Streptomycin sulfate applied as a water solution at the rate of 1, 3, and 9 lbs of antibiotic/acre to soil in which bean seedlings were growing failed to reduce the incidence of infection or severity of symptoms that developed on their primary leaves inoculated with the halo blight organism.

Bean plants treated with streptomycin sulfate failed to accumulate a sufficient amount of the antibiotic in their seeds to affect the susceptibility to the halo blight organism of seedlings that developed from these seeds. In this experiment, the antibiotic was applied as the paste previously described to those internodes of relatively mature plants which subtended fruit clusters. Other plants were sprayed twice with an aqueous solution containing 0.1% of streptomycin sulfate. Pods approximately 2–3 in. long at the time of treatment were harvested at maturity; the seeds in these were planted, and the first trifoliate leaves of the resulting seedlings were inoculated with the halo blight organism. There was no apparent difference between the severity of symptoms that developed on seedlings from treated and on those from untreated parent plants.

Several investigators have shown that roots of plants such as oats, lettuce, soybean, and lima bean can absorb antibiotics such as aureomycin, streptomycin, and griseofulvin from aqueous solutions of these compounds and translocate them to the leaves and stems (*1–3*). Boyle (*4*) reported that penicillin injected into cactus stems diffused into near-by infected areas and killed *Erwinia carnegieana* Lightle, Standring, and Brown. Others (*5, 6*) have reported that streptomycin, neomycin, bacitracin, chloromycetin, subtilin, and penicillin reduced the prevalence of halo blight when infected bean seeds were soaked in aqueous solutions of these antibiotics before planting.

Absorption and translocation of an antibiotic by an aboveground plant part in sufficient amounts to retard or inhibit a bacterial disease have not been reported previously. It is concluded from the experiments reported here that dihydrostreptomycin sulfate and streptomycin sulfate were absorbed by the stems of bean seedlings and translocated upward to the primary leaves where, within a period of 3–4 days, they accumulated in sufficient amounts to inhibit or prevent the growth and development of the halo and common blight organisms. Within a week after application of streptomycin sulfate to the stems of bean seedlings, a sufficient amount of the chemical had been absorbed and translocated to the first and second trifoliate leaves to suppress development of the halo blight organism.

References

1. ANDERSON, H. W., and NIENOW, I. *Phytopathology,* **37**, 1 (1947).
2. BLANCHARD, F. A., and DILLER, V. M. *Am. J. Botany,* **38**, 111 (1951).
3. BRIAN, P. W., *et al. Nature,* **167**, 347 (1951).
4. BOYLE, A. M. *Phytopathology,* **39**, 1029 (1949).
5. HILDRETH, R. C., and STARR, G. H. *Colo.-Wyo. Acad. Sci. J.,* **4**, 58 (1950).
6. SMITH, W. L. *Ibid.,* 49.

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Metabolism of Δ^1-Androstene-3,17-Dione

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Δ^1-Androstene-3,17-dione has been isolated from pooled urines from healthy and diseased individuals (*1*). Saturation of ring A has been shown to occur when this steroid is incubated with fermenting yeast (*2*), since the product androstane-3β,17β-diol was isolated. In the present study we have demonstrated that the oral administration of Δ^1-androstene-3,17-dione gives rise to relatively large amounts of androsterone, isoandrosterone, and $\Delta^{2 \text{ or } 3}$ androstene-17-one in the urine (Table 1). The $\Delta^{2 \text{ or } 3}$-androstene-17-one is considered to arise from androsterone, as a result of the chemical procedures employed during isolation (*3–5*).

TABLE 1

URINARY METABOLITES OF 3.2 G Δ^1-ANDROSTENE-3,17-DIONE FOLLOWING ORAL ADMINISTRATION

Compound isolated	Amount isolated (mg)	Melting points in ° C*			Percentage isolated
		Free	Acetate	Oxime	
Androsterone	210	184–5.5	163–4		10.9
	140	181–3	163–4		
$\Delta^{2 \text{ or } 3}$-Androstene-17-one	125	104–6.5		156–8.5	3.9
Isoandrosterone	55	175–7	115–7		1.7

* Fischer-Johns apparatus—uncorrected.

Over a 60-hr period, 3.2 g of Δ^1-androstene-3,17-dione[2] was administered orally in capsules to an adult male with rheumatoid arthritis. The control value for 17-ketosteroids for this individual was 9.8 mg/24 hr. The urine was collected during the treatment period and for 36 hr after treatment was stopped. The urine was extracted with butanol by the method of Venning (*6*), the butanol evaporated to dryness, and the residue subjected to simultaneous hydrolysis with HCl and extraction with benzene. The ketonic and nonketonic fractions were prepared using Girard's reagent T. The ketonic fraction was chromatographed, using aluminum oxide as adsorbant, and eluted with carbon tetrachloride, followed by carbon tetrachloride containing 0.1, 0.2, and 0.3% ethanol, respectively.

The ketonic fraction upon elution with carbon tetrachloride yielded 125 mg $\Delta^{2 \text{ or } 3}$-androstenone-17. Further elution with carbon tetrachloride containing 0.2% ethanol yielded 350 mg androsterone. The β-ketonic fraction precipitated with digitonin yielded 55 mg iso-

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[2] A gift from the Chemical Specialties Co., Inc., New York City.

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.

References

1. LIEBERMAN, S., FUKUSHIMA, D. K., and DOBRINER, K. *J. Biol. Chem.*, **182**, 299 (1950).
2. BUTENANDT, A., DANNENBAUM, H., and SURANYI, L. *Ber.*, **73**, 818 (1940).
3. VENNING, E. H., HOFFMAN, M. M., and BROWNE, J. S. L. *J. Biol. Chem.*, **146**, 369 (1942).
4. BUTENANDT, A., and DANNENBAUM, H. *Z. physiol. Chem.*, **229**, 192 (1934).
5. HIRSCHMANN, H. *J. Biol. Chem.*, **136**, 483 (1940).
6. VENNING, E. H. *Ibid.*, **119**, 423 (1937).
7. CALLOW, N. H. *Biochem. J.*, **33**, 559 (1939).
8. DORFMAN, R. I., and HAMILTON, J. B. *J. Biol. Chem.*, **133**, 753 (1940).
9. DORFMAN, R. I. *Proc. Soc. Exptl. Biol. Med.*, **46**, 351 (1941).
10. DORFMAN, R. I., WISE, J. E., and SHIPLEY, R. A. *Endocrinology*, **46**, 127 (1950).

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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