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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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.

The pure acid was tested on strains of *Escherichia coli* and *Micrococcus pyogenes* by incubating both organisms in antibiotic assay media fortified with serial dilutions of the acid. Comparison with penicillin (*M. pyogenes*) and chlortetracyclne (*E. coli*) indicated that this fatty acid was less than one-fourth as active as penicillin and less than one-fifth as active as chlortetracycline. In this procedure, the activity cannot be attributed solely to any change in *p*H produced by incorporating the acid into the medium, because each serial dilution was adjusted to *p*H 6.7 after the substance was added.

When 10-hydroxy-Δ^2-decenoic acid was evaluated by the paper disc method, neither of these organisms was able to grow in the presence of an impregnated disc during a 14-day period. However, when the salt was tested by the same procedure, the activity was considerably less than that of the free acid. Conversion of the acid to the salt may explain why royal jelly loses its bactericidal activity when neutralized or made slightly alkaline (*9*).

10-Hydroxy-Δ^2-decenoic acid also inhibited the growth of *Neurospora sitophila* as well as several unidentified molds. The antibiotic activity of this acid merits attention because it is active against both bacteria and fungi.

Townsend *et al.* (*10*) reported that admixture of royal jelly with tumor cells before inoculation completely suppresses the development of a transplantable mouse leukemia and the formation of ascitic tumors in mice. Fractionation showed this antitumor activity arises from the main fatty acid of royal jelly: 10-hydroxy-Δ^2-decenoic acid.

This growth inhibiting fatty acid is related to another natural product, the wound hormone of plants, traumatic acid (Δ^1-decene-1, 10-dicarboxylic acid) (*11*). Growth-inhibiting properties have also been demonstrated for other fatty acids (*12*), particularly for undecylenic acid against bacteria (*13*) and fungi (*14*).

It is not surprising that royal jelly contains a substance inhibiting the growth of microorganisms, since it would otherwise be an excellent substrate for bacterial growth. The origin of this fatty acid in royal jelly is now being investigated.

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Inhibition of O-Methyltransferase by Catechol and Sensitization to Epinephrine

Abstract. Catechol at equimolecular concentration inhibits the inactivation of adrenaline by O-methyltransferase in vitro about 50 percent. The inhibition is probably responsible for the sensitization of smooth muscles to epinephrine by various ortho di- or triphenols.

One of us (Z.M.B.) described in 1936 a remarkable potentiation of the effects of epinephrine on various smooth muscles of the cat (nictitating membrane, spleen, uterus, blood vessels) after injection of catechol or pyrogallol, but not of resorcinol (*1*).

The interpretation, at that time, was that the sensitizing phenols slowed down the oxidation of epinephrine by virtue of their well-known antioxidant action.

A new interpretation becomes highly probable after the demonstration by Axelrod *et al.* that epinephrine is inactivated by an O-methyltransferase which methylates the phenolic OH in position 3 (*2*). It seems that epinephrine is not a specific substrate, that other ortho-diphenols are also methylated by this enzyme. Thus, logically, catechol might sensitize in vivo by competitive inhibition of the methyltransferase.

The following simple test strongly suggests that such an inhibition occurs in vitro. In the reaction studied by Axelrod, the methyl donor is S-adenosylmethionine—that is, methionine activated by adenosine triphosphate (ATP). We have prepared a solution of enzymes containing the methyltransferase and the methionine-activating enzyme by precipitating a particle-free supernatant of rat liver homogenate (1 hour at 105,000 *g*) with ammonium sulfate (90 percent saturation) and dissolving the precipitate in $KHCO_3$. This solution, in the presence of a sufficiently high concentration of methionine and ATP, inactivates epinephrine.

The conditions are as follows: 0.6 to 0.4 μmole of adrenaline is incubated at 37°C for 45 minutes with 1 ml of the enzyme solution, in the presence of $MgCl_2$ (10 μmole/ml) in phosphate buffer (*p*H 7.6) (100 μmole/ml). The final volume is 2 ml. The quantities of ATP and methionine necessary to inactivate 70 percent of the epinephrine are about 10 μmole each; greater quantities do not increase the percentage of inactivation and interfere with biological titration. After incubation, proteins are precipitated by trichloracetic acid (5 percent) and centrifuged. Epinephrine is titrated by fluorescence (*3*) and by its action on the blood pressure and nictitating membrane in the cocainized cat.

During incubation, part of the epinephrine is inactivated, probably by auto-oxidation. If one adds an equimolecular amount of catechol to the system, the percentage of enzymatic inac-

Table 1. Influence of catechol on the inactivation of epinephrine by incubation with soluble enzymes of rat liver in the presence of ATP and methionine.

Experimental conditions				After incubation		
Tube No.	Epinephrine (μmole)	Catechol (μmole)	ATP and *l*-methionine (μmole)	Fluorescence (%)	Epinephrine (μmole/ml)	Inactivation (%)
1	0.4			61.5	0.08	0
2	0.4		10	18.5	0.024	71
3		0.4		2		
4		0.4	10	4		
5	0.4	0.4		62.5	0.081	0
6	0.4	0.4	10	36	0.047	42
7		0.8		2		
8	0.4	0.8		58	0.075	7.5
9	0.4	0.8	10	53	0.069	15