TABLE I. Response of *Lactobacillus bulgaricus* (ATCC 8001) to Uracil, Dihydrouracil and β-Ureidopropionic Acid.

Compound (γ/10 ml)	Turbidity (Klett-Summerson units)
0	42
5 γ uracil	51
10	74
15	78
20	88
50	178
100	315
500	387
10 to 500 γ dihydrouracil	43
10 to 500 γ β-ureidopropionic acid	43

I. It is interesting to note that while this strain, for example, can utilize dihydroorotic acid presumably by unsaturation to orotic acid and can utilize uracil as well as orotic acid(1) it does not accomplish the unsaturation of dihydrouracil. In this connection Lieberman and Kornberg recently have shown(3) that dihydroorotic dehydrogenase from a bacterial source that is concerned with the reversible reaction: Orotic acid + DPNH + H^+ ⇌ Dihydroorotic acid + DPN is inert towards uracil, cytosine, 5-methyleytosine, or thymine.

Summary. Dihydrouracil and β-ureidopropionic acid, the "uracil counterparts" of dihydroorotic acid and ureidosuccinic acid, intermediates in the biosynthesis of orotic acid, are inactive in promoting growth of 8 pyrimidineless lactobacilli.

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Effect of Diacetyl on Microorganisms.* (21054)

LOYD W. HEDGECOCK AND DAVID V. COHN. (Introduced by Tom Hamilton.)

From the Research Laboratory and Radioisotope Unit, Veterans Administration Hospital, Kansas City, Mo., and the Departments of Medical Microbiology and Pharmacology, University of Kansas Medical Center, Kansas City, Kans.

It is not clear whether diacetyl enjoys a major or relatively minor role in the metabolism of plants and animals. This compound occurs in a large variety of natural products such as tobacco, butter, coffee, cocoa, beer, and honey. The citric acid-fermenting streptococci produce diacetyl and cultures of these organisms are widely used for the production of desirable flavors in dairy products(1). This compound is metabolized by bacteria, bacterial extracts and mammalian tissue through

TABLE I. Inhibitory Concentration of Diacetyl for Microorganisms.

Organism	Diacetyl (μg/ml)	Organism	Diacetyl (μg/ml)
E. floccusum	200	*A. aerogenes*	400
A. niger	"	*P. vulgaris*	"
S. carlsbergensis	"	*B. mycoides*	"
S. griseus	"	*B. subtilis*	"
E. coli	300	*B. anthracis*	"
P. pyocyaneus	"	*Cl. tetani*	"
S. paradysenteriae	"	*S. viridans*	"
S. schottmuelleri	"	*M. pyogenes*	600
S. typhosa	"	*S. hemolyticus*	800
M. tuberculosis (607)	"	*S. fecalis*	"
M. phlei	"	*S. lactis* (8043)	1200
N. catarrhalis	"	*L. casei* (7469)	1600

Laboratory strains except where indicated.

* A portion of this work was done in the Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

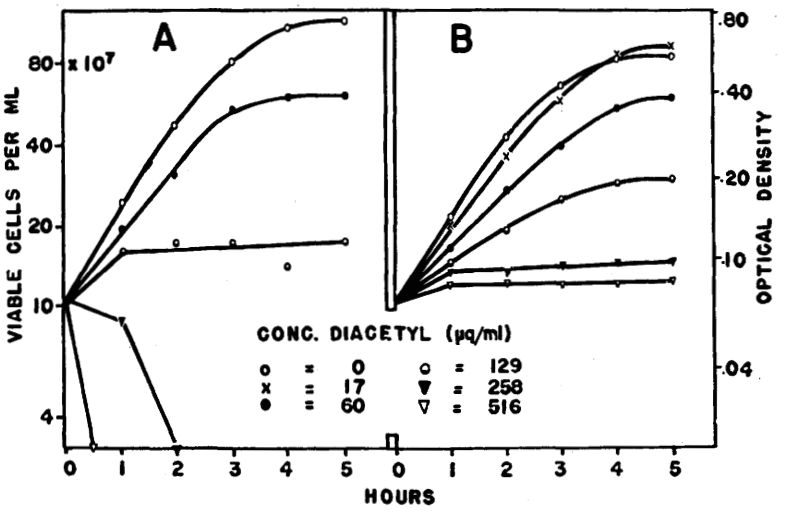

FIG. 1. Effect of diacetyl on microorganisms.

reactions in which diphosphothiamine is involved(2,3). In view of this information it seems surprising that little attention has been directed toward the antimicrobial properties of diacetyl which were first reported by O'Meara(4) and Jolander(5). The present paper describes the effect of diacetyl on the growth of a variety of microorganisms as well as a quantitative evaluation of its antibacterial action.

Experimental. The concentrations of diacetyl necessary to prevent growth of various microorganisms in Trypticase Soy broth for a 24-hour period are listed in Table I. The growth of all organisms tested was inhibited by diacetyl. Relatively small differences existed between the susceptibility of the fungi and many of the bacteria. However, the streptococci and lactobacilli, certain of which are known to produce substantial amounts of diacetyl, were the most resistant to its action. The values are maximal since an indefinite amount of diacetyl escaped from the unsealed tubes through volatilization and small amounts may have been inactivated by components of the medium. To evaluate the antibacterial action of diacetyl, *Escherichia coli* was chosen as the test organism because of its relatively simple growth requirements, its intermediate location in the diacetyl-sensitivity spectrum, and the availability of information concerning its metabolism. The medium used in this study was composed of the following ingredients per liter of solution: sodium phosphate, dibasic, 3 g; potassium phosphate, monobasic, 2 g; ammonium sulfate, 1 g; potassium nitrate, 1 g; dextrose,† 0.25 g; salts B†(6), 2.5 ml. An inoculum consisting of 6 x 10^6 organisms in the logarithmic growth phase was transferred to screw cap test tubes containing 6 ml of sterile media. Growth was followed turbidimetrically, and aliquots were plated for determination of viable cells.

Results. The rate of growth and viability of *E. coli* in the presence of varying amounts of diacetyl are shown in Fig. 1A and B. In the *logarithmic phase* the rate of growth of *E. coli* decreased progressively with increasing amounts of diacetyl. There was close agreement between viable cell counts and turbidity measurements when the concentration of diacetyl was 60 μg/ml or less. With an increase in the amount of diacetyl to 129 μg/ml a majority of the cells reproduced during the first hour only; however, a gradual increase in turbidity occurred throughout the 5-hour experiment. This increase may have been due to either slow reproduction of some cells balanced by death of others or may be explained by changes in the size of inhibited cells. A pronounced lethal action of diacetyl was effected in concentrations exceeding 129

† Solutions sterilized by use of sintered glass filter.

TABLE II. Effect of Concentration of Medium on Action of Diacetyl.

Conc. of medium	Optical density ($\times 10^{-3}$) Log phase (2 hr) Diacetyl (μg/ml) 0	17	60	Stationary phase (5 hr) 0	17	60	Final growth (22 hr) 0	17	60
4X	243	205	163	675	550	290	820	770	460
2X	266	238	195	685	660	420	770	770	630
1X	237	231	191	490	450	415	530	695	500
½X	215	209	179	345	395	345	390	510	425

μg/ml, although the increase in turbidity during the first hour indicated that initial reproduction had occurred. Thus, diacetyl appeared to exert an inhibitory action at lower and a lethal action at higher concentrations. To test the possibility that these effects might be influenced by the initial number of cells, the amount of inoculum was varied in 5 steps from 6 x 10^5 to 12 x 10^6 cells. The rate of cellular division was constant regardless of the size of inoculum. Restoration of viability of cells(7) which had been exposed to lethal concentrations of diacetyl for 22 hours could not be demonstrated following incubation of the cells for 24 hours in buffered solutions of a variety of metabolites.

As the *stationary phase* of growth was approached the effect of diacetyl at a concentration of 17 μg/ml differed from that of higher concentrations in that turbidity finally exceeded that of the controls. This observation suggested that the normal medium was deficient in amounts of components necessary for maximal growth, and that diacetyl was serving as a substrate for growth. To test this possibility the action of diacetyl was determined in ½, 1, 2, and 4 fold (X) concentrations of the medium used previously. The results of this experiment are summarized in Table II. At concentrations of both 17 and 60 μg/ml diacetyl enhanced final growth in the most dilute medium although this effect was not observed in the more concentrated media. That a deficiency in buffering capacity and dextrose content actually existed in the 1X medium was demonstrated in experiments in which the amounts of individual components of the normal medium were increased.

The postulate that diacetyl served as a substrate for growth was confirmed by a demonstration of its disappearance from the medium during growth (Table III). Furthermore, when the *utilization per unit turbidity* (K) was calculated this was found to be greatest in deficient media. For example, in dilute medium (½X) K equaled 52 after 24 hours growth, whereas, in a more concentrated medium (2X), K equaled only 38.

Discussion. The manner in which diacetyl influences growth of *E. coli* is not understood. It has been established that the inhibitory effects of this compound remained unaltered throughout an 8-fold range of medium-concentration. Hence, inhibition may not be explained by a non-reversible combination of diacetyl with an essential substance in the growth medium. Although the possibility exists that diacetyl reacts irreversibly to inactivate essential components of the cell, failure to observe any correlation between inhibition and size of inoculum does not support this concept. The initial reproduction of cells

TABLE III. Utilization of Diacetyl by *E. coli* during Growth in ½X Medium.

Incubation, hr	Exp. conditions	Diacetyl recovered* (μg/ml) 0°C	37°C	Δ (0°-37°)	Utilization over control (Δ_B-Δ_C)
5	A. Inoc. M†	0	0	—	
	B. Inoc. M + 60 μg/ml D	57	41	16	14
	C. M + 60 μg/ml D. No inoc.	57	55	2	
21	A. Inoc. M	0	0	—	
	B. Inoc. M + 60 μg/ml D	57	24	33	22
	C. M + 60 μg/ml D. No inoc.	61	50	11	

* Diacetyl determined by method of White, *et al.*(8). Individual tubes of inoculated and uninoculated ingredients were maintained at 0°C and 37°C to control for loss of diacetyl through volatilization and possible chemical reactions.

† Inoc. M = Inoculated medium; M = Medium; D = Diacetyl.

which occurred during the first hour in the presence of inhibitory amounts of diacetyl may indicate that in addition to its utilization in a normal metabolic pathway the compound may also interfere with the metabolism of an essential substance. Studies concerning this phase of the problem are now in progress.

Summary. Diacetyl prevented the growth of all of a variety of microorganisms in a range of 200-1600 μg/ml. In quantitative studies with a single strain of *E. coli,* diacetyl exerted an inhibitory action at low concentrations (< 129 μg/ml) and lethal action at higher concentrations (> 129 μg/ml). It was demonstrated that diacetyl was utilized during growth of *E. coli* and enhanced final growth when the medium was deficient in glucose.

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Inhibitory Effect of Cortisone Hyperlipemia on Arterial Lipid Deposition in Cholesterol-Fed Rabbits.* (21055)

HARRY H. STUMPF AND SIGMUND L. WILENS.

From the Departments of Pathology, Bellevue Hospital, and New York University College of Medicine, New York City.

Recent experiments on rabbits fed cholesterol and subjected to alloxan(1,2), detergents(3), malnutrition(4,5), and hyaluronidase(6) indicate that the amount of lipid deposited in arterial walls may be markedly at variance with the degree of hypercholesterolemia attained. In some of these experiments hypercholesterolemia was associated with increases of other serum lipid components. It has been suggested that arterial lipid deposition in cholesterol-fed rabbits is reduced if the ratio of these other lipid components, notably phospholipid, to cholesterol is increased. Administration of cortisone to cholesterol-fed rabbits, according to Oppenheim and Bruger(7), and Gordon, Kobernick, McMillan, and Duff(8), retards arterial lipid deposition. Cook *et al.*(9), however, did not find conclusive evidence of retardation of atherosclerosis in 3 cholesterol-fed rabbits that were given relatively small doses of cortisone. Kobernick and More(10) and others have observed rapid development of hyperlipemia in rabbits receiving cortisone. Rich(11) found that in cortisone hyperlipemia the increase was chiefly of fatty acids. The present experiment was designed to determine what role cortisone hyperlipemia plays in retarding arterial lipid deposition in cholesterol-fed rabbits.

Methods. Three groups, each of 6 young adult rabbits, were given daily intramuscular injections of cortisone (Cortone Acetate, Merck) and food supplements of 1% cholesterol in 3 courses according to the following scheme:

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