of cuttings and prolonged survival of rooted cuttings of the vine, fig, and other plants suggested that this compound might be used to delay wilting of cut flowers. This idea was tested experimentally with Shasta daisy (*Chrysanthemum maximum*) and with marigold (*Calendula officinalis*), both of which remained turgid 1–2 days longer upon addition of 1–10 mg of DTB/liter of tap water or of 0.1 M solution of KH_2PO_4 in tap water. A characteristic response of flowers with their stalks in solutions of DTB was an epinastic curvature beginning at the tips of the ray florets and progressing inward over a period of several days. It is known that solutions of molybdenum salts which form complexes with phenolic compounds favor survival of cut flowers. Likewise, DTB may be surmised to exert its favorable effect by protecting phenolic compounds against oxidation. In this connection it is interesting to note that another strong reducing agent, hydrazine sulfate, is advertised as preserving cut flowers (*Chem. Eng. News*, 1948, 26, 1452).

References

1. Chalopin, H., and Mudry, M. *Bull. Soc. Chim. Biol.*, 1945, **27**, 398.
2. Dufrenoy, J., Carlo, P., Pratt, R., Pickering, V. L., and Sah, P. P. T. *Growth*, 1948, **12**, 157.
3. Preisler, P. W., and Bateman, M. M. *J. Amer. chem. Soc.*, 1947, **69**, 2632.

Effect of Dietary Factors on Early Mortality and Hemoglobinuria in Rats Following Administration of Alloxan[1]

Paul György and Catharine S. Rose

Nutritional Service, Department of Pediatrics, and Gastro-Intestinal Section, Medical Clinic, School of Medicine, University of Pennsylvania

According to recent studies of Houssay and Martinez (*6*), alloxan diabetes and its accompanying toxic manifestations in the white rat may be considerably influenced by a variety of dietary factors. In the light of their experimental observations, they concluded that rations low in protein or high in lard reduced, and diets high in coconut oil, or supplements of methionine or thiouracil, increased the resistance of rats to alloxan. Substitution of butter or olive oil for lard or supplements of choline were without any appreciable effect. Oleomargarine and corn oil were slightly beneficial. It was previously known (*8*) that cystine (cysteine), when given just prior to alloxan, may also counteract the toxic effect of alloxan.

[1] Supported by a grant of Swift & Co., Chicago.

The grouping of the above noxious and beneficial dietary agents is reminiscent of, and almost analogous with, that which governs to a considerable extent the production of massive hepatic necrosis (cf. *1, 5*). There is only one important gap in this comparison. This concerns tocopherol, which is known (*2, 9*) to be an important additional protective factor in the etiology of massive hepatic necrosis. The experiments of Houssay and Martinez contain no specific information on the possible role of tocopherol in the prevention of alloxan intoxication. In fact, the rations used by them seemed to be rather low in tocopherol, and, in consequence, deficiency of tocopherol might have complicated the experimental findings.

Houssay and Martinez judged the beneficial or deleterious effect of a given ration by the number of rats which survived one week after administration of alloxan. They inferred that a lower mortality is a reliable indicator for increased resistance to the toxic and diabetogenic effect of alloxan.

The possibility that tocopherol may exert the same beneficial protective effect on necrotic changes in the pancreas (liver, kidneys, *4*) following administration of alloxan, as it does in the prevention of massive dietary hepatic necrosis in rats, made a special study of its role in alloxan intoxication desirable.

In accordance with the experimental technique followed by Houssay and Martinez, female rats weighing between 90 and 120 gm were divided into several groups and fed different rations for a period of one month. All rats were of the Sprague-Dawley strain and were housed in separate cages. After one month and an all-night fasting, the rats were injected intraperitoneally with 160 mg/kg of alloxan (Alloxan-monohydrate, Eastman Kodak), in the form of a freshly prepared solution. To make the alloxan experiments more comparable to those which dealt with dietary hepatic injury, slight modifications in the composition of the rations were introduced. Whereas the diets used by Houssay and Martinez were composed largely of crude natural food constituents, such as wheat flour, corn flour, yeast, and fats, we have substituted in our rations sucrose for flour and, in one group of the experiments, also omitted yeast from the diet. As vegetable fat we used, in addition to coconut oil, a partially hydrogenated shortening, Vream (Swift & Co.). Houssay and Martinez relied on yeast as a source of the vitamin B complex. We supplemented our diets, regardless of whether or not they contained yeast, as a matter of daily routine, with 20 μg of thiamine, 25 μg of riboflavin, 20 μg of pyridoxine, and 100 μg of calcium pantothenate, dissolved in 1 ml of water. The fat-soluble vitamins A and D were given, as in the experiments of Houssay and Martinez, in the form of cod-liver oil incorporated in the diet, except that the special rations (17–20) low in fat were supplemented with 3 drops of corn oil daily and 3 drops of percomorph oil once a week. The daily doses of tocopherol[2] in the respective groups were 3 mg; of methionine,[3] 50 mg; and of choline, 25 mg.

[2] Mixed natural tocopherols were kindly furnished by Distillation Products, Inc., Rochester, New York.

[3] Kindly furnished by Wyeth Inc., Philadelphia.