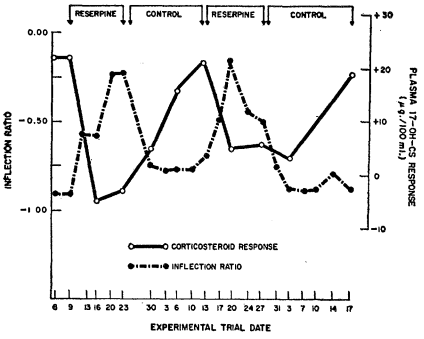

Fig. 2. Effects of reserpine on the emotional response and on the levels of plasma 17-hydroxycorticosteroid during anxiety conditioning sessions.

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

until a constant melting point was attained. The colorless platelets melted at 77°C and were soluble in chloroform, ethyl ether, water, and methanol. Sodium fusion indicated the absence of halogens, sulfur, and nitrogen. A molecular weight of 268 was obtained by the Rast camphor method, and elemental analysis showed that the compound contained 63.94 percent carbon and 5.86 percent hydrogen. These data suggest an empirical formula of $C_{15}H_{15}O_5$. The ultraviolet absorption spectrum in methanol revealed a maximum at 268 mμ and minima at 242 mμ and 287 mμ. The infrared spectral analysis in chloroform showed a maximum at 6.01 μ.

A search of the literature failed to reveal a discussion of a compound approximating $C_{15}H_{15}O_5$ and having the previously mentioned properties.

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8. Spots detected as blue fluorescence in the ultraviolet.

23 July 1956

New Chemical Method to Differentiate Human-Type Tubercle Bacilli from Other Mycobacteria

No morphological or immunological method to differentiate the various types of mycobacteria exists at the present time. All attempts to find a type-specific skin reaction have also failed. The only methods in current use are based on cultural and pathogenic characteristics. This paper describes chemical methods that are dependent on the metabolic properties of mycobacteria.

Pope and Smith (*1*) determined the vitamin-B production in the culture filtrate of human and bovine tubercle bacilli, respectively, grown on synthetic liquid media. Bird (*2*) also measured the vitamin-B content of human tubercle bacilli.

Konno and coworkers (*3*) reported on a marked quantitative difference in the niacin production by human-type tubercle bacilli and other mycobacteria when grown on synthetic culture media. This difference is strictly linked to the type of the bacillus.

The present investigation aims at simplification of the previous method by using colonies of bacilli taken directly from the routine solid diagnostic media. The following materials were used: 15 strains of standard laboratory mycobacteria (Table 1), 50 strains of tubercle bacilli obtained from tuberculous patients, including three isoniazid-resistant, catalase-deficient strains, which did not cause progressive tuberculosis in guinea pigs, and 10 strains of "atypical acid-fast bacilli," most of them chromogens (Table 2). These laboratory and clinical materials were inoculated on the Loewenstein-Jensen's solid culture media and allowed to grow from about 1 to 3 months.

For the estimation of niacin, the aniline and cyanogen bromide method (*4*) is used; a few colonies are carefully taken from the culture medium by a platinum loop and transferred into a test tube that contains 1 ml of 4-percent aniline solution (*5*). To this is added 1 ml of 10-percent cyanogen bromide (*6*). A positive test shows the development of an intensive canary-yellow color first noticed in the bacterial sediment of the test tube and later, after shaking, in the supernatant fluid. A positive test is developed only by human-type tubercle bacilli, whereas the other mycobacteria (bovine, avian, nonpathogenic mycobacteria and the group of "atypical acid-fast bacilli") do not develop an appreciable yellow color.

Controls with malachite green-containing medium showed a green discoloration of the fluid. Controls with aniline solution and bacilli or cyanogen bromide solution and bacilli show no color production. The color production by this aniline-cyanogen bromide method is more intense than the previously used metol and cyanogen bromide method (*7*) or the ammonia buffer and cyanogen bromide method (*8*).

As mentioned before only human-type tubercle bacilli give a positive test, irrespective of virulence. This was confirmed with standard laboratory strains as well as with strains obtained directly from patients; no difference in the test was noted between isoniazid-sensitive or resistant, catalase-producing or deficient strains. Bovine tubercle bacilli, whether highly virulent or as attenuated as the B.C.G., show a negative test. Avian tubercle bacilli, nonpathogenic bacteria, and all "atypical acid-fast bacilli" studied to date give negative tests.

Table 1. Niacin test of standard laboratory mycobacteria.

Type	Strain	Niacin test
Human tubercle bacilli	Virulent	
	1) H37 Rv	+
	2) Campbell	+
	3) Erdman	+
	Attenuated	
	4) H37 Ra	+
	5) JH 16 Ra	+
	6) JH 6 Ra	+
Bovine tubercle bacilli	Virulent	
	7) Vallée	−
	8) Ravenel	−
	Attenuated	
	9) B.C.G.	−
Avian tubercle bacilli	10) Sheard	−
	11) Camden	−
	12) Avian I	−
Nonpathogenic mycobacteria	13) *M. ranae*	−
	14) *M. phlei*	−
	15) M. 607	−

Table 2. Niacin test of tubercle bacilli and "atypical acid-fast bacilli" obtained from patients.

Fifty strains of tubercle bacilli including three isoniazid-resistant, catalase-deficient strains	Niacin test positive 50	Niacin test negative 0
Ten strains of "atypical acid-fast bacilli"	Niacin test positive 0	Niacin test negative 10

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5. Four-percent aniline in 96-percent ethylalcohol is practically colorless.
6. Dissolve 50 g of cyanogen bromide in 500 ml of distilled water. This reagent is colorless. Both reagents remain stable for several months if they are kept in a brown bottle and in the refrigerator.
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