ablation of all cortex except the frontal pole does not influence the responses. Bilateral, but not unilateral, ablation of specific sensory cortex has been reported to abolish these responses(2,3), but evidence has been presented demonstrating that this too represents only a rise in metrazol threshold, involving only the specific sensory system whose cortical representation has been ablated(4). In the case of the motor cortex, with unilateral ablation there is a depression of the response to all forms of stimulus.

It is evident that the motor cortex contributes but is not essential for reflex myoclonus. This infers that in some way the motor cortex exerts a facilitatory action on the neuronal mechanisms involved in the response. Whether this action is exerted at a spinal or supraspinal level is not yet established. Preliminary observations indicate the probability that metrazol has little influence on the spinal mechanisms in the nembutalized cat(5). If this be true, it is evident that the facilitatory influence of the motor cortex is to be attributed to activity at a supraspinal level, through its extrapyramidal projections.

The actual site and mechanism of action of metrazol are unknown. It is of interest that the myoclonic twitch of metrazolized man or animal is primarily a bilateral flexor response. Ablation of the motor cortex markedly diminishes these responses, and the participation of the affected extremity in a generalized seizure is characterized by a relative sparing of extensor mechanisms. The neuronal basis of such specificity of metrazol action is under investigation at the present time.

Summary. 1. Sharply limited ablation of the arm or leg area of the precentral motor cortex in the macaque produces a complete or partial depression of the reflex myoclonic responses of the affected extremities as elicited after subconvulsant metrazol dosage. This occurs in chronic, as well as acute preparations. 2. When responses are present, there is a predominance of extensor muscle activity, with marked diminution of finger- or toe-flexor responses. A similar pattern is observed when generalized seizures are elicited by repetitive acoustic stimulation.

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Estimation of Vi Antibody Employing Erythrocytes Treated with Purified Vi Antigen.* (20188)

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The occurrence of Vi antibody in the sera of individuals harboring typhoid bacilli was first observed by Felix(1,2). Reports from many laboratories have confirmed and generally established this relationship(3-5). Consequently, the presence of Vi agglutinins is now commonly considered to be correlated with the harboring of typhoid bacilli and may serve as a satisfactory means of detecting the carrier state. As a result of its relative simplicity and because it is a more practicable method than the bacteriological examination of stool specimens, the test for Vi antibody has been employed extensively as a screening procedure for the detection of typhoid carriers in large populations. The method enjoying the widest

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acceptance employs the Vi I strain of *S. typhosa* (Bhatnagar)(6). This culture is considered a pure Vi variant of *S. typhosa* free of H antigen and containing only a very small amount of O antigen, insufficient to interfere with its Vi agglutinating activity. In practice, however, it has been reported that the Vi I strain may yield an appreciable percentage of false positives(7) and most reports concerning its use emphasize the need for great care in its maintenance to obtain reliable results. While the value of the Vi agglutination test for the detection of typhoid carriers no longer is seriously questioned, it has been modified frequently by many workers in the field and the technic of its performance has not been fully standardized, although more than a decade has elapsed since its inception. The large number of publications concerning the technic, together with the fact that properties of Vi agglutination suspensions and the details of the test procedure differ widely, are all indicative of decided variations in the specificity of the test in the hands of various workers(5) and suggest that the procedure employed for determination of Vi antibody may be susceptible to improvement. Vi antigen was recently isolated by Webster, Landy, and Freeman(8) from V form *Escherichia coli* 5396/38, a non-typhoid enteric species rich in this antigen, by an ethanol-salt fractionation procedure, and it was shown to be a polymeric acid. Landy and Webster(9) in a study of its immunological properties, presented evidence indicating that the Vi antigen in the purified state had retained its distinguishing serological and immunogenic characteristics. Among other immunological attributes it was demonstrated that the purified Vi antigen could be adsorbed on human type O erythrocytes which then became specifically agglutinable by Vi antisera. This observation suggested that the isolated Vi antigen, possessing high potency and stability, might offer certain advantages in coping with the practical problem of detecting or measuring Vi antibody. It is the objective of this report to present a procedure for the preparation of a Vi agglutination reagent of high serological activity by the sensitization of human type O erythrocytes with purified Vi antigen and to indicate the results obtained with its use in the estimation of Vi antibody.

Materials and methods. Vi bacterial agglutination test. The Vi antigen suspension made from *S. typhosa* Vi I (Bhatnagar) and Vi antiserum† distributed by the Standards Laboratory of the Central Public Health Laboratory, London, were employed in accordance with the laboratory's procedure for conducting the Vi agglutination test. Agglutination tests were also performed with viable suspensions of *S. typhosa* Vi I freshly prepared for each test and checked with a reference (*ballerup*) Vi serum. These tests yielded essentially similar results. *Preparation of Vi antigen.* The purified Vi antigen employed in this investigation was prepared from acetone killed and dried V form *E. coli* 5396/38 by the method described by Webster *et al.*(8). Solutions of the antigen containing 10 μg per ml in 0.85% NaCl were stored at 4°C with 1:10,000 merthiolate as preservative. *Collection and preservation of erythrocytes.* Human type O erythrocytes were collected in equal volumes of modified Alsever's solution.‡ Erythrocytes were washed 3 times in 10 volumes of physiological saline, centrifuged each time in conical tubes at 1500 RPM for 5 minutes, and the supernatants discarded. The packed erythrocytes were resuspended in 10 volumes of saline and centrifuged at 1500 RPM for 10 minutes. The supernatant was discarded and the packed cells made up to a 10% suspension. *Sensitization of erythrocytes with purified Vi antigen.*§ Equal volumes of 10% erythrocytes and a solution

† The Vi reference serum (horse) distributed by Central Enteric Reference Laboratory for use in this test exhibits a Vi hemagglutination titer of approximately 1:10000. The serum dilution of 1:1400 is considered the "endpoint" for purposes of reading the results of the Vi bacterial agglutination test.

‡ Blood drawn by venipuncture was added to a flask containing an equal volume of sterile modified Alsever's Solution (dextrose 2.05%, NaCl 0.42%, trisodium citrate 0.055%). Contents were mixed and dispensed aseptically as 3 ml aliquots into sterile vaccine bottles fitted with rubber sleevecaps and stored at 4-8°C. Under these conditions of collection and storage, erythrocytes suitable for hemagglutination tests may be retained for at least one month.

of Vi antigen (10 μg per ml in saline)‖ were mixed and incubated in a water bath at 37°C for 2 hours with shaking every 30 minutes during incubation. Excess or unabsorbed Vi antigen was inhibitory and was removed by washing the suspension 2 times with 10 volumes of saline, centrifuging in conical tubes at 2000 RPM for 5 minutes and discarding supernatants. The packed cells following the second washing were made up to 10 times the original volume (cells plus antigen solution) and centrifuged at 2000 RPM for 10 minutes. The supernatant was discarded and the packed cells made up to a 1% suspension. When refrigerated at 4°C sensitized erythrocytes retain their specific agglutinative properties and are satisfactory for use in this test for 2 days.

Vi hemagglutination test. The test was set up to include reference Vi serum, known negative serum and serum and saline controls. Sensitized erythrocyte suspension (0.1 ml) was added to 0.2 ml volume of serial 2-fold dilutions of the test sera in 10 x 75 mm agglutination tubes. The tubes were shaken and incubated in the water bath at 37°C for 2 hours. Agglutination was determined by examining the pattern of settling of the erythrocytes on the bottom of the tube after the manner of Salk's technic for the titration of influenza hemagglutination. In the tubes containing a high concentration of immune serum a positive (complete) agglutination pattern appeared early and was most marked. This was in the form of a compact mass with ragged edges. Tubes containing lower concentrations of immune serum but sufficient to yield complete or almost complete agglutination exhibited a dispersed pattern of cells entirely covering the bottom of the tube. Tubes containing a still lower concentration of immune serum, and normal or negative sera, revealed a "doughnut" shaped pattern or a compact "button" of cells. The titer was expressed as the final dilution of serum in the last tube which showed complete or almost complete agglutination. This tube was the one immediately preceding the first tube showing a "doughnut" pattern of cells.

Results. It was considered desirable to compare the results of Vi antibody titrations by the procedure outlined above with the results obtained by other methods. Accordingly, hemagglutination titrations were made on 11 rabbit Vi antisera on which Vi bacterial agglutination titers and antibody N precipitable with the purified Vi antigen had been determined previously. The results of these tests are summarized in Table I.

The ratios of the hemagglutination to the agglutination titers and antibody N values for these sera are tabulated to indicate the extent to which these measurements of Vi antibody content are related. It will be noted that the ratios of the hemagglutination titers to the bacterial agglutination titers varied from 4.0 to 10.5, while the ratios of the antibody N values to hemagglutination titers ranged from 1.9 to 6.6. These respective 2.6 and 3.5 fold variations obtained for ths group of Vi antisera are moderate and are only slightly in excess of the experimental error common to tube agglutination tests. These results indicate that the three determinations, one of which is known to possess analytical accuracy, are correlated.

The application of the Vi hemagglutination technic to the measurement of antibody reactive with the purfied Vi antigen was studied with a variety of human sera which may be divided into four groups: normal, from individuals vaccinated with heat-killed typhoid vaccine, from individuals immunized with the purified Vi antigen and from typhoid carriers.

§ It was established that Vi antigen could also be adsorbed on erythrocytes of the mouse, guinea pig, rabbit, dog, sheep and horse. Of these species erythrocytes from sheep and man required the smallest quantity of antigen for sensitization. Human erythrocytes were employed exclusively in this study since, with their use, it was not necessary to absorb test sera for removal of normal hemagglutinins. For most laboratories they offer the added advantage of being more readily available than sheep erythrocytes.

‖ Serological activity of purified Vi antigen, *i.e.*, antigen titration, has been recorded elsewhere(9) and need not be repeated here. The quantity of Vi antigen suggested for sensitization of erythrocytes represents an amount considerably in excess of the "antigen titer". However, provided the antigen treated erythrocytes are washed, this excess is not inhibitory and provides a wide margin of safety in terms of sufficient antigen for maximum reactivity.

TABLE I. Correlation of Bacterial Agglutination, Quantitative Precipitin and Hemagglutination Determinations on Rabbit Vi Antisera.*

Hemagglutination titer	Agglutination titer	Hemagglutination titer / Agglutination titer	Antibody N/ml, μg	γ antibody N / Hemagglutination titer × 100
3210	680	4.7	215	6.6
6720	720	9.3	296	4.4
3210	400	8.0	115	3.5
960	140	6.8	28	2.9
840	80	10.5	16	1.9
940	150	6.3	33	3.5
600	150	4.0	27	4.5
3210	460	6.9	128	3.9
840	170	4.9	32	3.8
840	210	4.0	48	5.7
1680	170	9.8	46	2.7

* Rabbits were immunized with 1.5 mg of acetone killed and dried V form *S. typhosa*.

TABLE II. Results of Vi Hemagglutination Tests on Human Sera.

Sera	Normal: No history of typhoid or of typhoid immunization	Vaccinated: Received 3 inj. of heat killed typhoid vaccine	Vi immunized: Received single inj. of 40 μg purified Vi antigen	Typhoid carrier: Confirmed by isolation of *S. typhosa*
Total	252	251	220	20
No. positive Vi titer	4	18	213	19
% positive Vi titer	1.6	7.1	96.8	95

The results of these tests are given in Table II.

The 1.6% positive Vi reactors detected in normal individuals was in good agreement with the findings of 3.0% by Klein(5) and 2.9% by MacKenzie and Taylor(10). However, the incidence of Vi positives found in individuals immunized with heat killed typhoid vaccine (7.1%) was somewhat higher than the figure of 4.5% obtained by MacKenzie and Taylor. It generally has been assumed that the heat killed typhoid vaccine fails to elicit the production of Vi antibody. This probably is true if one considers the consistent failure to demonstrate Vi antibody by the use of the conventional bacterial agglutination tests. However, these results obtained with a more sensitive and specific test suggest that small, but measurable, amounts of Vi antibody occasionally are encountered in individuals immunized with heat killed-phenol preserved typhoid vaccine. This point requires further study.

Almost all individuals who received a single subcutaneous injection of 40 μg of purified Vi antigen and were bled 2 weeks later were found to possess antibody reactive with this antigen when their sera were tested by the hemagglutination technic(11). Eleven of the subjects possessed Vi antibody prior to immunization. However, all exhibited a significant rise in Vi titer following immunization.

Vi antibody was detected in the sera of 19 of 20 typhoid carriers tested by the hemagglutination procedure. The detailed breakdown of the figures on these sera and the comparison of titers with the Vi bacterial agglutination test in shown in Table III. Inspection of these data indicates that except for serums 19 and P-51, the bacterial agglutination and the hemagglutination tests were comparable in the detection of Vi antibody in the sera of typhoid carriers. However, the titers obtained with the hemagglutination test ranged up to 30 fold higher than the titers observed in the bacterial agglutination test. No explanation can be given at this time for these variations in Vi titers by the hemagglutination procedure. In contrast to this variation, it was observed that sera from individuals injected with purfied Vi antigen and tested by the two methods yielded values which were considerably more uniform. Table

TABLE III. Vi Antibody Determinations on Sera from Chronic Typhoid Carriers.

Bacteriologically proved duration of carrier state	Type*	Vi antibody titer (reciprocal) determined by: Bacterial agglutination test	Vi antibody titer (reciprocal) determined by: Hemagglutination test
30 mo	FT	40	30
29	UT	80	960
22	"	5	15
22	"	60	1920
24	"	80	240
23	"	5	Neg
23	"	40	120
22	"	80	240
21	"	30	240
22	"	160	2560
22	UC	20	60
23	UT	50	240
n.k.*	FT	Neg	7.5
n.k.	"	80	480
n.k.	"	20	120
n.k.	"	10	60
n.k.	"	20	60
10 yr	"	10	60
n.k.	"	Neg	Neg
4 mo	"	40	120

* Type FT, fecal typhoid; UT, urinary typhoid; UC, urinary paratyphoid C; n.k., not known.

Sera were kindly supplied by the late Surgeon Commander W. S. Miller, R. N., U. S. Naval Medical Research Unit No. 3, Cairo, Egypt; Dr. Daniel Widelock, Department of Health, City of New York and Dr. C. Gentzkow, Director, Bureau of Laboratories, Philadelphia, Pennsylvania.

TABLE IV. Vi Antibody Titers of Human Sera* Tested by the Hemagglutination and the "Standard" Bacterial Agglutination Procedures.

Vi titer by hemagglutination test	No. of sera tested	Reciprocal of "standard" Vi agglutination titer: 5	10	15	20	30	40	60	80
1:30	8	4	3	1					
1:60	6		1	4	1				
1:120	7				3	3	1		
1:240	6						1	3	2

* Individuals inj. subcut. with 40 μg of purified Vi antigen and bled 3 wk later.

IV gives the results of these comparative tests. Four groups of sera which had exhibited Vi titers of 1:30 to 1:240 by the hemagglutination procedure were tested by the conventional bacterial agglutination test. It will be noted that in every instance lower Vi titers were obtained and that these ranged from 1/3 to 1/6 of those recorded for the more sensitive hemagglutination test. However, the tests generally parallel one another with the hemagglutination test providing the more sensitive measure of Vi antibody.

The data presented are indicative, in a preliminary way, of what may be expected of the Vi hemagglutination technic for the estimation of Vi antibody. The most serious limitations of the Vi bacterial agglutination test have been the lack of a stable Vi agglutination suspension and the pronounced variability in the sensitivity of suspensions from lot to lot. British investigators, notably Felix and his associates at the Central Enteric Reference Laboratory and Bureau in London, have sought to standardize the Vi agglutination test by the use of a standard or reference Vi serum and issuance of a "standard" TVi suspension. In so doing, they have materially improved the performance of the test. However, little or no progress has been made in the development of a stable Vi antigen suspension and the product most widely used today (British) bears a 60-day expiration date.

On the other hand, the purified Vi antigen prepared from *E. coli* is a uniform product, is stable indefinitely and is reactive with Vi antibody only. The possibility of "non specific" reactions has been, as far as can be determined, eliminated. The potency of the product is so great that a single lot of 2 g, such as is presently prepared in these laboratories, would suffice for the sensitization of erythrocytes for 20 million tubes in the Vi hemagglutination test. Human type "O" erythrocytes are readily obtainable, may be preserved in Alsever's solution for periods of at least 30 days and give uniformly consistent results independent of their donor. The sensitization of erythrocytes with Vi antigen is a rapid and convenient procedure and requires only simple serological apparatus and technic.

Summary. 1. The preparation of a sensitive and specific Vi agglutination reagent by sensitization of human type O erythrocytes with Vi antigen isolated from *E. coli* has been described. 2. Vi antibody content of rabbit and human sera was determined by this method and the conventional bacterial agglutination test. The hemagglutination test provided a more sensitive measure of Vi antibody by a factor of 3 to 6 fold. Greater variability in Vi titers was encountered in testing the sera

of typhoid carriers; however, both methods were in good agreement in the detection of Vi antibody in these sera. The advantages inherent in the Vi hemagglutination procedure are discussed.

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Effects of p-Aminosalicylic Acid on Thyroid and Adrenal.* (20189)

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Recent clinical observations have indicated that goiter and, rarely, transient myxedema may occur in patients treated with p-aminosalicylate (PAS) over prolonged periods(1-3). Bavin(4) and Kjerulf-Jensen and Wolffbrandt (5) have shown that rats fed PAS developed cellular hyperplasia of the thyroid gland, and Rosenberg(6), in a study of a large group of peroxidase inhibitors, has reported diminished I^{131} uptake in rat thyroid glands after a single parenteral dose of PAS. Similarly, Hanngren (7) has reported a reduction in thyroid I^{131} uptake in 3 patients given a single intravenous injection of this compound.

Investigations over the past few years have suggested that salicylic acid and similar compounds exert a stimulatory effect on the pituitary-adrenal axis. Cronheim *et al.*(8) and Van Cauwenberge *et al.*(9) have shown that the administration of salicylic acid, aspirin and sodium salicylate to rats results in a marked eosinopenia and reduction of adrenal ascorbic acid and cholesterol concentrations. Whereas Cronheim did not observe these changes in the case of PAS, Van Cauwenberge reported a fall in circulating eosinophiles and ascorbic acid and cholesterol concentrations following a single parenteral dose of this compound. Bavin(4) on the other hand, found no eosinopenic response in mice following PAS administration. Because of these conflicting results and the widespread use of prolonged PAS therapy in tuberculosis, it was felt that further evaluation of the effects of prolonged administration of PAS on rat thyroid and adrenal functions was indicated.

Experimental. Male Sprague-Dawley rats weighing approximately 180 g were used. They were kept at a constant room temperature of 80°F, and were fed standard Purina chow diet, modified as indicated. All rats were weighed at the beginning and at the end of the experiment. The animals were divided into 4 groups of 10 rats each. One group of rats was fed 1% powdered PAS in the diet for 2 weeks and control and comparison groups were given a) stock diet alone, b) stock diet containing 0.2% powdered propylthiouracil and c) stock diet plus daily subcutaneous injections of 1 mg of ACTH gel.† At the end of the 2-week period, a tracer dose of I^{131} (6 μc) was injected intraperitoneally into all animals and at the same time food was removed from the cages. Four hours after in-

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