Technical Papers

Effect of Some Steroid Compounds on Ovine Rumen Function

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An implant of stilbestrol has been shown to increase the rate of gain and feed efficiency in lambs (*1–4*). Tests were designed to determine whether stilbestrol, cholestrol, or estrone would influence the digestion of cellulose by rumen microorganisms *in vitro* and to determine the effect of stilbestrol on the digestion of cellulose and crude protein by sheep.

Studies were made using the artificial rumen techniques described by Brooks (*5*). Stilbestrol was tested at levels of 2, 8, 10, 16, and 20 μg per artificial rumen, and estrone and cholesterol were each tested at the 20-μg levels. One microgram was equal to 1 ppm dry matter. Each artificial rumen contained 500 mg of cellulose and was paired with one containing the same ingredients other than the substances under test.

The effects of stilbestrol, estrone, and cholesterol on cellulose digestion in the artificial rumen are shown in Table 1. When stilbestrol was added at low levels (2 and 8 μg) in trial 1, there was a slight but nonsignificant increase in cellulose digestion. The addition of stilbestrol (16 μg in trial 1, or 10 and 20 μg in trial 2) increased cellulose digestion ($P < 0.05$). Twenty microgram of estrone increased cellulose digestion 63 percent ($P < 0.01$), and 20 μg of cholesterol increased it 35 percent ($P < 0.05$). The differences between cellulose digestion in rumina containing 20 μg of stilbestrol, estrone, and cholesterol were not significant.

The *in vivo* effect of stilbestrol on cellulose and protein digestion was studied in three lots of 5 crossbred yearling wethers. The basal ration provided 908 g cottonseed hulls, 95 g casein, 6 g Cr_2O_3, and 2500 IU vitamin A per sheep daily. The sheep had free access to salt and a mineral mixture of equal parts dicalcium phosphate, calcium carbonate, and sodium chloride containing 2 oz of cobalt sulfate per 100 lb of mixture. A 14-day preliminary period was followed by a 4-day collection period. The digestion trials and chemical determinations were conducted as described by Brooks *et al.* (*6*).

As is indicated in Table 2, the coefficient of digestibility of cellulose was increased 16 percent ($P < 0.05$), and the coefficient of digestibility of protein was increased 18 percent in animals that received 10 or 20 mg of stilbestrol per day. The differences in the coefficients of digestibility of cellulose between the basal lot and the stilbestrol-fed lots were significant ($P < 0.02$). The differences between the coefficients of digestibility of crude protein approach significance.

Table 1. Effect of some steroid compounds on cellulose digestion by ovine rumen microorganisms *in vitro*.

Trial	Mixture fermented	No. of rumina	Avg. cellulose digestion (%)
1	Basal ration	6	35.1
1	Basal ration + 2 μg stilbestrol	6	36.6
1	Basal ration + 8 μg stilbestrol	6	37.6
1	Basal ration + 16 μg stilbestrol	6	49.1*
2	Basal ration	10	36.7
2	Basal ration + 10 μg stilbestrol	10	44.7†
2	Basal ration + 20 μg stilbestrol	5	51.4*
2	Basal ration + 20 μg estrone	5	57.3†
2	Basal ration + 20 μg cholesterol	5	48.4*

* Difference in cellulose digestion (experimental vs. basal) significant ($P < 0.05$).
† Difference in cellulose digestion (experimental vs. basal) highly significant ($P < 0.01$).

Table 2. Effect of stilbestrol on the coefficients of digestibility of cellulose and protein in sheep.

Lot no.	Ration	Coefficients of digestibility	
		Cellulose	Crude protein
1	Basal	41.9	37.5
2	Basal + 10 mg stilbestrol per sheep per day	47.8	43.2
3	Basal + 20 mg stilbestrol per sheep per day	48.7	44.7

In a subsequent test, lot 3 sheep continued to receive 20 mg of stilbestrol per head daily. Two of them developed anorexia during the first week and appeared listless. An edematous swelling appeared around the anus of one sheep. Contractions of the abdominal muscles accompanied by apparent pain were observed 24 hr after the first symptoms had appeared. Digital examination indicated that the muscle tonus of the lower intestine was reduced and that the urethra and prostate had enlarged. Similar conditions have been described in Australia where sheep were pastured on swards high in subterranean clover (*7*). When the sheep was taken off the stilbestrol ration and given a subcutaneous injection of 100 mg of testosterone, it made a rapid recovery. Other sheep receiving stilbestrol showed mild symptoms similar to those seen in the sheep that was treated and were taken off the experiment.

Summary. (i) Stilbestrol (10 or 20 ppm) increased cellulose digestion by ovine rumen microorganisms *in vitro* and *in vivo* but could not be tolerated by wethers at these high levels. (ii) Cholesterol and estrone in-

creased cellulose digestion by rumen microorganisms *in vitro* (*8*).

References and Notes

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8. *Addendum.* Since this paper was submitted for publication Wise Burroughs *et al.* [*Science* **120**, 66 (9 July 1954)] have reported that the addition of diethylstilbestrol to the rations of fattening steers increased growth rate and feed efficiency.

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Particle Size and Shape of Purified Tomato-Ringspot Virus

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The particle size of the tomato-ringspot virus has been estimated at 50 mμ or less according to ultrafiltration techniques (*1*). The shape of such particles has not been described insofar as we are aware. Therefore, investigations were undertaken to determine the particle size with more precision and to ascertain its shape.

Purification of the virus. Tobacco plants of the Holmes' necrotic type with three or more fully developed leaves were inoculated with tomato-ringspot virus obtained from W. C. Price. Leaves showing typical symptoms after 18 days' incubation were frozen, minced, and extracted with dipotassium phosphate. The extract was then clarified by low-speed centrifugation. Initial concentration and purification were achieved by differential centrifugation in the preparative centrifuge, Spinco, Model L (*2*). The resultant pellets from both diseased and healthy tissues were suspended in 0.01*M* potassium phosphate buffer (*p*H 7) for each run. The pellet from the final centrifugation was resuspended in 0.1 ionic strength phosphate buffer (*p*H 6.5) for use in electrophoresis. Final purification was attained by electrophoresis for 312 min in the Tiselius apparatus.

Electron microscopy. Specimens for the electron microscope were prepared by the protein monolayer technique (*3*). This procedure involves the application of a suspension of virus particles in a dilute protein solution to a point on an aqueous surface. The protein spreads spontaneously and forms an insoluble monolayer in which the particles to be examined are uniformly distributed. The monolayer with its imbedded particles is then transferred to a celloidin-covered screen and shadowed with 8 A of uranium at a grazing angle of 16°. The magnification of an RCA-type EMU microscope was calibrated by a replica of a precision-ruled grating, consisting of 15,000 lines/in., manufactured by the Ford Company.

Particle size and shape. Preparations from the infected and virus-free tissue were examined with the electron microscope for differences in particle size and shape. Electron micrographs (Fig. 1) of preparations of an infective fraction from the electrophoresis apparatus indicate the presence of four- to six-sided particles having a cross section of 43 mμ when measured perpendicularly to the direction of the shadowing. The depth of the particle as obtained from shadow measurements was 13.5 mμ. Thus, the particles resemble flattened cylinders or pills. It is to be recognized that the measurement of shadows cast by individual particles is subject to uncertainty owing to local shadow angles, to the difficulty of making a precise determination of filament position, and to other factors. However, if it is assumed that this virus is not rigid and that the monolayer technique permits the distortion of the shape, then, from the dimensions given here, the volume may be equated to that of a sphere, in which case a diameter of 27 mμ is obtained.

Electron micrographs of preparations from virus-free plants contained many spheroidal particles approximately 14 mμ in diameter and much amorphous material. These particles were not observed in the electrophoretic fraction of extracts from virus-infected plants used for electron microscopy, but they were present in two of four other fractions. The polyhedral particles observed in infected tissue extracts as described in the preceding paragraph were not found in preparations from virus-free plants.

Infectivity. Aliquots of preparations from virus-infected plants containing the polyhedral particles were

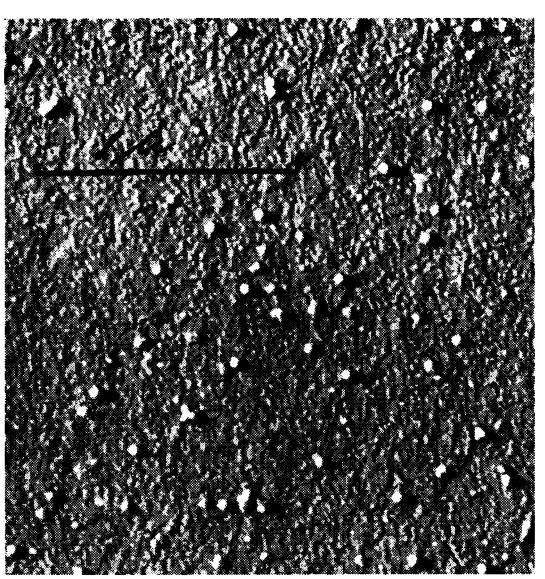

Fig. 1. Electron micrograph of particles from purified tomato-ringspot virus preparations (×33,000).