theoretical possibility of replacing experimental breeding by genetical analysis of somatic cells in culture, as discussed by Pontecorvo (*18*).

Although the phenomenon of symmetrical Qr formation between homologs has not yet been demonstrated in vivo and may be the result of some influence in vitro, its occurrence in these γ-globulin producing cells (*19*) is of interest in relation to some current concepts of autoimmunity. As a result of somatic crossing-over, a clone of cells could develop which (i) lacks certain genes (and antigenes) present in the other cells of the host, yet (ii) contains no genes not already present (Fig. 2, *d1* and *d2*). (As an example, a crossover in the lymphoid cell might result in homozygosity for some structural gene, perhaps blood group I^A/I^A, whereas the other cells of the heterozygous host would remain heterozygous I^A/I^B.) The host would to this degree become foreign to the lymphoid cell and its descendants. Somatic crossing-over provides a means for producing, by loss of genes, lymphoid cells with a genetic complement different from that of the host. It thus, in theory, is a cellular mechanism by which "the forbidden clone" of Burnet (*20*) might emerge. The genetic differences in the two-cell populations would be determined by the chromosomal site of the cross-over and the number of heterozygous allelic loci in the interchanged chromosomal segments.

Crossing-over, if it occurs in vivo in mammalian somatic cells, provides another theoretical mechanism by which such findings as genetic differences in monozygotic twins, mosaicism of tissue or blood cell type in a single individual (*21*), or antigen loss in neoplasms (*22*) might be explained. Since the crossover could be expected to include more than one genetic determinant, information concerning gene linkage might be obtained by the detection of multiple antigenic differences in the mosaic population. Although a number of genetic polymorphisms are now known in man, the rarity with which mosaicism of autosomally-determined traits has been detected, as well as the absence of reports of quadriradials in dividing cells taken directly from marrow or other tissues with a high mitotic index, suggest that somatic crossing-over in vivo is not a common occurrence in most individuals. However, unless stimulated to divide because of immunological mechanisms, the degree of maintenance and proliferation or perhaps inordinate overgrowth of a new clone—its fitness (Darwinian)—in relation to that of the cells of the host would vary with the relative values of homozygosity and heterozygosity in the tissue environment in which the new clone emerged.

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Estrone Inhibition of Cholesterol Biosynthesis at the Mevalonic Acid Stage

Abstract. *Studies on the synthesis of cholesterol with cell-free homogenates of liver from rats treated with graded doses of estrone show that, at doses of estrone which cause a reduction in blood cholesterol, there is an accompanying inhibition of cholesterol biosynthesis. Furthermore, this inhibition appears to occur at the stage of cholesterol biosynthesis at which mevalonate is decarboxylated.*

The hypocholesteremic effect of estrogens is well known (*1*) but the mechanism for this effect is obscure. Humber *et al.* (*2*) noted a series of estrogens that inhibited the biosynthesis of cholesterol from mevalonate-2-C^{14} in vitro. In contrast, Noble and Boucek (*3*) found no difference in cholesterol biosynthesis by liver slices obtained from female rats previously injected intramuscularly with water-soluble conjugated estrogens. Under the same conditions livers from males showed an increase in cholesterol synthesis. No correlation between decreased serum cholesterol and the in-

Table 1. Inhibition of the incorporation of mevalonate-2-C^{14} into the total nonsaponifiable lipids and digitonin-precipitable sterols by liver homogenates from estrone-treated rats. The reaction mixture contained 1.5 μmole of adenosine triphosphate, 1.5 μmole of nicotinamide-adenine dinucleotide, 1.5 μmole of nicotinamide-adenine dinucleotide phosphate, 80 μmole of nicotinamide, 7.6 μmole of magnesium chloride, 230 μmole of phosphate (K^+) *p*H 7.2, 1 ml of liver homogenate, and 2 μmole of mevalonate-2-C^{14} (0.2 μc) in a total volume of 2.5 ml. The reaction time was 15 minutes at 37°C. Radioactivity in the nonsaponifiable fraction and the digitonide was determined by liquid scintillation counting and was corrected by the addition of an internal standard.

Estrone treatment (mg/kg)	Serum cholesterol* (mg/100 ml)	Nonsaponifiable fraction			
		Lipids		Digitonides	
		Radioactivity (dpm)†	Inhibition (%)	Radioactivity (dpm)	Inhibition (%)
0	70, 92, 86	48,000		10,386	
2	38, 29, 46	17,650	63	4,290	59
4	32, 56, 46	22,550	53	5,310	49
8	24, 22, 44	12,720	73	3,060	70

* There were three rats on each treatment. Cholesterol values are for the individual rats. † Disintegrations per minute.

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