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).

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

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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.

Food intake and changes in weight were recorded for all rats throughout the whole experimental period. Determinations of blood sugar and nonprotein-nitrogen were repeatedly carried out, beginning usually on the third day after administration of alloxan. In all rats kept on rations containing yeast, and in 15 out of 20 in each group of rats receiving the other rations, special attention was paid to hemoglobinuria by placing sheets of white filter paper underneath the bottom screens of the single cages and analyzing the urine obtained for the presence of hemoglobin. In several instances intravascular hemolysis was tested in the blood serum and by repeated red blood cell counts.

Rats often died within the first two days after administration of alloxan, and several of these, as well as a fair proportion of the surviving animals, showed signs of more or less intensive hemoglobinuria. The effect of dietary factors on this early mortality and hemoglobinuria following administration of alloxan is summarized in Table 1. The relationship of dietary factors to longer survival and to diabetes, as well as the histological findings, will be discussed in a subsequent communication.

TABLE 1

EFFECT OF DIETARY FACTORS ON EARLY MORTALITY AND HEMOGLOBINURIA FOLLOWING ADMINISTRATION OF ALLOXAN

Diet	Casein	Sucrose	Fat	Cod-liver oil	Yeast	Salt mixture	No. of rats used	Rats dead in first two days		Hemoglobinuria (%)		
								No.	%	0	+	++
(1) High lard	20	36	38	2	..	4	20	11	55	0	33	66
(2) “ “ with tocopherol	20	36	38	2	..	4	20	5	25	100	0	0
(3) “ “ “ yeast	20	31	38	2	5	4	10	0	0	50	20	30
(4) “ “ “ “ and tocopherol	20	31	38	2	5	4	10	0	0	100	0	0
(5) “ “ “ methionine	20	36	38	2	..	4	20	10	50	6,6	6,6	86,6
(6) “ “ “ yeast and methionine	20	31	38	2	5	4	10	2	20	20	40	40
(7) “ “ “ choline	20	36	38	2	..	4	20	10	50	26,6	26,6	46,6
(8) “ “ “ yeast and choline	20	31	38	2	5	4	10	1	10	70	10	20
(9) “ vegetable shortening (“Vream”)	20	36	38	2	..	4	20	2	10	100	0	0
(10) “ “Vream” with yeast	20	31	38	2	5	4	10	2	20	100	0	0
(11) “ coconut oil	20	36	38	2	..	4	20	12	60	13,3	6,6	80
(12) “ “ “ with tocopherol	20	36	38	2	..	4	10	2	20	100	0	0
(13) “ “ “ “ yeast	20	31	38	2	5	4	10	0	0	80	0	20
(14) “ “ “ “ yeast and tocopherol	20	31	38	2	5	4	10	0	0	100	0	0
(15) “ protein and high lard	40	16	38	2	..	4	20	15	75	6,6	13,3	80
(16) “ “ “ “ “ with yeast	40	11	38	2	5	4	10	3	30	20	20	60
(17) Low fat	20	76	..	..	..	4	20	5	25	40	6,6	53,3
(18) “ “ with tocopherol	20	76	..	..	..	4	20	0	0	100	0	0
(19) “ “ “ yeast	20	71	..	..	5	4	10	1	10	10	20	70
(20) “ “ “ “ and tocopherol	20	71	..	..	5	4	10	1	10	100	0	0

The multitude of data contained in Table 1 may be summarized as follows: The highest early mortality was observed in rats fed rations free from yeast and high in lard or in coconut oil. This result was not alleviated by supplements of methionine or choline or by a higher proportion of protein in the diet. In contrast, a statistically significant reduction of early mortality was achieved by supplementing the rations high in lard or in coconut oil with tocopherol or by using rations with a vegetable shortening as the main source of fat. This vegetable shortening (Vream) is rich in tocopherol. An equally significant drop in the rate of early mortality was seen in rats receiving rations very low in fat. In general, rations containing yeast enabled rats to live longer—at least beyond the arbitrary two-day limit—than rats fed similar rations without yeast.

Hemoglobinuria was *never* observed in rats fed rations rich in tocopherol. On the other hand, a varying, but often high proportion of the experimental rats kept on rations free from, or very low in, tocopherol showed hemoglobinuria after intraperitoneal injection of alloxan. Here again, supplements of methionine or high protein-high lard diet were, in the absence of tocopherol, without any appreciable effect on hemoglobinuria. Supplements of choline, however, seemed to have a slight but definite beneficial effect on hemoglobinuria. Addition of yeast to the ration was followed in most instances by a reduction of hemoglobinuria. The yeast rations with high protein or with low fat were exceptions and did not decrease the incidence and intensity of hemoglobinuria.

Hemoglobinuria was accompanied by intravascular hemolysis and a rapid fall in the red blood cell count, occurring almost immediately, with a peak a few hours after injection of alloxan. Here are a few examples of the changes in red blood cell count:

Before alloxan	8.8, 7.4, 7.6, 8.5, 7.5, 7.5 million/mm^3
After "	1.9, 4.0, 1.0, 2.9, 1.7, 2.7 " "

In the past only Kennedy and Lukens (*7*) mentioned hemolysis as a complication of diabetes in rabbits. They implied that hemolysis and diabetes are closely related. In our observations, early mortality and hemoglobinuria (hemolysis) occurred independently from diabetes. The dietary factors, such as tocopherol or yeast, or a ration low in fat, which in our experience reduced the rate of early mortality and hemoglobinuria, had no influence on the incidence and intensity of diabetes. Thus, the pharmacological-toxicological action of alloxan seems to be based on two independent components, one being responsible for the hemolysis, the other for the diabetes. The preventive effect of tocopherol and related dietary factors on hemolysis caused by alloxan requires further elucidation.

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Growth and Fruiting of Tomato Plants as Influenced by Growth-regulating Substances Applied to the Soil[1]

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Hormones are used today for numerous purposes, including killing of weeds, thinning of blossoms, checking fruit drop, and increasing fruit set in various plants. Their application to flower clusters has been effective in increasing the fruit set of tomatoes during winter days of low light intensity in the north and also in hastening the maturity of the early summer crop. The addition of these growth regulators directly to the soil and their subsequent effect on the fruiting of plants does not seem to have received adequate attention. Zimmerman and Hitchcock (*6*) reported that "one to five mgms of 2.5-dichlorobenzoic acid per 4 inch pot of soil caused fruit set of flowers and buds present when the chemical was applied to the soil. It also caused parthenocarpic development of flower buds which were initiated after the soil was treated."

The following preliminary experiments were conducted in late spring of 1948 in the greenhouse with a view to studying the effect of application of various hormones to the soil on the growth and fruiting of the tomato plant. Seed of Valiant variety of tomatoes was sown on January 16, 1948; the seedlings were transplanted in flats 2″ × 2″ apart. Vigorously growing, uniform plants were transferred to 12″ pots on March 29. They were divided into 4 groups on April 16, when 2–3 flowers on the first cluster of most of the plants had opened. Aqueous solutions of hormones were applied as follows:

Treatment No. 1—	50 ppm	*o*-chlorophenoxyacetic acid, 100 cc thrice, at weekly intervals.
" No. 2—	50 ppm	α-*o*-chlorophenoxypropionic acid, 100 cc thrice, at weekly intervals.
" No. 3—	100 ppm	2,5-dichlorobenzoic acid, 100 cc twice, at weekly intervals.
" No. 4—		Check.

The general appearance of the treated plants was not much affected except in the case of those treated with *o*-chlorophenoxyacetic acid. These were yellowish-green and comparatively weak, resembling plants infected with virus mosaic. Their top growth was significantly poorer than the ones treated with α-*o*-chlorophenoxypropionic acid and 2,5-dichlorobenzoic acid (Table 1).

Although there was no statistically significant difference between root weight of different groups of plants, the data tend to indicate that there was some stimulation of root growth in the case of plants treated with α-*o*-chlorophenoxypropionic acid.

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