work of Tedeschi and Harris(2) and others, cited above.

Summary. The opacimetric and hematocrit methods for measuring relative mitochondrial volume are compared. Data from mitochondria swollen by exposure to hypotonic sucrose solutions demonstrate the validity of the simple opacimetric method, since, as osmolarity decreases, rate of increase in mitochondrial pellet volume approximates rate of decrease in optical density of the mitochondrial suspension. The results are discussed in terms of a current dispute regarding permeability of the mitochondrial membrane to sucrose.

1. Ponder, E., *Hemolysis and Related Phenomena,* Grune and Stratton, New York, N. Y., 1948, pp51-79.

2. Tedeschi, H., Harris, D. L., *Arch. Biochem. and Biophys.,* 1955, v58, 52.

3. Price, C. A., Fonnesu, A., Davies, R. E., *Biochem. J.,* 1956, v64, 754.

4. Jackson, K. L., Pace, N., *J. Gen. Physiol.,* 1956, v40, 47.

5. Recknagel, R. O., Malamed, S., *J. Biol. Chem.,* in press.

6. Hunter, F. E., Jr., Ford, L., *ibid.,* 1955, v216, 357.

7. Schneider, W. C., Striebich, M. J., Hogeboom, G. H., *ibid.,* 1956, v222, 969.

8. Werkheiser, W. C., Bartley, W., *Biochem. J.,* 1957, v66, 79.

9. Fonnesu, A., Davies, R. E., *ibid.,* 1956, v64, 769.

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Action of Colicines on *Shigella boydii* and on their Rough and Mucoid Variants. (23967)

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Certain antibiotic substances produced by strains of *Escherichia* and *Shigella* have been defined as colicines. A recent review of colicines(1) has been published by Fredericq, a pioneer in this particular field. Halbert and Gravatt(2) have reported on the relation of *Shigella* type specificity and susceptibility to antibiotic producing strains of *Escherichia coli.* As extension and partial confirmation of their report, 170 *Shigella boydii* cultures† consisting of serotypes 1 through 11 and the 5 provisional serotypes were tested for possible characteristic patterns of sensitivity to 28 colicines. Some correlation between serological type and sensitivity to colicines has been revealed by this study.

The present report deals with an observation made during the testing of the aforementioned *Shigella* cultures. It was noted that R (rough) and M (mucoid) colony variants seemed somewhat more susceptible to colicines than S (smooth) colonies of the same original culture.

Procedures. Further experiments were performed to establish the validity of these sensitivity differences. A group of colicine producing strains of *Escherichia* and *Shigella*‡ were tested against randomly selected *Sh. boydii* strains utilizing the chloroform-killed plate assay technic of Gratia and Fredericq(3). R variants were obtained from an S colony of *Sh. boydii* 1, Strain No. 1701, by the methods indicated in Table I, and simultaneously tested with the S parent organism against colicines. The results shown in Table I and Fig. 1 indicate the marked sensitivity of R variants to some colicines employed in contrast to the relative resistance of the S parent culture. Sensitivity differences between R or M variants and their corresponding S parent cultures were also demonstrated in 2 other

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‡ We wish to thank Dr. Pierre Fredericq, of University of Liege, for the majority of these strains.

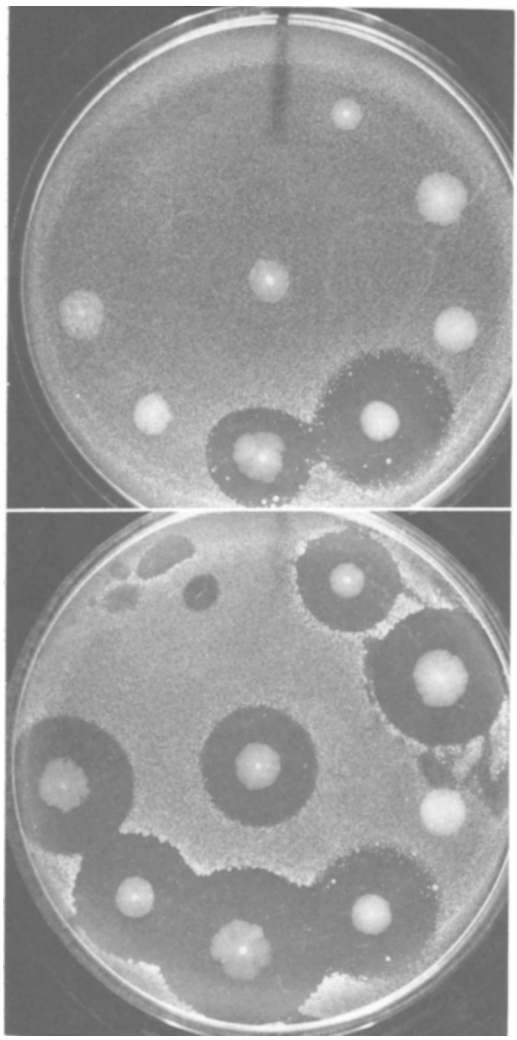

FIG. 1 (top). Colicine sensitivity of *Sh. boydii* 1, S parent culture.

FIG. 2 (bottom). Colicine sensitivity of *Sh. boydii* 1, R variant of culture in Fig. 1. Clockwise from top of plates, colicine producers are shown in the same order as listed in Table I.

strains of *Sh. boydii* 1 and in cultures of *Sh. boydii* types 2, 4, 5, 7 and provisional serotype 703. Some colicines were inactive against both S parent and their non-S variants, other colicines exhibited similar antagonism against S parent and variants, but in no instance was the S parent culture significantly more sensitive to a colicine than its variant progeny. The generally accepted criteria for R and M organisms(4,5), were satisfied by all variants utilized.

TABLE I. Colicine Sensitivity of S and Non-S Variants of *Shigella boydii* 1, Strain No. 1701.

Colicine	S parent	Rough variants by — Prolonged broth incubation	.2% phenol broth	Homologous antiserum, 1:5 in broth
S2	0*	6	6	7
L	0	7	7	7
H	0	0	0	0
C	6	4	5	8
F	5	7	6	8
E	0	6	1	7
D	0	7	7	8
B	0	5	5	5

* Zones of inhibition measured in mm.

Results. S and R cultures of one strain of *Sh. boydii* 2 (Strain 1810) were tested for susceptibility to carbomycin, chloramphenicol, chlortetracycline, dihydrostreptomycin, erythromycin, furadantin, gantrisin, neomycin, novobiocin, oleandomycin, oxytetracycline, penicillin, polymyxin B, and tetracycline. There were no differences in sensitivity between the S and R cultures. However, differences between S and R variants of other bacterial species in regard to their sensitivity to a variety of antibiotics and bacteriophages have been reported in other studies(5-8).

S (smooth) pathogenic bacteria generally undergo population changes involving establishment of spontaneously arising non-S (avirulent) variants under usual conditions of laboratory cultivation. Braun and his coworkers(9) recently reported *in vitro* non-S to S population changes in *Brucella,* pneumococci and typhoid bacilli, brought about under the influence of bacterial desoxyribonucleic acid (DNA) plus desoxyribonuclease (D-Nase), or 6-furfurylaminopurine (kinetin).§ Some of the M variants of *Sh. boydii* 5 isolated in our study were incubated in broth containing kinetin in concentrations of 10^{-3} and 10^{-4} μg/ml. Within 4 days S organisms were obtained which satisfied all the usual criteria for S cultures, although type specific anti-S serum titers were somewhat lower than those found in the original S parent cultures. Colicines were tested simultaneously against the S parent organism, the M variants and the

§ We are indebted to Dr. A. F. Langlykke and Miss B. Stearns of E. R. Squibb and Sons for a sample of kinetin.

TABLE II. Colicine Sensitivity of S, Induced M, and "Transformed" S Organisms of *Shigella Boydii* Type 5, No. 2132.

	S parent		Induced M variants				Kinetin-induced S variants			
			M1		M2		M1-Tr		M2-Tr	
Colicine	S1	S2	1	2	1	2	1	2	1	2
534	0*	0	9	10	10	6	6	7	5	4
V	0	0	10	10	10	8	3	3	2	1
CF1	0	0	7	0	12	10	5	3	1	5
K	0	0	7	9	9	12	2	2	1	2
67	1	1	11	10	10	10	5	7	5	4

* Zones of inhibition measured in mm.

S organism obtained from the kinetin-passed M variants. The test plates showed a sensitivity gradation in which the S parent organism displayed relative resistance, the kinetin-passed S organisms were distinctly more susceptible and the M variants were markedly antagonized by the same colicines (Table II).

Discussion. The protective function of smooth surface somatic antigen of *Shigella* seems to be the most obvious explanation for the difference in action of colicines against organisms with and without this antigen. Gradual loss of surface somatic antigen by population change, and gradual recovery of this antigen in variant cells whose establishment was promoted by kinetin, results in corresponding colicine sensitivity changes. The validity of any possible characteristic colicine sensitivity spectrum for a certain *Shigella* serotype, therefore, may be regarded as partially dependent upon the protective effect exerted by surface somatic antigen. The amount of this antigen present may account for some of the sensitivity fluctuations observed between cultures of identical serological composition.

Summary. In 58 experiments involving comparisons of 6 smooth parent cultures of various *Sh. boydii* serotypes and their corresponding non-S variants, the variants were significantly more sensitive to colicine action than the smooth parent strains. Reversal of the culture from the non-S to the S form was accomplished by use of kinetin, and the "transformed" cultures reflected, in part, the colicine spectrum of the original S parent culture and in part the spectrum of the non-S variants from which the "transformed" S cultures were immediately derived.

1. Fredericq, P., *Ann. Rev. Microbiol.*, 1957, v11, 7.

2. Halbert, S. P., Gravatt, M., *J. Immunol.*, 1949, v61, 271.

3. Gratia, A., Fredericq, P., 7 ème Congr. Intern. Chem. biol. Liege, 1946.

4. Burrows, W., *Textbook of Microbiology*, Saunders, Philadelphia, 1954, 152.

5. Braun, W. W., *Bacterial genetics, ibid.*, 1953.

6. Burnet, F. M., *J. Path. Bact.*, 1930, v33, 647.

7. Schmidt, W. H., Ward, G. E., Coghill, R. D., *J. Bact.*, 1945, v49, 411.

8. Gunnison, J. B., Shevky, M. C., Zion, V. K., Abbott, M. J., *J. Infect. Dis.*, 1951, v88, 187.

9. Braun, W., Firshein, W., Whallon, J., *Science*, 1957, v125, 445.

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Effects of Folic Acid Antagonist Therapy on Urinary Excretion of Formic Acid by Humans.* (23968)

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Although formic acid has been reported to occur in urine of normal human subjects on a mixed diet, the methods used for its determination have involved either extraction of urine with ether and subsequent distillation of volatile acids from acid solution(1,2), or direct distillation of the urine(3-6). Significance of values obtained using these methods is somewhat doubtful since many naturally-occurring substances are known to yield formic acid upon treatment with acid at elevated

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