male (age 46) and 1 I.U. in 3 kg of female (age 55) axillary fat, these concentrations are so low as to be of questionable significance. (The age of the subjects conceivably may have been responsible for the low androgen content.) This marked difference in androgen concentration between the woodchuck hibernating gland and human axillary fat suggests that a high androgen content is not a property of all fatty tissues but may reflect the part played by the hibernating gland in the sex cycle of the woodchuck.

Any theorizing as to the significance of the androgen in the woodchuck glandular adipose tissue must await confirmation of this finding and a study of its concentration in other tissues of the animal. In view of the functions already attributed to the hibernating gland: a protein-sparer;[3] a storage point for vitamins and lipoids;[4] and the source of a metabolism-depressing secretion,[5] we hesitate to attach any functional significance to the presence of androgen in the gland until further evidence is available.

*Summary.* The hibernating glands of woodchucks killed during the summer contained 1 International Unit of androgen, (100 $\mu$g androsterone) in 50 g of tissue, equal to the concentration in bull testis, the richest tissue source known. Human male and female axillary fat contained 1 International Unit in 1 kg and 3 kg respectively.

**11583**

## A Laboratory Method for the Soilless Growth of Grass.

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Fresh green plant material is frequently used to supplement standard stock diets in the raising of laboratory animals such as the rat. Recently, additional impetus has been given to the study of young green growing plants by observations on the previously unknown factor (factor *pl* of Wulzen and Bahrs,[1] grass juice factor of

3 Vignes, H., *C. R. Soc. Biol.*, 1913, **75**, II, 360, 397, 418.

4 Cramer, W., *Brit. J. Exp. Path.*, 1920, **1**, 184.

5 Wendt, C. F., *Z. physiol. Chem.*, 1937, **249**, 2.

1 Wulzen, R., and Bahrs, A. M., *Physiol. Zool.*, 1936, **9**, 508.

Kohler, *et al.*[2, 3, 4]) necessary for the normal growth of guinea pigs[1-5] and on the nutritive value of cereal grasses.[6] Such studies would be greatly facilitated if it should be possible to grow young plants in the laboratory under reproducible conditions at any time and in the desired quantities.

A method has been developed in this laboratory for the purpose of adding the grass juice factor to relatively purified breeding diets for nutrition studies on mice which are handled with bacteriological precautions. The following qualities are essential: (a) a product which will provide a potent source of the grass juice factor, (b) uniform seed, (c) a nutrient solution composed of chemically pure ingredients, (d) a planting base which is as inert as possible, and (e) general cleanliness, with emphasis on freedom from insects, vermin and harmful bacteria.

After more than a year of study, the following method has been evolved and has proved satisfactory. It consists essentially of spreading soaked seeds on a layer of cotton placed on a screen which has been suspended over a pan containing nutrient solution. The roots grow down through the cotton. The grass begins to sprout within 24 hours after planting, and reaches a height of about 5 inches by the seventh day (Fig. 1).

Before outlining the steps in the procedure, some essential features of the materials, equipment and environment suited to growing grass in the laboratory should be considered.

*Seed.* English rye grass was used during the first year but was discarded because it proved to be an unreliable source of the grass juice factor when assayed by the method of Kohler, *et al.*[4] Barley seed was next tried and was found to be satisfactory. A sufficient supply of first quality, fumigated seed for a series of experiments to be compared is desirable. It should be kept in a vermin-proof, cool, dry place.

*Nutrient Solution.* Culture Solution No. 1 was selected from the 8 formulas suggested by Ellis and Swaney[7] because its ingredients

2 Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1937, **14**, 131.

3 Kohler, G. O., Elvehjem, C. A., and Hart, E. B., *J. Nutr.*, 1938, **15**, 445.

4 Kohler, G. O., Randle, S. B., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 154.

5 Cannon, M. D., and Emerson, G. A., *J. Nutr.*, 1939, **18**, 155.

6 Graham, W. R., Jr., Kohler, G. O., and Schnabel, C. F., paper read at Cincinnati Meeting of Am. Chem. Soc., Apr. 8 to 12, 1940.

7 Ellis, C., and Swaney, M. W., *Soilless Growth of Plants*, Reinhold Publishing Company, 1938.

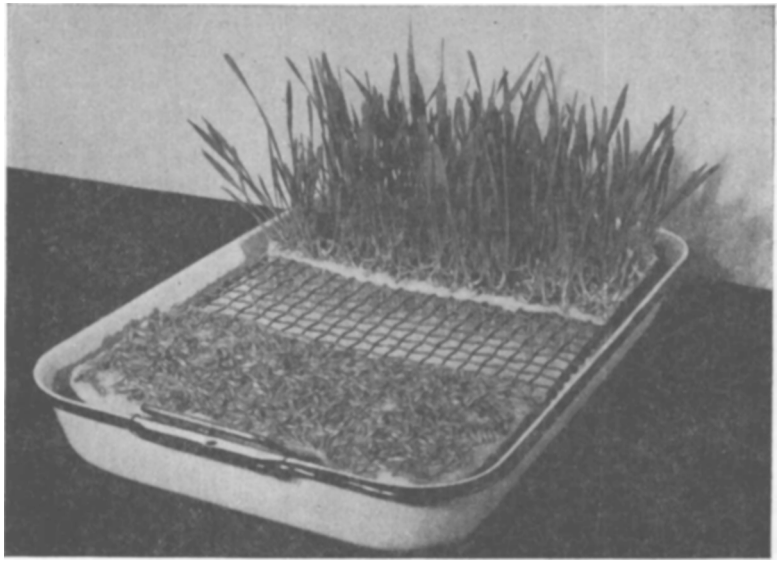

FIG. 1.
Planting pan with screen in place, showing seeds on day of planting and growing grass on the 7th day after planting.

are easily procured and it appeared to be equally adequate and less complicated than some of the others.

*Planting Pans.* Porcelain butcher trays 12″ wide, 17″ long and 2¼″ deep, of standard stock, are used. These trays are small enough to be easily handled, and large enough to grow about 75 g of barley grass per tray when it is cut on the seventh day.

*Planting Screens.* Cellar window wire which was galvanized, cadmium-plated or enameled with various finishes was tried and discarded because of corrosion. The corrosion not only contaminated the grass, but markedly retarded its growth. Stainless steel seems to be the material of choice at present. Two other qualities of the screen are important: (a) sufficient rigidity to prevent sagging, and (b) ample open space in relation to the wire to avoid interference with root growth. The stainless steel screens are half-inch mesh, and were made to fit the pans.*

*Suspension Wires.* 12-gauge aluminum wire is used for suspension of the screens because it is flexible enough to be adjustable and yet rigid enough to hold the screen in place. The need for this wire will be clear when the procedure for growing grass is considered below.

*Cotton.* Pieces from a pound roll of the cheapest absorbent cotton

* Made by H. T. Hernberg, 2012 North 32nd Street, Philadelphia.

are cut to fit the planting screens. Since a layer of one-half the original thickness is sufficient, each piece of cotton is divided horizontally, so that it furnishes material for 2 screens.

*Towel Supports.* Galvanized iron cellar window wire cut in pieces 12¾" x 18" is used.

*Towels.* Dampened turkish towels, 24" x 40", have been found satisfactory for keeping the seeds moist on the first and second days after planting.

*Temperature and Humidity.* The optimum temperature and humidity have not been determined. It is evident, however, that a temperature below 70°F and a relative humidity of less than 40 percent definitely retard the development of grass grown in this manner.

*Light.* The light that comes through ordinary glass appears to be adequate. It is important that the grass be uniformly exposed; therefore, the pans are turned around daily.

*Procedure* (for barley seed and the equipment described above).

*Day before planting:* Place 70 g of barley seed and 140 cc of distilled water in a container, mix thoroughly and allow to stand at room temperature for 18 to 24 hours.

*Day of planting:* 1. Lay cotton on the stainless steel screen, pour distilled water over it, patting gently so that all the cotton will become quickly saturated. 2. Spread the seeds evenly over the cotton. 3. Lay the towel support over the pan, resting it on the edges. This is necessary to prevent the damp seeds from adhering to the towel. 4. Place the double-folded towel, which has been lightly wrung out in distilled water, over the pan, taking care to cover the pan completely in order to prevent drying out of the seeds. No amount of soaking after the first 48 hours will fully overcome the ill effects of drying the seeds during the first 2 days.

*1st day after planting:* 1. Uncover the pan and sprinkle the seed-covered cotton generously with distilled water. 2. Replace towel support and towel which is moistened as on the previous day.

*2nd day after planting:* 1. Uncover the pan and sprinkle as on the preceding day. 2. Pour water from the pan. 3. Using the aluminum suspension wires, suspend the screen about 2 inches above the bottom of the pan (adjust the wires over the extended rims on the ends of the pan and bend both ends of each wire into a hook to grip the screen). 4. Pour nutrient solution into the pan to within about 1 cm of the screen.

*3rd to 6th days after planting:* 1. Sprinkle as on the preceding day. 2. Keep nutrient solution at about the same level by adding it

daily. 3. Keep screen raised so that roots clear the bottom of the pan.

*7th day after planting:* Cut and feed the green portion of the plants. In order to insure a uniform supply of fresh grass, only the first cutting is used. Seeds are planted at intervals to correspond to the days on which the grass is to be fed.

Kohler, Elvehjem and Hart[3] reported one experiment in which 5 g of greenhouse-grown oat grass per guinea pig per day did not furnish appreciable amounts of the grass juice factor. However, we have found that grass grown as described above and fed *ad libitum* in addition to the basal diet of Kohler and associates[4] completely protected guinea pigs against a deficiency of this dietary essential. Details of these experiments will be reported at a later time.

Although we were particularly interested in having a regular supply of the grass juice factor, grass for various purposes may be grown by this method.

## 11584

## Effect of Rammstedt Operation on Incidence of Cinchophen Ulcer.

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This study of the effect of the Rammstedt operation on the incidence of gastro-duodenal ulcer caused by the administration of large doses of cinchophen was undertaken for several reasons. First, it was observed that the ulcer caused by cinchophen is frequently located at the site of the pyloric sphincter, as well as in the duodenum, and along the lesser curvature of the stomach as has been observed by others.[1] Second, it was found that the intravenous injection of from 10 to 25 mg per kilo body weight of cinchophen caused an increase in the activity of the pyloric sphincter in 6 out of 14 dogs. The activity of the pylorus was recorded by a balloon made for the purpose. The increased activity of the pyloric sphincter, illustrated in Fig. 1, was abolished by atropine, but not by bilateral section of the vagi in the neck. Third, the results of studies by us on the effect of cinchophen (100 mg per kilo per day) on bilirubin clearance (4

1 Churchill and Van Wagoner, *Arch. Path.*, 1932, **14**, 860; Bollman and Mann, *Proc. Staff Mayo Clin.*, 1935, **10**, 580.