

duration of anesthetic and hypnotic action than the 2 non-methylated compounds. When one-half of AD<sub>50</sub> is intravenously injected at hourly intervals, all 4 N-methyl-substituted barbiturates show less cumulative action than sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and still less than thiopental. In anesthetized cats, 2 of the N-methyl barbiturates produce less hiccup, sneezing, and coughing than sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and thiopental, while the 2 others are free from such effects. Like all barbiturates, the 4 N-methylated compounds, when injected intravenously in anesthetized dogs, lower the blood pressure and depress respiration. They do not inhibit the vagal response in these preparations. They induce slight hypothermia and tachycardia in dogs following an AD<sub>50</sub> of each product.

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### *Escherichia coli* Hemagglutinin Response of Adult Volunteers to Ingested *E. coli* 055 B<sub>5</sub>. (20070)

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The concept of the possible etiological role of certain serogroups (0111, 055 and 026) of *Escherichia coli* in epidemic diarrhea of the newborn until recently was based largely on epidemiological data, namely, the presence in the feces of a single serogroup in a high percentage of infants with the disease in contrast to its rare occurrence in healthy infants and children suffering from other maladies. Furthermore, it is noteworthy that the particular antigenic serogroup of *E. coli* is frequently the predominant coliform organism in the feces of

affected patients. Recently, additional support was gained from feeding studies, indicating that the administration of *E. coli* 0111 and 055 to adult volunteers resulted in the development of mild gastrointestinal upset to severe gastroenteritis in the majority of volunteers; in contrast, ingestion of a "normal" strain of *E. coli* was not followed by ill effects (1-3). Furthermore, ingestion of *E. coli* 0111 by an infant resulted in severe diarrheal disease, whereas a "normal" strain in like amounts was well tolerated (4). Similar ob-

*Escherichia coli* HEMAGGLUTININ RESPONSETABLE I. *E. coli* Hemagglutinin Titers of Adult Volunteers Following Ingestion of *E. coli*.

| Group and No.<br>of volunteers | Ingested microorganism                    | Homologous <i>E. coli</i> hemagglutinin<br>titer in prefeeding<br>serum specimen | Incr. in titer<br>of postfeeding<br>serum specimen<br>(fold) |
|--------------------------------|-------------------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------|
| I A 8                          | <i>E. coli</i> 055 (16000*)               | —                                                                                | >4                                                           |
|                                |                                           | 1:100                                                                            | 16                                                           |
|                                |                                           | 1:50                                                                             | 4                                                            |
|                                |                                           | 1:100                                                                            | 16                                                           |
|                                |                                           | 1:50                                                                             | 8                                                            |
|                                |                                           | —                                                                                | >32                                                          |
|                                |                                           | —                                                                                | >8                                                           |
|                                |                                           | 1:50                                                                             | 8                                                            |
|                                |                                           | "                                                                                | 16                                                           |
|                                |                                           | 1:100                                                                            | 4                                                            |
| B 7                            | <i>E. coli</i> 055 (5330*)                | 1:50                                                                             | 8                                                            |
|                                |                                           | 1:100                                                                            | 4                                                            |
|                                |                                           | "                                                                                | 8                                                            |
|                                |                                           | —                                                                                | >16                                                          |
|                                |                                           | 1:100                                                                            | 8                                                            |
|                                |                                           | "                                                                                | 0                                                            |
| C 8                            | Control (milk only)                       | 1:50                                                                             | 0                                                            |
|                                |                                           | 1:200                                                                            | 0                                                            |
|                                |                                           | 1:100                                                                            | 0                                                            |
|                                |                                           | —                                                                                | 0                                                            |
|                                |                                           | 1:200                                                                            | 0                                                            |
|                                |                                           | 1:50                                                                             | 0                                                            |
| II A 7                         | <i>E. coli</i> 055 (1725*)                | "                                                                                | 0                                                            |
|                                |                                           | 1:100                                                                            | 2                                                            |
|                                |                                           | "                                                                                | 4                                                            |
|                                |                                           | —                                                                                | >8                                                           |
|                                |                                           | 1:100                                                                            | 8                                                            |
|                                |                                           | 1:400                                                                            | 2                                                            |
|                                |                                           | 1:50                                                                             | 0                                                            |
|                                |                                           | 1:100                                                                            | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | —                                                                                | >8                                                           |
| B 8                            | <i>E. coli</i> 055 (143*)                 | —                                                                                | 0                                                            |
|                                |                                           | 1:50                                                                             | 4                                                            |
|                                |                                           | —                                                                                | >8                                                           |
|                                |                                           | —                                                                                | 0                                                            |
|                                |                                           | —                                                                                | >8                                                           |
|                                |                                           | —                                                                                | >8                                                           |
| C 8                            | Control (milk only)                       | 1:100                                                                            | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | 1:200                                                                            | 0                                                            |
|                                |                                           | 1:100                                                                            | 0                                                            |
|                                |                                           | 1:200                                                                            | 0                                                            |
| III A 8                        | <i>E. coli</i> 055 (16275†)               | 1:100                                                                            | 0                                                            |
|                                |                                           | 1:400                                                                            | 0                                                            |
|                                |                                           | 1:50                                                                             | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | —                                                                                | 0                                                            |
|                                |                                           | —                                                                                | 0                                                            |
|                                |                                           | 1:100                                                                            | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
| B 8                            | "Normal" <i>E. coli</i> #5421<br>(15000*) | 1:100                                                                            | 0                                                            |
|                                |                                           | "                                                                                | 0                                                            |
|                                |                                           | 1:200                                                                            | 0                                                            |
|                                |                                           | 1:100                                                                            | 0                                                            |
|                                |                                           | —                                                                                | 0                                                            |

TABLE I (Cont.).

| Group and No. of volunteers | Ingested microorganism                                | Homologous <i>E. coli</i> hemagglutinin titer in prefeeding serum specimen | Incr. in titer of postfeeding serum specimen (fold) |
|-----------------------------|-------------------------------------------------------|----------------------------------------------------------------------------|-----------------------------------------------------|
| C 8                         | Control for "normal" <i>E. coli</i> #5421 (milk only) | 1:100                                                                      | 0                                                   |
|                             |                                                       | "                                                                          | 0                                                   |
|                             |                                                       | "                                                                          | 0                                                   |
|                             |                                                       | 1:50                                                                       | 0                                                   |
|                             |                                                       | "                                                                          | 0                                                   |
|                             |                                                       | "                                                                          | 0                                                   |
|                             |                                                       | 1:100                                                                      | 0                                                   |

• Million live organisms.

† Million killed organisms.

— = No hemagglutination with serum in dilution of 1:25 or higher.

servations were made on 2 additional infants(5).

The assumption of the etiological role of these serogroups of *E. coli* in epidemic diarrhea in infants would be strengthened, were it possible to demonstrate a specific antibody response during or following the illness. In the majority of infants suffering from gastroenteritis associated with these serogroups of *E. coli*, however, this has not been possible. The failure to demonstrate the development of homologous antibodies in these cases may be due to either lack of antibody response (immunogenic immaturity) or inadequate sensitivity of the conventional bacterial agglutination test. With the latter possibility in mind, a hemagglutination test was developed for the demonstration of antibodies to the serogroups of *E. coli* under discussion(6,7). It was shown that the group-specific antigens of *E. coli* 0111, 055 and 026 are adsorbed on red blood cells of various animal species; these modified erythrocytes then become specifically agglutinable by the homologous group-specific *E. coli* antisera obtained from rabbits. In order to determine whether this indirect bacterial hemagglutination test may be useful for the demonstration of *E. coli* antibodies of man, the *E. coli* hemagglutinin response of adult volunteers to ingested *E. coli* 055 B<sub>5</sub> was undertaken. The results of this investigation are summarized in this report.

**Material and methods.** All data connected with the feeding study have been described in detail elsewhere(3). It suffices therefore to briefly describe the technic used for the demonstration of the *E. coli* hemagglutinins. The

pre- and post-feeding (23 days apart) sera, designated by code numbers, were examined by two of us (E.N. and N.J.Z.) and only after the hemagglutination tests had been completed, was information made available as to which sera were obtained from volunteers who had ingested *E. coli* or had served as controls. Human red blood cells of blood group O were washed 3 times in physiological saline solution. The elimination of human plasma by washing was necessary, since it was shown previously (8) that human plasma interferes with the modification of red blood cells by *E. coli* antigen. The red blood cells (2.5%) were treated with boiled suspensions of the strains of *E. coli* 055 B<sub>5</sub> or control strain No. 5421 which had been used in the feeding studies, according to the method described in detail previously(7). The modified cells were then washed 3 times and mixed with equal amounts (0.2 ml) of the various sera in serial dilutions. The mixtures were incubated at 37°C for 30 minutes and centrifuged at 2000 r.p.m. for one minute. The resulting hemagglutination was read grossly. All tests were carried out 3 times; the titration end points were repeatable within a 2-fold dilution range.

**Results.** The results of this study on the *E. coli* hemagglutinin response of adult volunteers to ingested *E. coli* are summarized in Table I. It is evident that all 15 volunteers who ingested large numbers of viable *E. coli* 055 and 10 out of 15 individuals who received smaller numbers of this microorganism responded with an increase in titer of homologous *E. coli* hemagglutinins. The specificity of this antibody response is substantiated by

the observation that there was no increase in the titers of heterologous *E. coli* 0111 and 026 hemagglutinins. Furthermore, none of the volunteers who ingested killed *E. coli* 055 organisms showed an increase in the titer of homologous antibodies, nor did the individuals who received milk without *E. coli* and served as controls. It is interesting to note also that the homologous *E. coli* hemagglutinin titers did not rise in volunteers following ingestion of living organisms of a "normal" strain of *E. coli*. Finally, it can be seen from Table I that *E. coli* hemagglutinins were present in some of the volunteers prior to the feeding of the bacterial suspensions.

**Discussion.** The present study was undertaken as a first attempt to determine the potential usefulness of the bacterial hemagglutination test for the demonstration of antibodies to *E. coli* serogroups 0111, 055 and 026. These serogroups are of current interest, since they are associated with epidemic diarrhea of the newborn and sporadic diarrheal disease of infants.

It is clear that the hemagglutination technic proved to be useful for the demonstration of the increase in *E. coli* 055 hemagglutinins in sera of adult volunteers who ingested live 055 organisms and that no such increase was noted in the various control groups. Obviously, any new method must be compared, if possible, with an accepted conventional technic. A comparison of the results of the *E. coli* hemagglutinin titrations, reported here, and of the conventional bacterial agglutination tests, recorded in detail elsewhere(3), leads to the following conclusions. 1) All 15 volunteers who ingested large numbers of viable *E. coli* 055 responded with an increase in the titers of *E. coli* 055 hemagglutinins; 13 of them also developed bacterial O agglutinins, 2 did not. Among the individuals who ingested smaller numbers of viable *E. coli* 055, 10 responded with a rise in *E. coli* 055 hemagglutinins and developed bacterial O agglutinins. The same 5 volunteers who did not respond with a rise in hemagglutinin titers also did not develop bacterial O agglutinins. Thus, there is a good correlation between the two tests. There is also complete agreement with respect to the results obtained in the control

groups, including those who did not receive *E. coli* and those that ingested killed *E. coli* 055 organisms or living organisms of a "normal" strain. All these volunteers failed to show a rise in homologous *E. coli* hemagglutinins and bacterial O agglutinins could not be detected in the postfeeding specimens. 2) The hemagglutinin titers in the majority of postfeeding specimens of the volunteers who ingested live *E. coli* 055 organisms were approximately 5 to 20 times higher than the bacterial O agglutinin titers, indicating greater sensitivity of the former method. 3) Whereas bacterial O agglutinins could not be detected in the prefeeding specimens of any of the volunteers, the hemagglutination technic revealed the presence of *E. coli* 055 hemagglutinins in the same specimens of many of the subjects. 4) The observation that volunteers developed specific bacterial O agglutinins but not B agglutinins lends additional support to the previously made assumption(4) that the *E. coli* hemagglutination test detects O rather than B antibodies. These observations on the *E. coli* hemagglutinin response of adult volunteers to ingested live *E. coli* 055 organisms are sufficiently promising to warrant studies on infants with diarrheal disease associated with certain serogroups of *E. coli*.

**Summary.** The *E. coli* hemagglutinin response of adult volunteers to ingestion of *E. coli* 055 and a "normal" strain of *E. coli*, respectively, was determined. 1. Increase in *E. coli* 055 hemagglutinin titers was observed in all subjects following administration of living organisms in large numbers and in two-thirds of individuals who received smaller numbers. 2. None of the controls who received milk without *E. coli* showed such an antibody response, nor did the volunteers who ingested large numbers of killed 055 organisms or viable bacteria of a "normal" strain of *E. coli*. 3. Good correlation was found between increases of *E. coli* 055 hemagglutinin titers and the appearance of bacterial O agglutinins. 4. The *E. coli* hemagglutination test proved to be more sensitive than the conventional bacterial agglutination test, inasmuch as the former method with the postfeeding serum specimens yielded antibody titers which were from 5 to 20 times higher and

detected antibodies in prefeeding serum specimens which were not demonstrated by the latter method.

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## Ultrastructure of Two Invertebrate Synapses.\* (20071)

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The structure of the synapse has been the subject of numerous light microscope studies since the days of Cajal. These investigations have revealed many significant facts with regard to the grosser microscopic structural elements. However, the capriciousness with which synaptic structures appear in histological preparations has generally necessitated the use of one of the silver technics for their demonstration. These technics, while exhibiting quite well the grosser structures, appear to be subject to many severe technical limitations. They could hardly be expected to yield many significant facts about the finer structural elements intervening between pre- and post-synaptic axoplasm. Indeed, the resolution of the light microscope itself limits such studies. However, some speculation has appeared about the possible intervention of mitochondria, connective tissue, granules, or even about axoplasmic or neurofibrillar continuity(1).

The high resolution obtained by the electron microscope (EM) gives promise of a more fruitful approach. Accordingly, an investigation with this instrument has been instituted. The desire to conduct correlated physiological studies and the technical limitations thereby imposed led to the choice of an invertebrate synapse for the initial work. This paper constitutes a preliminary report of these studies.

The structure of several invertebrate synapses has been the subject of light microscope investigations(2-5) and related physiological studies(6-8) have rendered plausible their division into two general types. One type is physiologically polarized, introduces an appreciable conduction delay, fatigues and responds to drugs reversibly(6). A second type is physiologically unpolarized and seems to consist of a transverse septum in certain giant fibers(6-7). Structurally both types can be shown to be variants of the same general plan of side-to-side apposition of the axons of unipolar neurones.

*Materials and methods.* The physiologically polarized synapse between the median giant fibers and the third motor root giant fibers of the abdominal segmental ganglia of the crayfish (*Cambarus clarkii* and *Cambarus virilis*)(6) has been the primary subject of these studies. The polarized synapse between the second order giant fiber and the third

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