tomically, but the dynamics of the aqueous humor are equal in both. Therefore, it seems impossible to maintain the assumption that there is a predilection of Negro eyes for glaucoma.

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Staining Methods in Chromatography of Acidic and Neutral Mucopolysaccharides

Staining of complex carbohydrates following separation by paper chromatography or electrophoresis requires reagents other than those commonly used for detection of simple sugars. Such reagents as potassium permanganate (*1*), ammoniacal silver nitrate (*2*), acidic naphthoresorcinol (*3*), sodium metaperiodate, and lead tetraacetate (*4*) have been used to detect nonreducing sugars. The Hotchkiss periodate method has also been employed to stain protein-bound carbohydrates on filter paper after electrophoresis (*5*), although difficulty has been encountered in the rather intense staining of the paper itself and in applying this method to the detection of acidic mucopolysaccharides after chromatography (*6*). Acidic mucopolysaccharides have also been detected after paper electrophoresis (*7*) or ascending chromatography (*8*) by virtue of a metachromatic reaction with toluidine blue.

Mucoproteins of biological origin may consist of mixtures of acidic and neutral mucopolysaccharides. Toluidine blue stains substances metachromatically with free anionic groups. Neutral mucopolysaccharides and acidic mucopolysaccharides in which the anionic groups are combined—as, for example, with proteins, or otherwise masked—stain orthochromatically (*9*) or may fail to stain at all on paper.

Mucicarmine is frequently used empirically in staining procedures for the demonstration of "mucin" from various epithelial secretions (*10*). This stain may be effective histologically in sites where toluidine blue fails to stain (*11*). The chemical basis for its staining action is uncertain (*12*).

Mucicarmine has been adopted by us as a stain for locating the position of acidic and neutral mucopolysaccharides following paper partition chromatography. Heparin, potassium chondroitin sulfate from cartilage (*13*), and potassium hyaluronate from umbilical cord (*13*) served as acidic mucopolysaccharides. For a neutral mucopolysaccharide, dialyzed and concentrated blood group substances A and B (Sharp and Dohme) from hog and horse stomach were used. The product was subjected to hydrolysis with $2N$ H_2SO_4, and chromatographic examination for simple sugars revealed fucose, galactose, and hexosamine. Hexuronic acid was not detected on the chromatograms. Only traces of hexuronic acid could be detected by use of the naphthoresorcinol method (*14*).

Duplicate ascending chromatograms of solutions of blood group substances and acidic mucopolysaccharides were run on Whatman No. 1 paper for 22 hours at 23°C. The solvents found most satisfactory consisted of 55 percent of $0.05M$ Na_2HPO_4 and 45 percent of redistilled isopropyl alcohol, and 58 percent of a phosphate-sodium chloride buffer ($0.0007M$ NaH_2PO_4, $0.09M$ Na_2HPO_4, and $0.07M$ NaCl) (*15*) and 42 percent of redistilled isopropyl alcohol.

After the chromatograms have dried, fixation of the paper is desirable before staining. This was suggested by Leitner and Kerby (*6*) who used 95-percent alcohol and ether for this purpose. We have obtained somewhat sharper delineation of spots and more brilliant colors after staining by using formalin and ethanol as a fixative. Each chromatogram was immersed in a mixture of 20 ml of neutral formalin and 80 ml of absolute ethanol for 15 minutes. After drying, one chromatogram was sprayed with toluidine blue, the other with mucicarmine. Both sides of the paper were sprayed and care was taken to avoid running of the dye on the paper.

Recent reports have described many fractions in commercial toluidine blue of varying staining ability (*16*). In addition to a metachromatic staining fraction, much of the dye lot may either stain chromotropes orthochromatically or lack staining power entirely. Hence, commercial toluidine blue was recrystallized several times from hot water and alcohol before use. Forty milligrams of the recrystallized dye was added to 80 ml of acetone and 20 ml of distilled water. The solution was stored in a dark bottle at 5°C. Acetone effectively prevented spreading of the spots and did not inhibit metachromasy as alcohol does at this concentration. Other cationic dyes (pinacyanol, methylene blue, and crystal violet) were tried, but toluidine blue was the most satisfactory in brilliance of metachromatic color and in showing the least background discoloration of the paper.

Mucicarmine stock solution was prepared according to Lillie's method (*17*). The carmine was not recrystallized. Different dye batches gave similar staining results. One gram of carmine, 0.5 g of anhydrous $AlCl_3$, and 2 ml of distilled water were heated together in a porcelain crucible for 2 to 3 minutes. It was found desirable to drop the crucible directly into 100 ml of 50-percent ethanol. After 24 hours the solution was filtered and stored at 5°C. The spraying reagent was made up prior to use. Five milliliters of the stock solution were added to 17 ml of absolute ethanol and 3 ml of distilled water.

After the chromatograms had been sprayed, they were repeatedly rinsed in distilled water acidified with a small amount of glacial acetic acid (*6*) until the background discoloration was largely removed; then they were washed in distilled water and hung up to dry. More vivid metachromatic color changes were noted on chromatograms that had been sprayed with toluidine blue when they were dried in the cold.

Toluidine blue revealed acidic mucopolysaccharides as bright pink (heparin) or purple (chondroitin sulfate and hyaluronate) spots on a light blue background. Blood group substances did not stain. Mucicarmine stained both acidic mucopolysaccharides and blood group substance bright pink on a very light pink or colorless background.

The use of these two sprays on duplicate chromatograms thus permits the separate identification of acidic and neutral mucopolysaccharides in materials of biological origin (*18*).

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Technique for Assessing the Effects of Drugs on Timing Behavior

Before a drug can safely be recommended for relief of a specific physiological malfunction it must be checked for possible deleterious effects upon other systems. Careful consideration must also be given to the possibility of "toxic" effects upon normal adaptive behavior, particularly in the case of those drugs that are known to produce behavioral changes. In view of the importance of temporal orientation in normal behavioral functions, the effect of drugs upon timing would appear to constitute a primary area of investigation. The purpose of this report is to describe a method, based on earlier work by Skinner (*1*), for producing and measuring timing behavior in experimental animals, and to present some data resulting from the administration of amphetamine and alcohol (*2*).

White rats, deprived of water for 22.5 hours, were placed in a small chamber containing a lever and a mechanism for automatic delivery of a small drop of water. In the first session every depression of the lever by the animal produced the water reinforcement. In all following sessions, each 2 hours long, reinforcement was contingent on lever presses spaced at least 21 seconds apart. That is, a response produced the water only if it followed the preceding lever depression by at least 21 seconds. Programming and recording were accomplished automatically by timers, magnetic counters, and associated relay circuits.

Using this procedure, Wilson and Keller have demonstrated that the rate of lever pressing is inversely related to the length of the required delay between responses (*3*). Since drug-produced general excitatory or depressive effects might themselves alter the rate of lever pressing (*4*), a more useful measurement of the timing is the relative frequency distribution of interresponse times (intervals between successive responses). This distribution, which displays no consistent trend after the animals have been exposed to the experimental procedure for 30 to 60 hours, is illustrated by the "saline" control records of Fig. 1. The large proportion of responses that are spaced less than 3 seconds apart is typical and unexplained, but the remainder of the distribution provides a clear description of the timing behavior. The relative frequencies of interresponse times rise to a peak between 18 and 21 seconds and then display a gradual decline.

The center sections of Fig. 1 illustrate the effects of two relatively large doses of *dl*-amphetamine sulfate administered subcutaneously, 5 minutes prior to the experimental session, in a solution of 1 mg/ml of physiological saline. Increasing doses of this drug tend to move the peak of the distribution progressively toward the short interresponse times. That is, the animals press the lever more frequently before the required interval has elapsed.

In addition to the shifts in the relative frequency distribution, large increases in the total response output occurred (Table 1). In contrast, intraperitoneal injection of 3 ml of a 10-percent ethyl alcohol solution, representing a dose of 1 mg/kg, produced a decline of more than 50 percent in the rate of lever pressing. The relative interresponse time frequencies, however, as shown in the lower section of Fig. 1, displayed little change except for a slight leveling of the total distribution. Although alcohol, in the dose administered, produced a general depression of lever-pressing behavior, there was relatively little effect upon the timing.

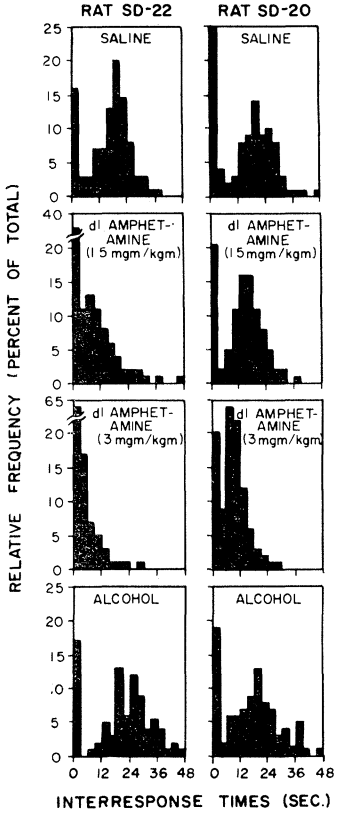

Fig. 1. Relative frequency distributions of the time intervals between successive lever presses. Each distribution represents one animal's performance in a single 2-hour session.

Table 1. Total number of lever presses emitted during each 2-hour session

Rat	Saline	*dl*-Amphetamine		Alcohol
		1.5 mg/kg	3.0 mg/kg	
SD-20	471	534	854	201
SD-22	433	820	1816	176

This technique for generating and measuring timing behavior is applicable to the minimally restrained individual organism, produces stable behavior over long periods of time, has procedural simplicity, and permits automatic programming and recording. The orderly response to drugs, illustrated by this data, indicates the feasibility of including this method in programs designed to screen drugs for their behavioral effects.

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Altering Juvenility with Auxin

One of the most carefully described instances of the juvenile phase in plant development is that of the Brussels sprouts (*Brassica oleracea gemmifera* L. var. Kolom) (*1, 2*). Plants of this species must reach a certain age—generally 11 weeks—before they are sensitive to the floral inductive experience of low temperature.

The effectiveness of low temperatures on flowering is known for many species of plants (*2–5*). The recent report by Moore (*6*) that auxin applied before low temperature may accentuate the bolting response of cabbage suggests the possibility that auxin levels insufficient for bolting may be a partial basis for juvenility.

This paper (*7*) deals with the influence of auxin on the low temperature induction of floral initiation in Brussels sprouts. It is shown that an application of auxin during the cold treatment has a promotive effect on flowering and can effectively shorten the juvenile phase.