been shown capable of propagation in mice. Quantitative studies may indicate whether modified growth cycles of "unadapted" type 1 poliomyelitis virus do in fact occur in the mouse brain. Infectivity for neither monkey nor tissue-culture fibroblasts is lost in the process.

There was failure to demonstrate interference in brains of mice between type 1 virus and mouse-adapted type 2 virus in the dosages and time intervals herein reported. When *incomplete* growth cycles of "unadapted" influenza virus occur in mouse brain(8) a variety of effects including interference with wholly unrelated viruses(9) may be demonstrated. Failure to demonstrate interference in our experiments is in harmony with previous findings of one of us(10) in which it was shown under the conditions therein employed that interference could not be demonstrated between type 2 (Lansing) virus and fecal and spinal cord materials subsequently(4) shown to be immunologically distinct type 1 viruses. It was also found that two immunologically *related* type 2 strains, *i.e.*, M.V. which is "unadapted" for mice and Lansing virus failed to exhibit interference.

Conclusions. Unadapted type 1 (Brunhilde) poliomyelitis virus inoculated intracerebrally in mice can be recovered 5 days and 10 days subsequently from the spinal cord of such mice as well as from their brains. Such virus does not appear to have undergone immunologic alteration and is neutralized by a type 1 (Brunhilde) monkey antiserum. The biological state of this unadapted virus in the central nervous system of the mice is such that it does not interfere with the propagation of mouse adapted type 2 (Lansing) poliomyelitis in such mice. It is suggested that the phenomenon may represent circumstances other than mere passive persistence of virus in the recipient mouse brain.

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A Turbidimetric Method for Assay of Vi Antigen. (19842)

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Recent studies(1,6) on the purification of Vi antigen from *Escherichia coli* required a rapid quantitative method for determining the antigen content of extracts and partially purified fractions. Since serological assays were tedious and quantitatively uncertain, a turbidimetric method was devised which took advantage of the propensity of acidic mucopolysaccharides and similar lyophils to form fine flocculent suspensions with albumin at low pH. This is analogous to the turbidimetric assay of hyaluronic acid reported by Kass and Seastone(2) and others(3,4). Although the exact chemical nature of this antigen has not yet been ascertained, its acidic nature, high molecular weight, and good correlation between the specific preciptin titers and the optical densities suggested the use of turbidity measurements as a quantitative measure of the antigen.

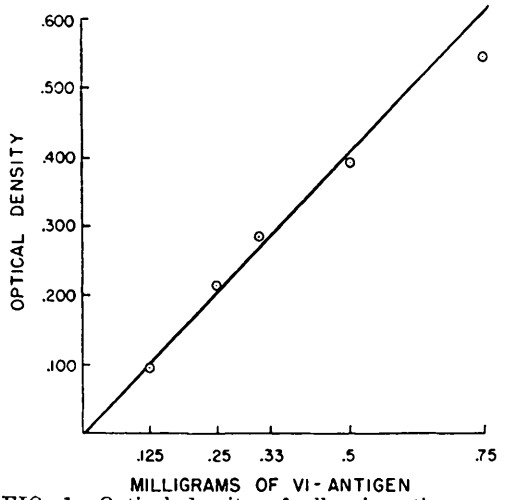

FIG. 1. Optical density of albumin-antigen suspensions for different concentrations of purified Vi antigen, at pH 4 and 26°C. Optical density was measured 10 min. after mixing.

Materials and methods. Acetone dried cells of V form *E. coli*, 5396/38; *Paracolobactrum ballerup*, 7851/39; *Salmonella typhosa*, Ty 2; and *S. typhosa*, 0-901 were prepared by methods detailed elsewhere(5). The last named, lacking Vi antigen, served as a negative control. The principal test organism, *E. coli*, was extracted, fractionally precipitated and concentrated as previously described(1). The purest of these preparations, electrodialyzed Vi antigen (Stage 6), was selected as a primary standard for relating albumin turbidity to immunological activity. Albumin solutions were prepared by dissolving crystalline bovine serum albumin (Armour), 2 mg/ml, in 0.1 N sodium acetate adjusted to appropriate pH values. Ten ml of albumin solution were rapidly mixed with 1 ml of antigen solution, held in a water bath at 26°C for exactly 9 minutes, and then mixed by inversion. Exactly 1 minute later, the optical density of the turbid suspension was measured at a wave length of 6000 Å, in a spectrophotometer previously adjusted to 100% transmission with the buffered albumin. A second sample of the antigen solution was mixed with the buffer solution without albumin, and its optical density was subtracted from the optical density of the antigen-albumin suspension. Antigen concentrations were read from standard curves, Fig. 1, relating mg of antigen to optical density, prepared by treating measured amounts of the selected antigen sample with albumin at pH 4.0. A satisfactory linear relation between optical density and mg of purified antigen was found up to concentrations of 0.5 mg of antigen. Above this value, linearity failed and samples containing more antigen were appropriately diluted.

Of the several conditions necessary for constant reproducible turbidities, acidity was the most critical. Albumin solutions, 2 mg/ml, were mixed with 0.33 mg of purified Vi antigen. After 10 minutes, maximum optical densities were found between pH 4.2 and 4.7, Fig. 2. However, these particles aggregated rapidly to a heavy coagulum with correspondingly rapid changes in optical density. Since this was unfavorable for accurate measurement, pH 4.0 was chosen where the optical density of the fine suspension changed slowly with time. At pH 4.0 and 26°C, 0.33 mg of purified Vi antigen treated with albumin reached a maximum turbidity in about 20 minutes, (Fig. 3). After 6 minutes, however, the optical densities changed more slowly; and a more convenient time of 10 minutes was used without entailing significant errors. Optical densities increased to maximum values more rapidly at high temperatures; while the slower aggregation of micelles at lower temperatures minimized the errors. A satisfactory

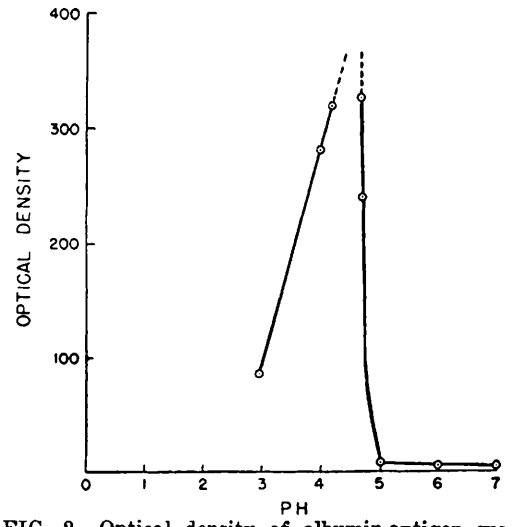

FIG. 2. Optical density of albumin-antigen suspensions, .33 mg purified Vi antigen, at different pH values.

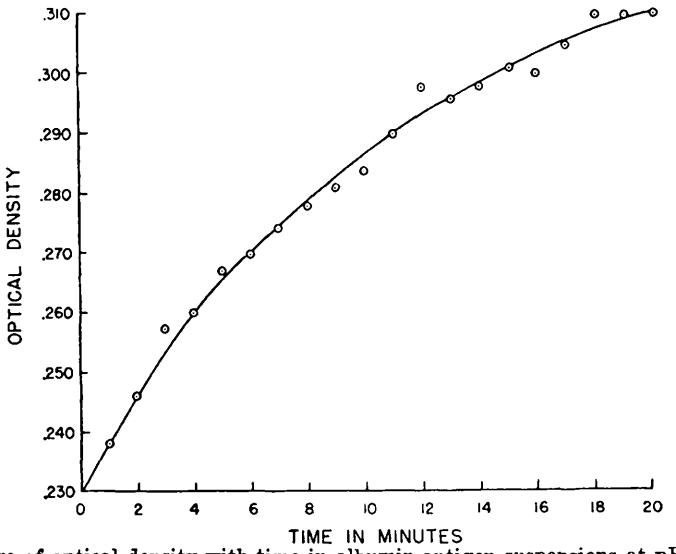

FIG. 3. Change of optical density with time in albumin-antigen suspensions at pH 4 and 26°C. Concentration of purified Vi antigen, .33 mg/ml.

compromise was taken at 26°C. The ionic strength did not have a profound effect under these conditions and distilled water was usually used to dissolve and dilute antigen samples. Two mg of albumin per ml exceeded the amount necessary to produce maximum turbidity with any of the antigen concentrations employed. However, different albumin preparations were found to yield different turbidities with the same antigen solution. Therefore, each new albumin solution was carefully standardized against a standard antigen sample if absolute values were desired.

Many other high molecular weight acids and polymers such as nucleic acids, hyaluronic acid, pectin, hemicelluloses, etc. are known to flocculate with albumin at low pH. Nucleic acids caused an insignificant error with the extracts from *E. coli* and *P. ballerup*, which contained relatively large amounts of Vi antigen. On the other hand, the extracts from *S. typhosa* contained such small amounts of Vi antigen that the contaminating nucleic acid contributed excessive errors and had to be removed by heating the extracts with *N* acetic acid for ½ hour in a boiling water bath and centrifuging.

TABLE I. Serological Activity and Optical Density of Vi Antigen-Albumin Suspensions.

Purified antigen	Optical density	Precipitin titer
E. coli	.004	1:8
	.026	1:128
	.138	1:256
	.320	1:512
*P. ballerup**	.268	1:256
	.430	1:256
S. typhosa, Ty 2	.073	1:64
	.380	1:512
0-901	.013	0

* Precipitin tests with antisera from rabbits immunized with *E. coli*.

Results. The procedure has given a satisfactory turbidimetric measure of Vi antigen in solutions and extracts. The relation of optical densities to specific precipitin titers is shown in Table I. The precipitin tests were carried out in the usual manner with antisera from rabbits immunized with *P. ballerup*. Within the limitations of this test, a quantitative relationship is evident. The turbidity method, however, has the great advantage of yielding a continuous series of quantitative values. It is conceivable that this procedure might also be of value in screening other bacterial extracts for the presence of similar acidic polymers and in facilitating their purification and identification.

Summary. It has been found that the Vi antigen content of extracts and partially purified fractions of *Escherichia coli, Paracolo-*

bactrum ballerup, and *Salmonella typhosa* can be determined by a turbidimetric procedure herein described.

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Production of Fever and the Shwartzman Phenomenon by Native Dextran.* (19843)

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Dextrans are polysaccharides, macromolecular polymers of glucose produced by bacteria, predominantly those belonging to the genus *Leuconostoc*, in culture media containing sucrose. These substances are presently of interest because of their use after partial hydrolysis as so-called plasma volume expanders.

The present report is concerned with some of the reactions noted after injection of unhydrolyzed or "native" dextrans in rabbits. These include leukopenia, fever, and the Shwartzman reaction.

Materials and methods. White male rabbits of mixed breed weighing 2-3 kg were used. Bacterial substances injected intradermally to prepare animals for the Shwartzman reaction were P-25 polysaccharide from *Serratia marcescens* (obtained from Dr. M. J. Shear) and a filtrate of agar-washings from a culture of *S. marcescens* prepared by the method of Shwartzman(1). The intradermal dose of P-25 was 0.1 mg in 0.5 ml of physiologic saline solution and of filtrate 0.5 ml of a 1:10 dilution. Leukocyte counts were made on blood obtained from fresh cuts over a small vein in the ear. In certain instances, cover-slip smears stained with Wright's stain were examined for differential leukocyte counts. All fever tests were conducted in an air-conditioned room using methods previously described(2). Several native dextrans obtained through the courtesy of Dr. Walter L. Bloom were used. These included samples from lots N-112 and N-316 from Commercial Solvents Corporation, lot 84506A1 from another commercial source, and three lots of crude dextran designated I, II, and III prepared in Dr. Bloom's laboratory at Emory University. In addition, a few experiments using unhydrolyzed dextran LM512, obtained from Dr. Virginia Whiteside-Carlson at the University of Alabama were done. Dextrans were injected in a solution of 25 mg/ml in physiologic saline. Two specimens of clinical dextran were employed for comparative experiments. These were that produced by Commercial Solvents Corporation and "Macrodex", a Swedish product (Pharmacia Laboratories). All glassware and needles were sterilized for 2 hours at 170° C to destroy any contaminating bacterial pyrogen. Saline solutions were tested frequently in rabbits and were always pyrogen-free.

Experimental. Leukopenia. The injection of as little as 10 mg of unhydrolyzed dextran produced transient leukopenia followed by leukocytosis in rabbits. With doses of 100-200 mg, leukopenia due to decrease in circulating heterophiles was invariably present within 5 minutes after injection and persisted for as long as 2 hours, finally giving way to heterophile leukocytosis after a few hours. This alteration of the peripheral leukocytes is

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