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A Practical Method for Routine Blood Cultures in Brucellosis.

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Various methods have been used in our laboratory for the isolation of Brucella organisms from the blood of patients. Only a minor proportion of the cultures are positive since we are dealing with unselected cases which include patients with other diseases; and because on the brucellosis cases serial blood cultures are performed during the course of the disease. The time-consuming bacteriological procedure and the waste of material entailed in such a large percentage of negative cultures made it advisable to look for other dependable methods for the detection of positive blood cultures avoiding troublesome secondary bacteriological transplants. The method as devised also minimizes the handling of possibly infective material, since such handling has proved dangerous to the technician who sooner or later becomes infected with the melitensis strain. Various procedures were investigated and the one described below has given the most satisfactory results.

The medium used is prepared with bactotryptose. The formula follows:

Bactotryptose	2.0 g
Sodium chloride	0.5 "
Sodium citrate	0.5 "
Agar	3.0 "
Distilled water	100 ml

Fifteen ml of the medium are placed in 100 ml flat-sided, rectangular bottles. The mouth of the bottle (about 1¼ cm in diameter) is covered with thick paper and sterilized at 15 lb for 15 minutes. The bottles are placed on their sides so that the agar sets on one of the narrow sidewalls forming an even, transparent layer. The paper is then lifted, using aseptic technic, and 10 cc of bactotryptose broth are added. The flask is then closed with sterile rubber stoppers and the bottle again covered with the same paper cap. The broth contains 2% bactotryptose and 2% sodium citrate and is sterilized in separate containers at 15 lb for 15 minutes. The air within the bottles can be replaced by suitable mixtures of CO_2 and air by any mechanical device which avoids contamination of the double medium.

Before using this double medium, tests for sterility are made at 37°C for 3 or 4 days. The agar surface is wet by the broth at 24-hour intervals.

The double medium may be inoculated with 10 cc of blood, the patient usually being bled with a 20 cc autoclave-sterilized syringe. The needle is inserted through the rubber stopper after the careful removal of the paper cap. The mixture of blood and broth is spread over the agar layer for a moment and the bottle incubated in a vertical position at 36°C. Every other day the broth is allowed to flow over the agar layer and the bottle replaced in the incubator, again in a vertical position.

Results. When the agar layer shows colonies after 24-48 hours, it is likely that the culture has been contaminated. When the colonies appear 24 or 48 hours after the second inoculation, the cultivated organism has usually been found to be a Salmonella, less frequently Staphylococcus or Streptococcus, but may be Brucella. Positive cultures of Brucella are detected more frequently after the third agar inoculation, that is on the sixth day of cultivation. After 20 days the negative cultures may safely be discarded. The number of colonies and time of their appearance on the agar layer vary from one case to another and serve as a good indication of the intensity of the bacteriemia. Serial cultures performed twice a month have been of assistance in roughly estimating the course of the bacteriemia. During the acute phase of the disease the early appearance of innumerable colonies is the usual finding. In the chronic phase when positive, the culture shows few or sometimes a single colony in 10 or more days.

The colonies of Brucella are clearly seen through the agar layer and are easily transferred for further study. With some experience one may differentiate them from contaminants, Salmonella and other organisms.

We consider that the ease with which posi-

tive cultures are detected and the reduction of bacteriological manipulation and material, plus the increased safety to the technician, compensate for the trouble taken in the preparation of the double medium. Furthermore, the technician in charge of identification of the organism receives only positive cultures and need not be warned about careless handling of the material. The possibilities of accidental infection are thereby reduced. The increase in the proportionate amounts of agar in the solid medium is necessary in order to prevent the deterioration of the agar layer. The rather high citrate content is intended not only to prevent clotting of the blood but also to reduce the phagocytic activity of polymorphonuclear leucocytes to a minimum. It is thought that the ease with which the Brucella colonies appear on the agar layer is due at least in part to the absence of plasma, thus reducing the bactericidal effect.

The 100 cc bottles have been found suitable for inoculation with 100 ml of blood. However, 200 or even 500 ml bottles may be used if one desires to culture larger amounts of blood.

Summary. A double medium for blood cultivation in brucellosis is described. The method saves time and material by avoiding transfers and affords increased safety against accidental infection to the personnel.

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Hematologic Effects of Pteroylglutamic Acid (Folic Acid) in Man.*

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Pteroylglutamic acid, commonly known as folic acid or *L. casei* factor, has been shown by several groups of investigators to be an effective hematopoietic substance in human macrocytic anemia.[1,2,3] Since 1945 when synthetic pteroylglutamic acid became available, 23 patients with macrocytic anemia have been treated with this material in our clinic. The findings obtained in 15 patients during the first few months of therapy have been reported.[4,5,6] Five patients have now been followed for approximately one year and 2 additional patients for over 6 months. In each instance, the clinical and hematological improvement which followed the administration of pteroylglutamic acid has been maintained.

Seventeen of the 23 patients studied were benefited by therapy. Hematologic findings at the initial and most recent visits to the clinic, and the amount and method of administration of pteroylglutamic acid are given in Table I. In all patients with pernicious anemia and nutritional macrocytic anemia there was a marked increase in the erythrocyte count, in hemoglobin and in the volume of packed red blood cells. In patients with sprue the blood picture was affected in only 3 of 5 cases. However, each patient improved in other respects including relief of glossitis and diarrhea, gain in weight, better absorption of nutrients from the intestinal tract as indicated by the glucose tolerance test, and diminution in steatorrhea. In one patient with cirrhosis of the liver there was a slight increase in the blood count after pteroylglutamic acid was administered while in 5 patients with macrocytic anemia of varied

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