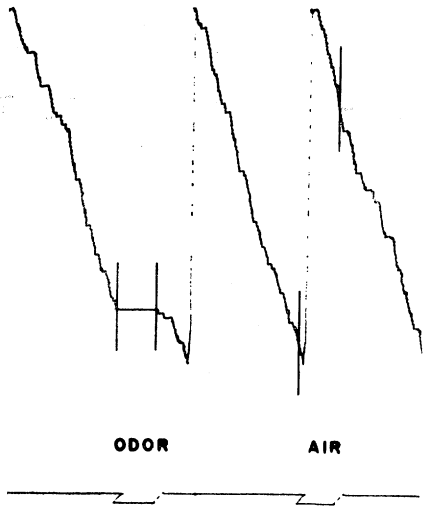

Fig. 2. Sample record of odor discrimination (cumulative response record of bar pressing). The pen moves from the top to the bottom of the record and resets automatically. Note that there is complete cessation of response during the introduction of odor but no diminution of response during the introduction of air from an empty control channel. The pairs of vertical lines on the record were drawn in to show the onset and duration of stimulation indicated by the signal marker.

odor bottles are maintained at a constant temperature by water bath *g*. The outflow from each odor bottle is controlled by a three-way stopcock so that the odor can be added to the main stream or shunted through a bypass (not shown) to an exhaust. This provides a continuous flow of air through the odor bottle when the input stopcock to the odorant bottle is opened, to insure equilibrium when the odor is added to the main stream. The concentration of odorant in the odor stream is a function of the rate of air flow over the odorant surface, the height of the odorant in the odor flask, and the vapor pressure of the odorant. The concentrations of odorant can be computed from the weight of odorant lost for given durations and flow rates. The final concentration of odor can be computed from the relative flow rates of pure and odorized air and may be specified in milligrams per liter, moles per liter, or number of molecules.

All parts of the olfactometer are made of Pyrex glass, including the first 5 in. of the "wind tunnel" section (*j*). The liberal use of ground glass joints makes the entire system easily demountable for cleaning. Section *k* is made of brass tubing 4 in. in diameter and contains the bar-pressing platform and dipper mechanism, which projects 3 in. into the glass part (*b*) of the response chamber. The platform, bar, and dipper and the anterior two-thirds of the animal are visible to the experimenter. A new model of the apparatus has been built (*3*) in which the response chamber is made entirely of glass and in which the bar and drinking font project into the chamber from below, through small ports.

The training procedure is typical of operant discrimination training in which responses are occasionally reinforced in the absence of odor (S^D) but never during the presence of odor (S^Δ). The onset of odor signals the "no-reinforcement" condition. A control training procedure is employed in which clean air from an empty odor bottle is presented, *with reinforcement,* to insure that discrimination is learned on the basis of odor and not of other cues incidental to turning stopcocks, to changes of air pressure, and so on. Odor training (S^Δ) and control training are interspersed randomly. After discrimination has been established, test trials consisting of a 10-second period during which odorized air is introduced and a 10-second odorless control situation are presented, without reinforcement.

In the early experiments we employed a discrimination criterion of fewer than six responses during the 10-second odor test with no diminution in response rate during the 10-second odorless-air control procedure. In order to maintain discrimination at this level, discrimination training trials were continued and interspersed among test trials. Training trials usually consisted of 30 seconds or more of odor (S^Δ) or of odorless control procedure. Responses during the 10-second control test numbered approximately 30 (mode), with a range of from 20 to 40, depending on the animal. A well-trained animal usually stopped bar-pressing immediately in response to a suprathreshold odor, with a negligible latency after the odor stopcock was turned (see Fig. 2). The time before resumption of bar-pressing after the odor is turned off may be variable, but this was not important in the present testing procedure. This method has proved useful for studies of threshold in normal animals after ablation of the olfactory bulbs and in experiments on animals with altered endocrine balance.

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1. B. F. Skinner, *Behavior of Organisms* (Appleton-Century-Crofts, New York, 1938).
2. This study was carried out in the psychology laboratory of Brown University, under a grant from the National Science Foundation.
3. Victor Bartosewitz of the Brown University chemistry department constructed the glass components of this olfactometer.

29 July 1958

Chromatography of Molten Salts on a Glass Powder Column

The physicochemical similarity of glasses with zeolithes and with the noncrystalline oxides (silica, alumina) used in chromatography induced us to test the ion-exchange and adsorptive properties of glass under conditions in which these properties might be expected to be of practical use. As a medium, molten salts were selected.

A Pyrex column of inner diameter 8 mm and length 20 cm was filled with Pyrex glass powder (100 to 140 mesh); the column could be slowly charged with the melt. As stopcocks, connecting tubes of length 10 cm and inner diameter 2.5 mm were used. The tubes were placed in sections of the (vertical) oven that could be cooled.

In order to avoid chemical attack on the glass, only salts melting below 400°C were used, such as alkali nitrates and mixtures of alkali halides (preferentially the eutectic mixture of lithium and potassium chlorides, which melts at 352°C).

To this mixture, from 0.1 to 1 percent of chlorides of heavy metals, such as Cr^{III}, Cu^{I}, Ni^{II}, Co^{II}, and Mn^{II}, were added. Separation in zones was observed, which compared in sharpness favorably with those obtained by chromatography in aqueous solution. With the above column and the eutectic mixture used, the adsorption of the heavy metals at 400°C decreased in the above order.

Elution (with pure molten salt) is as easy as in the chromatography from aqueous solutions.

When a small particle of the eutectic mixture of lithium and potassium chlorides, containing one or more heavy metals, is slowly heated to the melting point on a porous Pyrex filter plate, concentric colored circles, showing the differential adsorption of the heavy metals, are observed.

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Nonpolar Transport of Gibberellin through Pea Stem and a Method for Its Determination

Gibberellin was found to differ from auxin in many physiological effects (*1*). Hence, it is of interest to see whether the transport of gibberellin through the stem is polar or not. As to gibberellin transport, it is only known that gibberellin is transported lengthwise twice as rapidly as transversely (*2*).

A method of determining gibberellins is needed for transport experiments, as

well as for many others. The fluorometry by Phinney *et al.* (*3*) and the method of Bird and Pugh (*4*) are not quantitative. In this report (*5*) a method of using ultraviolet absorption is presented. The basipetal and the acropetal transports of gibberellic acid were compared with each other by means of this method. The dwarf-corn method was also used.

Figure 1 represents the ultraviolet absorption curves of alcohol solutions of gibberellic acid, gibberellin A, and a crude gibberellin preparation (*6*). Gibberellic acid has two peaks of absorption, at 210.5 and 253.5 mμ, while gibberellin A has only one, at 210.0 mμ. The crude gibberellin preparation used also had peaks corresponding to these, with two additional peaks between them.

It was determined by means of ethanol solutions that Beer's law holds for the absorbency at 253.5 mμ of gibberellic acid, at least for concentrations of up to 200 μg/ml, but does not hold for the absorbency of gibberellic acid at 210.5 mμ or for gibberellin A. The absorption curve and the absorbency at 253.5 mμ of gibberellic acid did not change, at least during 24 hours at room temperature. Hence, gibberellic acid may be measured with the absorbency, if proper precautions are taken.

In order to apply to gibberellic acid the method used for the transport of

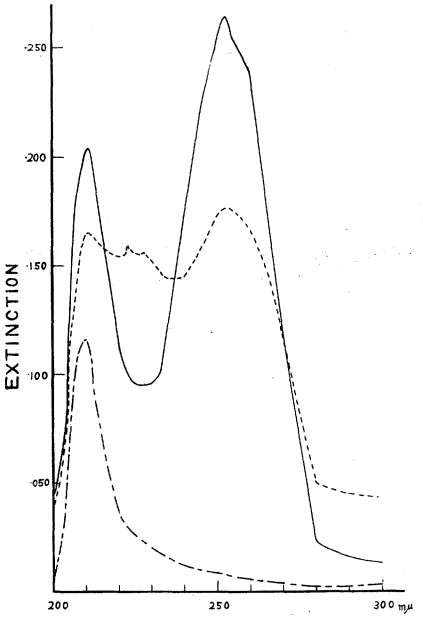

Fig. 1. Ultraviolet absorption curves of ethanol solutions of gibberellic acid (dash-dot line), gibberellin A (broken line), and a crude gibberellin preparation (solid line).

Table 1. Amount of gibberellic acid transported through the pea stem section in the acropetal and in the basipetal directions.

Position of stem section	Gibberellic acid content (μg/block)				Gibberellic acid (μg/stem hr)	
	Donor		Receiver		Disappearing from donor	Transported into receiver
	Initial	Final	Initial	Final		
Normal	11.1	7.0	0	0.87	1.37	0.29
Inverted	11.1	6.4	0	1.02	1.57	0.34

auxin (*7*), the acid contained in agar blocks should be measured. Hence, the most suitable solvent for the extraction from agar was looked for, as follows.

Well-washed agar blocks 35 by 35 by 20 mm³ in size were soaked in 70-percent ethanol for a month, with occasional renewal of ethanol in order to wash out soluble substances from them. They were washed again with distilled water. Thirty-six pea stem sections were put on each agar block, in the normal position or inverted, just as they would be placed in the transport experiment to be conducted. After 3 hours' incubation at 25°C, the blocks were steeped for 24 hours in ethanol, acetone, ether, and acidic ether at 4° to 5°C. A number of blocks were extracted, one by one, separately for each solvent in order to see the probable error. Extracts were evaporated, and ethanol solutions of the residues were assayed spectrophotometrically. Acetone extraction gave the smallest probable error and seemed to be the most suitable. Recovery by acetone from the agar block in which 100 μg of gibberellic acid had been dissolved was about 80 percent. Weak absorbence was detected, even with the extracts made from agar blocks without any treatment.

The extracts from the agar blocks which had been in contact with stem sections, as well as the extracts from the blank blocks, showed no gibberellin reaction in the dwarf-corn test.

Pea seedlings (var. Usui) were grown for 7 days in a darkroom at 25°C. A 5-mm section was excised from the third internode, which was from 15 to 20 mm long. These sections were laid on moistened filter paper for 3 hours in order to let exuding substances out. The sections were then put standing on agar blocks prepared as mentioned above, 36 on each block, in the normal position and inverted. Upon each of the stem sections an agar block 2 by 2 by 2 mm³ in size and containing 11.1 μg of gibberellic acid was placed as the donor. After 3 hours at 25°C the donor blocks and the receiver blocks were extracted with acetone for 24 hours at 4° to 5°C. The extracts were evaporated, the residues were dissolved in ethanol, and the absorbency at 253.5 mμ was measured. The changes in gibberellic acid content of the donor and the receiver blocks are given in Table 1. The acropetal transport was only a little larger than the basipetal.

Bioassay of gibberellic acid in receiver blocks was also made. After 3 and 24 hours of standing, the receiver blocks were extracted as described above. Each dried residue was mixed with 1 g of lanolin. About 60 mg each of the pastes thus prepared was smeared on the seedling of dwarf corn grown in a seeding box under field conditions of from late spring to summer. Gibberellin reaction was positive only with the paste prepared from the blocks which had received gibberellic acid for 24 hours. No difference with respect to the direction of the transporting pea stem sections, however, was observed.

It is thus confirmed that the gibberellic acid transport through pea stem is not notably polar, as auxin transport is known to be (*8*).

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4. H. L. Bird, Jr., and C. T. Pugh, *Plant Physiol.* 33, 45 (1958).
5. This report is No. 4 in a series of studies on the physiological effect of gibberellin.
6. The gibberellic acid used in this study was provided through the courtesy of Dr. C. Leben, of Eli Lilly and Company. The gibberellin A and the crude gibberellin were provided through the courtesy of Prof. Y. Sumiki.
7. F. W. Went and K. V. Thimann, *Phytohormones* (Macmillan, New York, 1937).
8. I wish to thank Prof. Joji Ashida for his cordial guidance in the course of this work and Dr. Bernard O. Phinney, associate professor, University of California, for kindly providing me with dwarf corn seeds.

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