tions are a factor in the development of new races. Other forms of radiation having greater penetration, such as x-rays and thermal neutrons, may be more effective mutagens. The methods used in these tests are readily adapted to screening large numbers of spores. Therefore it should be possible to determine the relative mutation rates of the genes that condition pathogenicity (*4*).

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Effect of Free Radicals on Chromosomes of Barley

Early work on the radiation chemistry of water and aqueous solutions led investigators to conclude that the hydrogen peroxide found in such solutions might also be present in the cells of irradiated biological material and cause at least some of the genetic and physiological effects of radiation (*1*). This contention seems to have gained ground with the findings that organic peroxides and H_2O_2 increased the number of mutations in bacteria (*2*) and that the curves for inactivation of enzymes (carboxypeptidase) and production of H_2O_2 in irradiated aqueous solution were inversely related (*3*). Structural damage to chromosomes in *Tradescantia* microspores that had been irradiated with x-rays was attributed by Gray (*4*) to the effect of H_2O_2.

Recent investigations, however, indicate that hydrogen peroxide per se probably has little or no effect on the biological system and that it is the precursors of H_2O_2, H, OH, and HO_2, that produce the effect sometimes ascribed to H_2O_2. Barron *et al.* (*5*) found the effect of H_2O_2 on cell metabolism, proteins, and enzymes to be negligible unless the concentration was far above that found in the cytoplasm of irradiated cells. They asserted that a large number of cellular oxidations that had previously been traced to H_2O_2 were actually caused by free radicals evolved by the action of catalytic amounts of Cu^+ and Fe^{++} on H_2O_2.

Weiss (*6*) was one of the first workers to ascribe biological significance to radiochemically excited H, OH, and HO_2 radicals, suggesting that such radicals (especially OH) could be considered as the reactive entities in the target theory of mutation and chromosome breakage. Since that time, the role of the various radicals in biological systems has received considerable attention in the literature. Scholes and Weiss (*7*) noted fragmentation of the polynucleotides and increase in titratable acid groups when deoxyribonucleic acid was treated with chemically and radiochemically derived HO_2 radicals. Collinson *et al.* (*8*) showed that OH radicals completely inactivated solutions of ribonuclease.

This study (*9*) was designed primarily to assess the relative effects of H_2O_2 and free radicals on chromosome breakage. Of secondary interest was a comparison of the types of aberrations induced in this experiment with those produced by irradiation on the one hand and by radiomimetic substances on the other. Dormant barley seeds, variety Himalaya, were exposed to H, OH, and HO_2 radicals produced by combining dilute solutions of hydrogen peroxide and ferric sulfate. The seeds were treated for 16 hours and germinated on blotting paper. After fixation in Carnoy III (3 parts ethanol, 4 parts chloroform, and 1 part acetic acid), acetocarmine smears of shoot tips were examined cytologically for chromosome bridges and fragments at late anaphase of the first cycle of mitoses. Photomicrographs of such aberrations may be seen in Caldecott and Smith (*10*). Table 1 presents a condensed summary of numbers of chromosomal aberrations in the four treatments. Three concentrations of H_2O_2 (2, 4, and 6 percent), two concentrations of $Fe_2(SO_4)_3$ (0.01 and 0.1 percent), and the six resultant combinations of H_2O_2 and $Fe_2(SO_4)_3$ (2 percent H_2O_2 and 0.01 percent $Fe_2(SO_4)_3$; 4 percent H_2O_2 and 0.01 percent $Fe_2(SO_4)_3$; and so forth) were used, but since the differences between subtreatments were not significant, they have been combined in Table 1.

As indicated in Table 1, the frequency of chromosome fragments in the seeds treated with hydrogen peroxide and ferric sulfate is 15 to 20 times the frequency found in those treated with either hydrogen peroxide or ferric sulfate alone. It is quite clear that while hydrogen peroxide may play a minor role in chromosome fragmentation [even the small increase in fragment frequency owing to peroxide as compared with the H_2O control might be due to free radicals arising from the splitting of H_2O_2 by endogenous catalysts in the seed (*5*)] the preponderance of bridges and fragments must have been caused by the free radicals derived from the catalytic splitting of the H_2O_2 molecule.

Since H radicals will either combine in pairs to form molecular hydrogen or combine with O_2 to form HO_2 (*11*), it is the OH and HO_2 radicals that must be responsible for the observed effect. Both of these free radicals have been shown to degradate nucleic acids, nucleotides, and nucleosides (*1*).

Table 1. Chromosome bridges and fragments resulting from treatment of barley seeds in H_2O_2, $Fe_2(SO_4)_3$, and H_2O_2 and $Fe_2(SO_4)_3$.

Treatment	Cells (No.)	Bridges (No.)	Fragments (No.)	Fragments/cell
H_2O (control)	400	0	1	0.0025
H_2O_2	1200	0	6	0.005
$Fe_2(SO_4)_3$	800	0	3	0.00375
H_2O_2 and $Fe_2(SO_4)_3$	2400	13	211	0.0875

In order to assess fully the relative frequencies of chromosome and chromatid breaks, one must score cells for types of reunion as seen at metaphase as well as at anaphase. As mentioned in a previous paragraph, chromosome as well as chromatid breakage was estimated from bridge and fragment frequency at anaphase only. But by assuming that paired acentric fragments and paired dicentric bridges reflect a chromosome break, and that single fragments (or single, isolated fragments when there is more than one fragment in a cell) and single bridges indicate origin in a chromatid break, an approximation of the relative frequencies of chromosomes and chromatid breaks can be made.

Based on this assumption, 39 percent of the induced aberrations were of the chromosome type and 61 percent were chromatid breaks. This relationship of chromosome-chromatid breakage is more typical of that induced by x-irradiation than that caused by other radiomimetic chemicals (*3, 12*). Most chemicals have been found to inhibit or upset DNA synthesis, the result becoming manifest as chromosome breaks. The action of x-rays takes place later in interphase of the mitotic cycle after chromosome reduplication and results in a predominance of chromatid breaks (*13*).

This experiment produced two results that seem to be of particular interest. (i) Whereas H_2O_2 per se was shown to be ineffective in causing chromosomal aberrations, the treatment by the free radical precursors of H_2O_2 resulted in chromosome breakage. (ii) Chemically derived free radicals have much the same effect as x-irradiation in the production of chromosome aberrations. This aspect gains importance in the light of recent studies that attribute a major role to organic and inorganic free radicals in the x-ray induction of

mutations and chromosome damage. The action of these irradiation by-products (indirect effect) is becoming increasingly important in explaining irradiation phenomena that were once attributed solely to a direct effect (target theory) of ionizing radiation.

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Larval Stages and Phylogeny as Exemplified by the Lung Fluke of Turtles

Embryonic and larval forms as clues to relationships have been criticized largely because their application to phylogeny has been misinterpreted as acceptance of the recapitulation hypothesis of Haeckel. That larval stages may be highly specialized has been well established but, as de Beer (*1*) has stated, "although it can in many cases be shown that these larval forms could not represent the adult ancestral forms, this does not detract from their value as evidence of affinity between the organisms that possess any particular type." That such value not only applies to relationships between phyla and classes but also may extend to less inclusive categories as well is shown in a striking manner by the digenetic trematodes. A larval stage common to all of them is the cercaria (cercariaeum, if tailless) of which there are several types. The discovery that certain very similar adult trematodes have utterly different types of cercariae and that very dissimilar adults may have the same larval type has required drastic revision of existing concepts of relationships and phylogeny within the group. For example, immature stages have demonstrated that trematodes once thought to form a single family, the Heterophyidae, actually belong to three families in two orders (*2*). On the other hand, life-history studies promise to consolidate major groups and, more importantly, indicate lines of descent among them.

It thus was particularly desirable to investigate the life-history of *Heronimus chelydrae,* a common parasite in the lungs of fresh-water turtles and so different from other trematodes that it has long been the sole occupant of a distinct family. It has been found that the miracidium penetrates *Physa* sp. and develops into a sporocyst which produces cercariae directly without the interpolation of an intermediate sporocyst or redial generation, as is the case in most known life-histories. The cercaria differs from the larvae of the paramphistomes only in lacking eyespots and possessing a pair of flame cell groups in the tail. However, these differences are known to occur in larvae belonging to the same superfamily, and the fundamental resemblances between the cercaria of *H. chelydrae* and that of the paramphistomes, especially in the embryology and form of the excretory system, justify the allocation of that species to the superfamily Paramphistomatoidea. The cercaria has a powerful ventral sucker which disappears before the adult stage is attained, and the unique features of that stage, notably the unusual position of the excretory pore and form of the genital glands, may be attributed to differential growth after the cercaria leaves the snail.

From the phylogenetic standpoint, the cercaria of the turtle lung fluke is of much interest. The presence of flame cells in the tail, the posterior position of the definitive excretory pores in that structure, the thin-walled excretory vesicle, and absence of an intermediate generation in the molluscan host are all characteristics that in combination have been reported only for certain fork-tailed cercariae, the larvae of the order Strigeatoidea. Although the amphistomes have been placed heretofore in the order Prosostomata, they thus may be the extant group closest to the trematodes from which the two orders evolved. Furthermore, the life-history of *H. chelydrae* lends support to the view that the fasciolids, echinostomes, and many monostomes, as well as the paramphistomes, trematodes that have never fitted comfortably into the Prosostomata, actually are closer to the Strigeatoidea.

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Effects of Certain 19-Nor Steroids on Reproductive Processes in Animals

In previous publications we have reported that progesterone and certain of its chemical relatives are effective inhibitors of ovulation in rabbits, rats, and women (*1–4*). Among the most potent of a large number of compounds tested in animals have been a series of 19-nor steroids. These have proved to be effective ovulation-inhibitors by both oral and parenteral routes of administration. We have recently concentrated our attention on four of these compounds in a series of studies designed to reveal their action in various mammalian reproductive processes (*5*). The compounds are 17α-ethinyl-19-nortestosterone (I), 17α-ethinyl-5(10)-estraeneolone (II), 17α-ethyl-19-nortestosterone (III), and 17α-methyl-19-nortestosterone (IV).

The following assays with progesterone and these compounds have been performed in female mammals: (A) the Clauberg assay for endometrial stimulation in immature female rabbits, (B) the Rubin assay for uterotrophic activity in immature mice, (C) the deciduomagenic activity in primed, ovariectomized rats, (D) the conception-inhibiting activity in mature female rats caged with fertile males, (E) the ovulation-inhibiting activity in postpartum female rabbits mated to fertile males, and (F) the ability to induce and sustain implantation of the fertilized ovum in female rabbits castrated 1 day after a fertile mating. The details of the methods employed in these assays are being published elsewhere (*6*). In Table 1 we present the calculated minimum effective dose (M.E.D.) in each of the tests. Except where noted in the table, subcutaneous administration of the compounds was practiced.

The data demonstrate, first of all, that each of these compounds possesses progestational activity by virtue of its ability to induce pseudopregnant proliferation in the Clauberg assay (A). Of them II has approximately one-half of the activity of progesterone, whereas I, III, and IV are 5 to 10 times as active.

All of the substances are uterotrophic (B), but they exhibit marked quantitative differences, II being approximately 350 to 400 times as active as progesterone, and III, I, and IV being intermediate in activity. Actually there is a qualitative difference between these compounds in this test in the sense that the slope of the dosage/response curve for I, III, and IV resembles that of progesterone, whereas that of II more nearly resembles that of estrone, and comparison of the curves gives II an activity equivalent to one-fortieth to one-eightieth of that of estrone (*6*). In the spayed female rat test