Table 2. Inhibition of the decarboxylation of mevalonate-1-C^{14} by liver homogenates from estrone-treated rats. Each Warburg vessel contained 230 μmole of phosphate (K^+) *p*H 7.5, 2.85 μmole of adenosine triphosphate, 1.9 μmole of nicotinamide-adenine dinucleotide, 1.9 μmole of nicotinamide-adenine dinucleotide phosphate, 6.75 μmole of magnesium chloride, 65 μmole of nicotinamide, and 0.5 ml of rat liver homogenate. Reaction time was 15 minutes at 30°C. After temperature equilibration with the manometers open to the air, the stopcocks were closed and the reactions were started by adding 2 μmole (0.2 μc) of mevalonate-1-C^{14} from a side arm into the reaction mixture. The $C^{14}O_2$ was trapped in the center well which contained 0.3 ml of 20 percent ethanolamine in methyl cellosolve. The reactions were stopped by the addition of acid from a second side arm into the main compartment. The trapped $C^{14}O_2$ was measured by liquid scintillation counting and the counts were corrected by the addition of an internal standard.

Estrone treatment (mg/kg)	Serum cholesterol* (mg/100 ml)	$C^{14}O_2$ evolved (dpm)	Inhibition† (%)
None	75, 68, 68	17,780	
None	78, 75, 70	15,120	
1	72, 56, 45	14,100	14.3
2	52, 52, 36	10,000	39.2
4	57, 24, 16	5,445	67.0

* There were three rats on each treatment. Cholesterol values are for the individual rats. † Based on the average of the two control values.

hibition of the biosynthesis of chlosterol in vitro was reported in either of these studies. We have now administered estrone orally at doses that produce hypocholesteremia in the rat and have shown that in homogenates prepared from the livers of these animals, the incorporation of mevalonate into the total nonsaponifiable lipids and beta-hydroxy sterols was less than that of normal controls. There was also a decrease in the decarboxylation of mevalonate-1-C^{14}, a key intermediate in the biosynthesis of cholesterol.

Estrone suspended in 1 percent gum tragacanth was administered daily for 4 days to groups of three rats (about 200 g each). Control rats received 1 percent gum tragacanth only. On the 5th day the rats were bled by heart puncture and killed. The livers were removed and immersed in cold homogenizing medium (*4*). A random portion of each liver was excised and pooled by group. Homogenates were prepared by the method of Bucher (*4*). Serum cholesterol was determined on each rat by the method of Turner and Eales (*5*) with *p*-toluenesulfonic acid as the catalyst. The homogenates were tested for the incorporation of mevalonate-2-C^{14} into total nonsaponifiable lipids and sterols and for the decarboxylation of mevalonate-1-C^{14}.

Table 1 shows that treatment with estrone resulted in diminished incorporation of mevalonate-2-C^{14} into the nonsaponifiable fraction and into the digitonin precipitable sterols. The nonsaponifiable fraction was obtained by extraction with light petroleum ether after saponification of the reaction mixtures. Separately, fractionation of the total nonsaponifiable fractions from control and treated systems on alumina showed that there was no accumulation of radioactivity in squalene, the beta-hydroxy sterols, or a fraction tentatively identified by Holmes and DiTullio (*6*) as a 3-keto intermediate participating in the conversion of lanosterol to zymosterol. This suggests that the inhibition in the pathway of the biosynthesis of cholesterol takes place prior to the cyclization of squalene.

Table 2 shows the data obtained by manometric methods of the decarboxylation of mevalonate-1-C^{14}. The data show that one point of inhibition of cholesterol synthesis is in the reactions leading to the decarboxylation of mevalonic acid.

Since Noble and Boucek (*3*) did not show that their water-soluble conjugated estrogens were given in doses that produced hypocholesteremia, the reason their estrogen did not decrease cholesterol synthesis may simply have been due to the possibility that although the doses were estrogenic they may not have been hypocholesteremic. Under these conditions no inhibition of cholesterol biosynthesis would be expected.

From the data given it may be seen that homogenates prepared from estrogen-treated rats synthesize cholesterol at rates below those of homogenates from untreated rats. This decrease in the rate of cholesterol synthesis is shown to be due to interference with the decarboxylation of mevalonic acid.

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Schistosomiasis: Age of Snails and Susceptibility to X-irradiation

Abstract. *Studies on sensitivity of* Australorbis glabratus *to x-rays have defined the chronological and physiological age at which the snail is most sensitive to radiation damage. Results showed that the dose producing 50-percent mortality at 30 days after irradiation increased with age but that at 90 days it was practically constant from 2 to 210 days of age. In view of the available data on recovery from radiation damage caused by doses from 6000 to 9000 roentgens it is suggested that doses above those causing 50 percent lethality at 60 days but below those causing 50 percent lethality for 30 days should be considered in setting up "radiation barriers" to control snails in water-distribution systems.*

The well-known limitations for controlling the snail vectors of schistosomiasis in moving waters make it necessary to develop more efficient and less costly methods of reducing the numbers of these snails. That snails in open waters might be destroyed by suitable shielded irradiators appears possible, but such a procedure requires information on the effect of radiation on the population dynamics of the snail host.

The literature on radiation sensitivity of fresh-water snails is rather limited. Several studies (*1*) have been made of age changes in sensitivity during the period of prenatal development.

This is the first series of reports to deal with the effects of radiation on the population kinetics of *Australorbis glabratus*. The age-dependence in acute radiosensitivity was studied, the dose producing 50 percent mortality being taken as a criterion of radiation damage.

The snails used were from a nonpigmented strain derived from a Brazilian × Puerto-Rican cross. The experiments were performed with 10 groups from 2 to 210 days of age; the radiation dosage was from 3000 to 30,000 r; in each age group there was a similar number of nonirradiated specimens. The biological and physical methods have already been described (*2*). Snails were irradiated with x-rays produced by a Van de Graaff generator operating at 2 Mev. Each radiation dose was delivered at a rate of 1000 r/min for lower amounts of radiation and at a rate of 1500 r/min for the larger ones. Deaths were counted in the control and test groups at the end

of the 1st, 2nd, and 3rd month after exposure and the mortality due to irradiation was estimated by applying Abbott's formula (*3*). In estimating the LD_{50} the Spearman-Karber method (*3*) was used wherever appropriate, otherwise the moving average method (*3*) was employed. Sets of LD_{50}'s were examined for trend with the use of Spearman's rank correlation test (*4*).

The results show (Table 1) that there was considerable variation in the LD_{50} estimated from deaths at 30 days, this variation apparently being related to the age of the snail at the time of exposure. There was less variation at 90 days estimated in the same way. Although results obtained for the 11-day-old specimens require further testing, the general conclusion was that 50-percent mortality at 30 days was influenced by the age at the time of exposure. The newborn animals, 2 to 7 days old, were most sensitive to radiation damage. An increase in age resulted in an increase of LD_{50} at 30 days. Higher radiation doses were required to produce 50-percent mortality in juveniles (18 to 44 days old) than in the newly born snails. The dose required by the young and old adults was practically twice that needed to kill 50 percent of the snails approaching sexual maturity (about 44 days). However, while the upward trend of 50-percent mortality at 30 days [$P < .01$, $r = .842$, r being Spearman's rank correlation test (*4*)] and at 60 days ($P < .01$, $r = .745$) was significant, the trend was not significant at 90 days ($P < .20$, $r = .358$). This may suggest that sensitivity to x-rays remained constant over the age range from 2 to 210 days, but the period of survival may be dependent on the age of the snail. Furthermore, if the death rate is a function of time, regardless of age at exposure, conclusions concerning the interdependence of age and radiosensitivity will vary according to criteria used in determining radiation-induced injury.

Results indicate that with increasing age at exposure there is a significant downward trend in the difference between corresponding LD_{50} at 30 and 60 days ($P < .01$, $r = .842$), as well as between 60 and 90 days ($P < .05$, $r = .717$). Since there was also a significant downward trend in the difference between the foregoing differences ($P < .01$, $r = .883$), the decline of LD_{50} with the passage of time was not linear but appeared to be somewhat greater from the 1st to the 2nd month than from the 2nd to the 3rd month after exposure.

Table 1. The LD_{50} (10^3 r) for *Australorbis glabratus* as a function of age at exposure and of time after exposure.

Age at irradiation (days)	No. of snails	1 month		2 months		3 months		Cumulative control deaths
		LD_{50}	S.D.	LD_{50}	S.D.	LD_{50}	S.D.	3 months (No. dead/ No. obs.)
2–3	166	7.6*	1.7	7.0	0.9	5.9	2.3	5/24
4	162	7.5	1.4	7.1	†	7.0	0.5	4/24
6–7	174	8.7	3.1	6.4	2.6	6.7	1.8	4/32
11	242	17.0	5.4	11.5	4.0	10.1	4.3	9/32
18	264	12.5*		7.2	3.4			
32	204	14.3*		9.2	3.8	8.0	3.5	3/25
44	235	11.9*		8.4	3.4	8.0	2.9	3/30
55	82	24.9*		9.1	8.3	2.9*		1/10
97	99	26.8*		9.3	4.6	5.3	2.8	1/11
210	16	23.8*		16.3*		13.8	2.6	0/4

* Moving average method used to obtain estimate. † Standard deviation not available.

Table 2. The LD_{50} as a function of size at exposure.

Age at irradiation (days)	Size range* at irradiation (mm)	No. of snails	1 month		2 months		3 months	
			LD_{50}	S.D.	LD_{50}	S.D.	LD_{50}	S.D.
			Larger specimens					
18	2–3	84	14.2†		8.1	3.8		No data
32	5–6	87	17.5†		10.7	3.8	9.3	4.1
44	6–7	80	9.6†		11.1	1.8	8.0	3.5
97	16.5–19	39	>30‡		10.0	3.4	4.8	2.5
			Smaller specimens					
18	1–1.5	180	11.0	5.1	6.7	3.0		
32	2–3	117	11.8	7.0	7.9	3.3	7.0	2.7
44	1.5–2	155	12.3	8.3	7.0	3.2	7.9	2.3
97	6–7	60	23.1†		8.8	5.2	5.6	3.1

* Five of the largest and five of the smallest specimens measured in each group. † Moving average method used to obtain estimate. ‡ Sight estimate.

Table 3. Mortality among control snails.

Age at start of experiment (days)	Cumulative mortality at		
	1 month	2 months	3 months
2–18	3/156*	17/156	22/112
32–210	1/80	3/80	8/80

* No. dead/No. observed.

The results shown in Table 2 suggest that the larger specimens are more resistant than the smaller ones of the same chronological age.

The results summarized in Table 3 show the principal trends of mortality among control snails. There was an increase in cumulative mortality with the passage of time and a decrease with the increase of age of specimens under observation.

One of the reasons for making this study of 50-percent mortality at increasing periods of time was to test the adequacy of a single dose of radiation in inducing lethal damage in snails of different ages and sizes as they occur in natural habitats. The results indicate that a dose from 6000 to 9000 *r* produced 50 percent of the deaths in a mixed population of young, old, small, and large snails, within 3 months after exposure. This finding, to the extent that it may be applied, represents an obstacle to forecasting the degree of success of control. There is reason to believe that survivors from treatment with 6000 and 9000 *r* are able to recover at least partially from radiation damage (*5*).

In order to avoid the risk of repopulating any treated place by undamaged or partially damaged offspring from irradiated parent snails, radiation doses smaller than those determined for LD_{50} at 30 days but higher than those obtained at 60 days should be considered in setting up "radiation barriers" in water distribution systems.

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Audiogenic Seizures, the Dilute Locus, and Phenylalanine Hydroxylase in DBA/1 Mice

Abstract. *Reduced audiogenic seizure susceptibility in dilute mice was associated with enhancement of phenylalanine hydroxylase activity, previously reported as low in dilute mice. Though nutritional changes complicate the interpretation, evidence exists for a mutation linked with dilute which modifies susceptibility.*

The mechanism of genetic control of susceptibility to audiogenic seizures has not been satisfactorily explained. One hypothesis was suggested by Coleman (*1*) who demonstrated a relationship between the dilute locus and phenylalanine hydroxylase activity. The relatively low activity of this enzyme in dilute mice could reduce synthesis of serotonin or other neurohormones, thus modifying seizure susceptibility. For example, reduced enzyme activity could directly decrease production of serotonin from tryptophan (*2*); or an increase in phenylacetic acid (*1*), resulting from the lowered conversion of phenylalanine to tyrosine, could decrease synthesis of serotonin, adrenalin, and γ-aminobutyric acid through inhibition of their respective decarboxylases. Large amounts of phenylalanine have been shown to decrease the concentration of serotonin in the brain (*3*). This postulated mechanism is similar to that known to occur in the human condition, phenylketonuria. Busnel and Lehmann (*4*) have shown that a number of drugs that lower serotonin metabolism potentiate audiogenic seizures.

We, therefore, hypothesized that dilute (*dd*) mice would be more prone to seizures than nondilute (*Dd* or *DD*) mice which were maintained constant at other loci. The multiple factor determination of seizure susceptibility has been demonstrated (*5*), so that the effects of a single locus might be difficult to demonstrate in genetically heterogeneous populations. Nevertheless, some evidence for association of increased susceptibility and dilute coat color has been obtained (*6*).

Investigations were undertaken to determine the effect of two alleles, *D* and *d*, at the dilute locus maintained on a common genetic background by repeated backcrossing in stock mice of strain DBA/1 (*dd*) (*7*). The *D* gene, which is phenotypically indistinguishable from wild type, arose as a mutation from *d* in this stock. Matings of, *DD* × *DD*, *Dd* × *dd*, and *DD* × *dd* were made in order to obtain a large group of animals that were tested for seizure susceptibility at 30 days of age. Each subject was exposed once for 90 seconds to a door bell (sound level approximately 100 db above 0.0002 dyne/cm^2). Only actual convulsions were counted as positive responses.

The results of this experiment, which are shown in Table 1, did not support the original hypothesis. However, the low incidence of seizure in all three genotypes compared with the 80 percent expected in mice of the DBA/1 strain suggested that unknown factors were operating. Measurement of phenylalanine hydroxylase (*8*) in all three genotypes showed that individuals had enzyme activity ranging between 48 and 100 percent of the "standard" C57BR/cd strain. Most of them were close to 100 percent. Previously, enzyme activity in all DBA/1 mice had been low, only 51 percent of the standard activity found in *dd* individuals.

The association in these mice of an increase in enzyme activity with decreased susceptibility to audiogenic seizures is consistent with the original hypothesis, but is not conclusive proof of it. One can only be certain that the dilute phenotype is not invariably a marker for low phenylalanine hydroxylase activity.

Further experiments were conducted to determine the physiological nature of the change in susceptibility. A group of mice not convulsing at 30 days was tested at 40 days. The population incidence was similar to that found previously when subjects were classified as susceptible if they convulsed on either the first or the second trial (Table 2). The increase on the second trial could have been caused by a sensitization produced by the first trial, or by an age shift in susceptibility. In order to distinguish between these possibilities mice were tested first at 40 days and later at 50 days with the results shown in Table 3. First-trial seizure incidence was similar at 30 and 40 days; both groups showed enhanced susceptibility after 10 days, although the increase was less for the 40- to 50-day subjects. These DBA/1 mice differed from the usual stock, not in age of maximum sensitivity, but in requiring a sensitizing stimulation before becoming highly susceptible. A similar phenomenon was observed in the HS strain formerly maintained by one of us (J.L.F.).

The concurrent change in seizure susceptibility and enzyme activity could be fortuitous, or both could be dependent upon some common factor. Between the time of Coleman's study (*1*) and ours, the standard colony diet was shifted from Purina Lab Chow to

Table 1. Occurrence of convulsions on first trial in DBA/1 mice at 30 days of age.

Genotype	Male		Female	
	N	Convulsions (%)	*N*	Convulsions (%)
DD	50	26	63	26
Dd	114	17	96	15
dd	78	8	63	14

Table 2. The number of DBA/1 mice that convulsed when tested at 30 days and 40 days (first and second trials combined).

Genotype	Male		Female	
	N	Convulsed (%)	*N*	Convulsed (%)
DD	28	88	33	81
Dd	62	83	50	85
dd	39	67	31	87

Table 3. The number of DBA/1 mice that convulsed when tested at 40 days and 50 days.

Genotype	*N*	Convulsed at 1st trial (%)	Convulsed at 1st and 2nd trial (%)
DD	29	28	36
Dd	24	13	52
dd	7	0	57

Table 4. Effect of selection based on the occurrence of audiogenic seizures during the first trial.

	Genotype	
	DD	*dd*
Generation O		
Number of mice	113	141
Seizures (%)	26	11
F1 selected for resistance		
Number of mice	35	23
Percentage susceptible	26	13
F1 selected for susceptibility		
Number of mice	28	33
Percentage susceptible	71	15
Mice not selected		
Number of mice	29	97
Percentage susceptible	34	11