receptors of *Anthopleura* show specificity for the size and shape of activator molecules. Glutamate, although it induces no positive feeding responses in *Anthopleura* (Table 1), is an effective competitive inhibitor of the ingestion response of anemones both to reduced glutathione and to *Artemia* homogenate (*10*). This points to GSH as the specific component of *Artemia* tissue fluids that induces ingestion. The same type of inhibition of the response to GSH by glutamate is seen in *Hydra* (*11*). Therefore, like *Hydra*, the GSH receptors of *Anthopleura* must be most sensitive to the γ-glutamyl moiety of the molecule. Unlike the case in *Hydra* (*12*), in *Anthopleura* the thiol group of GSH cannot be methylated. The molecule's primary chain length also appears to be a very important feature. Loomis (*1*) found that *Hydra* gives no response to asparthione (next smaller homolog of GSH). Likewise, the sea anemone *Boloceroides* gives no response to leucine, the next larger homolog of its feeding activator, valine (*5*). *Anthopleura* is sensitive to asparagine, but glutamine (next larger homolog of asparagine) does not elicit positive feeding responses (Table 2).

Preliminary results (*10*) indicate that reduced glutathione may affect the cilia of the mouth and actinopharynx. Experiments with colloisol dyes (Farbwerke Howchst, Frankfurt) have shown that nonfeeding anemones in clean seawater have ciliary currents flowing out of the mouth and across the oral disk toward the tentacles. Dye squirted above the oral disk does not enter the coelenteron but is eventually rolled up in mucus and moved off the oral disk. This current is reversed in the area of the mouth when animals are placed in solutions of glutathione. Dye enters the coelenteron and is retained there for over an hour (*10*). Reversal of ciliary currents in coelenterate feeding behavior was proposed in 1896 by Parker (*13*).

The effects of asparagine and reduced glutathione on the behavior of *Anthopleura* are very different. Asparagine is a feeding incitant, that is, a substance that induces the initial contact between mouth and food, or tasting (*14*). Reduced glutathione is a feeding stimulant, that is, a substance that induces ingestion or continued feeding (*14*). Asparagine induces tentacle bending and transfer of treated paper from tentacle to mouth. Ingestion of this paper does not occur, however, unless glutathione is present. Glutathione induces swallowing but only when present directly on the mouth.

The experiments with *Anthopleura* illustrate an extension of the role played by chemical activators in the feeding behavior of a coelenterate, the division of the feeding response into two phases, each phase controlled independently by a different chemical activator. It is probable that other phases of coelenterate feeding behavior, for example, the "preparatory feeding" phase described by McFarland (*15*), will be found to be controlled by specific chemical activators as well.

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8. Approximately 50 to 100 nauplii were washed several times in distilled water and homogenized in 1 ml of water. This homogenate was spotted and dried on Whatman No. 1 filter paper.
9. A small percentage of anemones (less than 20 percent) ingested untreated filter paper. An even smaller percentage (5 to 15 percent) rejected paper treated with *Artemia*.
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Genetic Aspects of Increase in Rat Liver Aldehyde Dehydrogenase Induced by Phenobarbital

Abstract. *In the supernatant fraction of homogenized rat liver, the activity of aldehyde dehydrogenase that is dependent on nicotinamide adenine dinucleotide (E.C. 1.2.1.3) is increased up to tenfold after administration of phenobarbital for 3 days. The effect is genetically controlled and is inherited as an autosomal dominant characteristic. The mechanism is apparently unrelated to other drug-induced increases in enzyme activity such as that which occurs in the hepatic microsomal systems for drug metabolism.*

Aldehyde dehydrogenases that are dependent on nicotinamide adenine dinucleotide (NAD) are enzymes of broad substrate specificity located in highest concentrations in the liver although they exist in other tissues in smaller amounts (*1*). The enzyme activity is present in both the supernatant and mitochondrial fractions of liver homogenates.

There is evidence that the enzymes recovered from these two subcellular fractions differ in their substrate specificities (*2*) and in their physical properties (*1*). However, neither has been completely purified. The enzymes are capable of the oxidation of a large number of aldehydes to the corresponding acids. Among these aldehydes are glyceraldehyde, an intermediate in fructose metabolism (*3*); acetaldehyde, a product of ethanol metabolism (*4*); the aldehydes generated from biogenic amines as a result of monoamine oxidase activity (*5*); and many other aromatic and aliphatic aldehydes (*6*).

Recently Redmond and Cohen (*7*) reported that repeated injections of phenobarbital into mice resulted in a twofold increase in aldehyde dehydrogenase activity in whole liver homogenates. This report describes a similar but much greater effect in rat liver and demonstrates that it is confined to the soluble enzyme present in the supernatant and is inherited as a dominant characteristic in this species.

Rats of the Fischer, Long-Evans, and Sprague-Dawley strains were obtained from Simonsen Laboratories. The optimum weight of the rats used in these studies was about 200 g. Rats were injected intraperitoneally with phenobarbital (20 mg/ml) in a dose of 100 mg per kilogram of body weight, once daily. Three doses were required for a consistent response, and even a dose of 100 mg/kg was not sufficient to elicit the maximum response. Controls

either were not injected or were injected with saline. For liver biopsies the rats were anesthetized with ether, the abdominal cavity was opened, and a small lobe of liver was ligated and excised. The abdominal incision was closed with sutures; the skin was closed with wound clamps. Few animals were lost as a result of bleeding, and none were lost from infection.

Activities of aldehyde dehydrogenase were determined in the supernatant and mitochondrial fractions of homogenates of the biopsied livers, with propionaldehyde and NAD being used as the substrates (*8*). The supernatant was prepared from either whole homogenates or the supernatant left after removal of the mitochondrial fraction. The preparations were centrifuged at 120,000*g* for 1 hour to ensure removal of all microsomes and mitochondria. Protein content was determined by the biuret method with bovine serum albumin used as the standard.

Only the activity of supernatant aldehyde dehydrogenase increased in response to treatment of the animal with phenobarbital (Table 1). Fischer rats were uniformly nonreacting (that is, there was no increase in enzyme activity in response to phenobarbital), whereas few Long-Evans rats were nonreactors. Throughout, I shall use R to represent a dominant, autosomal gene that confers ability to respond to phenobarbital with an increased activity of liver aldehyde dehydrogenase activity.

Breeding experiments were undertaken with Long-Evans, Sprague-Dawley, and Fischer animals whose genotypes were predicted from the liver biopsy studies (Table 2). In no case are the observed numbers of offspring in each genotype significantly different from the expected number as determined by the chi-square test. The gene is not sex-linked and does not segregate with coat color in the Long-Evans strain. The Fischer animals were most useful in these experiments because they are apparently genetically homogenous and are recessive for the trait. Thus, any question about the genetic constitution of a Long-Evans animal could be resolved by examination of the distribution of the ability to react to phenobarbital in the hybrid offspring of that animal and a Fischer mate. This was sometimes necessary because there was some overlap in the values for aldehyde dehydrogenase activity for heterozygous reacting animals (Rr) and values for those that are homozygous and reacting (RR).

Table 1. Effect of phenobarbital (100 mg kg^{-1} day^{-1}) on aldehyde dehydrogenase activity in livers of three strains of rats. Rats were treated with phenobarbital for 3 days. Livers were taken 16 hours after the last dose. Sprague-Dawley and Fischer rats treated with phenobarbital and having a specific activity of the enzyme of less than 50 nmole of reduced NAD formed were designated nonreactors, those which formed from 50 to 120 nmole of reduced NAD were designated intermediate reactors, and those producing reduced NAD in excess of 180 nmole were designated high reactors. These groups are designated rr, Rr, and RR, respectively. For Long-Evans or crosses of Long-Evans and Fischer these values are 100 nmole, 101 to 400 nmole, and above 400 nmole, respectively. Activity is measured as nanomoles of NADH produced per milligram of protein per 5 minutes; means and standard errors of the mean are given. The number of animals used is given in parentheses.

Strain	Preparation	Enzyme activity: Control	Enzyme activity: Treated with phenobarbital		
			rr	Rr	RR
Sprague-Dawley	Supernatant	16.7 ± 1.47 (17)	20.6 ± 0.76 (64)	72.4 ± 3.02 (25)	153.8 ± 10.5 (4)
	Mitochondria	144.8 ± 10.3 (17)	143.8 ± 4.8 (8)	150.6 ± 6.9 (7)	141.7 ± 10.1 (4)
Fischer	Supernatant	13.3 ± 0.4 (7)	15.4 ± 1.2 (18)	(0)	(0)
Long-Evans	Supernatant	44.7 ± 4.2 (7)	65.1 (2)	318.9 ± 8.7 (10)	625.0 ± 35.5 (9)

The evidence indicates that the ability of rats to respond to phenobarbital treatment with an increased activity of aldehyde dehydrogenase in liver supernatant is a dominant characteristic that is inherited autosomally according to classical Mendelian genetics. The magnitude of the change indicates that whatever mechanism is involved, it is a very sensitive control point in aldehyde dehydrogenase activity. As such it offers a valuable tool for the study of the physiological importance of this reaction. It is different from the classical induction of drug-metabolizing enzymes by phenobarbital in that at least 2 to 3 days of treatment with phenobarbital are required for the increase of the liver enzyme whereas microsomal enzymes will respond to a single injection of phenobarbital. Also the dehydrogenase involved is soluble, linked to NAD, and present in the supernatant; it is not a particulate enzyme like the microsomal enzyme system. Other barbiturates such as barbital and pentobarbital are relatively ineffective in elevating activities of this enzyme although diphenylhydantoin is somewhat stimulatory (*9*). Increase of aniline hydroxylase activities in response to treatment with phenobarbital is not under the same genetic control as aldehyde dehydrogenase. This observation would indicate that the genetic differ-

Table 2. Genetics of increased activity of liver aldehyde dehydrogenase in response to treatment with phenobarbital in crosses of Fischer (F) and Long-Evans (LE) rats and in Sprague-Dawley (SD) rats. Liver biopsies were taken after 3 days of treatment with phenobarbital (100 mg kg^{-1} day^{-1}). None of the Fischer rats exhibited any increase in liver aldehyde dehydrogenase after treatment with phenobarbital. Brothers and sisters of the F_1 generation were inbred to produce the F_2 generations. Offspring in each genotype are from more than one set of parents in most cases. In parentheses are given the numbers of offspring expected on the basis of classical Mendelian genetics.

Parents: Female	Parents: Male	Offspring (No.): rr	Offspring (No.): Rr	Offspring (No.): RR
F (rr)	LE (rr)	22 (22)	0 (0)	0 (0)
F (rr)	LE (Rr)	3 (4)	5 (4)	0 (0)
F (rr)	LE (RR)	0 (0)	11 (11)	0 (0)
LE (RR)	F (rr)	0 (0)	23 (23)	0 (0)
SD (rr)	SD (rr)	20 (20)	0 (0)	0 (0)
SD (Rr)	SD (Rr)	9 (9)	17 (18)	10 (9)
LE (RR)	LE (rr)	0 (0)	19 (19)	0 (0)
LE (RR)	LE (Rr)	0 (0)	7 (6)	5 (6)
F_1 Generation			*F_2 Generation*	
LE-F (Rr)	LE-F (Rr)	8 (6)	10 (12)	6 (6)
LE-F (Rr)	LE-F (rr)	6 (6)	3 (3)	0 (0)
SD (RR)	SD (RR)	0 (0)	0 (0)	4 (4)

ence does not lie in different rates of absorption or elimination of phenobarbital. Several other NAD-linked enzymes such as alcohol, lactic, malic, and α-glycerophosphate dehydrogenases are not materially affected.

The increase in capacity for aldehyde oxidation in whole liver after treatment with phenobarbital is of the order of three- to fivefold in animals whose supernatant enzyme activity increases by tenfold. This is because the mitochondria contain from 50 to 75 percent of the total capacity for aldehyde oxidation in the liver when propionaldehyde (*10*) or acetaldehyde (*11*) is used as substrate (*1*). Redmond and Cohen (*7*) indicate a twofold increase in the capacity for aldehyde oxidation in whole homogenates of mouse liver after treatment with phenobarbital. I have found that after treatment with phenobarbital there is a twofold increase in enzyme activity in mouse liver supernatant which is not dependent on genotype. This is a quantitatively but not qualitatively different response from that obtained by Redmond and Cohen (*7*) and may be due to the strain of mice used or to the difficulty in measurement of aldehyde dehydrogenase activity in homogenates of whole liver.

It seems unlikely that this reaction to phenobarbital has any relation to cross tolerance of barbiturate and ethanol as has been suggested (*7*). The response is present only in some animals; it is not brought about by other barbiturates; and, it does not extend to the brain aldehyde dehydrogenase (*9*).

The fact that a tenfold increase in aldehyde dehydrogenase in liver supernatant can be brought about by treatment of selected animals with phenobarbital, indicates that this is a system in which may be studied the function of this enzyme in alcohol, aldehyde, and amine metabolisms and the relationship of the enzyme to ethanol intoxication and addiction. Animals that do not respond to treatment with phenobarbital with an increased activity of aldehyde dehydrogenase make ideal controls for these experiments. The mechanism of the increase in aldehyde dehydrogenase activity is unknown, but the genetic basis for the effect provides a possible means for its discovery. Phenobarbital induces microsomal enzymes (*12*) and a mitochondrial enzyme, δ-amino levulinic acid synthetase (*13*). My studies indicate that administration of phenobarbital is associated with increased activity of a soluble aldehyde dehydrogenase found in the liver supernatant and that this responsiveness is inherited as a dominant characteristic in accordance with classical Mendelian genetics.

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[^{3}H]Adenosine Triphosphate: Release during Stimulation of Enteric Nerves

Abstract. *The isolated taenia coli of the guinea pig takes up tritiated adenosine, adenosine monophosphate, adenosine diphosphate, and adenosine triphosphate, in preference to tritiated inosine and adenine. After uptake, [^{3}H]adenosine is converted and retained primarily as [^{3}H]adenosine triphosphate. Tritium is released from taenia coli treated with [^{3}H]adenosine upon activation of the nonadrenergic inhibitory nerves. These results are consistent with the previous evidence that adenosine triphosphate may be the transmitter from the nerves.*

Although adrenergic inhibitory nerves in the gut are well known, the existence of nonadrenergic inhibitory nerves supplying the mammalian gut was not established until recent years (*1*). The cell bodies of these nerves are localized in Auerbach's plexus, and their axons supply both longitudinal and circular muscle coats; they are controlled by preganglionic vagal fibers in the stomach, but in the colon they are without extrinsic nerve connections (*2*). In ultrastructural studies, the nerves appear to be represented by profiles containing a predominance of large unique granular vesicles (1000 to 2000 Å), which distinguish them from adrenergic nerves (*3*).

Evidence has been presented recently that adenosine triphosphate (ATP) or some related nucleotide is the transmitter substance released by nonadrenergic inhibitory nerves in the gut (*4*). The results of experiments were described which, in broad outline, satisfied the criteria summarized by Eccles (*5*) for the establishment of a neurotransmitter substance: ATP and the enzymes necessary for its formation are present in nonadrenergic inhibitory nerves; ATP or its breakdown products (or both) are released on nerve stimulation; enzymes which inactivate ATP are present in gut; some drugs produce parallel effects on responses to both

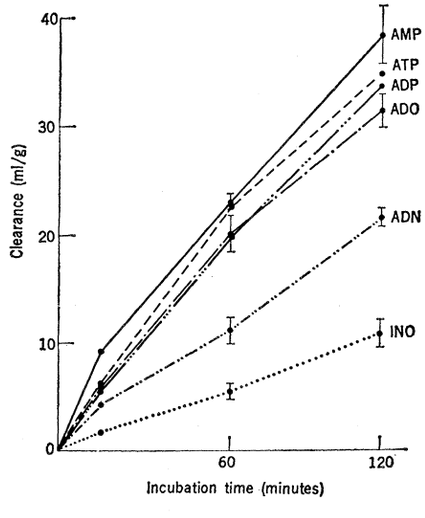

Fig. 1. Uptake of purine derivatives by guinea pig taenia coli. The tissue was incubated with the tritiated derivatives (10^{-7} mole/liter), digested, and assayed for tritium. Each point represents the mean value of six to eight preparations; the vertical bars represent 2 S.E.M. After incubation for both 60 and 120 minutes, the uptake of adenine (ADN) was significantly smaller than that of adenosine (ADO), AMP, ADP, or ATP ($P < .02$) and larger than that of inosine (INO, $P < .01$) as determined by the *t*-test.