pared, in 2 runs of 5 l and 10 l. The purity, as determined by mass spectrographic analyses, was 98.1% and 97.0%, respectively.

Thirteen liters of this material was fractionated at liquid hydrogen temperature in 3 batches. The still was that employed by Scott and Brickwedde in their earlier

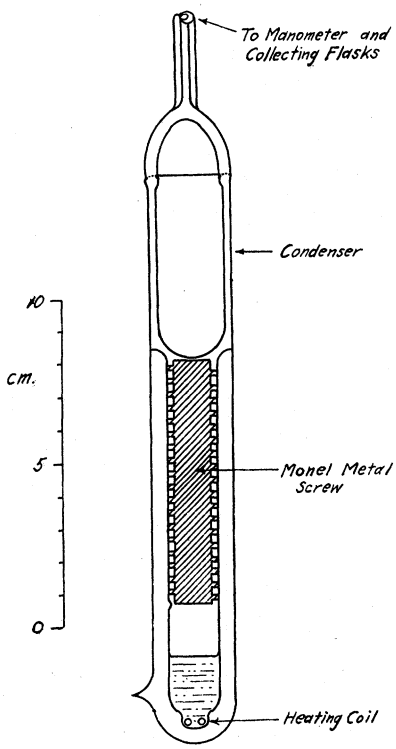

FIG. 1.

work (Fig. 1). It consisted of a boiler of about 5 ml capacity, a monel helix rectifying section, and a cold-finger type dephlegmating condenser. The boiler and rectifying section were vacuum-jacketed; heat was provided by a constantan heater in the boiler, the leads for which emerged through the top of the still through a wax seal. The entire still was immersed in liquid hydrogen, thereby maintaining a constant temperature throughout the distillation. The progress of the distillation was followed by means of a manometer which registered the pressure of the vapor in equilibrium with the boiling charge at liquid hydrogen temperature. The points at which fractions should be cut were thereby indicated.

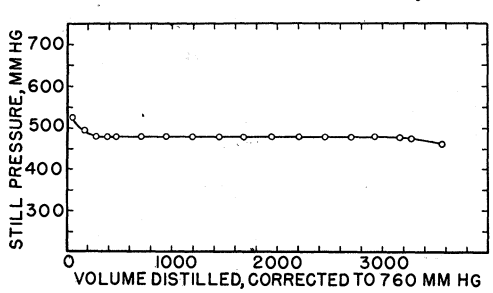

FIG. 2.

This is shown on the accompanying curve (Fig. 2) for the distillation of a charge of about 4,500 ml of crude hydrogen deuteride.

Employing a boil-up rate of 11 ml of liquid per hr and a reflux ratio of the order of 15: 1, there was obtained 10 l of hydrogen deuteride of 99.8% purity in terms of hydrogen isotopes. Because of the difficulties inherent in the mass spectrographic method for measuring trace impurities in hydrogen isotope samples, it is believed that the purity may be even higher. This degree of purity, obtained by means of one distillation of the crude, compares very favorably with that previously obtained from the equilibrium mixture by Scott and Brickwedde in two distillations. This is undoubtedly due to the fact that the starting material used by them was much poorer in hydrogen deuteride than that used in this work.

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The Effect of 2,4-D on Potassium Nitrate Levels in Leaves of Sugar Beets[1]

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As early as 1946 research workers in the U. S. Department of Agriculture showed that 2,4-D in pure form at relatively high levels was not toxic to cattle. They concluded that the amount of 2,4-D that might be consumed by cows or sheep in grazing pastures sprayed with this material to kill weeds would not be injurious (*1*). Extensive grazing trials undertaken at the Michigan Agricultural Experiment Station in 1949 substantiated these earlier observations (*2*), and indicated that cattle grazed 2,4-D-sprayed and unsprayed areas indiscriminately. It has been commonly observed that weeds such as pigweed (*Amaranthus* spp.), ragweed (*Ambrosia* spp.), and even such plants as Jimson weed (*Datura stramonium*), which under normal conditions are not grazed by cattle, are eaten with relish after they have been treated with 2,4-D. A number of cases have been reported of cattle becoming ill, or dying, after eating

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the above species of weeds that had been treated with 2,4-D. Reports of cattle poisoning, or death, following the grazing of 2,4-D-treated weeds, have to our knowledge, in no case been definitely and authentically identified with the direct or indirect action of 2,4-D.

The poisoning of livestock through grazing of oats hay, or straw, and other plants, under unfavorable conditions of development of the plants, has been well established by a number of workers (*3, 4, 5*), who have tentatively set the lower toxic limit of potassium nitrate in forage on a dry weight basis as 1.5%. The potassium nitrate or other nitrates commonly found in plant bodies are not in themselves toxic to ruminants, but the nitrates are changed to nitrites as a result of the action of microorganisms in the rumen. The nitrites cause the formation of methemoglobin in the blood stream, which interferes with the transfer of oxygen from the lungs to the body tissues, and may cause death of animals through anoxia within a few hours. Animals affected with nitrate poisoning show general symptoms associated with oxygen deficiency, such as the distinctive brown color of the blood, which is recognizable by the gray-tan appearance of the mucous membranes of the nose and mouth. This symptom indicates the presence of methemoglobin, which gives the blood the brown-red color instead of the bright-red color normally derived from the oxyhemoglobin. A 2% solution of methylene blue in water is a recognized counteractive treatment for nitrate poisoning.

Several researchers have shown that sublethal dosages of 2,4-D increase protein content of wheat (*6, 7, 8*). Hullinger (*9*), in a preliminary study of the effects of 2,4-D on nitrogen and carbohydrate metabolism of the corn plant, showed marked changes in these constituents in various parts of the corn plant following 2,4-D absorption. The data of these investigators clearly indicate that sublethal dosages of 2,4-D may markedly affect the metabolism of treated plants, and that this upset in the normal metabolism may result in accumulation of toxic quantities of nitrates similar to those established for plants growing under abnormal conditions, such as drought.

Sugar beets are more sensitive to 2,4-D than most field crops. Concentrations of 100 ppm will kill beet seedlings. Beets beyond the four-leaf stage of development are markedly affected by concentrations of 2,4-D at this level (*10*). These abnormal growth characteristics resulting from light applications of 2,4-D were well described by Tanner (*11*) in a recent article. Beets beyond the four-leaf stage of development are killed by concentrations of 2,4-D between 500 and 1,000 ppm, and death at these more advanced stages is slow. Sugar content is reported by Tanner to be markedly lowered, and reductions in yield vary with date of treatment preceding normal harvesting of the beets. In mid-August in 1949, 335 acres of beets belonging to 7 growers in North Dakota were sprayed with a supposedly Toxaphene mixture to control a late brood of webworm. After application it became evident that this insecticide had been contaminated or mixed with a high percentage of 2,4-D. This matter was brought to our attention by J. C. Tanner (*11*), and samples of beet leaves from each of the 7 treated farms, together with 3 samples of leaves from untreated beet fields on adjacent farms, were secured through the courtesy and assistance of E. A. Helgeson and his associates, of the Botany Department, North Dakota Agricultural Experiment Station. These samples were air-dried and analyzed for potassium nitrate by the Chemistry Section of the Agricultural Experiment Station of South Dakota during the winter of 1950.

Table 1 shows that potassium nitrate in dry leaves of normal sugar beets averaged 0.22%. This level of potassium nitrate is considered within the normal range for dried beet leaves and is well below the minimum lethal toxic level. The potassium nitrate level in dried leaves of beets from the 7 farms where 2,4-D had been accidentally applied, was at an average level of 4.5%, with a variation of 1.81–8.77 (Table 1). All samples of 2,4-D-treated beet leaves analyzed showed potassium nitrate at a level well above that considered as the minimum lethal concentration. Leaves of beets from the fields on which these samples were taken were not fed to livestock. It is evident that feeding of the material would have been highly hazardous, certain to be followed by considerable losses.

TABLE 1

EFFECT OF 2,4-D ON THE POTASSIUM NITRATE CONTENT OF LEAVES OF SUGAR BEETS

Sample no.	Treatment	KNO_3 per cent of dry weight of leaves	
1	None	0.20	
2	"	0.22	
3	"	0.25	
	Average of untreated		0.223
4	2,4-D	1.81	
5	2,4-D	2.26	
6	2,4-D	4.41	
7	2,4-D	4.65	
8	2,4-D	4.68	
9	2,4-D	5.01	
10	2,4-D	8.77	
	Average of treated		4.50

The data of Table 1 indicate clearly that variously reported cases of livestock losses following grazing of 2,4-D-treated weeds and crops may well have been caused by potassium nitrate poisoning, resulting from accumulation of nitrates in sprayed plants as a secondary response of the plants to the action of 2,4-D. It is evident that investigation is necessary in order to establish the varietal reaction of weeds and crop plants to 2,4-D, with special emphasis on accumulation of potassium nitrate where postgrazing of the treated plants occurs. A review of the literature and data of this study indicates further that, where cases of poisoning of livestock following 2,4-D treatment are reported, the distinctive symptoms of nitrate poisoning should be diagnosed, with the remedial action or measures developed by earlier workers employed. Further, grazing or feeding of sugar-beet tops, oats hay and straw, corn sorghum, pigweed, or lambsquarters, and other closely related species of weeds should be undertaken with caution following intentional

or accidental spraying with 2,4-D until the role of 2,4-D in the accumulation of toxic quantities of nitrates in these species has been more fully determined.

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An Analysis of the Enzyme Activity of the Conditioned Salivary Response in Human Subjects

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In the early work on conditioning, it was generally assumed that the conditioned response was identical with the original or unconditioned response, both in a qualitative and a quantitative sense. Later, many workers, including Pavlov, found that the conditioned response did differ in a quantitative way from the original response. As a rule, it was found to be less vigorous (motor responses) or associated with a decreased amount of saliva (glandular response). The aim of the present experiment was to determine whether there is any chemical difference in the saliva of human subjects between the original and the conditioned response.

Eleven female college students were used in the experiment. Four specimens of saliva were taken from each subject: previous to the experiment, during the response to a bell before conditioning, during the presentation of food, and after the process of conditioning had been completed. The saliva was collected from the sublingual spaces by means of a glass pipette, so that the secretion from all the salivary glands would be represented.

The tests were made in an isolated room, with the blinds drawn to eliminate gross nonexperimental stimuli. Each subject was tested individually during a single session of about 3 hr. The unconditioned stimulus was a Cryst-O-Mint candy wafer, which induced a free flow of saliva and was neutral with respect to the chemical techniques later applied. The conditioned stimulus was an electric bell. The subjects were instructed not to eat or smoke for 4 hr previous to the series of tests and reported that they had adhered to this schedule.

The bell preceded the wafer by 10 sec, and the latter was held on the tongue for 20 sec during each trial, following the suggestion by Razran (*1*). The paired stimuli (bell–wafer) were given at short, irregular intervals, so that the time interval itself could not operate as a conditioned stimulus. The time interval between presentations of the pair ranged from 30 to 90 sec, and the series of presentations was randomized. The conditioning phase consisted of 150 paired presentations.

At ½-hr intervals, each subject was asked to report any change in the amount of the salivary secretion noticed. The 11 subjects utilized in this experiment reported a definite increase in saliva from the first interval onward.

The amount of amylase in the saliva was measured for each condition. This substance was chosen because it is the starch-hydrolyzing enzyme and the most active component of saliva (*2*). The results are reported in units of amylase activity per ml of saliva. One unit of amylase may be regarded as the amount required to digest 5 ml of 1% soluble starch to the achromic point in 10 min under the conditions of the standard analysis as presented by Hawk, Oser, and Summerson (*3*). The analytical reagents employed were: (1) light-yellow aqueous iodine solution, (2) 1% aqueous solution of soluble starch, (3) 1% aqueous solution sodium chloride, and (4) phosphate buffer ($K_2HPO_4 + KH_2PO_4$) adjusted to pH 6.6.

The results of this experiment are: (1) With respect to amylase activity, there is a statistically significant quantitative difference between the salivary secretion in response to a conditioned stimulus and the reflex response. (2) There is more amylase activity in the salivary component during the conditioned response phase than in the unconditioned response. There is a mean gain of 31.7 in units of amylase activity in the salivary conditioned response over that of the unconditioned response. This difference is significant at the 0.01 level of confidence. (3) The amylase activity in the determination of experimental conditions (1) and (2) is consistently close.

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Spectrophotometric Assay of Ascorbic Acid with Peri-Naphthindanetrione Hydrate

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When peri-naphthindanetrione hydrate (I) (*1, 2*) is allowed to react with ascorbic acid (II), the reaction is of a reddish color owing to the formation of dihydroxy-peri-naphthindone (III) (*3*). This reaction is an oxidation reduction system in which the stage of oxidation stops at the point of the formation of dehydroascorbic acid (IV).