TABLE 2

OBSERVATIONS OF TUMOR DEVELOPMENT, ADRENAL AND MAMMARY HYPERPLASIA IN CASTRATED FEMALES

| Stock | Number sacrificed | Number of tumors | Adrenal hyperplasia | Mammary nodules |
|---|---|---|---|---|
| A .... | 13 | 0 | slight | absent |
| Z .... | 4 | 4 | present | present |
| High tumor hybrids . | 27 | 15 | present | present |
| Low tumor hybrids . | 11 | 0 | present | absent |

The animals of the A strain showed great individual variation in the extent of growth of their mammary glands. No precancerous lesions have been detected.

It is apparent that there is a relationship between the incidence of breast tumors in mice and the degree of hormonal stimulation. The development of mammary cancer in virgin females of cancerous strains has been demonstrated to be partially dependent upon an inherited character. This inherited hormonal influence may also be responsible for the adrenal cortical hyperplasia in castrated mice. The adrenal changes are apparently followed by hormonal stimulation of the uterus resulting in estrus, mammary gland development with precancerous nodules, and eventually the appearance of mammary tumors in those animals with the active milk agent. In the castrated animals without the active milk agent there were adrenal changes but precancerous lesions and mammary tumors have not appeared. Castrated animals having the milk agent but not the inherited hormonal influence may show modified adrenal changes but no other evidence of hormonal stimulation.

FERN W. SMITH

DEPARTMENT OF PHYSIOLOGY,
UNIVERSITY OF MINNESOTA MEDICAL SCHOOL

## QUININE ACTION IN BACTERIAL GROWTH AND DISINFECTION[1]

RECENT studies of the concentration, temperature and pressure relationships of quinine action on bacterial luminescence have indicated that the drug promotes a reversible denaturation of the protein catalyst concerned in light emission.[2] The effects of quinine on dehydrogenase activity in washed cells of *E. coli*, in relation to drug concentration, temperature and presence of bacterial extracts and coenzyme, have given no clear evidence of a protein-denaturing action of quinine on these systems except at relatively high temperatures.[3] Slight inhibitions of dehydrogenase activity, observed throughout the low temperature range, appeared to result from the formation of a loose complex between the drug and a coenzyme. Both types of effects, *i.e.*, in relation to protein and to coenzyme, are evidently involved in the action of quinine on growth rates and viability of bacteria, as indicated herein.

With *E. coli* as a test organism, growing in a glucose-asparagine "synthetic" medium,[4] the addition of quinine causes an immediate slowing of growth or a disinfection, depending on a narrow range of concentration added (Fig. 1). The bacteriostatic or disinfecting action ceases at once on dilution of the medium, and growth is resumed at approximately the rate of the control (Table 1).

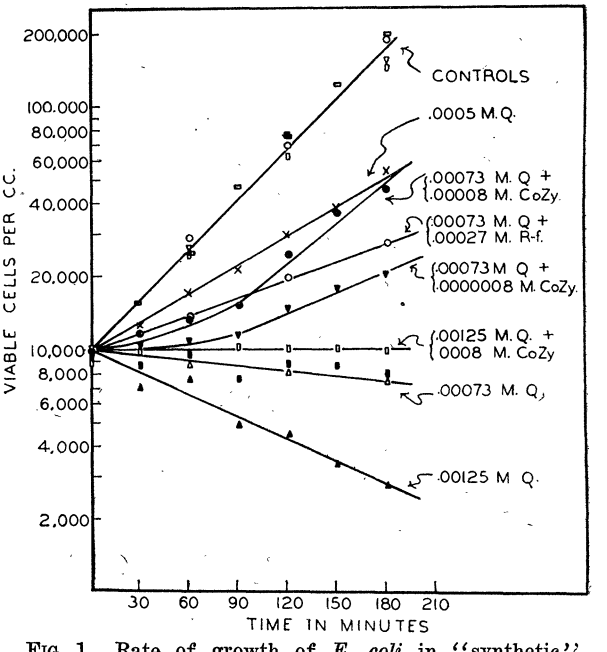


FIG. 1. Rate of growth of *E. coli* in "synthetic" medium at 37° C in relation to quinine (Q), cozymase (CoZy) and riboflavine (R.f.). Results of four experiments. Some of the curves have been extrapolated a short distance on the ordinate to coincide with 10,000 viable cells per cc at zero time in each case. No increase in rate of growth of controls without quinine was caused by these concentrations of cozymase and riboflavine, respectively.

Antagonism of the growth-inhibiting and the disinfecting effects may be brought about either by the addition of pure riboflavine or of partially pure cozymase[5] preparations. The latter are more potent and the amount needed depends upon the amount of quinine present (Table 2). To antagonize the disinfection, a ratio of roughly one molecule of coenzyme to between one and four molecules of quinine is re-

[1] This study is carried on with the aid of a grant from the Cinchona Products Institute, Inc.

[2] F. H. Johnson and L. Schneyer, *Amer. Jour. Trop. Med.*, 24: 163, 1944.

[3] The results of this investigation will be presented in a subsequent publication.

[4] The medium consisted of: 0.2 per cent. glucose, 0.2 per cent. asparagine, 0.47 per cent. $(NH_4)_2SO_4$, 0.5 per cent. NaCl, 0.27 per cent. $KH_2PO_4$, trace of $FeCl_2$, $CaCl_2$, and $MgCl_2$, plus NaOH to make pH equal to 7.15.

[5] Preparations of cozymase, about 50 per cent. pure, were kindly supplied by Dr. F. Schlenk. Although impurities may be concerned, the results shown in Fig. 1 and Table 2 are given with respect to the amount of cozymase added.

TABLE 1

REVERSIBILITY OF BACTERIOSTATIC AND DISINFECTING ACTION OF QUININE ON *E. coli*, BY DILUTION OF THE MEDIUM. TEMPERATURE 37° C.

| Growth period: | | | 0′–120′* | | | | 120′–240′* |
|---|---|---|---|---|---|---|---|
| Molar conc. of quinine | Viable cells per cc at 0′ (B) | Viable cells per cc at 120′ (b) | Rate of growth (+) or disinfection (−) $k=\frac{1n\ b-1n\ B}{t}$ k | Molar conc. of quinine after dilution with quinine-free or quinine-containing medium | Viable cells per cc at 120′ (B) | Viable cells per cc at 240′ (b) | $k=\frac{1n\ b-1n\ B}{t}$ k |
| 0 | 6,450 | 42,000 | +.0156 | 0 | 5,800 | 47,000 | +.0174 |
| .00013 | 7,550 | 54,500 | +.0164 | .00013 | 6,900 | 56,000 | +.0174 |
| .00073 | 8,400 | 8,400 | 0 | .00012 | 1,025 | 8,650 | +.0178 |
| .00081 | 7,800 | 3,700 | −.0062 | .00013 | 775 | 5,400 | +.0157 |

* Plate counts made every 30′ indicated a straight line relation between log of cell-numbers and time. In similar experiments, but with larger cell populations, direct microscopic counts indicated no change of total cell numbers in fully bacteriostatic or in disinfecting concentrations of quinine.

quired under the conditions employed. Much smaller ratios, *viz.*, one cozymase to nearly 1,000 quinine molecules, are detectable in growth rates, as shown in Fig. 1. In the concentrations used, cozymase has no effect on the growth rate of the control. Peptone or blood serum, on the other hand, accelerate the growth rate of both control and quinine-containing cultures, and in this sense may be considered as antagonists of the drug's action. Unlike cozymase, however, peptone in concentrations as much as 1 mg per cc does not counteract the disinfecting action of high concentrations (0.00125 M.) of quinine. Although a precise interpretation requires extensive data, these results are consistent with the view that the effects of quinine on both growth and viability result in part from either a competition or a loose combination with cozymase. Loose combinations between quinine and substances not metabolized by the organism, including inhibitory substances, may also give rise to phenomena of antagonism.[2] A similar mechanism possibly accounts for antagonism of atabrine in bacterial growth by diverse agents.[6]

TABLE 2

ANTAGONISM OF THE QUININE DISINFECTION BY COZYMASE

| Molar concentration of quinine | Molar concentration of cozymase | Rate of growth (+) or disinfection (−) $k=\frac{1n\ b-1n\ B}{t}$ k | Ratio of cozymase to quinine |
|---|---|---|---|
| .000875 | ..... | −.0055 | |
| .000875 | .00024 | +.0048 | 1 : 3.6 |
| .001 | ..... | −.0074 | |
| .001 | .0004 | +.0015 | 1 : 2.5 |
| .00125 | ..... | −.0071 | |
| .00125 | .0008 | 0 | 1 : 1.5 |

The effect of a given concentration of quinine on the rate of growth and on viability increases with rise in temperature (Table 3) in a manner resembling the evidently protein-denaturing action of this drug in bacterial luminescence. An apparently similar action is exerted by atabrine on the flavoprotein component of certain extracted and purified systems.[7] In the present instance, the rate of growth in controls rapidly decreases with rise of temperature above 39° C., and growth ceases at 45.2° C. During short exposures to 45.2° C, the number of viable cells remains constant, and growth is immediately resumed, at very close to the control rate, on cooling to 37° C. In the presence of quinine, similar reversible effects take place, but they occur at lower temperatures. These phenomena again resemble those observed with luminescence in relation to both temperature and quinine, as well as certain other drugs. Thus, the fundamental theoretical chemistry that has been extensively discussed in connection with the influence of temperature, pressure and drugs on luminescence[8] should be useful in furthering an understanding of the action of such factors on the systems controlling microbial growth rates and viability.

TABLE 3

RELATION OF THE GROWTH-INHIBITING AND DISINFECTING ACTION OF QUININE TO TEMPERATURE

| Temperature °C. | Growth rate, k | | Per cent. inhibition |
|---|---|---|---|
| | Control | .00063 M.Q. | |
| 18.0 | .0021 | .0016 | 23.8 |
| 27.1 | .0068 | .0045 | 33.8 |
| 37.0 | .0169 | .0071 | 58.0 |
| 39.4 | .0172 | .0051 | 70.4 |
| 41.4 | .0132 | 0 | 100 |
| 45.2 | −.0023 | −.0115 | Disinfecting |

FRANK H. JOHNSON
ISAAC LEWIN

DEPARTMENT OF BIOLOGY,
PRINCETON UNIVERSITY

[6] M. Silverman and E. A. Evans, Jr., *Jour. Biol. Chem.*, 154: 521, 1944.

[7] E. Haas, *Jour. Biol. Chem.*, 155: 321, 1944.

[8] F. H. Johnson, D. E. Brown and D. A. Marsland, SCIENCE, 95: 200, 1942; *Jour. Cell. Comp. Physiol.*, 20: 269, 1942; D. E. Brown, F. H. Johnson and D. A. Marsland, *ibid.*, 20: 151, 1942; H. Eyring and J. L. MaGee, *ibid.*, 20: 169, 1942; F. H. Johnson, H. Eyring and R. W. Williams, *ibid.*, 20: 247, 1942; F. H. Johnson, H. Eyring and W. Kearns, *Arch. Biochem.*, 3: 1, 1943; F. H. Johnson, H. Eyring, R. Steblay, H. Chaplin, C. Huber and G. J. Gherardi, *Jour. Gen. Physiol.*, in press.