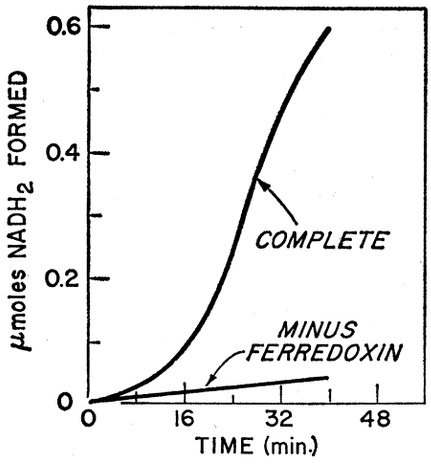

Fig. 2. Ferredoxin dependency of formate oxidation. Each cuvette contained NAD, 1 µmole; sodium formate, 100 µmole; phosphate buffer at *p*H 7.5, 100 µmole; and treated extract (Sephadex and diethylaminoethyl-cellulose), 11 mg of protein. Partially purified ferredoxin (0.07 mg of protein) from *M. omelianskii* was used.

may be transferred enzymically to such substrates as pyridine nucleotides (*6, 7*), nitrite (*8*), hydroxylamine (*9*), and urate (*2*), or may accept protons to form hydrogen (*1*). Examples of electron donors are light-activated chlorophyll (*5*), α-ketoglutarate (*10*), pyruvate (*1*), hydrogen (*6*), and hypoxanthine (*2*).

Cell-free extracts of *Methanobacillus omelianskii* were prepared as described by Wolin *et al.* (*11*). The endogenous pyridine nucleotides and low molecular weight substrates were removed by adding 10 ml of the crude extracts (40 mg/ml) to a Sephadex G-25 column (24 × 2.75 cm) at 4°C and then eluting with demineralized water. Pyridine nucleotide reduction was assayed in an anaerobic cuvette which had been evacuated and flushed with argon several times.

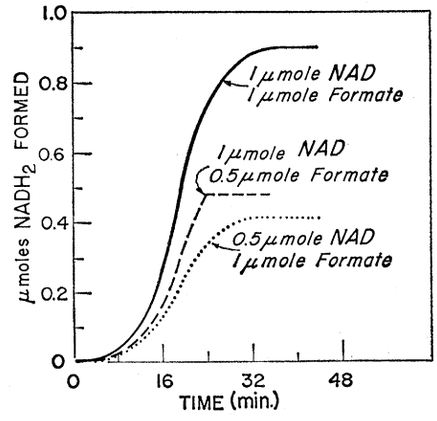

Fig. 3. Stoichiometry of formate oxidation. Each cuvette contained NAD and formate as indicated; potassium phosphate buffer, 100 µmole, *p*H 7.5; and Sephadex-treated extract, 7.0 mg of protein.

The main compartment of the cuvette contained, in a total volume of 3 ml, 1 µmole of nicotinamide-adenine dinucleotide (NAD) or nicotinamide-adenine dinucleotide phosphate (NADP), 100 µmole of potassium phosphate buffer at *p*H 7.5, and a suitable amount of cell-free extract. Protein was assayed by the biuret method (*12*). Sodium formate (100 µmole) was tipped from the side arm to initiate the reaction which was followed in a Cary model 14 recording spectrophotometer by measuring absorbancy at 340 mµ. As shown in Fig. 1, the formic dehydrogenase is NAD specific.

The ferredoxin dependency of formate oxidation is shown in Fig. 2. When ferredoxin was removed from the crude extract by passage through a small diethylaminoethyl-cellulose (phosphate) column (1 × 4 cm), as described previously (*1*), the ferredoxin-free extract did not appreciably reduce NAD from the formate, but when ferredoxin was added back to the system, $NADH_2$ was formed. Partially purified ferredoxins from *C. pasteurianum, M. omelianskii,* and *Clostridium acidi-urici* were interchangeable in this reaction. We have obtained no evidence to support the possibility that hydrogen is a product of formate cleavage in *M. omelianskii.* Certain aged, frozen extracts showed negligible hydrogenase activity; yet NAD reduction by formate occurred rapidly, showing that hydrogen is not an intermediate in the reduction.

Evidence to show that both formate and NAD are quantitatively utilized is presented in Fig. 3. The reaction also was studied using Warburg vessels, showing the dependency of carbon dioxide evolution on ferredoxin and NAD.

The formic dehydrogenase of *C. acidi-urici* has been studied (*13*), but was not found to reduce pyridine nucleotides. Using the techniques presented here, we have shown this organism to have an NAD-specific, ferredoxin-dependent formate oxidation system similar to that of *M. omelianskii.* The mechanism by which *M. omelianskii* is able to reduce carbon dioxide to methane is still an unsolved problem. Perhaps formate or an active formyl complex is a direct intermediate in the reduction of carbon dioxide.

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References and Notes

1. L. E. Mortenson, R. C. Valentine, J. E. Carnahan, *Biochem. Biophys. Res. Commun.* **7**, 448 (1962).
2. R. C. Valentine, R. L. Jackson, R. S. Wolfe, *ibid.*, p. 453.
3. B. B. Buchanan, W. Lovenberg, J. C. Rabinowitz, *Proc. Natl. Acad. Sci. U.S.* **49**, 345 (1963).
4. C. C. Black, C. A. Fewson, M. Gibbs, *Nature* **198**, 88 (1963).
5. K. Tagawa and D. I. Arnon, *ibid.* **195**, 537 (1962).
6. R. C. Valentine, W. J. Brill, R. S. Wolfe, *Proc. Natl. Acad. Sci. U.S.* **48**, 1856 (1962).
7. R. C. Valentine, W. J. Brill, R. D. Sagers, *Biochem. Biophys. Res. Commun.* **12**, 315 (1963).
8. L. E. Mortenson, *Bacteriol. Proc.* **1963**, 117 (1963).
9. R. C. Valentine, L. E. Mortenson, H. F. Mower, R. L. Jackson, R. S. Wolfe, *J. Biol. Chem.* **238**, pc 857 (1963).
10. R. C. Valentine and R. S. Wolfe, *J. Bacteriol.* **85**, 1114 (1963).
11. E. A. Wolin, M. J. Wolin, R. S. Wolfe, *J. Biol. Chem.* **238**, 2882 (1963).
12. A. G. Gornall, C. J. Bardawill, M. M. David, *ibid.* **177**, 751 (1959).
13. D. H. Hug and R. D. Sagers, *Bacteriol. Proc.* **1957**, 111 (1957); R. D. Hamilton and R. S. Wolfe, *J. Bacteriol.* **78**, 253 (1959).
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Cytological Evidence for Crossing-Over in vitro in Human Lymphoid Cells

Abstract. *In human blood cells dividing in vitro two chromosomes are occasionally found intimately associated in a quadriradial configuration (Qr). A Qr by traditional interpretation is the result of chromatid interchange. Since the configurations in blood cells often are equal and symmetrical and are composed of homologous chromosomes, they are considered cytological evidence that somatic crossing-over may occur in mammalian cells.*

In meiosis, in addition to chromosomal segregation during reduction division, crossing-over normally contributes additional genetic diversity to gametes. As reported in his classical paper of 1936, Stern (*1*) demonstrated by genetical methods that crossing-over

occurs also in the somatic cells of *Drosophila*. In recent years the possibility of the occurrence of somatic crossing-over in mammalian cells has been speculatively discussed. The cytological observations reported here give direct support to this possibility.

Among human nucleated blood cells dividing in vitro, there is found occasionally a cell in prophase or metaphase in which two chromosomes are intimately associated in a quadriradial configuration (Qr) (Fig. 1). Twenty-one such cells from eight different individuals, two of whom were considered normal, have been found in this laboratory during the past 4 years. The Qr's occurred in 3-day cultures of cells which had been stimulated by phytohemagglutinin to enter DNA synthesis and which were treated terminally with colcemide for 1 hour. Two cells with Qr's were found in a culture of cells from the translocation-bearing mother of a mongol and one in a cell from her brother, whose chromosomal complement did not include the translocation (see Figs. 4*a* and 6 of ref. *2*). Quadriradial configurations were present with varying frequency in each of four separate cultures derived from a patient with Bloom's syndrome (*3*), 13 Qr's having been discovered during the examination of several hundred cells (Fig. 1, *b*, *c*, and *e*). This individual, who was examined repeatedly at yearly intervals because of an increased incidence of broken chromosomes, will be described extensively elsewhere (*4*). One Qr was found in cultures of cells from each of the following individuals: a normal female laboratory employee [the Qr was in a nonradioactive cell from a culture which had been treated briefly with H^3-thymidine (Fig. 1*d*)]; a true hermaphrodite (*5*); two patients with macroglobulinemia of Waldenstrom (*6*) (Fig. 1, *a* and *f*); and a patient with carcinoma who had received 180 rad of total body irradiation 28 days earlier.

These configurations represent discoveries made during the microscopic examination of satisfactory cultures prepared from the blood of 200 different individuals, 49 of whom were considered clinically normal. During this same period, in addition to the Qr's, two cells with triradials (Tr) were found.

In the 21 cells under consideration there usually were, in addition to the Qr, 44 chromosomes of normal appearance, although small acentric fragments were also seen in four, possibly five, cells. The Qr itself regularly appeared to be composed of two entire chromosomes. Its sister chromatids were approximated to the same degree as that in the other chromosomes of the cell, except that in the immediate vicinity of the change of partner of the figure, there was often a failure of close pairing (Fig. 1, *c*, *e*, and *f*), in agreement with an observation by Fabergé (*7*). This area at times appeared to be expanded by a material which took less orcein stain than the cytoplasm generally, and which resembled the material which normally encircles and indents the centromeric area of metaphase chromosomes (*8*) [recently discussed by Lubs (*9*)]. The junction of the four arms of the Qr was at or close to the centromeres in 12 cells (as in Fig. 1*d*). In four of the five cells in which the No. 1 chromosomes were joined, the crossing was at the region of the secondary constriction which is characteristically observed near the centromere of this chromosome (Fig. 1, *a*, *b*, and *c*); this region of chromosome No. 1 has already attracted attention because of its association with small acrocentrics (*10*) and because of its asynchronous timing of DNA replication (*11*). In each of the eight cells in which the centromeres were not at or near the crossing, they

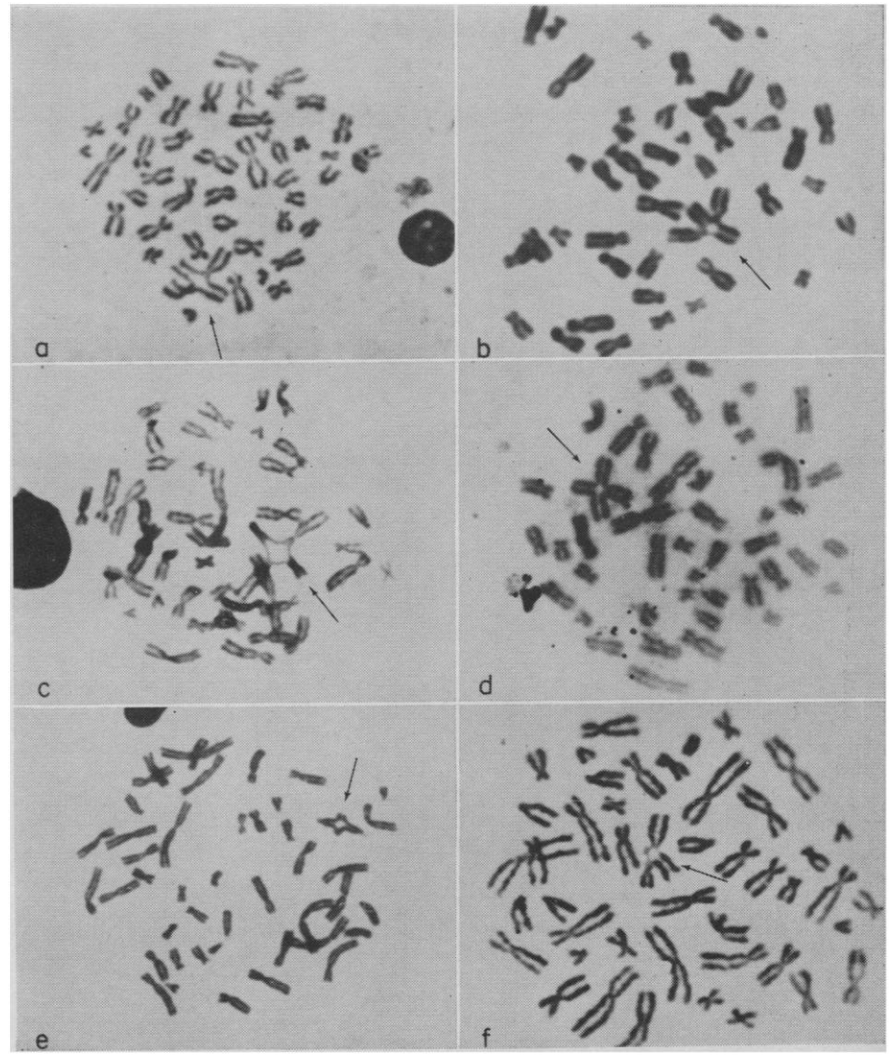

Fig. 1. Six of the 21 cultured human nucleated blood cells found to contain a quadriradial figure composed of two chromosomes in intimate association. Equal and symmetrical configurations are formed by the No. 1 chromosomes in *a*, *b*, and *c*, the No. 3 chromosomes in *d*, and two of group 13–15 in *e*. In *f* the Qr is composed of nonhomologs; the chromatid breakage and interchange had occurred in the SAT-zone of a chromosome of group 13–15 and in an area of secondary constriction in one of group 6–X–12. (Orcein, × 1320 for *a* and *c-f*; × 2000 for *b*).

appeared to lie in opposite arms of the Qr (Fig. 1*e*).

There was a striking frequency (19 of the 21 cells) not only with which two homologous chromosomes participated in the formation of the Qr, but also with which the opposite arms of the Qr appeared to be equal and symmetrical (Fig. 1, *a–e*). In seven cells, homologous chromosomes clearly were joined, the No. 1 chromosomes in five cells, and the No. 2 and the No. 3 chromosomes in one cell each. In 12 other cells homologs appeared to be joined, but this could not be determined with certainty because the chromosomes involved were from groups in which the individual members cannot be definitely delineated—group 6–X–12 in six cells, group 13–15 in two cells (Fig. 1*e*), and group 19–20 in four cells. In the remaining two cells (one is shown in Fig. 1*f*), the only cells in which there was clearly an unequal and assymmetrical Qr, and in one of the two cells containing Tr's, nonhomologous chromosomes appeared to be joined.

The formation of Qr's observed in plant material after treatment with agents which break chromosomes has generally been interpreted as being the result of chromatid interchange (*12–16*). In *Vicia* treated with nitrogen mustard, the sites of breakage and interchange were discovered by Ford (*13*) to be nonrandom; however, there was no evidence that interchanges took place between homologs any more often than would be expected by chance (*14*). McLeish (*15*) found that there is an increased tendency for particular regions close to heterochromatic segments to break in the chromosomes of *Vicia* after treatment with maleic hydrazide; in this species interchanges often occurred at these regions, and consequently symmetrical Qr's composed of homologous chromosomes were frequently seen.

The quadriradial configurations in the human cells under consideration were indistinguishable in general form from the chromatid interchanges described by students of spontaneous and induced chromosomal rearrangements, and it may be assumed, therefore, that they resulted from such events. In human cells it was possible to discriminate between two classes of Qr's: (i) those clearly composed of nonhomologs and which were nonsymmetrical (Fig. 1*f*), and (ii) those in which the two members of a chromosomal pair had participated and which, in addition, exhibited symmetry (Fig. 1, *a–e*). The symmetrical appearance of the configuration in 19 of the 21 cells indicated that the breakage and rejoining had taken place at or near corresponding sites on the two homologs of the pair. These are precisely the configurations that would be expected in metaphase as a consequence of somatic crossing-over earlier in the cell cycle. In respect to their symmetry and to the fact that interchanges took place between undoubted or apparent homologs, these configurations stand in striking contrast to the chromatid interchanges recorded in the earlier literature, in which there was rarely an exact symmetry and homologs were joined no more frequently than could be ascribed to chance proximity. In the human cells considered here, different pairs of chromosomes formed Qr's in different cells. If sites of breakage and chromatid interchange had been randomly distributed, there would have been a very small probability that homologous chromosomes would have formed the Qr's in so high a proportion of the cells. Therefore, it appears that during the preceding intermitotic period of these particular cells a degree of pairing or association of at least portions of homologous chromosomes, perhaps in the chromocenters or nucleoli, was prerequisite. In fact, the two chromosomes of certain autosomal pairs have been shown to lie nearer one another than could be expected from random positioning in the metaphase of nucleated blood cells in culture (*17*). It seems probable that symmetrical and equal Qr formation in this type of cell results from a form of homolog pairing in the preceding intermitotic period, chromatid breakage, homologous chromatid interchange, and persistence into metaphase of the cross-like configuration as a result of sister-chromatid attraction. The observations are therefore interpreted as evidence that under certain conditions, not yet defined, somatic crossing-over may occur in human blood cells in culture.

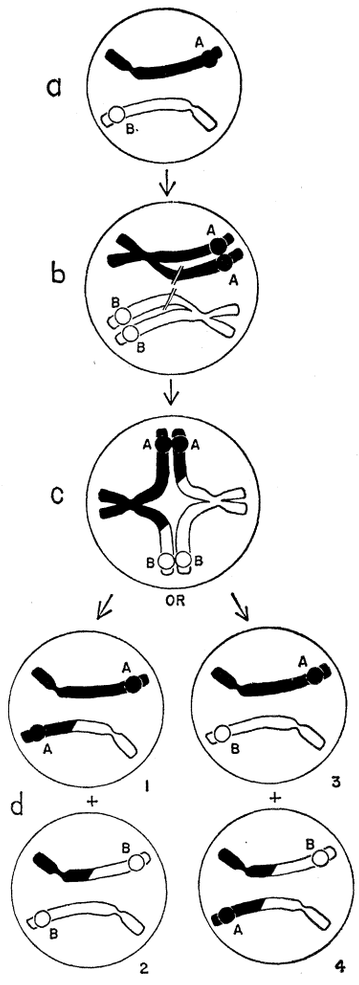

Fig. 2. Schematic representation of (*a*) two homologous chromosomes containing alleles A and B near end of long arms. In (*b*), after replication, breaks occur in comparable sites in a chromatid of each, and chromatid interchange ensues. In metaphase (*c*) the result is a quadriradial configuration of symmetrical proportions with centromeres in opposite arms. The progeny (*d*) may be like the parent cells genetically (*d3*), perhaps with new positioning (*d4*), or may, as result of crossing-over, be homozygous AA (*d1*) or BB (*d2*). The last two cells could give rise to clones to which certain genes of the host (B or A, respectively) would be foreign.

Possible genetic consequences (Fig. 2) would be essentially the same as those expected following crossing-over, "illegitimate" (*16*) only because the cell is somatic rather than germinal. The genetic constitution of the daughter cells and of the clone descending from each would be dependent on the pattern of chromatid segregation. The phenomenon demonstrated here is one cellular mechanism by which mammalian cells in vitro may develop a genome which differs from that of the parental strain as well as from other cells in the culture. (Figure 2 shows how daughter cells of a heterozygous parent might become homozygous for the hypothetical genes at the "AB" locus.) The cytological evidence of somatic crossing-over in these cells suggests the feasibility of detecting a recombination of genes in vitro in mammalian cell systems of certain types, pertinent to the

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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References and Notes

1. C. Stern, *Genetics* **21**, 625 (1936).
2. J. L. German, A. P. DeMayo, A. G. Bearn, *Am. J. Human Genet.* **14**, 31 (1962).
3. D. Bloom, *Am. J. Diseases Children* **88**, 754 (1954).
4. D. Bloom and J. German, in preparation.
5. J. L. German, A. G. Bearn, J. H. McGovern, *Am. J. Med.* **33**, 83 (1962).
6. W. Siegenthaler and J. German, unpublished data.
7. A. C. Fabergé, *J. Genet.* **43**, 121 (1942).
8. J. L. German, unpublished observations.
9. H. A. Lubs, Jr., and S. Blitman, paper presented at the Mammalian Cytogenetics Conference, Vergennes, Vermont, 26–28 Sept. 1963.
10. M. W. Shaw, *Lancet* **1961-I**, 1351 (1961); M. A. Ferguson-Smith and S. D. Handmaker, *Ann. Human Genet.* **27**, 143 (1963).
11. J. L. German, *Trans. N.Y. Acad. Sci.* **24**, 395 (1962).
12. D. E. Lea, in *Actions of Radiations on Living Cells*, L. H. Gray, Ed. (Cambridge University Press, New York, ed. 2, 1956), chap. 6.
13. C. E. Ford, "Proc. Intern. Congr. Human Genet. 8th, Lund," *Hereditas Suppl.* 570 (1948).
14. C. E. Ford, personal communication.
15. J. McLeish, *Heredity Suppl.* **6**, 125 (1953).
16. S. H. Revell, *ibid.*, p. 107.
17. L. H. Schneiderman and C. A. B. Smith, *Nature* **195**, 1229 (1962).
18. G. Pontecorvo, *Brit. Med. Bull.* **18**, 81 (1962).
19. M. W. Elves, S. Roath, G. Taylor, M. D. Israels, *Lancet* **1963-I**, 1292 (1963).
20. I. R. Mackay and R. M. Burnet, *Autoimmune Disease* (Thomas, Springfield, Ill., 1963), pp. 37–40.
21. R. R. Race and R. Sanger, *Blood Groups in Man* (Davis, Philadelphia, ed. 4, 1962), pp. 40–43.
22. G. Klein and K. E. Hellstrom, *J. Nat. Cancer Inst.* **28**, 99 (1962).
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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.