ing of soluble materials from chloroplasts may be reduced by the presence in the isolating medium of high-molecular-weight substances such as polyethylene glycol (Carbowax 4000, molecular weight 2500) (*1, 2*). McClendon (*2*) has shown that a buffered solution of Carbowax (40 g/100 ml) is beneficial in maintaining the morphologic integrity of chloroplasts during isolation. In addition, Carbowax tends to prolong the time that such isolated chloroplasts are active in the Hill reaction.

In the hope that Carbowax might prove useful in a study of the intracellular location of enzymes in leaves, its effect on the apparent distribution of amylophosphorylase was investigated (*3*). Although Carbowax proved unsuitable for this purpose, the results obtained are of interest in view of the use of Carbowax in the isolation of chloroplasts and mitochondria (*2, 4*). Phosphorylase was chosen as a test enzyme because it is highly soluble and almost always found in the supernatant liquid derived from the centrifugation of sucrose homogenates of starch-free leaves (*5*). Logically, its presence would be expected within the chloroplasts where starch is usually formed.

One explanation offered for the distribution observed in sucrose homogenates is that phosphorylase diffuses from the chloroplasts during the isolation procedure (*6*). If this explanation is correct, then a study of the distribution of phosphorylase should be a good index of the effectiveness of any particular isolation method in preventing the loss of highly soluble enzymes from the chloroplasts during their isolation.

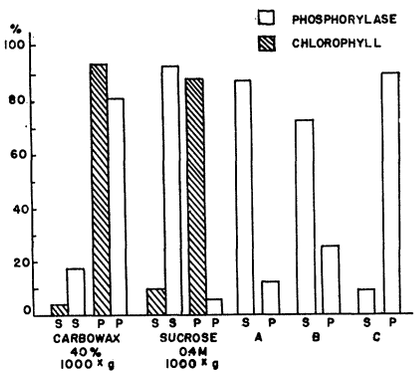

Fig. 1. Effect of Carbowax on the distribution of phosphorylase in leaf homogenates. Phosphorylase and chlorophyll reported as percentage of the total found in the supernatant and the precipitate. (*S*) Supernatant after centrifugation; (*P*) precipitate after centrifugation; (*A*) phosphorylase distribution after the 1000 *g* sucrose supernatant was centrifuged for 10 minutes at 20,000 *g*; (*B*) the same, to which 4 g of Carbowax had been added to 20 ml prior to the final centrifugation; (*C*) the same, to which 8 g of Carbowax had been added to 20 ml prior to the final centrifugation.

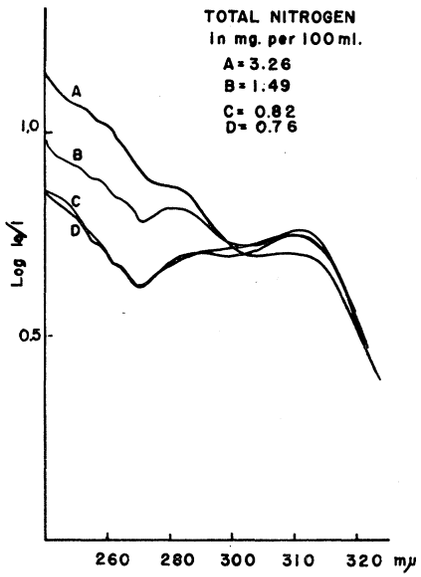

Fig. 2. Ultraviolet-absorption spectra of the supernatant obtained from leaves homogenized in sucrose (0.4*M* sucrose, 0.1*M* citrate, *p*H 6). (*A*) Supernatant after 20,000 *g* centrifugation for 10 minutes; (*B*) the same, to which 2 g of Carbowax per 20 ml had been added before centrifugation; (*C*) 4 g of Carbowax added per 20 ml; (*D*) 8 g of Carbowax added per 20 ml.

When starch-free leaves of young tobacco plants were ground in iced Carbowax (Carbowax 4000, 40 g/100 ml of 0.1*M* citrate, *p*H 6), more than 80 percent of the phosphorylase was found in the particulate fraction obtained after centrifuging at 1000 *g* for 10 minutes (Fig. 1). In contrast, when a parallel experiment was run with a sucrose medium (0.4*M* sucrose, in 0.1*M* citrate *p*H 6), more than 90 percent of the enzymatic activity appeared in the supernatant fluid after centrifugation at 1000 *g*. However, when a solution of Carbowax was added to this supernatant, phosphorylase was precipitated. When the final Carbowax concentration was 20 g/100 ml, approximately 17 percent of the phosphorylase was precipitated. A final concentration of Carbowax (40 g/100 ml) precipitated 90 percent of the phosphorylase (Fig. 1). Qualitative tests indicated a similar precipitation of catalase.

The precipitation of soluble enzymes by Carbowax renders this material of doubtful value in studies of the intracellular distribution of enzymes. Certainly the occurrence of phosphorylase in the chloroplast fraction isolated in Carbowax solution is not evidence of the natural distribution of this enzyme.

McClendon (*2*) observed, during plastid isolation, that Carbowax precipitated ultraviolet-absorbing material, which he presumed to be nucleoprotein. Figure 2 shows the effect of adding Carbowax in solution to the supernatant obtained from leaves that were homogenized in sucrose solution and centrifuged at 1000 *g* for 10 minutes. After the addition of the Carbowax, the solutions were centrifuged at 20,000 *g* for 10 minutes, and the ultraviolet-absorption spectra of the resulting supernatant solutions were determined with the Cary recording spectrophotometer. It was evident from the decreased absorption at 280 mμ that Carbowax 4000 in concentrations of 20 and 40 g/100 ml precipitates protein from the leaf homogenate. The decrease in absorption at 260 mμ is in agreement with McClendon's observations.

These results indicate that, although it may be useful in maintaining the apparent morphologic integrity of the chloroplasts and in stabilizing certain of their biochemical reactions, notably the Hill reaction, Carbowax is not suitable for studies of the intracellular distribution of enzymes. Soluble enzymes and other proteins are precipitated by Carbowax and may contaminate the particulate fractions. Unless a method is employed to remove the precipitated material, chloroplasts isolated in Carbowax will be associated with enzymes not present as normal *in vivo* chloroplast constituents. This effect should be taken into consideration by investigators who use chloroplasts or other cellular bodies isolated in Carbowax solutions.

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29 December 1955

Assessment of Drug Effects on Emotional Behavior

Recent developments in the use of chemicotherapeutic agents for clinical psychopathology have stimulated renewed interest in laboratory-testing methods for assessing behavioral changes associated with such drug administration. Animal-conditioning experiments promise to provide the behavioral control techniques that are basic to such an approach,

although the selective assessment of specific emotional or affective responses that are of primary interest in this area has continued to present both methodological and theoretical problems. The purpose of the present report is to describe a method, based on earlier animal experimental work (*1*), for producing and selectively measuring emotional behavior in experimental animals and to present some data that illustrate the use of this method for investigating the behavioral effects of amphetamine and reserpine (*2*).

Rats and monkeys that had been deprived of solid food and liquids for 24 hours or more were trained to press a bar for a reward of water (rats) or sugared orange juice (monkeys). Initially, the animals received a drop of the liquid reward every time they pressed the lever (continuous reinforcement), although they were rapidly shifted to a schedule on which the bar-press produced the reward only occasionally (average, once in 60 seconds). When the response rates had stabilized on this variable-interval reinforcement schedule during experimental sessions that lasted several hours or more, a conditioned emotional response of the "fear" or "anxiety" type was superimposed upon the lever-pressing behavior (*3*). Briefly, this conditioned "anxiety" response consisted of suppression of lever pressing, crouching, defecation, and immobility upon presentation of a clicking noise that had previously been paired with a painful electric shock to the feet. In the present study, the clicking noise was presented at 7-minute intervals during the experimental session and continued for 3 minutes before termination with the grid shock (approximately 1.5 ma) to the feet. Programming of the experimental procedure and recording of the animals' behavior were accomplished automatically by timers, magnetic counters, cumulative-work recorders, and associated relay circuits.

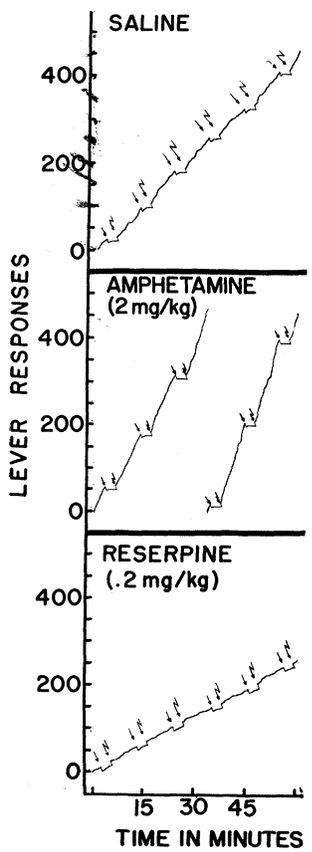

Fig. 1. Sample cumulative-response curves for rat AA-26 showing the effect of amphetamine and reserpine on lever pressing and on the conditioned emotional response. The oblique solid arrows indicate the onset of the conditioned auditory stimulus, and the oblique broken arrows indicate the termination of the conditioned stimulus contiguously with the brief, unconditioned grid-shock stimulus to the feet.

The behavior pattern that develops as a consequence of this procedure is illustrated for one of the rats by the cumulative-response record in the top ("saline"-control) section of Fig. 1. A marked depression in lever-pressing rate is apparent during the 3-minute clicker periods, which are indicated by the short offset sections of the cumulative curve between the straight ("clicker") and broken ("shock") arrows, although the stable lever-pressing rate is maintained throughout the 7-minute intervals between emotional-conditioning trials. After establishment of this pattern, the ratio of the number of lever responses during the clicker periods to the number of lever responses during the nonclicker periods has been found to remain stable (showing no consistent trend) during more than 80 to 100 experimental hours.

The center section of Fig. 1 illustrates the effects of a relatively large dose of amphetamine administered intraperitoneally to the same animal 1 hour prior to this behavior sample. The total number of lever responses during this 1-hour period shows more than a 100-percent increase over the saline-control session, although the rate increase is accounted for completely by increased lever pressing in the 7-minute periods between emotional conditioning trials. The number of lever responses during the 3-minute clicker periods is actually seen to decrease under the influence of the drug.

In contrast, daily intraperitoneal injections of 0.2 mg/kg of reserpine were found, after 4 days, to produce a decrease of more than 50 percent in the total number of lever responses during the 1-hour session for this same animal, although the conditioned suppression of responding during the 3-minute clicker periods was virtually eliminated. The lower section of Fig. 1 shows that, despite the over-all depression in lever pressing, the animal, under the influence of this drug, continued to respond throughout the 3-minute clicker presentations at the same rate as during the 7-minute intervals between conditioning trials, even though the pain shock continued to be paired with termination of the clicker.

The results obtained with this technique have been replicated with several animals (rats and monkeys) (*4*). It is clear, however, that the method described does provide an approach to the selective assessment of specific drug-behavior relationships in the affective sphere while providing a control for the general behavioral and motor disturbances that frequently develop as nonspecific side effects of such drug administration.

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5 January 1956

Cork Virus Leafspots on Triumph Sweetpotato Contain Separated Parenchyma Cells

In the autumn of 1954, leafspotting was observed on specimens of the Porto Rico and Triumph varieties of sweetpotato that were growing beside one another on the same bench in a greenhouse at Beltsville, Md. Close examination of the specimens revealed a distinction between the kinds of spots on the different kinds of specimens.

On the Porto Rico variety there were some leaves with few or many chlorotic spots that were later surrounded for a time by a rather sharp, purple ring. This foliage symptom was transmitted within a month by approach and cleft grafting and is now known to be typical of internal cork virosis on this pigmented variety.

On the Triumph variety, a starchy, nonpigmented type, the spots were chlorotic at first, tending to enlarge and become translucent, and were followed later by necrosis. Microscopic examination of free-hand sections revealed the striking fact that the parenchyma cells were uncemented or free and easily separated from one another (Fig. 1). Pressure on the coverslip made the free cells, with their complement of chloroplastids, move apart and separate. Obviously the something or entity causing the spots