A few samples of human malignancies have also been examined, and here too significant thioglycosidase activity was found.

Some representative values for thioglycosidase activities of normal mouse tissues are: small intestine, 5370; spleen, 2895; liver, 2100; stomach, 1626.

Mammalian thioglycosidase activity is inhibited by delta-gluconolactone at relatively high concentrations. Twenty-five-percent inhibition is produced at $10^{-3}M$, and 50 percent, at $10^{-2}M$. A number of related compounds were studied as inhibitors, including D-glucurono-δ-lactone, D-gluconic acid, D-galacturonic acid, D-galacturono-δ-lactone, D-gulono-δ-lactone, D-arabono-δ-lactone, calcium-D-arabonate, isoascorbic acid, iso-inositol, D-ribono-δ-lactone, and D-glucoheptono-δ-lactone. Of these, all were inactive with the exception of D-galactono-δ-lactone. Various other substances have been examined for activity on mammalian thioglycosidase [enzyme inhibition or no activity are indicated by (+) or (−), respectively]: $HgCl_2$ (+); NaN_3 (−); $CuSO_4$ (+); NaF (+) Na_2SO_3 (+); H_2O_2 (+); 2,4-dinitrophenol (+); 2,4-dinitrothiophenol (+); Na_3AsO_4 (+); glutathione (−); H_2S (−); cysteine (−); EDTA (−).

Further studies of the properties of the enzyme and its substrate specificity were undertaken with acetone powders of mouse intestine, hog liver, and hog pancreas (which are quite similar in thioglycosidase activity). These may be stored for at least 15 weeks at −10°C, after which time the activity slowly declines. The optimum *p*H curve is rather broad, with the maximum activity at about *p*H 5.8. The enzyme is sensitive to elevated temperatures, and heating for 5 minutes at 60°C results in a loss of 50 percent in activity; heating for 5 minutes at 70°C causes almost total inactivation. The rate of hydrolysis decreases with time; after about 3 hours under the conditions given above, the reaction finally ceases entirely, although excess enzyme and substrate are present, as shown by the fact that addition of either more substrate or more enzyme to the reaction mixture causes further hydrolysis. However, the enzymatic hydrolysis is not inhibited by the hydrolysis products.

A number of purine, pyrimidine, and other thioglycosides (*13*) have been examined as substrates for the mammalian thioglycosidase. The results are summarized in Table 1. From these results, certain generalizations may be made. The active substrates include derivatives of D-glucose, D-galactose, L-arabinose, and D-xylose, while the D-arabinoside is virtually inactive. The essential configuration required for activity is that of β-D-glucopyranose or β-D-galactopyranose. The hydroxymethyl group of C-5 may be replaced by —H, but replacement by —COOH (as in the thioglucuronides) results in loss of activity. The 6-purinyl structure is the most active aglycone found to date, but a rather wide range of aglycone variation is permitted, including purines, pyrimidines, and pyridazines.

Thus, the enzyme appears to resemble the thioglycosidase enzymes previously found only in plants and is similar to the classical β-glucosidase found in plants and animals in the structural requirements for the glycosyl residue. Furthermore, both classes of enzymes are inhibited by delta-gluconolactone (*14*).

When the presence of purinethioglycosidase at high levels of activity in tumors was first discovered, it was hoped that MPG might serve as a relatively nontoxic carrier of 6-MP, which thus would be released *in situ* in the tumor, and that the differential effect of 6-MP on tumors might thus be increased. The pharmacological properties of MPG, its rapid clearance, and its poor cellular penetration (*4*) appear to interfere with the realization of this aim. Thus, to explain the significant antitumor effects of MPG and related compounds on the basis of tumor thioglycosidase levels would appear to be premature at this point, for when MPG was administered intraperitoneally to the mouse, no evidence of cleavage to 6-MP was observed in any tissue, in spite of the demonstrated presence of thioglycosidase activity in nearly all mammalian tissues. However, orally administered MPG is rapidly hydrolyzed, liberating 6-MP in the gut.

No clues to the occurrence of natural substrates for this thioglycosidase have yet been found. Its high level of activity and wide distribution are all the more puzzling since MPG appears to possess the optimal substrate configuration.

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10. We gratefully acknowledge the valuable technical assistance of Mr. Ludwig Salce in these studies.
11. Absorption maxima and molecular extinctions at *p*H 6: for 6-MP; $\lambda_{max.} = 320$ mμ, $E_m = 18.9 \times 10^3$; for MPG, $\lambda_{max.} = 280$ mμ, $E_m = 16.0 \times 10^3$.
12. Resting cells of *Escherichia coli* B showed no thioglycosidase activity, whereas a sonicated cell-free extract of this organism showed marked activity. This observation may have bearing on the cell permease hypothesis of Monod *et al.* [G. N. Cohen and J. Monod, *Bacteriol Revs.* 21, 169 (1958)].
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27 March 1959

Learning Differences in Two Separated Lines of Mice

Abstract. Significant differences in conditioning and response topography were obtained with two lines in C57BL/10 mice, suggesting the occurrence of a behavioral mutation. It is suggested that the two lines be classified as substrains. The two substrains would appear to be useful in experiments on the genetics of behavior.

In the summer of 1957 while working at the Behavior Laboratory of the Roscoe B. Jackson Memorial Laboratory (*1*), I found that 150-day-old C57BL/10 mice had a significantly higher conditioning score than did equivalent mice tested the previous year in a different study (*2*). In trying to determine the cause of this difference, I noted that the mice used in 1957 had come from the colony at the Main Laboratory while the mice tested in 1956 had come from the Behavior Laboratory. Mice from the Behavior Laboratory colony which were 150 days old were then tested and found to have a mean conditioning score very similar to the mean obtained the previous year with the Behavior Laboratory animals. A number of 50-day-old mice were available from both laboratories and were also tested. The same phenomenon was obtained: the mean score of the Behavior Laboratory mice in 1957 was not significantly different from that of the Behavior Laboratory mice tested in 1956, while the Main Laboratory animals were again significantly better on the conditioning task.

These findings suggested either that there had been some genetic change or that the environmental rearing conditions in the two laboratories were different enough to affect conditioning ability differentially. To determine whether these differences were simply correlated

Table 1. Summary statistics on conditioning of 50-day-old mice by year of testing, laboratory strain, and filial generation.

Year	$\bar{X}$	s	n	Generation
Behavior Laboratory				
1956	1.80	2.30	10	*
1957	2.17	2.23	6	F_{64}, F_{65}
1958	1.45	1.13	11	F_{67}
Total	1.74	1.83	27	
Main Laboratory				
1957	4.21	1.93	14	F_{57}, F_{58}
1958	4.44	1.51	9	F_{60}, F_{61}
Total	4.30	1.76	23	

* Unknown.

with place of rearing, pregnant mice from the Main Laboratory colony were brought to the Behavior Laboratory in the summer of 1958, and their offspring were reared and tested under the same environmental conditions as the Behavior Laboratory animals. Both groups were tested at 50 days of age.

The conditioning procedure, described fully elsewhere (*2*), consisted essentially of presenting a 3-second buzzer which was always followed immediately by 1 second of shock. Forty-one seconds later the buzzer-shock sequence was repeated. Ten such trials were given. The conditioned response was defined as any gross movement by the mouse during the interval that the buzzer was on. For each trial the animal received either a score of 0 for no response or a score of 1 for a conditioned response. Since the mouse could not make a conditioned response on the first trial, the maximum possible score was 9. Table 1 summarizes the pertinent statistics by year, laboratory strain, and filial generation. The mice from the Behavior Laboratory were all reared and tested there. The Main Laboratory mice in 1957 were reared there and then sent over to the Behavior Laboratory, where they were tested; in 1958 they were reared and tested at the Behavior Laboratory. Within each laboratory strain the means do not differ significantly, while the mean difference between laboratory strains is significant beyond the .01 level.

A further analysis of the 1958 data showed that the type of conditioned response differed between the strains. Of the 16 responses made by the Behavior Laboratory mice, 13 were runs and three were jumps. This finding is consistent with the results obtained in 1956 (*2*). However, of the 40 responses elicited by the Main Laboratory mice, 30 were jumps and ten were runs.

The Behavior Laboratory and the Main Laboratory mice were separated in 1947 at F_{32}, and each strain has passed through approximately 30 generations since then, during which time a mutation is likely to have occurred which could have caused the obtained behavioral differences. Since the strains have been apart for more than the 15 to 20 generations of separated breeding which has been specified in the definition of a substrain (*3*), it is evident that these two groups should be considered substrains. It is suggested that the Main Laboratory mice be designated C57BL/10Jax, while the Behavior Laboratory mice maintained by J. P. Scott would continue to be called C57BL/10Sc.

The results suggest that a behavioral mutation has occurred which affects learning capacity and response topography. Thus, these two substrains would appear to be useful for experiments on the genetics of behavior. Another implication of these data is the important methodological consideration that, even when highly inbred animals are used, care should be taken to use as experimental subjects only animals which are closely related in terms of filial generation (*4*).

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24 December 1958

10-Hydroxy-Δ^2-Decenoic Acid, an Antibiotic Found in Royal Jelly

Abstract. 10-Hydroxy-Δ^2-decenoic acid, the major component of the lipide fraction of royal jelly, exhibits antibiotic activity against many bacteria and fungi. This fatty acid is less than one-fourth as active as penicillin against *Micrococcus pyogenes* and less than one-fifth as active as chlortetracycline against *Escherichia coli*. It also slows the growth rate of *Neurospora sitophila* and some unidentified molds. The salt of this compound is considerably less active than the free acid.

The presence of antimicrobial activity in the royal jelly of the honey bee (*Apis mellifera* L.) has been known for 20 years. McCleskey and Melampy (*1*) demonstrated that, although royal jelly inhibited the growth of both Gram-positive and Gram-negative bacteria, it was about twice as effective against the Gram-positive organisms. More recent investigations (*2*) have shown that both royal jelly and its extracts exhibit antibiotic activity against a variety of microorganisms. Other investigators (*3*) have been unable to demonstrate antibacterial activity in royal jelly. This discrepancy may be explained by the fact that the various investigators have tested royal jelly samples of different ages and that the antibacterial activity of royal jelly diminishes with age (*4*).

McCleskey and Melampy (*1*) showed that the antibacterial component of royal jelly was soluble in either acetone or alcohol and was present in concentrates of the fatty acid and nonsaponifiable fraction. Abbott and French (*5*) suggested that a fatty acid was at least partially responsible for the ability of royal jelly to resist bacterial and fungal infestations. They further suggested that the high sugar content of royal jelly may also contribute to its ability to inhibit the growth of microorganisms. Fractionation of royal jelly in this laboratory has shown that its antibiotic properties result solely from the presence of an unusual fatty acid, 10-hydroxy-Δ^2-decenoic acid, isolated from royal jelly by Butenandt and Rembold (*6*) and more recently by Barker *et al.* (*7*), who reported little activity against bacteria and fungi.

Royal jelly was collected from queen cells and immediately frozen (−16°C). The frozen royal jelly (90 g) was lyophilized, yielding a cream-colored powder (30 g) which inhibited the growth of *Escherichia coli* and *Micrococcus pyogenes* when tested by the paper disc method. The powder was then extracted for 72 hours with ethyl ether in order to remove the lipide components (2.9 g). The remaining powder was inactive against the test organisms, but the ether extract was active. The ethereal solution was extracted with 2*N* KOH. This alkaline extract was acidified with 0.1*N* H_2SO_4, and the free acids were extracted from it with ethyl ether. Removal of the ether on a steam bath yielded a thick yellow gum (2.59 g). The neutral lipide fraction was inactive, proving that the antibiotic principle found in royal jelly is in the fatty acid fraction.

The fatty acid fraction was dissolved in warm 50-percent ethyl alcohol and kept at −10°C for 24 hours. The acid precipitated as thin white crystals which were collected and recrystallized from 50-percent ethyl alcohol. The acid melted at 56° to 57°C. Carbon-hydrogen analyses indicated an empirical formula of $C_{10}H_{18}O_3$. The infrared spectrum of the acid showed the presence of a hydroxyl band at 2.97 μ and an unsaturation band at 6.05 μ, which is characteristic of a hydroxy-unsaturated acid. All data are in agreement for 10-hydroxy-Δ^2-decenoic acid. By means of a mixed melting point and an infrared comparison with a pure sample of this acid (*8*), its structure was confirmed.