ever, when 0.27*M* acetaldehyde in tritium oxide was incubated for 30 minutes at room temperature in the presence of aldolase, no tritium was detected in the isolated dimedon derivative. This finding, taken in conjunction with the observed failure of fructose-1,6-diphosphate to incorporate tritium in the presence of aldolase (*2*), indicates that the enzyme does not, either in the presence or absence of DHAP, significantly exalt the polarization of the aldehyde carbonyl function. From the foregoing considerations it appears that the formation of C ⟷ E from DHAP is the sole detectable activating function of aldolase.

The observation has been made (*1*) that when fructose-6-phosphate is incubated with phosphoglucose isomerase in deuterium oxide the product, glucose-6-phosphate, contains one atom of deuterium per mole. This finding reveals that the isomerization is mediated by labilization of a proton leading to C ⟷ E formation rather than through a hydride ion migration. The data in Table 1 and results reported recently (*3*) demonstrate that approximately one atom of tritium is incorporated per mole of DHAP incubated with phosphotriose isomerase. Thus it would appear that the mechanism of isomerization of triosephosphate (Eq. 4) is identical with that of hexose phosphate. The same stereochemical relationships as described for the hexose isomerase reactions (*1*) would apply here; that is, either the *cis* or the *trans* enediol, but not both, is the intermediate in the interconversion of DHAP and D-glyceraldehyde-3-phosphate. The formation of L-glyceraldehyde-3-phosphate, if it occurs, would presumably involve the other enediol.

Mechanistically the aldolase and phosphotriose isomerase reactions have two features in common; that is, both involve C ⟷ E formation and both activate only one hydrogen atom per mole of substrate. The latter observation suggests that both enzymes are capable of distinguishing between the two hydrogen atoms on the primary carbinol. It was of further interest to determine whether the hydrogen which is labilized by aldolase is the same as that labilized by phosphotriose isomerase. When DHAP containing carbon-bound tritium, introduced by the action of aldolase in tritium oxide, was again subjected to the action of aldolase in hydrogen oxide a complete loss of tritium occurred. A similar incubation of labeled substrate with phosphotriose isomerase resulted in no significant loss of tritium. These results and those previously reported (*3*) clearly show that each enzyme acts on a different alpha hydrogen. Here then, are two more examples of substrate-enzyme relationships in which the Ogston concept applies; that is, at least three sites are implicated in complex formation. Two of these sites must involve the hydroxyl and one of the hydrogen atoms of the carbinol.

As is pointed out in a foregoing paragraph, C ⟷ E formation is a common feature of the aldolase and phosphotriose isomerase reactions. This finding, taken together with the observation related to the stereospecific character of these enzymes suggests that in the one case a *cis*-enediol is involved, while in the other a *trans*-enediol is implicated (*1*).

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Plasma 17-Hydroxycorticosteroid Changes Related to Reserpine Effects on Emotional Behavior

Recent studies of adrenal-pituitary activity and behavior have shown that marked elevations of plasma 17-hydroxycorticosteroid (17-OH-CS) levels can be selectively related to conditioned emotional response patterns of the "fear" or "anxiety" type in laboratory animals (*1*). Disruption of lever-pressing behavior for a liquid reward by thirsty monkeys in response to the presentation of an auditory stimulus that had previously been paired with an electric shock was found to be accompanied by plasma 17-OH-CS increase at the rate of 20 to 25 μg percent per hour. A closely related series of experiments has also shown that chronic administration of reserpine can markedly attenuate the suppressing effect of the conditioned anxiety stimulus on lever-pressing behavior (*2*). This series prompted a further analysis of the interrelationships between such drug activity and the adrenalcortical response to emotional conditioning.

Rhesus 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 sugared orange juice. When the response rates had stabilized, the conditioned emotional response was superimposed on the lever-pressing behavior by repeated presentation of a 5-minute clicking noise that was terminated contiguously with a painful electric shock to the feet. After a few such pairings, the anxiety response appeared as a disruption of the stable lever-pressing pattern, accompanied by crouching, trembling, piloerection, and frequently urination and defecation.

Experimental sessions lasting 1 hour consisted of six such 5-minute clicker presentations with 5-minute periods of no clicker between each trial. Blood samples were drawn before and after each session, and the plasma was analyzed for 17-OH-CS concentrations by the Nelson-Samuels method (*3, 4*). Base lines were determined for plasma 17-OH-CS responsiveness to the anxiety stimulus alone and to control procedures including lever-pressing alone after conditioning. Figure 1 shows the selective increases in steroid output related to the anxiety conditioning situation as compared with the control procedures.

Following several such base-line determinations and unequivocal establishment of the relationship between plasma corticosteroid increase and the emotional behavior, daily intramuscular doses of reserpine (0.75 mg/kg) were administered 20 to 22 hours before the time of the experimental sessions (*5*). Figure 2 shows the effects of these treatments on the behavioral response to the conditioned anxiety stimulus and on the corticosteroid response during such experimental sessions for one monkey. The strength of the anxiety response is indicated on the left ordinate of the curve in terms of "inflection ratios" (*6*), a

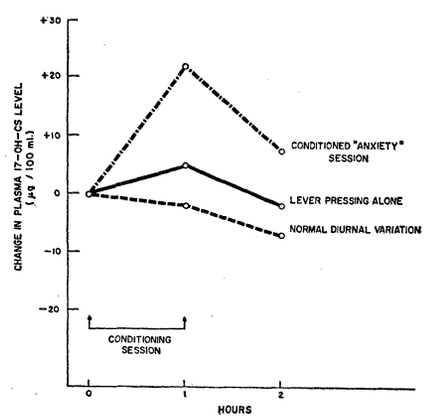

Fig. 1. Plasma 17-hydroxycorticosteroid levels during anxiety conditioning sessions compared with control levels during lever-pressing sessions without the emotional stimulus and compared with normal diurnal levels.

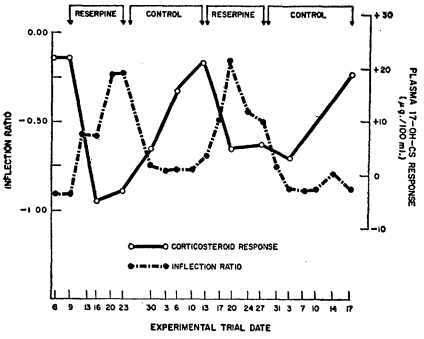

Fig. 2. Effects of reserpine on the emotional response and on the levels of plasma 17-hydroxycorticosteroid during anxiety conditioning sessions.

measure derived from comparing the number of lever responses during the 5-minute periods with lever responses during the 5-minute intervals between clickers. Inflection ratio values near 0.00 indicate that an approximately equal number of lever responses were emitted during and in the absence of the clicker (no disruption of the rate by the anxiety stimulus), and values near – 1.00 reflect virtually complete suppression of the lever-pressing rate during the operation of the clicker. Inflection ratio values between 0.00 and – 1.00, then, indicate progressively greater degrees of suppression of lever-pressing as a consequence of the clicker presentations. The corticosteroid response is indicated on the right ordinate of the figure; it is expressed in terms of micrograms per 100 ml of plasma change during the 1-hour experimental session.

The control points on the figure taken before reserpine treatments were initiated show the relationship between suppression of lever pressing in the anxiety situation and elevation of corticosteroid levels during such a session. Within 1 week following daily reserpine administration, however, suppression of the lever-pressing response by the anxiety stimulus had virtually disappeared (indicated by the inflection ratio values near 0.00), and corticosteroid levels showed no elevation during the experimental sessions. As a matter of fact, even the slight rises in corticosteroid levels that usually accompanied control lever-pressing sessions without clicker after emotional conditioning failed to appear. The levels during this treatment period more closely approximated the normal diurnal variation (Fig. 1).

When the drug treatments were discontinued after 2 weeks, suppression of lever pressing by the anxiety stimulus reappeared (inflection ratios approximating – 1.00), and corticosteroid elevations of about 20 μg/100 ml of blood were again recorded during the experimental sessions. Finally, readministration of reserpine following recovery of both the behavioral and the steroid response to the anxiety stimulus again produced attenuation of both the psychological and physiological reactions, with subsequent recovery following withdrawal of the drug.

Clearly, the striking correlation between the alterations induced by reserpine in lever-pressing rate and in adrenal-cortical activity during anxiety sessions suggests that the relationship between these behavioral and endocrine responses is an intimate one. There is some indication in the present data, however, that recovery of the plasma corticosteroid response following withdrawal of reserpine may proceed somewhat more slowly than the reappearance of the emotional response to the anxiety stimulus.

Although the suppression of lever pressing was again apparent within 1 week after withdrawal of the drug, the steroid response to the anxiety stimulus did not completely return to normal until the third week after cessation of treatment. The fact that the recovery periods differed somewhat in length could indicate that the neural mechanisms underlying the two responses may be, at least in part, independent. The precise definition and specification of such psychophysiological relationships, however, remain for future research to delimit (7).

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Occurrence of a Bitter Principle in Carrots

Bitterness in carrots is a serious problem in some of our vegetable-producing states and is annually causing a considerable loss to the industry. Bitterness has been associated with carrots having a low alpha-carotene content (1), with carrots infected with aster yellow virus (2), and with stored carrots that have been grown in muck soils (3). Although no bitter principle has been isolated from carrot roots, a syrupy bitter glycoside has been isolated from carrot leaves (4), and another bitter glycoside has been isolated from the seeds of red carrots (5). Neither of these substances was crystalline, and therefore the identity of these materials remains doubtful.

Table 1. R_f values of a crystalline bitter principle of carrots and the acetone extracts of bitter carrots on Whatman No. 1 filter paper (8).

Solvent	R_f values	
	Crystalline principle	Acetone extracts of bitter carrots
Water	0.44	0.44
Phenol saturated with water	0.97	0.97
Acetic acid (15 percent)	0.74	0.74
Heptane: 1-butanol: water (29:14:57 by vol.)	0.58	0.58
Ethylacetate: ammonia (2*N*) (1:1 by vol.)	0.55	0.55

Because of the current interest in the flavor problem of this important vegetable crop, an investigation was begun to isolate and characterize compounds that are responsible for bitterness (6).

Previous workers have reported two methods for measuring the bitterness of carrots (7), both based on absorption in the ultraviolet region. In an attempt to identify the bitter principle, R_f values on paper chromatograms were determined in five different solvents for acetone extracts of bitter carrots, and the crystalline compound was isolated from bitter carrots (Table 1). The isolated crystalline compound has the characteristic absorption in the ultraviolet region used in the methods previously mentioned (7), the same R_f values as the substances in the bitter carrot extract, and the identical flavor of bitter carrots.

Fourteen bushels of bitter carrots were dried in a forced-draft oven for 3 hours at 60°C. The dried carrots were ground in a Wiley mill to pass a 2-mm sieve. Twenty pounds of the ground material was extracted in a large Soxhlet unit with acetone for 8 hours. The acetone was removed under reduced pressure until crystals began to separate from the solution. One hundred milligrams of bitter crystals were obtained; these were recrystallized from aqueous methanol