above the pressure inversion point among patients studied to date has been over 9 cm. However, it should be emphasized that variations in height of reflux in the same individual, tested at different times, are great.

Results. Fig. 1 illustrates a normal record. The tracing was started with sensing elements placed 3 cm below the hiatus. Thereafter, the tube was withdrawn one cm at a time. So long as it remained in the stomach, pH remained low and intraluminal inspiratory deflections were upward or positive. However, as pressure inversion point was passed, deflections became negative and pH changed rapidly toward neutrality. It is also apparent that intraesophageal pressures are lower than intragastric pressures, a finding that has been noted by all workers.

Fig. 2 illustrates acid reflux. The pH remained strongly acid for several cm above pressure inversion point, and only as the sensing device was removed far above the hiatus did it return to neutrality. A dry swallow had little effect on pH. In contrast, swallowing water (not shown) usually induced a rapid but transitory rise in pH which persisted for 5 to 30 seconds. On the recording, to right of pressure inversion point, there is a distinct increase in intraluminal pressure. Fyke, Code, and Schlegel(4) have recently described this phenomenon which they believe represents an intrinsic gastroesophageal sphincter. Similar zones have been noted in over half of our tracings, but no correlation between their existence and the presence or absence of acid regurgitation could be found.

To date, 90 subjects have been tested for evidence of gastroesophageal reflux, and acid regurgitation has been demonstrated in 54. Of this latter group, all but 2 had clinical, endoscopic, or radiographic evidence of esophageal inflammation. In contrast, no subject with normal records had either symptoms or findings referable to the esophagus. These findings lend support to the concept that regurgitation of gastric contents plays a dominant role in pathogenesis of esophagitis. In addition, it is felt that this new method will provide further information about the mechanisms whereby gastric contents are normally prevented from entering the esophagus, and the nature of the defect which permits such regurgitation.

Summary. Simultaneous intraluminal pressure and pH recordings at various levels within the esophagus and stomach were performed in 90 subjects. Evidence of esophageal inflammation was present in 52 of 54 individuals manifesting gastroesophageal acid regurgitation but was absent in all of those with normal records. A zone of increased pressure at the diaphragmatic hiatus could not be correlated with presence or absence of acid reflux. The evidence suggests that reflux of gastric contents is important in pathogenesis of esophagitis.

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Received February 28, 1958. P.S.E.B.M., 1958, v98.

Metabolic Inhibition Test for Determination of Antibodies to Group B Coxsackie Viruses.* (23999)

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The metabolic inhibition test of Salk, Youngner and Ward(1) has found extensive use in assay of poliovirus neutralizing antibodies. Several changes in technic of this

* Technical assistance was rendered by H. D. Huff and O. W. Dandridge.

test have been suggested(2-6), and recently Archetti *et al.*(7) have reported its use in the study of ECHO viruses(8). This paper outlines modifications of previously described procedures which permit determination of neutralizing antibodies for Group B Coxsackie viruses. The test has been evaluated by its performance in serodiagnosis of 55 cases of human infection with Group B Coxsackie viruses. Metabolic inhibition test depends upon appearance of demonstrable acidity as an indication of tissue culture cell viability. During these studies on Coxsackie viruses, Eagle's KB cell strain(9) was found preferable to monkey kidney cells as the host system.

Materials. Sera employed in evaluation of the test were obtained from 55 patients proved by appropriate studies to have Group B Coxsackie virus infections. In 41 of these cases the clinical syndrome was aseptic meningitis or "non-paralytic poliomyelitis." Most of remaining cases were diagnosed clinically as pleurodynia. The following materials were obtained from Microbiological Associates, Bethesda, Md.: 1) Medium A, lactalbumin hydrolysate and calf serum in Hanks' basic salt solution; 2) trypsinized first passage monkey kidney cells suspended in Medium A at concentration of 1,200,000 cells/ml; 3) trypsinized KB cells in Medium A supplemented with 10% horse serum, used at concentration of 100,000 cells/ml; 4) monkey kidney and KB cell stationary tube cultures; 5) Coxsackie virus strains B1, B2, B4 and B5, and 6) rabbit antisera to all 5 Coxsackie viruses. The Coxsackie B3 virus used was isolated in this laboratory from patient with pleurodynia. "Drakol," a heavy white mineral oil, was obtained from Pennsylvania Refining Co., Butler, Pa. Medium A with addition of 100 units of penicillin and 100 μg of streptomycin/ml was used as basic diluent for all tests. Stock virus suspensions were prepared from infected monkey kidney and KB cell cultures and maintained at −70°C between tests. *Methods.* The metabolic inhibition test as performed in this laboratory, utilizes sterile unstoppered Wasserman tubes in 90-hole wire serology racks. All sera are inactivated at 56°C for 30 minutes. To each of 5 replicate 2-fold dilutions of serum (1:10 through 1:640 distributed in 0.25 ml amounts), an equal amount of one of the 5 virus types is added. Virus suspensions are diluted to contain approximately 300 ID_{50}/0.25 ml. After incubation of the serum-virus mixture at room temperature for 1 hour, the KB (or monkey kidney) cell suspension is added in 0.25 ml amount to each tube. Controls for each test include: (1) tubes corresponding to 2 lowest dilutions of each serum incubated with cells but without virus to detect serum toxicity; (2) titration of standard rabbit antisera for each virus; and (3) serial 10-fold titrations of each stock virus, 5 tubes/dilution. Virus titer is calculated by the Reed-Muench formula(10). To obtain an approximation of metabolic activity of cells, 4 replicate 2-fold dilutions of the cell suspension are incubated without addition of virus or serum. Finally, each tube's contents are "sealed" with 0.5 ml of "Drakol." To maintain sterility each rack of tubes is covered with sterile aluminum foil at all times except during addition of reagents. Tests are incubated at 35°C until color changes are complete. Readings are usually taken after 5 and 7 or 6 and 8 days of incubation. Growth of virus is signaled by destruction of cells and preservation of red or alkaline color of the phenol red indicator in the medium. Absence of virus, or protection of cells from existing virus by antibody is made apparent by change in color of the indicator to yellow or acid form. Any detectable reddish or reddish-orange tint is considered indicative of virus action; when KB cells are used equivocal tubes can be jarred and observed macroscopically to determine whether cells are attached to the glass (alive) or free in the medium (killed).

Results. Virus titers: Table I summarizes

TABLE I. Range of Stock Virus Titers in KB Cell Metabolic Inhibition Tests.

Virus type	No. titrations	Titer variation*
B-1	13	7.8 to 8.5
2	13	6.7 7.7
3	13	6.8 7.8
4	15	6.7 7.7
5	13	6.9 8.1

* Expressed as the negative log ID_{50}/.25 ml.

TABLE II. Titer of Rabbit Antiserum for Type B-1 Coxsackie Virus in Tests Using KB Cells.

Date of test	Virus dose*	Serum titer†
4/25/57	2.6	1280
5/ 9	2.8	"
6/ 6	2.5	2560
13	2.5	"
27	2.5	"
7/11	2.6	"
25	2.8	"
8/22	3.0	"
29	2.7	"
9/12	2.5	"
10/ 3	2.5	"
7	2.4	"
11/ 7	2.2	1280

* Expressed as log index of test dose.
† Expressed as reciprocal of highest serum dilution causing neutralization.

data with respect to the reproducibility of the KB cell test. This Table shows range of titers obtained on serial testing of identical stock virus suspensions. The results obtained on 5 typical stock viruses, tested 13 to 15 times, are shown. In general, the titers from same virus did not vary by more than 1 log unit but in certain instances might exceed this slightly.

In addition, during the investigation it was noted that when KB cells were used the virus titers were consistently higher and more readily reproducible than when monkey kidney cell suspensions were used.

Serum titers. Table II displays the control rabbit antiserum titers in all tests performed to date with KB cells where B-1 Coxsackie virus dose fell between $10^{2.0}$ and $10^{3.0}$ ID_{50}. Similar results have been obtained with control antisera for the other 4 serotypes also, regardless of type of cell suspension used. At a given virus dose, serum titers are quite reproducible from test to test.

Table III shows the relation of a typical human convalescent serum titer to a varying virus dose in tests employing different cell types. While the same stock virus was used in both tests, it should be noted that only a limited comparison of serum titers was possible between the 2 systems, since the highest virus dose obtained in monkey kidney cells was only $10^{3.0}$ ID_{50}.

In Table IV the ability to demonstrate a significant (4-fold or greater) antibody increase with the metabolic inhibition test is correlated with the phase of disease during which the initial serum was drawn. It is apparent that the diagnostic efficiency of the test was impaired when the earliest serum available was drawn after the first week of disease.

TABLE III. Effect of Coxsackie B-3 Virus Dose on Neutralizing Titer of a Human Convalescent Serum.

Test performed with KB cells		Test performed with monkey kidney cells	
Virus dose (log_{10} ID_{50} per .25 ml)	Serum titer*	Virus dose (log_{10} ID_{50} per .25 ml)	Serum titer*
.7	10,240		
1.3	5,120	1.5	>1,280
1.7	1,280	2.0	640
2.3	640	2.5	320
2.7	320	3.0	320
3.3	320		
3.7	160		
4.3	160		

* Expressed as reciprocal of highest serum dilution causing neutralization.

TABLE IV. Coxsackie Virus Serodiagnosis: Importance of Early Collection of Acute Phase Serum.

Days after onset earliest serum drawn	No. of serum pairs	% successful diagnoses
0- 4	19	84
5- 7	9	78
8-14	26	46
14 or more	15	20

In 40 of the 55 cases tested the specific infecting Group B Coxsackie virus serotype was apparent, either because the agent had been isolated from stool, throat washings, or spinal fluid, or because there was antibody present for only one of the 5 serotypes. Thirty-three of these cases were examined for neutralizing antibodies by 3 separate technics, a metabolic inhibition test with monkey kidney cells, a metabolic inhibition test with KB cells, and a microscopic cytopathogenicity-neutralization test(11) using monkey kidney tissue culture tubes inoculated with a standard amount (approximately 300 ID_{50}) of the infecting virus. Results of all 3 methods were comparable, and neutralizing antibody increases were demonstrable with all 3 methods in 80-90% of the cases where the acute phase serum was drawn within 4 days of the onset of illness.

Heterologous antibody. As expected, the occurrence of antibody to other than the infecting Coxsackie virus serotype correlated with the age of the patient. Of the 6 preschool children tested, all had antibody to one type only. Heterologous antibody was present in only 3 of 10 cases aged 6 to 18 years in contrast to its presence in 16 of 24 patients over the age of 18. When heterologous antibody was present, a significant increase during the course of the infection occurred in 10 of the 24 older patients. In those cases with a significant increase in antibody to more than one serotype, from which virus could not be isolated, the specific infecting virus type could not be determined.

Discussion. The metabolic-inhibition test described has proved useful for the diagnosis of Group B Coxsackie virus infections. Laboratories already engaged in the study of poliomyelitis by this method will find no difficulty in adapting their procedure to this end. Eagle's KB cells were deemed more satisfactory than monkey kidney cells for this test on the basis of the higher and more reproducible virus titers obtained; either cell line gave satisfactory results in practice, however.

Other tests for Group B Coxsackie virus antibodies exist. A neutralization test in tissue culture tubes employing the microscopic observation of cytopathogenicity has been described(11). This procedure was used to corroborate the metabolic-inhibition test results obtained for this paper, but it is too time-consuming for routine application in the examination of large numbers of sera. Complement-fixation tests using antigens derived from infected suckling mice have also been described for this family of viruses(12-16). It is important to recognize, however, that problems relating to the occurrence of heterologous antibody increases may appear with this procedure(17) as well as with the neutralization test.

Data have been presented on the effect of the virus dose on serum titers. The logarithm of the serum titer is in inverse linear relation to the log dose but the data are insufficient to allow one to state categorically that the curve is straight line. Further observations will be necessary to establish the precise relationship.

Studies of normal human populations(18) have demonstrated that antibodies for Coxsackie viruses increase in prevalence with age. It is notable that in the present small series no children under 6 years of age possessed antibody for heterologous group B Coxsackie virus serotypes. Older children and adults frequently showed heterologous antibody and heterologous antibody increases. These data, like those obtained from the study of complement-fixing antibodies(17), support the hypothesis that increases of heterologous antibody for Coxsackie group B strains occur only in patients who have previously been infected with the heterologous serotype. There is no evidence that non-specificity plays a part in this phenomenon.

Summary. A metabolic inhibition test for detection of antibodies to 5 group B Coxsackie virus serotypes has been described. A serologic diagnosis could be made in as high as 84% of cases. Persons possessing neutralizing antibodies from previous group B Coxsackie virus infections frequently showed heterologous as well as homologous antibody increases.

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Received November 21, 1957. P.S.E.B.M., 1958, v98.

Colloidal Zirconyl Phosphate-P-32. Preparation and Tissue Distribution Following I. V. Administration. (24000)

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Radiocolloid therapy was first reported by Hahn and co-workers(1). Of the many radiocolloids employed since, radiogold and chromic phosphate-P-32 have been most widely accepted. Chromic phosphate is advantageous because of its pure, high-energy β-emanation as contrasted to the undesirable hard γ-ray from Au-198. Preparation of colloidal chromic phosphate has been achieved but proved time-consuming(2). Colloidal zirconyl phosphate-P-32, first described by Mayer and Morton(3), exhibits low solubility even in concentrated acids and is easy to prepare. However, as reported, the product is unstable and precipitates from neutral media.

This investigation was undertaken 1) to find a means of dispersing and stabilizing zirconyl phosphate colloid, 2) to investigate its fate in man and experimental animals, 3) to establish particle size, and 4) to determine its rate of excretion and radiation toxicity in experimental animals.

Methods. Preparation. By addition of zirconyl ion to aqueous orthophosphate a milk-white colloid is formed. In neutral media this sol is unstable and precipitates unless a suitable dispersant is added. Of the many substances tested for colloid stabilizing action, only 3 have merit: dextran, soluble starch, and glucose. Two of these, glucose and dextran, have been used routinely in the preparation of injectable zirconyl phosphate-P-32 sols.

Dextran-stabilized zirconyl phosphate: 2 ml .1 M K_2HPO_4 was equilibrated with the desired amount of radiophosphorus† (100 mc P-32 in not more than 25 ml) for 10 minutes.

A drop of phenolphthalein indicator and 64 ml 6% dextran‡ were added, and the solution neutralized with 1 N NaOH. After 5 minutes, 7 ml .1 N $ZrOCl_2$ was added dropwise with simultaneous adjustment of pH with NaOH. Volume made to 100 ml with saline. The colloid was then autoclaved for 20 minutes at 15 p.s.i. *Starch-stabilized zirconyl phosphate*: This procedure was similar to that for dextran-stabilized colloid. A 2.5% solution of soluble starch (Merck, according to Lintner) in .9% saline was centrifuged at 5,000 rpm for 20 minutes. Sixty ml of supernatant was employed for 100 ml of colloid. *Glucose-stabilized zirconyl phosphate*: 1) 2 ml .1 M K_2HPO_4 was equilibrated with 100 mc H_3PO_4-P-32 for 10 minutes. 2) 9 ml .1 M $ZrOCl_2$ was mixed with 50 ml 50% dextrose solution. 3) The equilibrium mixture, 1), was added slowly and with constant stirring to 2), followed by 3 rinsings of the container with 50% dextrose. 4) After 10 minutes the solution was titrated with 1 N NaOH to phenolphthalein end point. Volume was adjusted to 100 ml with distilled water, container sealed, and product pasteurized for 1 hour at 65°C. *Fate in Experimental Animals.* Studies were made of tissue localization of zir-

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† Available as H_3PO_4-P-32, carrier-free, from Oak Ridge National Laboratories, Oak Ridge, Tenn.

‡ *Plavoflex*, 6% aqueous, Wyeth Labs.