EFFECT OF DEOXYCORTICOSTERONE ON THE GROWTH OF MICROORGANISMS[1]

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It has been shown that deoxycorticosterone markedly inhibits the growth of *Neurospora crassa* (Lester *et al.*, 1958). This effect was highly specific since small alterations in the deoxycorticosterone molecule greatly diminished or abolished its inhibitory activity. It was also shown that deoxycorticosterone inhibited the uptake of sugars, amino acids, and rubidium by *N. crassa*. These effects on *N. crassa* appeared to be analogous to those on the cells and tissues of many higher organisms, and suggested the possibility that the action of this mammalian hormone (and perhaps others) could be expressed and studied in mircoorganisms. The general value of such a proposal would, in some measure, depend on whether a hormonal effect is unique for a certain microorganism, or occurs in many microbial species. It is the purpose of this communication to present a survey of the effect of deoxycorticosterone on the growth of a large variety of microorganisms. The data obtained indicate a possible correlation between gram-positivity and deoxycorticosterone sensitivity.

MATERIALS AND METHODS

Media. Bacteria and yeasts were cultured in synthetic (S) and complex (C) media whose compositions have been previously described (Lester, 1958), and also in nutrient broth (N). The molds were cultured in the Fries synthetic medium (F) commonly used for *N. crassa*. Deoxycorticosterone (DOC) in ethanol-chloroform, 1:1, was added to sterile tubes (bacteria or yeasts) or flasks (molds) and the solvents were evaporated overnight at 30 C. Sterile medium was then aseptically added, 4 or 5 ml per tube, and 20 ml per flask. Controls were prepared similarly, with and without solvent alone, and these showed no appreciable differences in growth.

Inoculation. The tubes were inoculated with a loopful, or a drop of a fresh culture of bacteria or yeast grown on a similar medium; the flasks were inoculated with a suspension of mold spores (or hyphal fragments) scraped from a slant. Each experiment was set up either in duplicate (bacteria or yeasts) or triplicate (molds).

Incubation. All cultures were incubated at 30 C, with vigorous agitation for bacteria and yeasts, and gentle agitation for the molds.

Growth determination. The growth of bacteria and yeasts was estimated by turbidimetric determination with the Klett-Summerson colorimeter equipped with a red (660) filter. When necessary the cultures were diluted so that the readings did not exceed 250 colorimeter units. Mycelial dry weights were used as the growth values for the molds.

Agreement between replicates was very good, but with some organisms there was some variation between experiments. However, the variations encountered could not be correlated with the medium used, inoculum size, incubation time, or the level of deoxycorticosterone used; for comparative purposes the results are given in terms of percentage of inhibition, rather than as the growth values actually obtained.

RESULTS AND DISCUSSION

The effects of deoxycorticosterone on the growth of various species of microorganisms are summarized in table 1, which shows that the growth-inhibiting action of this steroid is not restricted to a few unique species. These data show that the growth of all of the gram-positive bacteria, and yeasts, and the molds is significantly inhibited by deoxycorticosterone. On the other hand, all of the resistant organsims are gram-negative. It might appear that a clear-cut relationship between gram-positivity and deoxycorticosterone sensitivity is precluded since the normally gram-negative species, *Neisseria catarrhalis* and *Agrobacterium radiobacter* are inhibited. However, Neisseria species are well known for their variability with respect to gram-staining

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TABLE 1

Effect of deoxycorticosterone on bacteria, yeasts, and molds

Species	No. of Expts	Incubation	Medium*	DOC	Inhibition Range
		hr		*mg/ml*	%
Gram-negative bacteria:					
Escherichia coli	6	9–18	NSC	0.15–0.25	<5
Aerobacter aerogenes	3	16	NSC	0.15–0.25	<5
Salmonella enteritidis	1	16	N	0.08	<5
Shigella sonnei	1	16	N	0.08	<5
Proteus vulgaris	2	18	SC	0.25	<5
Serratia marcescens	6	16–18	NSC	0.08–0.25	<5
Erwinia carotovora	5	18–24	NSC	0.15–0.25	<5
Pseudomonas fluorescens	2	18	SC	0.25	<5
Pseudomonas aeruginosa	1	16	N	0.08	<5
Agrobacterium radiobacter	6	18–24	NSC	0.15–0.25	12–30
Neisseria catarrhalis	2	48	C	0.25	95–99
Gram-positive bacteria:					
Micrococcus aureus (white variant)	3	16–24	NC	0.08–0.25	73–94
Sarcina lutea	2	18–24	NC	0.15–0.25	90–100
Bacillus megaterium	5	16–24	NSC	0.08–0.25	49–100
Bacillus subtilis	7	11–24	NSC	0.15–0.25	50–100
Corynebacterium pseudodiptheriticum	1	42	C	0.25	
Mycobacterium ranae	7	24–72	NSC	0.15–0.25	97–100
Yeasts:					
Torulopsis utilis	4	16–18	NS	0.08–0.17	25–33
Saccharomyces cerevisiae	4	18–42	NSC	0.17–0.25	65–94
Molds:					
Neurospora crassa	17	72	F	0.25	40–65
Aspergillus niger	4	72	F	0.25	56–76
Cunninghamella blakesleana	2	72	F	0.25	38–63
Curvularia lunata	3	96	F	0.25	60–100
Penicillium lilacinum	2	96	F	0.25	71–86
Penicillium puberulum	4	120	F	0.25	76–100

* The letter designation of the types of media employed are explained in the section on Materials and Methods.

reaction (e. g., Mitchell and Moyle, 1950); and Rhizobium species, to which members of the genus Agrobacterium are closely related (Breed *et al.*, 1948), have been claimed to have gram-positive affiliations (Bisset, 1955; Hale, 1957). Indeed, in view of the otherwise excellent correlation between gram-positivity and deoxycorticosterone sensitivity, the sensitivity of species of the genera Agrobacterium and Neisseria to deoxycorticosterone could be adduced as evidence in support of their gram-positive affiliations.

The nature of the action of deoxycorticosterone on the growth of the species examined has yet to be determined. However, the aforementioned observations with Neurospora (Lester *et al.*, 1958) suggest the possibility that the observed inhibition of growth by deoxycorticosterone is due to its interference with the uptake of certain essentual nutrients. Recent work (Lester and Hechter, 1958) indicates that deoxycorticosterone appears to inhibit the intracellular concentration of rubidium (and possibly other substances) rather than its entry into Neurospora. This observation, together with those reported here, might permit the speculation that a functional basis for the Gram reaction might be found in cellular concentration systems. Also, as a conjectural corollary, it would follow that the concentration

systems of gram-positive and gram-negative organsims are mechanistically and materially different, with gram-variable organisms representing transition forms.

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SUMMARY

The effect of the mammalian steroid deoxycorticosterone on the growth of bacteria, yeasts, and molds has been examined. No stimulation was observed, but deoxycorticosterone was found to inhibit the growth of the gram-positive bacteria, yeasts, and molds tested; gram-negative bacteria were generally insensitive to deoxycorticosterone.

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