phatic or cyclic groups, marked activity was found against all the fungi under test when R_1 = dodecyl, R_2 = methyl, and when R_1 = naphthyl, R_2 = hexyl, heptyl, octyl, nonyl, and decyl (.25-8 mg %). Low or moderate activity was found when R_1 = naphthyl, R_2 = methyl, ethyl, and propyl (4-64 mg %).

No activity was observed among the lowest members of the series of aliphatic dimethylaminoethylamines. Using the compound $\begin{matrix}R_1\\R_2\end{matrix}> N\text{–}CH_2\text{–}CH_2\ N(CH_3)_2$, and replacing R_1 and R_2 in the aliphatic series, no activity appeared until $R_1 = R_2$ = butyl, when *M. tuberculosis* H37RV was inhibited by .5 mg %. The compounds $R_1 = R_2$ = methyl, $R_1 = R_2$ = ethyl, $R_1 = R_2$ = propyl, R_1 = allyl, R_2 = isopropenyl, R_1 = butyl, R_2 = ethyl, and R_1 = amyl, R_2 = methyl were all without effect on all organisms tested. When R_1 = amyl, R_2 = propyl, isopropenyl, or butyl, the compounds showed activity against *K. pneumoniae* and *Sh. sonnei* (.25 mg %) and weaker activity against gram positive organisms. When $R_1 = R_2$ = amyl antibacterial activity was low, as was the case when R_1 was hexyl, R_2 was methyl, ethyl, or propyl. In contrast R_1 hexyl, R_2 isopropyl inhibited *K. pneumoniae* and *Sh. sonnei* at .25 mg %. In ascending this series, when R_1 was amyl and R_2 was propyl, activity against gram negative organisms appeared. Bacteriostatic effects were maintained until R_1 was hexyl and R_2 was allyl. Activity then diminished, to reappear with a broader spectrum when R_1 was octyl and R_2 was hexyl. Maximal potency against both gram negative and gram positive organisms was obtained when R_1 was hendecyl or above. The most active members of this series of compounds were also effective against *Trichophyton mentagraphytes*. Optimal activity appears to be associated with a long chain hydrocarbon radical, but any such generalization would exclude the hexyl allyl derivative and would not account for the effectiveness of the lower members of the series against gram negative organisms.

Discussion. The more active members of the series of substituted dimethylaminoethylamines inhibited the growth of 4 g positive organisms, including aerobic and anaerobic spore formers, 4 g negative bacteria, and 2 varieties of tubercle bacilli. In addition, they were found to be active against a representative spectrum of pathogenic fungi. Although tests for bacteriostatic effects give no indication of germicidal activity, the above data indicate which of the compounds should be studied further. Work designed to evaluate the therapeutic potentialities of these compounds will be reported separately.

Conclusions. (1) No appreciable antimicrobial activity was found in an *in vitro* examination of a series of dimethylaminoethyl ethers. (2) Significant activity was observed in various long-chain di-aliphatic dimethylaminoethylamines. (3) When one of the aliphatic substituents was replaced by a naphthylene group marked antimicrobial activity was found.

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Thermolabile Antigens of *Shigella boydii* 2 Cultures with Special Reference to an Encapsulated Culture. (18988)

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The purpose of this paper is to report the natural occurrence of an encapsulated culture of *Shigella boydii* 2 (P288) and to describe the relationships of the thermolabile substance of the capsule to thermolabile somatic antigen in other *S. boydii* 2 strains and in *Klebsiella* and *Escherichia* cultures.

Fletcher(1) reported the occurrence of

mucoid variants in paratyphoid and dysentery bacteria but did not describe capsules in the dysentery bacteria nor the nature of the mucoid material. Various investigators reported that some strains of *Shigella dysenteriae* 1 (Shiga bacillus) are inagglutinable and Bauch(2) and Schuetze(3) noted that such cultures were made more readily agglutinable after they were heated at 100°C for ½ hour. Archer(4) described a thermolabile factor in Alkalescens-Dispar 01 *(Shigella alkalescens)* cultures which inhibited O agglutination but did not determine the nature of the thermolabile substance. Barnes(5) mentioned that inagglutinable cultures of *Shigella dysenteriae* 2 *(Shigella ambigua)*, *Shigella flexneri* 6, *S. boydii* 2, and *Shigella boydii* 5 (P143) had been encountered. However, a search of the literature failed to reveal any previous description of the occurrence of encapsulated shigellae. Kauffmann(6) described thermolabile envelope antigens in coliform bacteria which later were called L antigens. Envelope antigens of the L variety were encountered in *Escherichia coli* cultures that belonged to various O antigen groups. Kauffmann(7) reported that the thermolabile substance in Alkalescens-Dispar (A-D) cultures that inhibits O agglutination is an L antigen which occurs in certain *E. coli* cultures. Also, the relationship of the L antigen of A-D and *E. coli* cultures was mentioned by Frantzen(8). Kauffmann(9) reported that some *Escherichia* cultures contained an antigen that differed from L antigen in certain characteristics. Knipschildt(10) studied this antigen further, found that it was a relatively thermostable capsular substance, and designated it as A antigen. A third variety of envelope antigen, designated B was described by Knipschildt (11). Subsequently, these three thermolabile antigens were grouped together under the designation K (Kauffmann)(12). All 3 varieties, L, A, and B, are envelope antigens which inhibit O agglutination but the 3 differ in respect to the degree of their thermolability, antibody binding power after heating, and certain other characteristics(12,13). Utilizing the methods for determination of K antigens advised by Kauffmann, Madsen(14) studied *Shigella flexneri* cultures and reported that some *S. flexneri* 6 cultures contain a thermolabile antigen similar in its behavior to that of the B antigen of Knipschildt(11). *S. boydii* 2 is not one of the common *Shigella* types. It was described first from the United States by Morris, *et al.*(15). Twelve cultures of the type were employed in the present investigation. These included a type culture (P288, Poona), received from Brigadier J. S. K. Boyd in 1944; a culture isolated in Italy in 1944; one from Dr. W. W. Ferguson, and 9 cultures that were sent to this laboratory for identification between July, 1948 and May, 1951. These 9 cultures were isolated in Arizona, California, and New Mexico.

The biochemical reactions of the 12 *S. boydii* 2 cultures are as follows: Glucose and mannitol are utilized without gas formation within 24 hours incubation and maltose is fermented after 9 or 10 days incubation; lactose, sucrose, salicin, adonitol, and dulcitol are not utilized within a 30-day period; the bacteria are nonmotile, do not produce acetylmethylcarbinol, do not grow on Simmons' citrate agar nor hydrolyse urea, do not utilize

1. Fletcher, W., *Lancet*, 1918, 102 (July 27).
2. Bauch, R., quoted by Kauffmann, 1951.
3. Schuetze, H., *J. Path. Bact.*, 1944, v56, 250.
4. Archer, G. T. L., *J. Roy. Army Med. Corps*, 1942, v79, 109.
5. Barnes, L. A., *U. S. Naval Med. Bull.*, 1944, v43, 1178.
6. Kauffmann, F., *Acta Path. et Microbiol. Scand.*, 1943, v20, 21.
7. Kauffmann, F., *Acta Path. et Microbiol. Scand.*, 1949, v26, 879.
8. Frantzen, E., *Acta Path. et Microbiol. Scand.*, 1950, v27, 236.
9. Kauffmann, F., *Acta Path. et Microbiol. Scand.*, 1944, v21, 46.
10. Knipschildt, H. E., *Acta Path. et Microbiol. Scand.*, 1945, v22, 44.
11. Knipschildt, H. E., *Acta Path. et Microbiol. Scand.*, 1946, v23, 179.
12. Kauffmann, F., *J. Immunol.*, 1947, v57, 71.
13. Kauffmann, F., Enterobacteriaceae. Ejnar Munksgaard, Copenhagen, 1951.
14. Madsen, S., on the classification of the *Shigella* types. Ejnar Munksgaard, Copenhagen, 1949.
15. Morris, J. F., Brim, A., and Sellers, T. F., *Am. J. Public Health*, 1944, v34, 1277.

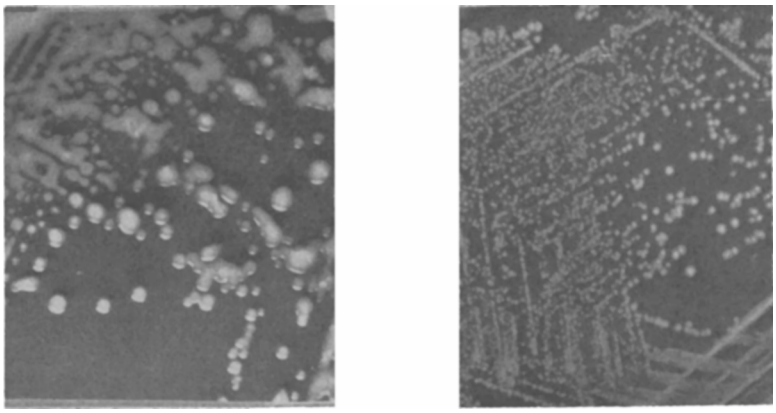

FIG. 1. Colonies of encapsulated and non-encapsulated *S. boydii* 2 cultures. Left, colonies of encapsulated culture. Right, colonies of non-encapsulated culture (reduced 1/10).

citrate in the medium of Christensen(16), and do not produce indol; all cultures are methyl red positive and reduce nitrates to nitrites.

The methods employed for the determination of O and K antigens were those advised by Kauffmann(12,13) for study of these antigens in *Escherichia* and *Klebsiella* cultures. Antiserums that were used for determination of the heat stable somatic antigens of the bacteria were prepared from 18-hour broth cultures which were heated for 2.5 hours at 100°C. Antiserums for the K, or thermolabile somatic, antigens were prepared by injection of unheated formalinized broth cultures that were incubated for 4 hours at 37°C. Agglutination tests for K antigens were made with formalinized (0.5%) 4-hour broth cultures and incubation was at 37°C for 2 hours followed by overnight incubation at 4 to 5°C. Tests for O agglutination were made with 18-hour broth cultures which were heated for 1 hour at 100°C and the tests were read after overnight incubation in a 52°C water bath. Examinations for capsules and for quellung reactions were made with living 4-hour broth cultures. The presence of capsules was determined in moist mounts with Pelican India ink. Living cultures of 5 of the 9 more recently isolated *S. boydii* 2 strains were inagglutinable in O antiserum when received. All cultures were agglutinated to the titer of O antiserum for *S. boydii* 2 when heated suspensions were used as antigens. One of these cultures, 4158/50, was mucoid and upon examination of a young broth culture was found to be encapsulated. The capsules were as large as those usually seen in *Klebsiella* cultures. Plating of this culture on infusion agar resulted in the appearance of 2 colony forms (Fig. 1). The first was an opaque, glistening, mucoid colony, and the second a translucent, smooth, and somewhat less raised colony form. Living cultures, made from the translucent O colony forms, were readily agglutinable in O antiserum while living cultures from the opaque, or K, colony forms were inagglutinable. The other inagglutinable cultures of *S. boydii* 2 also gave rise to 2 colony variants upon plating but the opaque, inagglutinable K forms were not mucoid or encapsulated. Upon plating, all of the cultures gave rise to these 2 colony forms, but in the case of those cultures that were agglutinable in the living state when received, K forms were few in number. However, by plating and selection, K colony forms were derived from all of the *S. boydii* 2 cultures including the older stock cultures.

Heated suspensions and O colony variants of the 12 *S. boydii* 2 cultures were agglutinated to the titer of an O antiserum for the type. Furthermore, heated suspensions of the encapsulated culture, 4158/50, removed all agglutinin from a *S. boydii* 2 antiserum. Reciprocally, all O agglutinin for culture 4158/50 was removed from an antiserum pre-

16. Christensen, W. B., *Res. Bull. Weld. Co., Colo. Health Dept.*, 1949, v1, 3.

TABLE I. Quellung Reactions of Encapsulated *S. boydii* 2 Culture and *Klebsiella* Types.

Suspensions*	Antiserums		
	S. boydii 2 K antiserum	*Klebsiella*, Type 21	*Klebsiella*, Type 11
S. boydii 2 (4158/50, encapsulated)	16†	4	1
Klebsiella, Type 21 (1702/49)	4	32	2
Klebsiella, Type 11 (390)	1	4	16

* Broth cultures incubated 4 hr at 37°C then formalinized.

† Figures represent reciprocal of highest dilution that gave positive reaction.

pared with that culture when aliquot portions of the antiserum were absorbed with each of the 11 other *S. boydii* 2 cultures. Thus, it is clear that the O antigens of culture 4158/50 and the other *S. boydii* 2 strains are identical as demonstrated by reciprocal absorption tests. An antiserum prepared with a young living broth suspension of the encapsulated culture, 4158/50, reacted in a dilution of 1:320 with a living broth suspension of the homologous culture. The type of agglutination that occurred with typical K agglutination, firm discs were formed similar to those seen in capsular agglutination of other bacteria. This antiserum gave a positive quellung reaction in a dilution of 1:16 with the encapsulated form of the homologous culture (Table I). The homologous O agglutinin titer of this antiserum was 1:2560 to 1:5120 and heated suspensions of all 12 *S. boydii* 2 strains reacted in it to titer.

By selection of opaque colony forms, it was possible to demonstrate the presence of K antigen in all of the *S. boydii* 2 cultures. The 12 cultures gave K agglutination in dilution of 1:40 to 1:320 in K antiserum prepared with culture 4158/50. These same antigens were tested in *S. boydii* 2 O antiserum to determine the extent of inhibition of O agglutination. O agglutination was either partially or completely inhibited in all cases. Culture 4158/50 did not react even in a dilution of 1:40, while the other cultures were agglutinated partially in dilutions of 1:80, 1:160 and 1:320 in an antiserum the O titer of which was 1:10,240. The results are summarized in Table II.

Several attempts were made to prepare a pure K antiserum by absorption of the KO antiserum prepared with encapsulated culture 4158/50. This antiserum was absorbed with suspensions of the homologous culture and with several other *S. boydii* 2 cultures which were heated for 2.5 hours at 100°C and washed twice by centrifugation. In each instance all agglutinin for both O and K antigens was removed from the antiserum. Also, attempts were made to prepare a pure K antiserum by absorption of antiserum with minus forms (O forms) of the homologous and other *S. boydii* 2 cultures. These attempts failed because it appears that the absorbing suspensions still contained some K antigen and thus the colonies were not true minus forms. The results of these experiments indicate that the K antigen of culture 4158/50 is probably a B antigen because its antibody binding power was not destroyed by heating the suspensions at 100°C.

The results of direct K agglutination tests indicated that the 11 other *S. boydii* 2 cultures contained a K antigen related to that found in the encapsulated 4158/50. In order to determine whether or not the K antigen was the same in all strains examined, aliquot portions of antiserum prepared from the capsulated form of 4158/50 were absorbed with living suspensions of each of the 11 other *S. boydii* 2 cultures. In all cases, the K agglutinin was removed from the antiserum and the antiserum no longer gave a quellung reaction. This indicated that the K antigen was the same in all of the cultures examined. However, it is clear that there is considerable difference in the amount of K antigen present in the various cultures. Culture 4158/50 contains the greatest amount of K antigen as manifested by complete inhibition of agglutination of living suspensions in O antiserum and by the presence of capsules. It can be argued that the presence of a visible capsule on culture 4158/50 is not due to the production of a larger amount of K antigen, but simply due to the fact that it accumulated on the cells of 4158/50 but not on the cells of the other cultures. If this were true, a larger amount of K antigen should be found in the supernatant fluid of broth cultures of the

TABLE II. Serological Reactions of *S. boydii* 2 Cultures.

S. boydii 2 cultures	*S. boydii* 2 antiserums: O antiserum, Living antigen	O antiserum, Heated antigen (1 hr 100°C)	KO antiserum, Living	KO antiserum, 100°
4158/50 (encapsulated)	0*	10,240	320	2,560
66 (representative of non-encapsulated strains)	160†	10,240	160	5,120

* O equals no agglutination in dilution of 1:20 or higher.
† Figures represent reciprocal of highest dilution in which agglutination occurred.

non-encapsulated strains. Such is not the case. The supernatant fluid of culture 4158/50 produced more voluminous disc precipitates in precipitin tests and was active in much higher dilution than the supernatant fluids of the remaining 11 cultures. It was necessary continually to select moist, opaque colonies if culture 4158/50 were to be maintained in the encapsulated state. Serial transfer on the usual stock mediums resulted in a rapid disappearance of the capsule.

The relationship of the K antigen of *S. boydii* 2 culture 4158/50 to those of *Klebsiella* and *Escherichia* types was also investigated. The capsular material of culture 4158/50 was found to be related closely to the capsular antigen of Klebsiella type 21 (1720/49) and in a lesser degree to *Klebsiella* type 11 (390) (Table I). No reaction was obtained in other *Klebsiella* antiserums. The possibility of the coexistence of an O antigen relationship between the two *Klebsiella* types and *S. boydii* 2 was investigated, but it was found that only insignificant minor O antigen relationships existed. Living suspensions of 59 *Escherichia coli* cultures, containing the known 59 K antigens of that group, were tested in the KO antiserum for culture 4158/50. No relationship was noted between the K antigen of culture 4158/50 and any of the 59 K antigens of the *E. coli* group.

Comment. The writers found no other report in the literature of the presence of capsules in bacteria of the genus *Shigella*. It is clear, however, that occasional encapsulated *Shigella* cultures do occur and that living cultures of encapsulated strains are inagglutinable in O antiserums. Also, the data given above indicate that other inagglutinable *S. boydii* 2 cultures possess a similar antigen though they may not possess enough of the antigen to manifest itself in the form of a capsule. Studies are in progress to determine whether or not the thermolabile somatic antigens of other *Shigella* types may appear as capsules. An encapsulated culture of *Shigella boydii* 1 (Boyd 170) has been found. Living suspensions of this culture are inagglutinable, while heated suspensions are agglutinated to the titer of *S. boydii* 1 antiserum. The capsular antigens of *S. boydii* 1 and *S. boydii* 2 are not related.

Extragenetic relationships of the capsular antigens of members of different groups of Enterobacteriaceae is not a new finding. Kauffmann(17) described mucoid (M) forms of certain *Salmonella* types, notably *Salmonella paratyphi* B. The mucoid antigen inhibited O agglutination and gave typical capsular agglutination in antiserum prepared with living cultures of the M forms. Perch(18) demonstrated relationship between the M antigen of *S. paratyphi* B and *Klebsiella* type 13. Recently Edwards (unpublished data) demonstrated that the M antigen of an encapsulated culture of *Salmonella bovis-morbificans* is also related to the capsular material of *Klebsiella* type 13. The capsular antigen of *S. boydii* 2 is not related to that of *Klebsiella* type 13, but is related closely to that of *Klebsiella* type 21.

Summary. 1. Encapsulated cultures of *Shigella* types are described, apparently for the first time. 2. An encapsulated culture of *Shigella boydii* 2 is described. The occurrence of the same K antigen in other non-encap-

17. Kauffmann, F., *Z. f Hyg. u Infectionskr.*, 1935, v116, 617.

18. Perch, B., *Acta Path. et Microbiol. Scand.*, 1950, v27, 565.

sulated *S. boydii* 2 cultures is reported. An encapsulated culture of *Shigella boydii* 1 also is mentioned. The capsular antigens of *S. boydii* 1 and *S. boydii* 2 are not related. 3. The antigenic relationship of the capsular antigen of *S. boydii* 2 and the capsular antigens of *Klebsiella* types 11 and 21 is reported. This relationship was demonstrated by both agglutination and quellung reactions. No relationship was found between the K antigen of *S. boydii* 2 and any of the 59 K antigens of the *Escherichia* coli group.

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Effect of Cortisone, Related Hormones, and Adrenalectomy on Susceptibility of Mice to Virus Infections.* (18989)

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Cortisone treatment may affect the course of virus infection to the disadvantage of the host. This has been demonstrated for influenza A, influenza B, and mumps viruses in chick embryos(1); for influenza A virus in mice(2); for strain MEF 1 poliomyelitis virus in mice and hamsters(3); for a Coxsackie virus in adult mice(4), and for vaccinia intradermally in the guinea pig(5).

The present work demonstrates that cortisone increases the susceptibility of mice to certain neurotropic viruses, and further investigates the nature of this effect by parallel studies with other steroid hormones, adrenocorticotropic hormone (ACTH), and adrenalectomy.

Methods. Mice. Banks (Swiss) mice weighing 18 to 24 g (6 to 10 weeks old) were used, except for experiment III-12 in which Rockefeller Institute mice of similar age were used. Female mice were used throughout except that males were used for the testosterone treated groups in Exp. IV-27. Adrenalectomies were done about a week before virus inoculation, using ether anesthesia and a dorsal approach. Adrenalectomized mice were given isotonic saline instead of water to drink, but received no other treatment. Autopsies on 38 of the adrenalectomized mice revealed small fragments of adrenal tissue in 11, indicating that about 70% of the mice had been completely adrenalectomized. *Viruses.* All viruses were obtained from Dr. Koprowski of Lederle Laboratories, and were carried in this laboratory by serial intracerebral passage in mice. In the experimental mice all virus inoculations were intraperitoneal using a volume of 1.0 ml of serial 10-fold dilutions of virus-infected mouse brain suspension. Five mice were inoculated with each dilution. Routine virus titrations were performed by intracranial inoculation of Banks mice with 0.03 ml of 10-fold serial dilutions of the material being tested, usually using 3 mice per dilution. Titers, either intraperitoneal or intracranial, are expressed as the LD_{50} dilution using the above volumes. In calculating titers the Reed-Muench formula(6) was used. In Exp. VI-5 the 10^{-1} virus dilution killed only one of the 5 control mice, so in order to calculate an

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1. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 116.

2. Kass, E. H., Ingbar, S. H., Lundgren, M. M., and Finland, M., *J. Lab. and Clin. Med.*, 1951, v37, 780.

3. Schwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

4. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 135.

5. Kligman, S. M., Baldridge, G. D., Rebell, G., and Pillsbury, D. M., *J. Lab. and Clin. Med.*, 1951, v37, 615.

6. Reed, L. J., and Muench, H., *Amer. J. Hyg.*, 1938, v27, 493.