androsterone. No other products were identified from the various fractions. Endogenous excretion of androsterone and isoandrosterone during this period would be expected to be in the range of 8 mg and 0.4 mg, respectively.

The main urinary metabolites isolated following administration of testosterone and Δ^4-androstene-3,17-dione are androsterone, etiocholane-3-(α)-ol-17-one and isoandrosterone (*7–10*). The amounts of androsterone and isoandrosterone excreted following the administration of Δ^1-androstenedione-3,17 are of the same order as those found following testosterone and Δ^4-androstene-3,17-dione administration. Etiocholane-3(α)-ol-17-one was not isolated in this experiment.

The significance of the Δ^1-C_{19} steroids is at present not clear. These studies indicate, however, that the *in vivo* reduction of the double bond at carbon 1 in ring A is accomplished in good yield, comparable to the *in vivo* reduction of the double bond at carbon 4 in ring A.

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Protection of *Escherichia coli* against Ultraviolet Radiation by Pretreatment with Carbon Monoxide[1]

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Radiation damage to biological systems can be reduced by manipulations which lower the oxidation state within the cell or its environment. This may be accomplished by removing the oxygen, by the addition of various reducing agents, or by the use of metabolic inhibitors (*1–3*). Glycolytic inhibitors generally do not protect (*4*). Cyanide and azide have been shown to impart increased resistance to rodents (*1, 4*) and *Drosophila* (*2*) if applied prior to x-irradiation. Table 1 demonstrates the protection rendered aerobic, log phase *Escherichia coli* cells against ultraviolet irradiation following pretreatment with 0.05 *M* NaCN for 30 min. Due to the established mutagenicity of cyanide (*5, 6*) and to its frequently irreversible inhibitory nature, light-reversible inhibition by carbon monoxide was utilized for further experiments. The intent was to block the terminal respiratory enzymes involved in passing electrons to oxygen. It seemed reasonable to believe that such a block should result in an accumulation of reduced enzymes in the cell and, thereby, in an increased resistance to radiation.

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TABLE 1

PROTECTION OF *Escherichia coli* FROM ULTRAVIOLET RADIATION BY CYANIDE*

Seconds ultraviolet		0	15	30
Control	Cells/ml†	155.0	5.1	0.03
	Percentage surviving	100.0	3.3	.019
NaCN‡	Cells/ml†	131.0	6.6	.19
	Percentage surviving	100.0	5.04	0.145

* Multiply all figures by 10^6.
† Washed log phase cells. Decrease in numbers of the cyanide-treated culture accounted for by manipulations and cyanide toxicity.
‡ 0.05 *M* for 30 min prior to washing and irradiating.

Cultures were grown at 37° C to equal turbidity (log phase) in flasks of Difco nutrient broth through which gases were bubbled to yield conditions of high aerobiosis (95% oxygen and 5% carbon dioxide), medium aerobiosis (air), mild anaerobiosis (methane), and strict anaerobiosis (methane atmosphere in a Brewer anaerobic jar heated for 45 min). A 20-ml aliquot of each culture was placed in a sterile Petri dish, gently agitated for 20 min to allow it to equilibrate with air, and then irradiated with ultraviolet light. Another aliquot of 100 ml of each culture was placed in a darkened vessel through which carbon monoxide (generated by dehydrating formic acid with concentrated sulfuric acid) was bubbled for 5 min. Controls remained constant at this time interval, but more extensive treatment yielded irreversible inhibition. Immediately after the monoxide treatment two 20-ml samples were placed in sterile Petri dishes. One covered plate was kept darkened, and the other was exposed to fluorescent light. After being gently agitated for 20 min, each was immediately irradiated with ultraviolet light. The equilibration served two purposes. The first was to allow diffusion of the various gases (CO, CH_4, O_2) from the media so that irradiation could be effected in air alone. The second was to reverse the monoxide inhibition in the samples exposed to light to enable the cells to respire, and in this manner regain a higher oxidation state prior to irradiation, while those held in the dark remained in a reduced (inhibited) condition. This exposure to air increased the sensitivity of the strictly anaerobic cells to some extent, but was essential in order to obtain comparable results.

Cultures were exposed to a quartz ultraviolet lamp delivering radiations of about 90% 2537 A within a darkened chamber (35° C), which was sterilized by operating the lamp for 30 min prior to exposing the suspensions. Cultures were irradiated in open Petri dishes, which were gently agitated to ensure uniform exposure of the cells. Aliquots were withdrawn at intervals, diluted, and immediately plated in replicate

in Difco nutrient agar for determination of survivors. All operations were carried out in a darkened room to limit photoreactivation. Plates were counted after 3 days' incubation at 37° C. Data obtained from averaging replicate experiments are presented in Fig. 1.

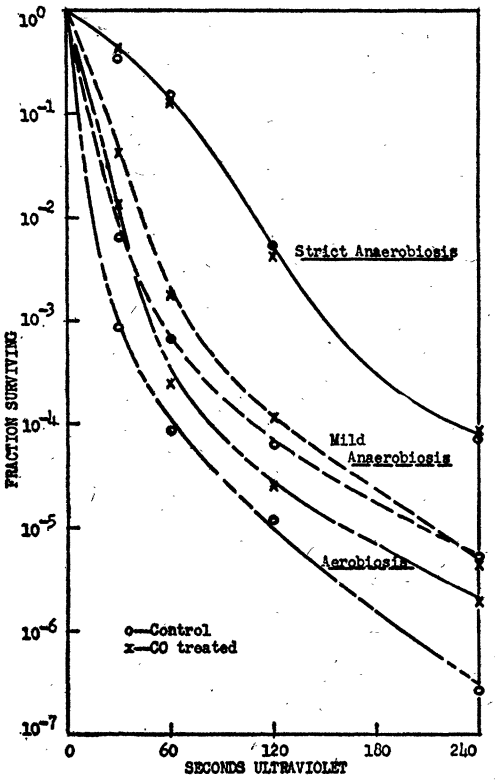

FIG. 1. Comparative sensitivities of aerobic and anaerobic cells treated with carbon monoxide prior to ultraviolet irradiation. Cultures were grown in the following: Aerobic, 95% oxygen, and 5% carbon dioxide; mildly anaerobic, methane; strictly anaerobic, methane in Brewer anaerobic jar.

Results from the monoxide-inhibited aliquots which were exposed to visible light are not reported in the figure, since the results were almost identical with those of the controls. The protection accompanying monoxide inhibition is progressively lost following its reversal by light, if the cells are allowed to respire during this time. Short intervals of exposure to light prior to irradiation only partially reversed the inhibition, but 20 min exposure resulted in a loss of almost all the protective effect. No recovery occurred upon post-irradiation treatment with the monoxide. Hollaender *et al.* (*7*) reported that anaerobically cultured *E. coli* cells exhibited a decreased sensitivity to x-rays. In our experiments we noted that anaerobic cultures were also more resistant to ultraviolet rays. Aerobic cells were markedly protected by carbon monoxide treatment. This is evident from the fact that monoxide-treated aerobic suspensions had approximately 13 times as many survivors as the nontreated controls at 30 sec irradiation. On the other hand, mildly anaerobic cells were protected to a lesser extent and strictly anaerobic cells not at all. In the latter case, since the cells are already in a highly reduced state, a further reduction is impossible. This failure of anaerobic cells to derive additional protection, coupled with the protection rendered respiring (aerobic) cells, by monoxide- and cyanide-inhibition substantiates the thesis that this protective mechanism is due to the reduced state of the enzymes which result from the treatment.

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Selection for DDT Resistance in a Beneficial Insect Parasite[1]

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Biological control of insects is often partially nullified by the effects of chemical control; beneficial parasites, as well as pests, are destroyed by the insecticides. Experiments are in progress in this laboratory to see whether it is possible to produce, by selective breeding, appreciable DDT resistance in insect parasites of pest insects.

The work is being done with *Macrocentrus ancylivorus* Rohw. (Hymenoptera: Braconidae), an effective parasite of the larvae of the oriental fruit moth *Grapholitha molesta* (Busck). Mass propagation of this parasite is carried out by the method of Finney, Flanders, and Smith (*1*). The life cycle of the parasite is approximately 21 days, but this method involves continuous rearing with overlapping generations, so that a fresh crop of newly emerged adults is produced each day. Every day for 3 min the insects are exposed to the insecticide in the form of a thin film of celloidin in which DDT crystals have been deposited (*2*). The survivors are counted 40 hr later and are then returned to the breeding unit to oviposit on young host larvae. Selection has been continued daily in this way for 9 months; during this period the films in use contained 23 μg of pure *p-p*-DDT/cm².

Survival was initially about 30%. It increased rapidly and at the end of the first 4 months it was about 80%; this figure approximates to that for control insects exposed on plain glass surfaces. This high level of survival persisted, under selection, for the remainder of the period. Selection is now being carried out at a higher DDT concentration.

A more exact estimation of relative survival has

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