in diameter. Larger bodies, such as might correspond to initial bodies, are rare although at least one 770 mμ in diameter has been noted. There are two possible reasons for the paucity of initial bodies. They may be largely discarded with the low speed sediment or they may fragment, at least to the extent of losing their capsular material, in the process of repeated differential centrifugation. There is evidence that both occur. Thus examination with the light microscope of low speed supernates of preparations, originally rich in initial bodies, show complete disappearance or marked decrease in numbers—to between 1% and 2%. Such supernates are then further treated, by repeated high speed sedimentation and resuspension, and those showing any initial bodies suffer a further 90% decrease.

Pairs, chains and groups of elementary bodies are to be found (Figs. 1-4). Fairly dense intercellular bridges stretching between bodies are not infrequent and may be enclosed by limiting membranes (Fig. 4). The picture is reminiscent of that shown by certain bacteria.[4]

Summary. Electron microscope studies of the agent of feline pneumonitis suggest that the elementary bodies in nature have properties similar to those of jelly-filled sacs which in course of preparation for examination in the microscope settle to a form similar to a wrinkled pea with one flattened side. Such distorted bodies are approximately 465 mμ in diameter. They have several characteristics which resemble rickettsiae and bacteria.

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Pathogenizing Effect of Different Carbohydrates on *Eberthella typhosa.*

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It has long been known that the pathogenicity of bacteria for laboratory animals can be enhanced by addition of certain substances to the injected suspensions (cf. references in papers of McLeod,[1] Ercoli *et al.*[2]). Several investigators have used carbohydrates for this purpose. No systematic study of the relation between carbohydrate structure and pathogenizing potency has, however, been reported. The present communication reports experiments which form part of an investigation designed to clarify the chemical basis of pathogenizing activity in the carbohydrate group.

The experiments define the activity of selected sugars and polysaccharides as pathogenizing agents for *E. typhosa* in the mouse. Special attention is directed to the pathogenizing activity of several levans, including 2 natural sources and a preparation synthesized from sucrose by a cell-free system. The information obtained is inadequate to define finally in a positive sense the features of molecular pattern which determine pathogenizing activity in the carbohydrate group. It suffices, however, to show that several features of the chemical structure and physical character of a carbohydrate are unimportant to its rôle as a pathogenizer.

Test organism. E. typhosa (0901) served as the test microorganism. The bacteria were grown in plain broth at 37°C, the incubation lasting 24 hours.

Test of pathogenizing activity. The experiments were designed to show whether intraperitoneal injection of carbohydrate into

[1] McLeod, Ch., *Am. J. Hyg.*, Sec. B, 1941, **34**, 41 and 51.

[2] Ercoli, N., Lewis, M. N., and Harker, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 273.

a mouse has a pathogenizing effect. White mice of laboratory stock served as the experimental animal. The mice were at the time of the tests 4-5 weeks old and averaged 18 g in weight. The animals were injected intraabdominally with 0.5 cc of a suspension which consisted of a mixture of 0.05 cc of a selected dilution in broth of a 24-hour broth culture and 0.45 cc of a sterile-aqueous solution of the tested pathogenizing substance. Where no pathogenizing effect was observed, the carbohydrate concentration was increased in successive tests up to a concentration of 10% or until a concentration which gave a solution too viscous to be injected was reached. Where a pathogenizing effect was obtained the number of injected bacteria was decreased by power of 10 in successive tests until a nonlethal dose was reached. Groups of 5 mice were used at each assay level. The findings are recorded in terms of the quotient—number of animals which died within 48 hours after injection/5 (total number of mice in group). The pathogenizing activity is expressed in terms of the quotient, MLD of broth culture without added pathogenizer/MLD of broth culture in presence of added pathogenizer. In order to exclude error due to accidental death, the MLD is defined as that dose which produces a mortality rate of at least 2/5. The MLD of the broth cluture without added pathogenizer was found to be 0.4 cc. In repeats of assays of the pathogenizing potency of different substances, the maximum variation in mortality between replica tests was $\pm$ 1/5. Values of pathogenizing potency were reproducible within the limits of one logarithm integer of the broth dilution.

Materials. Sugars: pure crystalline preparations.* Galactomannan:† a preparation from carob seed. Mannan: prepared from carob galactomannan by selective hydrolysis according to the method of Wailew and Gortner.[3] Dextran: prepared in powder form from sucrose broth culture of *Leuconostoc mesenteroides* by repeated precipitation in alcohol with stirring in a Waring blendor. Levans: prepared in powder form from sucrose cultures of *Aerobacter levanicum* and *Bacillus subtilis* as described by Hestrin *et al.*[4] Cellulose I: fibril suspension prepared from cultures of *Acetobacter xylinum* as described by Aschner and Hestrin.[5] Cellulose II: acid-degraded cotton cellulose prepared by exposure of one part ether-extracted cotton wool to the action of 9 parts 60% sulfuric acid for 10 minutes at room temperature. The suspension after dilution and neutralization was dialyzed and obtained in the form of a powder by evaporation over a boiling water bath. Other materials employed: glycogen,‡ inulin,§ gum acacia,‖ agar powder,¶ mucin,** kaolin,†† rice starch, soluble starch, and pectin.‡‡

Survey of pathogenizing activity of different carbohydrates. The following carbohydrates were found to be entirely lacking in pathogenizing activity in a concentration range of 0.2-10%: pentoses—arabinose and xylose; hexoses—glucose, fructose and galactose; oligosaccharides—maltose, trehalose, lactose, cellobiose, sucrose, and raffinose; polysaccharides — galactomannan (0.2%), soluble starch (5%), rice starch (5%) and pectin (5%). The MLD in the presence of these carbohydrates except in the case of pectin was equal to that found in absence of carbohydrate. Pectin was found to be bactericidal at a concentration of 5%.

Polysaccharides which showed measurable or marked pathogenizing activity under the conditions of the test are listed in order of decreasing activity in Table I. On the basis of the maximum pathogenizing effect (pathogenizing potency) obtained 3 classes of carbohydrates can be distinguished: (a) pow-

* British Drug Houses, London.

† Kindly supplied by Dr. E. Simon, Daniel Sief Research Institute.

[3] Wailew, B., and Gortner, R., *Arch. Biochem.*, 1943, **1**, 325.

[4] Hestrin, S., Avineri-Shapiro, S., and Aschner, M., *Biochem. J.*, 1943, **37**, 450.

[5] Aschner, M., and Hestrin, S., *Nature*, 1946, **157**, 659.

‡ Rhône-Poulenc, Paris.

§ British Drug Houses, London.

‖ Pharm. central de Belgique.

¶ May and Baker, London.

** Burroughs Wellcome, London.

†† Merck, Darmstadt.

‡‡ Commercial source unknown.

TABLE I.
Pathogenizing Activity of Polysaccharides in Presence and Absence of Kaolin.

Substances tested	Pathogenizing activity expressed as ratio: MLD without pathogenizer/MLD with pathogenizer. Concentration of carbohydrate 0.2%	0.3%	0.5%	1.0%	2.0%	5.0%	10.0%
Agar	—	$8x10^3$	—	—	—	—	—
" with kaolin 1%	—	$4x10^3$	—	—	—	—	—
Levan *(A. levanicum)*	—	$8x10^2$	$8x10^3$	$8x10^4$	$8x10^5$	—	—
" with kaolin 1%	—	—	—	T	T	T	T
Levan *(B. subtilis)*	—	—	—	$8x10^3$	$8x10^4$	—	—
" with kaolin 1%	—	—	—	$4x10^3$	$4x10^4$	—	—
Mucin	—	—	—	—	—	$8x10^4$	$8x10^5$
" with kaolin 1%	—	—	—	—	—	T	T
Dextran *(L. mesenteroides)*	—	—	—	$8x10^2$	$8x10^3$	—	—
" with kaolin 1%	—	—	—	—	$4x10^3$	—	—
Cellulose I *(A. xylinum)*	—	—	—	—	8	—	—
" with kaolin 1%	—	—	—	—	$4x10^2$	—	—
Cellulose II (Cotton)	—	—	—	—	—	80	—
" with kaolin 1%	—	—	—	—	—	$4x10^2$	—
Gum acacia	—	—	—	—	—	1	80
" with kaolin 1%	—	—	—	—	1	4	$4x10^3$
Mannan (carob)	—	—	—	—	—	8	—
" with kaolin 1%	—	—	—	—	—	4	—
Glycogen	—	—	—	—	1	1	8
" with kaolin 1%	—	—	—	—	40	40	$4x10^3$
Inulin	—	—	—	—	1	1	4
" with kaolin 1%	—	—	—	—	1	40	$4x10^2$
Dextrin	—	—	—	—	—	—	1
" with kaolin 1%	—	—	—	—	—	—	40

— = No test.
1 = MLD not lowered; 8 = MLD 8-fold lowered, etc.
T = Toxic action in absence of bacteria.

erful pathogenizers; (b) moderate pathogenizers and (c) nonpathogenizing carbohydrates. Class (a) includes 2 extracellular bacterial polysaccharides (levan and dextran), mucin and agar-agar. Members of this class showed high activity as pathogenizers also at fairly low concentration. Class (b) comprises the 2 cellulose preparations, gum acacia, glycogen, mannan and possibly should also include inulin. Class (c) comprises all the tested crystalline sugars and several of the polysaccharides (starch, galactomannan). It may be noted that the above classification of carbohydrates on the basis of pathogenizing potency is consistent with subdivision of the carbohydrates on the basis of other criteria of their pathogenizing action as described in following sections.

Copathogenizing effect of kaolin. Kaolin alone at a concentration of 1% produced no significant pathogenizing effect, the MLD being in absence of kaolin 0.4 cc and in its presence 0.2 cc. Kaolin is, however, in certain cases a powerful copathogenizer, *i.e.* it enhances the pathogenizing potency of carbohydrates which also show pathogenizing activity in its absence. The influence of kaolin on the pathogenizing potency varies consistently with the classification of carbohydrates already described. Kaolin exerts a marked effect on the pathogenizing activity of carbohydrates of class (b), but has little or no effect in class (c) and (a).

Effect of pathogenizer concentration. The effect of pathogenizer concentration has been investigated in the following: levan, mucin, gum acacia, inulin and glycogen. It is evident from the values shown in Table I that the pathogenizing activity increases at an increasing rate as the concentration of pathogenizer is increased. There is in every case a threshold level below which no activity can be found. The threshold varies markedly in the different carbohydrate classes. It is relatively low ($<2\%$) in (a), and fairly

high (5-10%) in (b). The existence of the threshold places doubt on the general significance of negative results which may be obtained when possible pathogenizers are tested at low concentrations.

Pathogenizing activity of synthetic levan preparation. The observations on the pathogenizing activity of levan from 2 different bacterial sources suggest that levan from *A. levanicum* has significantly greater pathogenizing potency than levan from *B. subtilis*. This observation is of particular interest in view of the fact that no chemical or serological difference between different bacterial levans has hitherto been detected.[6,7] The pathogenizing action of levan prepared by the action on sucrose of a cell-free enzyme solution was therefore undertaken.

In preliminary experiments it was found that levan-free levan-sucrase solution prepared from *A. levanicum* by autolysis as described by Hestrin *et al.*[4] is toxic to mice in a dosage of 0.1 cc. Since the toxin is heat-stable and derives from a Gram-negative organism, it is probably to be regarded as endotoxin. The toxin of the enzyme preparation is precipitated by alcohol. It accompanied levan in the alcohol precipitate when levan was formed from sucrose added to the enzyme solution. Methods accordingly were sought which would permit the preparation of a nontoxic synthetic levan preparation. Two suitable methods are described below.

In one case, levan was synthesized from sucrose by incubation with levan-free levan-sucrase at 37°C for 2 days in a mixture of the following composition: 100 cc enzyme solution, 100 cc acetate buffer pH 5.0, 100 cc 15% sucrose. Toluol was added to maintain sterility. The material was precipitated with 2 parts alcohol, purified by repeated precipitation with alcohol, and dried in a vacuum desiccator. About 1.2 g of dry material was thus obtained, whereas a control mixture of enzyme and buffer without sucrose afforded 0.3 g by the same process. The yield of levan was therefore 0.9 g. To remove possibly present endotoxins the 2 products were treated according to Furth and Landsteiner[8] with hot 0.5 N NaOH for 30 minutes. The solutions were neutralized and dialyzed against tap water in cellophane bags for one day. The dialyzed solutions were precipitated with 2 volumes of alcohol. The precipitates were dried and aqueous solutions of 1 and 2% concentration were set up. Solutions of the enzyme-buffer mixture product proved to be nontoxic and nonpathogenizing, whereas the solutions of the enzyme-buffer-sucrose product, *i.e.* the synthetic levan preparation, showed no toxicity but high pathogenizing activity. The pathogenizing activity rated $8x10^2$ and $8x10^3$ at the concentration levels 1% and 2% respectively.

In a second case, advantage was taken of the fact that dilution improves the levan yield per unit of enzyme employed.[9] The reaction mixture was composed as already described except that the enzyme solution was diluted 10-fold. The yield of levan was decreased by only one-half by this 10-fold decrease of the enzyme. A levan preparation was obtained which showed no toxic effect in 2% solution even in absence of alkali pretreatment.[8] The pathogenizing activity was found to rate $8x10^2$, $8x10^2$ and $8x10^3$ at the concentration level 0.5, 1.0 and 2.0% respectively.

It may be concluded therefore that levan synthesized *in vitro* by the action of cell-free *Aerobacter* levan-sucrase on sucrose exhibits a marked pathogenizing activity independent of the presence of any alkali-labile toxic component in this preparation. The "synthetic" levan shows slightly less activity than levan prepared from a living *Aerobacter* culture.

Discussion. The experiments presented show that pathogenizing activity towards *E. typhosa* is not uncommon in polysaccharides. Of a total of 15 polysaccharides investigated, high pathogenizing potency was found in 5, moderate activity in 6, and little or no activity in the remainder. On the other hand,

[6] Genghof, D. S., Hehre, E. J., and Neill, J. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 339.

[7] Hehre, E. J., Genghof, S. D., and Neill, J. M., *J. Immunol.*, 1945, **51**, 5.

[8] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.

[9] Avineri-Shapiro, S., and Hestrin, S., *Biochem. J.*, 1945, **39**, 167.

not one member of a group of 10 oligosaccharides and simpler sugars examined showed pathogenizing activity. It is appropriate in the light of these findings to inquire what physical and chemical properties in a carbohydrate determine its activity.

The hypothesis has been presented that viscosity is an overriding determinant of the pathogenizing activity of an injected substance. Experiments by several investigators have tended, however, to throw doubt on this conclusion. The experiments here reported confirm this criticism. Among the carbohydrates examined by us, no consistent relationship between viscosity and pathogenizing activity can be observed. In the polysaccharide group with marked pathogenizing activity, dextran with a viscosity at a concentration of 2% about 10 times that of levan shows far less pathogenizing activity than does levan at the same concentration. Again, in the group of polysaccharides with low pathogenizing activity, a pathogenizing potency which is roughly constant is associated with widely fluctuating viscosities. Finally, it may be noted that the inactive polysaccharide group includes several substances of very high viscosity (galactomannan, mannan, starch). It is clear, therefore, that viscosity is not an overriding determinant of pathogenizing activity and that it does not *per se* confer pathogenizing activity on an injected substance.

Since a pathogenizing activity which is absent in simple sugars and oligosaccharides can be present in their polymers, it is suggested that pathogenizing activity is a function of patterns which are specific to the colloidal state. It is significant in this respect that different polysaccharides with a common hexose unit and similar features of chemical structure can present widely different pathogenizing activity (levan from *A. levanicum* vs. levan from *B. subtilis* vs. inulin; starch vs. dextran, etc.)

Pathogenizing activity seems to be dependent only in a minor degree on the structure, configuration and manner of linkage of the individual repeating unit or on the presence of polar groups in a carbohydrate polymer. Thus it has been found that either high or moderate pathogenizing activity may be manifested by simple polysaccharides of such groups as glucan (dextran, cellulose preparations, glycogen), fructan (levans, inulin), and mannan (carob preparation) and by complex polysaccharides with polar groupings (agar, gum acacia, mucin). Our pathogenizing carbohydrates include both α and β linkage types, furanosidic as well as pyranosidic units, and intersaccharidic C-C linkages of the 1-4 (cellulose, glycogen), 1-6 (dextran), 2-1 (inulin) as well as 2-6 (levan) types.

In an investigation of the pathogenizing potency of different fractions of mucin, Anderson and Oag[10] found that whereas marked activity is shown by protein fractions no pathogenizing activity is presented by the purified carbohydrate fraction of mucin. This result suggests that the pathogenizing activity of certain carbohydrates may be due to the presence of foreign substances in these preparations. In view of the fact that pathogenizing activity is manifested by polysaccharides from widely different sources, this possibility must be considered as most unlikely. In the case of levan it has been possible to exclude it. The demonstration of pathogenizing activity in a levan obtained in defined reaction conditions by the action of cell-free enzyme on pure sucrose affords proof that pathogenizing activity is an intrinsic property of levan.

Summary. The pathogenizing action of 15 polysaccharides and of 11 simple sugars and oligosaccharides on *Eberthella typhosa* in the mouse has been described. High pathogenizing activity was shown by the following carbohydrates: levan, dextran, mucin, and agar-agar. Moderate activity was shown by the following: cellulose preparations, gum acacia, glycogen and mannan. Inclusion of kaolin in the injected suspension enhanced the pathogenizing action of this group. Levans from 2 bacterial sources *Aerobacter levanicum* and *Bacillus subtilis* showed different degrees of pathogenizing activity. Levan synthesized *in vitro* by the

[10] Anderson, C. G., and Oag, R. K., *Brit. J. Exp. Path.*, 1939, **20**, 25.

action of cell-free *Aerobacter* enzyme showed marked pathogenizing activity. The pathogenizing activity must be, therefore, an intrinsic property of this levan.

It is suggested that the pathogenizing activity of a polysaccharide is a function of patterns which are specific to the colloidal state. The activity depends only in minor degree on the structure, configuration and manner of linkage of the individual repeating unit or on the presence of polar groupings in the carbohydrate polymer. There is no consistent relationship between the viscosity of the injected carbohydrate solution and the pathogenizing activity which it manifests.

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Electroshock Therapy by Stimulation of Discrete Cortical Sites with Small Electrodes.*

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Among the many phenomena observed during and following electroshock therapy are convulsion, loss of memory and profound stimulation of the autonomic nervous system. By using small electrodes (8 mm in diameter as contrasted to conventional electrodes of 5 cm^2) it was found that no convulsion resulted when the stimulus was applied through regions some distance from the motor strip, although the current used was sufficient to produce convulsions when applied to the motor strip itself. The conventional shock machine was used in the experiment. Fairly accurate control of current was possible by measuring the resistance of the subject.

Some degree of localization of the stimulation was possible. Observations made following stimulation of several chosen sites indicated that stimuli over the various regions produced different results. Correlating these observations with clinical results and information concerning other organic treatments used in mental disorders, has made possible some speculation concerning the factors which make electroshock treatment effective. It has also offered directions for further investigations into the mechanism of the psychoses.

Sites for stimulation through the frontal area were chosen in accordance to external skull landmarks. Site A was high on Area 4 (Brodmann) just posterior to the interauricular line. Site B was roughly through Brodmann's Area 11. Site C was roughly through Brodmann's Areas 9 and 10. Site D was a point midway between the orbit and auditory canal and 1 cm above the upper border of the zygomatic process. (Brodmann Area 38).

Observations of results following stimulation of the selected sites will be presented according to:

(A) Motor phenomena
(B) Autonomic nervous system phenomena
(C) Disturbance of intellectual function
(D) Clinical results

(A) Motor phenomena. Stimulation of Site A or Area 4 of Brodmann resulted in a typical tonic and clonic convulsion lasting 40 seconds to one minute. Stimulation of the other sites produced with considerable regularity a single extension "jerk" of all extremities.

(B) Autonomic nervous system phenom-

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