of the plot. Soil was obtained to plow depth from the area of maximum whiptail and placed in ½-barrel steel drums that had previously been painted with a nontoxic asphalt varnish. These large containers were used in an attempt to avoid frequent watering, which it was felt might have been responsible for the failure of Wessels' greenhouse plants to show whiptail, as they did in the field.

The soil was allowed to dry in the drums until February 8, 1950. On February 9, 2 cauliflower plants of a strain of Super Snowball, seeded December 6, spotted into similar soil December 22, and grown under fluorescent lights, were planted in each container and fertilized with a small amount of KNO_3. On February 24, reagent grade KH_2PO_4 in solution to supply 500 lb to the acre and borax to give 10 lb to the acre were added in trenches 2½ in. deep and 4 in. from the plants. Six of the drums were supplied with ammonium molybdate at the rate of 1 lb to the acre in the trenches, and 4 received no molybdenum. Additional drums could not be placed in the greenhouse space available. KNO_3 at the rate of 200 lb to the acre was added, and the soil watered. On February 27, the soil in all drums was thoroughly wet, and 5 of the drums were covered with wet burlap bags that dipped into water in jars beside the drums. The bags covered practically all the soil in the drums but did not touch the plants or the soil. The wet bags reduced water loss from the soil, and these drums were not watered during the course of the experiment. The other 6 drums were watered heavily, but not leached, whenever the soil became dry.

The plants in all the drums turned somewhat yellow following the heavy watering prior to and including that of February 27. On March 17, it was noted that those in both series receiving molybdenum were greener than those without molybdenum. Differences in growth and color became more marked daily and were very marked on March 29, when the experiment was terminated because the plants in the dry soil were wilting slightly during the warm part of the day. All the plants lacking molybdenum showed marked interveinal chlorosis, as noted by Davies and Waring and Wilson and Shirow (*3*, *6*). Growth was very upright, and the leaf tissue appeared brittle. A few of the smaller leaves showed parts of the laminae greatly reduced, suggesting a condition similar to whiptail. A few leaves had dead tissue at the edges. All plants supplied with molybdenum were of dark-green color and growing normally. The average weight of the plants cut off at the soil surface from soils with molybdenum was 429.5 g; the weight of those not supplied with molybdenum was 310.7 g. There was little difference between plants in the two moisture treatments. It is probable that the plants not supplied with molybdenum obtained some molybdenum from the drums in spite of the coating of varnish. The pH at the end of the experiment was 4.60.

C. B. Raymond, after seeing the marked response of the cauliflower to molybdenum, stated that on June 1, 1948, he had noted areas of very poor growth in red clover seeded for a cover crop in the same field. He made a map of the field and obtained pH readings. Areas with good clover averaged pH 5.40, whereas those with poor clover averaged pH 4.90. A check with Raymond's map disclosed that the soil for this cauliflower experiment was obtained in the center of one of the largest areas that showed poor clover growth in 1948. Much of the literature on molybdenum deficiency indicates that clovers are especially susceptible.

Experiments on this plot with cauliflower plants from the same seed lot as used in the greenhouse show a marked response to molybdenum. On August 1, 1950, it was evident that practically all the plants not supplied with molybdenum would be so badly whiptailed as to produce no marketable cauliflower.

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A Simple Apparatus for Producing Droplets of Uniform Size from Small Volumes of Liquids

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Frequently in the study of sprays of plant-growth regulators, fungicides, and insecticides it is pertinent to observe the behavior of droplets on the vegetation. For a long time it has been observed that foliage is wetted more readily by aqueous sprays containing certain surface-active agents and by oils than by wholly aqueous sprays. Moreover, the leaves of different species vary in their ability to retain droplets of various kinds of liquids. Conventional spray equipment is unsuitable for studying the action of individual droplets impinging on the leaves of different species. Likewise, the usual spray equipment is not satisfactory for applying low volumes of liquid (.01–.1 ml) as small individual droplets of uniform size to individual leaves or plants. By utilizing a principle devised by Lane (*1*) and Levvy (*2*) for producing small drops of liquid, a simple glass apparatus has been designed which produces in quantity individual droplets of uniform size and is adaptable for delivering very small volumes of aqueous and nonaqueous solutions. The apparatus has been termed a droplet sizer.

One end of a glass tube was drawn into a very fine capillary, and the tip was broken off at a point where its diameter was just small enough that water would not run through except under low pressure (Fig. 1); a tube 11 mm OD by 145 mm long had about 5 ml capacity. The outside diameter of the capillary tip, *B*, influences the range of droplet sizes that can be produced. Three holes, *G*, of

approximately 3 mm diameter were made in the liquid tube, *D*, near the air inlet, *F*, and then the tube was sealed into an air jacket (20 mm OD) having the shape of *E*. A larger glass tube may be used in making the liquid chamber if it is desirable to work with larger volumes of liquid. In the construction of the air jacket it is important that the constricted portion, *C*, be of sufficient length to insure a smooth flow of air, which picks off the droplets formed on the capillary tip as they reach the desired size. A constricted portion about 1½ in. long and 2.5–3 mm ID was adequate to produce uniform droplets that could be directed on the desired surface area with accuracy. The protective rim, *A*, helps prevent accidental breakage of the capillary tip.

Liquid to be discharged can be placed into the fluid tube, *D*, with a pipette. If it is desired to discharge completely a measured volume of liquid of high surface tension, care should be exercised in introducing the pipette to prevent adherence of separate droplets to the fluid tube wall. All liquids should be free of solid particles or lint because the small capillary can be easily blocked.

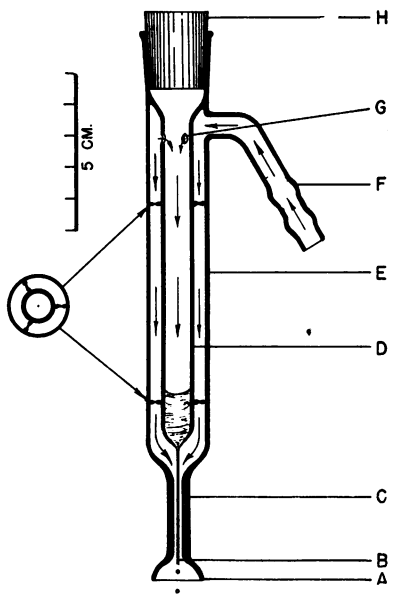

FIG. 1. Glass droplet sizer.

If the capillary becomes blocked, the foreign material may sometimes be removed by inserting a tightly fitting rubber tube to a level below the holes in the liquid tube and applying negative pressure by attaching the other end of the rubber tube to a laboratory suction pump. When not in use the apparatus should be stored upright in a covered vessel containing enough ethyl alcohol to cover the capillary tip.

Filtered air is introduced at *F*; pressure is measured manometrically. The pressure is adjusted to give a steady formation and removal of uniform droplets from tip *B*. Both rate of production and size of droplets are governed by this single pressure adjustment. The pressure employed in certain studies ranged from 2 to 30 mm mercury at *F*, depending upon the character of the liquid and the size of the fine capillary. Liquids with a high surface tension may not flow readily at first because of the presence of small air bubbles near the end of the capillary. Initially, to remove these small air bubbles, a higher pressure may be required; once they are eliminated, the pressure can be quickly readjusted to the desired level. If the air pressure is too great, extremely small droplets of mixed sizes are produced. Typical droplet patterns obtained with the droplet sizers are shown in Fig. 2. With different droplet sizers, aqueous droplets

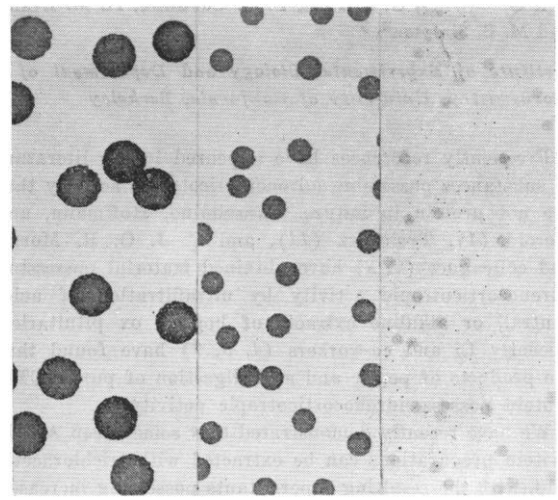

FIG. 2. Typical droplet patterns. × 1.

ranging from .22 to 1.82 mm in diameter have been produced. It has been found that with the same liquid used in the same apparatus droplet size is reproducible at the same pressure.

Data obtained with one droplet sizer indicate that within limits the diameter of the droplets produced is inversely but not linearly proportional to the air pressure (Table 1). It has been found that change in liquid

TABLE 1

DIAMETER OF AQUEOUS DROPS PRODUCED AT DIFFERENT AIR PRESSURES. OUTSIDE DIAMETER OF CAPILLARY TIP WAS .22 MM

Air pressure, mm Hg	Diameter drops, mm
4	1.40
9	0.72
12	0.54
23	0.45
28	0.40

level in the inner tube affects the size of the droplets to a slight extent. If greater accuracy is required, variation in droplet size can be avoided by maintaining a constant level of liquid. This may be done by replenishing the supply with a syringe inserted through stopper *H*.

The apparatus has been found satisfactory for delivering known small volumes of liquid as uniform droplets without calibration of delivery rate. The system may also be calibrated if required for a particular purpose.

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Preparation of Nonprotein Fractions Possessing Adrenocorticotropic Activity from Fresh Sheep Pituitary Glands[1]

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Frequently references have appeared in the literature to substances possessing adrenocorticotropic activity that are not protein in nature. Anselmino, Hoffmann, and Herold (*1*), Tyslowitz (*11*), and C. J. O. R. Morris and colleagues (*2, 8*) have obtained material possessing adrenocorticotropic activity by ultrafiltration of acid, neutral, or alkaline extracts of hog or ox pituitaries. Recently Li and co-workers (*4, 5, 7*) have found that the products of peptic and acid digestion of pure ACTH protein possess adrenocorticotropic activity.

We have recently demonstrated that some sheep ACTH protein preparations can be extracted with trichloracetic acid, with the resulting supernatants possessing increased amounts of activity per unit of nitrogen. These preparations may be dialyzed at certain pH's, and the activity is shown to pass through the dialysis membrane (*3*).

The preparation, from sheep pituitary glands, of nonprotein fractions possessing adrenocorticotropic activity has been undertaken in the hope that good amounts of activity may be so obtained. Five percent trichloracetic acid (TCA) was used as the solvent. In a typical experiment, 250 g of fresh sheep pituitary glands were ground in the cold (5° C) with 175 ml of water. To this mixture 375 ml of 10% TCA was added, and the resulting mixture was stirred for 4 hr in the cold, and then allowed to settle out overnight. The precipitate was removed by centrifugation in the cold, and discarded. The TCA supernatant so obtained was extracted 10 times with 200 ml aliquots of diethyl ether in order to remove the TCA. The solution was then lyophilized, and 2.9 g dry weight of a brownish, hygroscopic powder were obtained. Kjeldahl analyses revealed the powder to contain 10% nitrogen, of which about 10% was ammonia and amide nitrogen, and 43% was amino nitrogen. When an aliquot of the powder was subjected to 2-dimensional paper chromatography in phenol-water followed by lutidine-water (1 : 1), and the paper then sprayed with ninhydrin, it was found that its free amino acid pattern was similar to the patterns obtained from the hydrolysis of casein. In addition, several spots undoubtedly due to other components (peptides?) were obtained.

The powders were then subjected to assay for adrenocorticotropic activity by the ascorbic acid method of Sayers *et al.* (*9*) and by the repair test of Simpson *et al.* (*10*), and were shown to be active. By the ascorbic acid depletion method, a response equivalent to 10 γ of an ACTH protein isolated by Li was obtained from the injection of 0.5 ml/100 g body weight of a solution containing 0.65 mg of the powder per ml. Thus an activity equivalent to 360 mg of an ACTH protein could be obtained from 1 kg of fresh sheep glands.[4]

Previous experiments have shown that peptic hydrolyzates of ACTH, consisting of polypeptides, when chromatographed on paper using phenol-water as solvent, yield among others a fluorescent area, with an R_F value of 0.95, which when eluted and assayed showed considerable adrenal-stimulating activity, whereas the rest of the paper was almost devoid of activity (*6*). This was demonstrated, too, with whole ACTH protein (*3*). TCA supernatants of whole sheep pituitaries, when chromatographed as above, presented a similar fluorescent area at the front (Fig. 1), which, when eluted, was shown to have considerable adrenocorticotropic activity by the ascorbic acid test, whereas the rest of the paper contained minimal amounts of activity. In one such experiment a total of 28 mg of the powder was run on a series of papers, and the fluorescent areas were eluted, combined, and lyophilized, and then dissolved in 4 ml of water. The solution was assayed at a level of 0.5 ml/100 g body weight, and a maximal response was

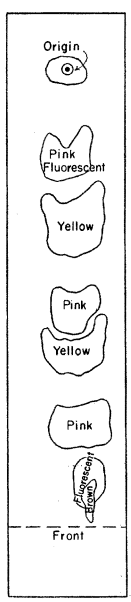

FIG. 1. Ascending chromatogram of 2 mg of the 5% TCA extract of fresh sheep pituitaries run in phenol-H_2O and developed with a 0.2% solution of ninhydrin in ethanol.

[1] Aided by grants from the U. S. Public Health Service RG-409 (C-2).

[2] With the technical assistance of Betsey S. Williams.

[3] We should like to acknowledge the support of C. H. Li in our experimental work, and the advice of John H. Northrop, who was kind enough to read this paper.

[4] In order to demonstrate further the nonprotein nature of the active material, 200 mg of the TCA extract powder was dialyzed for 3 days in the cold through a Visking sausage casing. The dialysate was lyophilized, and the resulting powder was made up to a concentration of 34 mg of the original powder per ml of solution. When assayed at 0.5 ml level, it showed a maximum response.