multiplied. The inoculum for this tissue has always been undiluted infected fluid from cultures of virus adapted to bovine kidney cells. The type of cytopathogenic damage in lung and heart tissue was different and apparently less severe than in kidney cells, and in no case a total destruction of the cell outgrowth was detected during 48 hours of observation. The great number of cells that remained unaltered at 12 hours, when a relatively high virus activity had been shown, led us to make several whole harvests of fluid at 6 hour intervals until 72 hours.

Each of these harvests was titrated in kidney cells, and their titers fluctuated around $10^{-6.5}$ and $10^{-4.5}$ ID/ml until 66 hours. Beyond this time the activity detected fell to 10^{-2}.

Virus of the C type from kidney cells, and heart and lung cultures has been titrated simultaneously in slant tubes, and in cattle by intralingual inoculation. No difference in titers was apparent in the 10-fold dilution method used in both titrations. The usefulness of the heart and lung tissue culture, as a source of f. and m. virus for vaccination purposes is now being tested. A detailed investigation of the development of cytopathogenic activity of the f. and m. virus in bovine kidney cell cultures is now also under way.

Summary. Foot and mouth disease virus has been multiplied in bovine tissue cultures. When embryonic heart and lung bovine tissue cultures were inoculated with foot and mouth disease virus, it was possible to collect virus at 6 hour intervals until 66 hours without great decrease of virus activity.

1. Sellers, R. F., *Nature,* 1955, v176, 547.
2. Bachrach, H. L., *et al., Science,* 1955, v122, 1269.
3. Mazzaracchio, *et al., Zooprofilassi,* 1955, v11, 277.
4. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
5. Bodian, D., *Virology,* 1956, v2, 575.
6. Ramos Alvarez M., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 655.
7. Freund, J., and Thompson, K. J., *Science,* 1945, v109, 468.

Received June 3, 1957. P.S.E.B.M., 1957, v95.

Potassium Acetate Inhibition of *Lactobacillus casei* and Its Reversal by Lithium, Sodium and Fatty Acids.* (23334)

M. N. CAMIEN AND M. S. DUNN
Chemical Laboratory, University of California, Los Angeles

In a previous communication from this laboratory it was reported that although *Lactobacillus casei* 280-16A fails to grow in conventional D-α-hydroxy acid-free media(1,2), its growth in the absence of a D-α-hydroxy fatty acid supplement may be promoted either by reducing the potassium content of the medium or by supplementing it with either lithium or (at considerably higher concentrations) sodium ion(3). Similarly, it was reported(3) that growth of *L. casei* 280-16B, which normally does not require a D-α-hydroxy fatty acid supplement(4), is inhibited by potassium in D-α-hydroxy acid-free media, but not in D-α-hydroxy fatty acid-supplemented media, and that the inhibition may be reversed by either lithium or sodium. The parent strain of *L. casei* (strains 280-16A and 280-16B are mutants) was not inhibited by potassium under these conditions(3), presumably because it (but not the mutants) is able to elaborate D-lactic acid, which serves as an adequate D-α-hydroxy fatty acid source, but it has now been found that this organism is markedly inhibited by potassium when acetate is simultaneously supplied at elevated concentrations. This synergistic inhibition of *L.*

* Paper 119. This work was aided by grants from Natl. Multiple Sclerosis Soc., the U.S.P.H.S., and University of California. The authors are indebted to Evelyn Brown and Tibor Batonay for technical assistance.

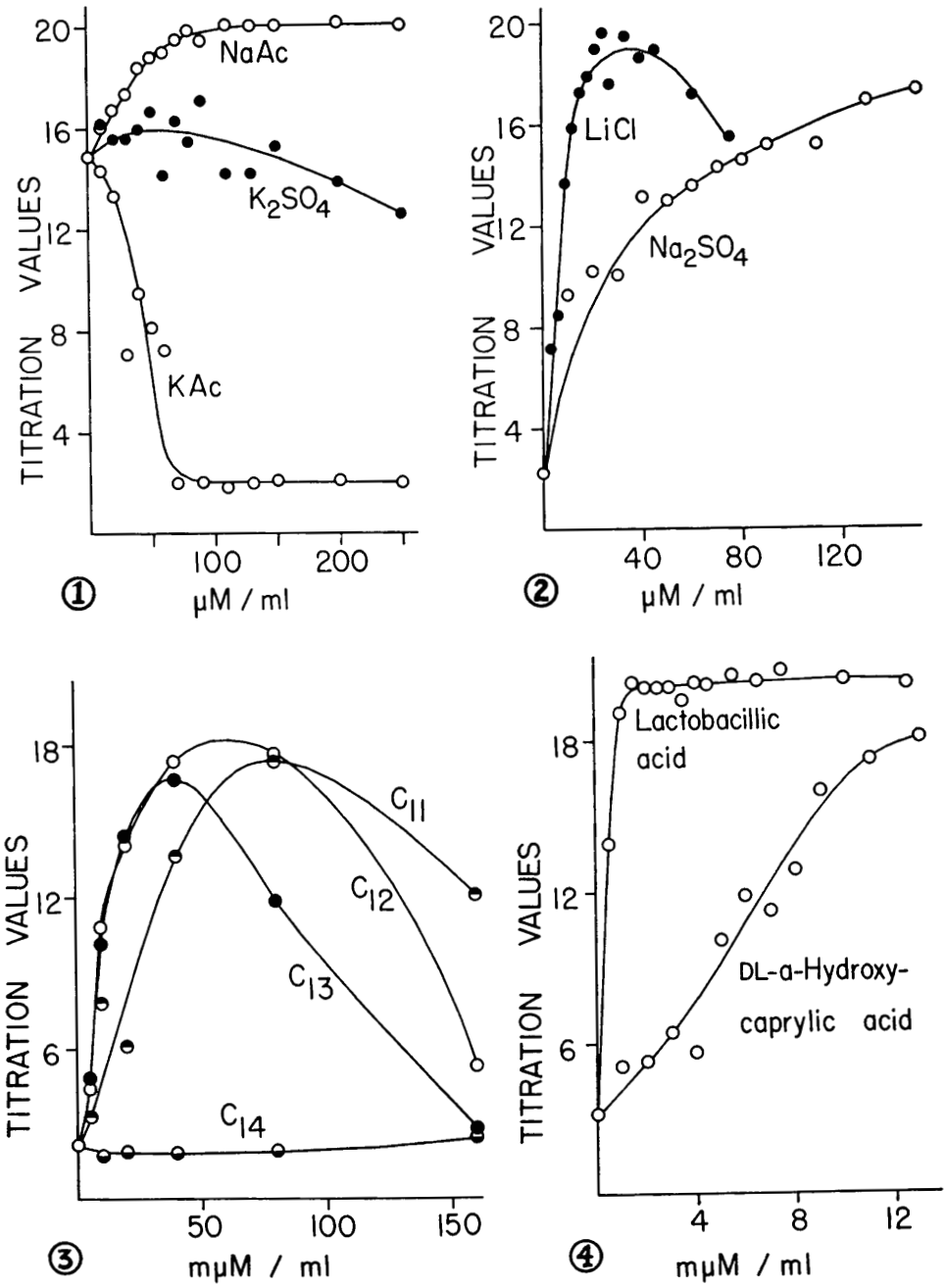

FIG. 1. Responses of *L. casei* to sodium acetate, potassium sulfate, and potassium acetate, respectively. Values on horizontal scale represent test concentrations of acetate and potassium ion (not potassium sulfate) in μmols/ml. These concentrations are in addition to those initially present in the test medium (see *Methods*). Titration values (vertical scale) are calculated as ml of 0.01 *N* sodium hydroxide required to titrate 1 ml of test culture.

FIG. 2. Responses of *L. casei* to lithium chloride and sodium sulfate, respectively, in the test medium supplemented with 150 μmoles of potassium acetate/ml. Values on horizontal scale represent test concentrations of lithium and sodium ion (not sodium sulfate) in μmols/ml. These concentrations are in addition to those initially present in test medium (see *Methods*). Titration values (vertical scale) are calculated as in Fig. 1.

FIG. 3. Responses of *L. casei* to hendecanoic (C_{11}), lauric (C_{12}), tridecanoic (C_{13}), and myristic (C_{14}) acids, respectively, under test conditions stipulated in Fig. 2. Test concentrations (horizontal scale) are given in mμmols/ml.

FIG. 4. Responses of *L. casei* to lactobacillic and DL-α-hydroxycaprylic acids, respectively, under test conditions stipulated in Fig. 2. Test concentrations (horizontal scale) are given in mμmols/ml.

casei by potassium and acetate is the subject of the present report.

Methods. *Lactobacillus casei* 7469 (American Type Culture Collection number) was employed, following essentially the experimental procedures described previously(1-4). The test medium contained casein digest† 0.6%, glucose 2%, vitamin-mineral mixture (6) 20.6 mg %, buffer solution‡ 5 ml %, and Tween 40§ 4 mg %. Its pH, before sterilization, was 6.5. This medium, when not supplemented additionally, provided potassium at 80 μmols/ml, acetate at 146 μmols/ml, and sodium at 9.5 μmols/ml. An additional 4.5 μmols of sodium/ml (final concentration) was included with the inoculum suspension, which was made up in sterile 0.8% sodium chloride solution. Lithium was not deliberately provided in the basal medium, but traces of this element were probably present as an impurity. The results to be discussed were obtained with an incubation period of 40 to 42 hours at 35°.

Results. Typical responses of *L. casei* to potassium and acetate are shown in Fig. 1. It may be seen (Fig. 1) that these agents together (as potassium acetate), but not separately, were markedly inhibitory, effecting 100% inhibition at 80 μmols/ml. Inhibition resulting with 150 μmols of potassium acetate/ml was completely reversed by lithium at 10 μmols/ml (Fig. 2), by sodium at 85 μmols/ml (Fig. 2), by hendecanoic, lauric and tridecanoic acids at 48, 25 and 23 mμmols/ml, respectively (Fig. 3), by DL-α-hydroxycaprylic acid at 8.8 mμmols/ml (Fig. 4) and by lactobacillic acid‖ at 0.6 mμmols/ml (Fig. 4). The 18-carbon unsaturated acids, oleic, linoleic and linolenic acids, were very effective in reversing potassium acetate inhibition, their activities in this respect being approximately the same as that of lactobacillic acid (Fig. 4). Erucic, licanic and *trans*-vaccenic acids had activities of a lower order of magnitude approximating that of DL-α-hydroxycaprylic acid (Fig. 4). The percentage activities¶ of the straight chain DL-α-hydroxy fatty acids according to chain length and with the value of 100 arbitrarily assigned to DL-α-hydroxycaprylic acid (Fig. 4) were as follows: C_3 7, C_6 73, C_7 89, C_8 100, C_9 89, C_{10} 67, C_{11} 57, C_{12} 95, C_{13} 130, C_{14} 220, C_{15} 220, and C_{16} 130. The percentage activities of the straight chain non-hydroxylated saturated acids, on the same basis, were: C_{10} 5, C_{11} 20, C_{12} 50, C_{13} 50, C_{14} 0, C_{15} 0.

Discussion. These results strongly suggest that potassium acetate inhibits growth of *L. casei* 7469 by interfering with fatty acid biosynthesis, and it is curious in this regard that acetate, which is normally a fatty acid precursor, should under the present conditions assume the apparent role of an antimetabolite. The function of potassium as a co-inhibitor in these experiments is also apparently incongruous since this element is generally thought to be relatively non-toxic(8) to lactobacilli as compared to sodium and lithium, which promote growth under the present conditions. These considerations suggest that perhaps potassium and acetate do not inhibit directly, but rather effect the over-production of some normal metabolite, which at excessive

† Prepared from vitamin-free casein essentially as described by Roberts and Snell(5) except that hydrochloric acid was employed in place of acetic acid to neutralize the alkaline mixture after digestion and the charcoal treatments were omitted.

‡ Same as that described previously(6) except that sodium acetate was replaced by equivalent amount of potassium acetate.

§ Atlas Powder Co. product employed as a 10% stock solution in 50% ethanol.

‖ A derivative of *L. casei* lipid(7). This material was kindly supplied by Professor Klaus Hofmann, Univ. of Pittsburgh School of Medicine.

¶ Calculated as 100 times the quotient of molar concentration of test acid effecting 50% reversal of inhibition divided by molar concentration of DL-α-hydroxycaprylic acid effecting 50% reversal of inhibition in a simultaneous test.

concentrations is able to inhibit growth. Myristic acid, for example, could be derived from acetate and is known to effect a marked inhibition of *L. casei* reversible by either lactobacillic acid or unsaturated fatty acids at relatively low concentrations(9). The effects of sodium and lithium according to this hypothesis would be to relieve the inhibition by impairing synthesis of the toxic product. It is entirely possible, on the other hand, that either sodium or preferably lithium activates an enzyme system concerned with essential fatty acid biosynthesis and that potassium inhibits the system by competition with sodium and lithium. The inhibitory effects of acetate in such a system might be due to competition of acetate with fatty acid intermediates.

Superficially the synergistic inhibition of *L. casei* 7469 by potassium and acetate appears to be closely related to the genetic block in *L. casei* strains 280-16A and 280-16B, since growth of the latter strains is promoted by either lithium or sodium(3) and by a variety of DL-α-hydroxy fatty acids(2). Further investigations are being undertaken to define this possible relationship more exactly.

Summary. Growth of *L. casei* 7469 in a low-sodium, fatty acid-free medium is inhibited synergistically by potassium and acetate. Neither potassium nor acetate is significantly inhibitory when tested separately. The inhibition is readily reversed by either lithium or sodium and by a wide variety of fatty acids, the most effective of which are lactobacillic, oleic, linoleic, and linolenic acids.

1. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1953, v201, 621.
2. ———, *ibid.*, 1954, v211, 593.
3. ———, *Science*, 1957, v125, 1149.
4. ———, *Arch. Biochem. and Biophys.*, 1956, v60, 452.
5. Roberts, E. C., and Snell, E. E., *J. Biol. Chem.*, 1946, v163, 499.
6. Camien, M. N., and Dunn, M. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 177.
7. Hofmann, K., and Sax, S. M., *J. Biol. Chem.*, 1953, v205, 55.
8. Sirny, R. J., Braekkan, O. R., Klungsøyr, M., and Elvehjem, C. A., *J. Bact.*, 1954, v68, 103.
9. Camien, M. N., and Dunn, M. S., *Arch. Biochem. and Biophys.*, 1957 (Paper 112, in press).

Received June 4, 1957. P.S.E.B.M., 1957, v95.

Action of Diphtheria Toxin on Cells Cultivated *in vitro*. (23335)

E. S. LENNOX* AND ALBERT S. KAPLAN (Introduced by L. M. Henderson)
Departments of Bacteriology and Chemistry, University of Illinois, Urbana

Tissue culture technics that have recently proved useful in quantitative virology can also be profitably applied to the study of some toxins. This note is a preliminary report of experiments on the action of diphtheria toxin on mammalian cells cultivated *in vitro*(1).†

Methods and materials. Most of the experiments were done with monolayer cultures of cells in glass tubes. Cultures of rabbit kidney cells in tubes were prepared by the technic of Rappaport(2) and cultivated in Earle's saline containing 0.5% lactalbumen hydrolysate (Nutritional Biochemical) and 3% bovine serum. HeLa cells (strain obtained from Tuskegee Institute); 2 kinds of monkey kidney cells, a normal strain and several altered derivatives, from Dr. Raymond C. Parker (3); human epithelial carcinoma cells, HEP #2 from Dr. Alice Moore were grown in Earle's saline containing 0.5% lactalbumen hydrolysate, 0.1% yeast extract, and 20% horse serum. Additions of diphtheria toxin‡

* Fellow of the National Foundation for Infantile Paralysis until June 1956.

† The authors gratefully acknowledge support from American Cancer Soc. (grant to Dr. S. E. Luria recommended by Committee on Growth), USPH Grant, and the the Univ. of Illinois Research Board.

‡ We are indebted to Eli Lilly and Co. for generous supply of diphtheria toxin and antitoxin.