

**Summary.** Thyroid gland of guinea pig was subjected to low temperatures ( $-70^{\circ}$ , or  $-190^{\circ}\text{C}$ ) and subsequently autotransplanted. In some instances pituitary thyrotropin was administered before or after transplantation in order to increase the growth potential of the transplant and overcome the injurious effects incident to transferring the tissue. There was one successful transplantation of thyroid gland in 12 attempts after exposure at  $-70^{\circ}\text{C}$  and one parathyroid transplant.

Eight viable thyroid grafts in 12 attempts were obtained after immersion at  $-190^{\circ}\text{C}$ . The significance of these findings with respect to rapidity of freezing is discussed in their relation to the mechanism of survival of mammalian cells and tissues exposed to extremely low temperature. The relation of these results to evidence purported to indicate a viral transmission of transplanted frozen-dehydrated tumor material is also pointed out.

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### Comparative Study of Agglutinins and Complement-Fixing Antibodies for *Brucella* in Blood and Cerebrospinal Fluid. (17577)

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Although brucellosis commonly causes symptoms referable to the nervous system, objective evidence of the results of invasion of the central nervous system is frequently lacking. Over a period of 12 years at the University of Minnesota and cooperating hospitals, a total of 161 cases of active brucellosis, 86 of which were culturally proved, have been studied, and the diagnosis of encephalomeningitis was made in four cases.(1) A fifth case exhibited a severe cerebral vascular accident secondary to subacute bacterial endocarditis, which was due to *Br. abortus*.(2) Except for the cases cited, there was no evidence of organic cerebral changes in the remaining cases, although psychic disturbances with emotional instability and mental depression were commonly encountered. Apter and his associates(3) were impressed by the

"disturbances in the adaptive capacities" of patients having chronic brucellosis and carried out a battery of neuropsychologic studies, as outlined by Halstead,(4) on a series of 10 patients. As a result of their investigations, it was concluded that organic damage to the frontal lobes had occurred in 7 of the 10 patients. While such a study suggests a reasonably high incidence of brain damage in patients with chronic brucellosis, there remains an urgent need for acquiring more information on the frequency with which the central nervous system is invaded by *Brucella*. Since serologic techniques are applied successfully to an examination of the cerebrospinal fluid in individuals with suspected syphilis, there was a possibility that serologic methods utilized for the diagnosis of brucellosis might be of some aid in resolving the problem of neurobrucellosis. Simultaneous tests on the blood and cerebrospinal fluid of patients with brucellosis were performed, utilizing procedures for detecting the presence of agglutinins and complement-fixing antibodies.

**Methods.** Specimens of venous blood and

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1. Spink, W. W., and Hall, W. H., *Trans. Am. Clin. and Climat. Assn.*, in press.

2. Spink, W. W., Titrud, L. A., and Kabler, P., *Am. J. Med. Sci.*, 1942, v203, 797.

3. Apter, N. S., Halstead, W. C., Eisele, C. W., and McCullough, N. B., *Am. J. Psych.*, 1948, v105, 361.

4. Halstead, W. C., *Brain and Intelligence*, Chicago, The University of Chicago Press, 1947.

TABLE I.  
Comparative Results of Agglutination and Complement Fixation Tests on Blood and Cerebrospinal Fluid.

| Case No.                                                                        | Duration of illness | Titer of agglutinins |      | Titer of complement-fixing antibodies |      |
|---------------------------------------------------------------------------------|---------------------|----------------------|------|---------------------------------------|------|
|                                                                                 |                     | Blood                | CSF  | Blood                                 | CSF  |
| A. Patients with <i>Br. abortus</i> recovered from blood or cerebrospinal fluid |                     |                      |      |                                       |      |
| 1                                                                               | 6 mo.               | 160                  | Neg. | 20                                    | Neg. |
|                                                                                 |                     | 1280                 | "    | 10                                    | "    |
| 2                                                                               | 2 yr                | 640                  | 10   |                                       |      |
| 3                                                                               | 3 "                 | 640                  | 2    | 320                                   | 4    |
| 4                                                                               | 4 "                 | 640                  | 320  | 160                                   | 128  |
| B. Patients with illness due to brucellosis but with sterile cultures           |                     |                      |      |                                       |      |
| 5                                                                               | 2 mo.               | 640                  | Neg. | 40                                    | Neg. |
| 6                                                                               | 3 "                 | 640                  | "    |                                       |      |
| 7                                                                               | 5 "                 | 320                  | "    | 2.5                                   | "    |
| 8                                                                               | 6 "                 | 2560                 | "    | 320                                   | "    |
| 9                                                                               | 1 yr                | 640                  | "    | 160                                   | "    |
| 10                                                                              | 1 "                 | 640                  | "    | 40                                    | "    |
| 11                                                                              | 2 "                 | 320                  | "    | 20                                    | "    |
| 12                                                                              | 3 "                 | 160                  | "    | 5                                     | "    |
| 13                                                                              | 6 "                 | 320                  | "    | 5                                     | "    |

cerebrospinal fluid were obtained from the patients on the same day. Tests for the presence of agglutinins and complement-fixing antibodies were determined simultaneously on the blood and cerebrospinal fluid. In performing the agglutination test, the macroscopic tube method was employed in which falling concentrations of serum up to a dilution of 1 to 5120 were used. The antigen was obtained from the Bureau of Animal Industry, Beltsville, Md., and was prepared from Strain 1119-3 of *Br. abortus*. This antigen has proved satisfactory for diagnostic purposes over a period of several years in the Laboratory of the Minnesota State Department of Health and at the University Hospitals. Mixtures of the serum or cerebrospinal fluid and antigen were incubated overnight at 37°C. for 18 hours, and then placed in a refrigerator at 4°C. for one hour. The tubes were then examined macroscopically. Antigen for the complement-fixation test was obtained from Dr. C. W. Bonyne of Los Angeles, Calif. This Brucella antigen had proved to be quite satisfactory for detecting complement-fixing antibodies in bovine and human serum. The Kolmer-Wassermann(5)

technique for detecting complement-fixing antibodies in syphilis was used, substituting the Brucella antigen.

*Results.* Studies were carried out in 13 patients with brucellosis, and the results are presented in Table I. In group A, there were 4 patients from whom *Br. abortus* was obtained. Cases 2 and 4 were instances of encephal meningitis with isolation repeatedly of *Br. abortus* from the cerebrospinal fluid. The fluid in both cases showed an exceedingly high protein content with an increase in cells and a reduction in sugar. Although in case 2 Brucella agglutinins were found in the spinal fluid in a titer of 10, unfortunately no test for complement-fixing antibodies was carried out. Case 4 had had evidence of chronic encephal meningitis for 4 years. Evidence of diffuse cortical damage was demonstrated in the electroencephalogram, and he had acquired complete nerve deafness. It was in this patient's fluid that a high titer of Brucella agglutinins and complement-fixing antibodies was demonstrated. Case 3 was of interest in that the patient had been ill for 3 years. *Br. abortus* was isolated from blood cultures, but the cerebrospinal fluid remained sterile. A complete examination of the fluid removed from the lumbar area revealed 2 mononuclear cells

5. Kolmer, J. A., and Boerner, F., *Approved Laboratory Technic*, Fourth Edition, New York, Appleton Century Co., 1945.

and normal protein and sugar values. A paravertebral abscess was demonstrated roentgenologically in association with a destructive process of the seventh and eighth thoracic vertebrae and the intervening intervertebral disc.

There were 9 patients in Group B. Although brucellosis was not proved bacteriologically in these cases, the clinical course and the titer of *Brucella* agglutinins in each of the cases was compatible with the diagnosis of brucellosis. Many of these patients had somatic complaints. The nature and chronicity of their symptoms were consistent with those observed in psychoneurotic individuals. It was in this group of cases that serologic data were highly desired. But in every instance antibodies were absent in the cerebrospinal fluid by both technics. Case 13 was of particular interest in that he had contracted his illness 6 years before being studied. He had had narcolepsy for 3 years. An electroencephalogram was within normal limits, as were the findings in his cerebrospinal fluid.

**Discussion.** Most authorities agree that for diagnostic purposes with human serum in brucellosis the complement fixation test does not possess any advantage over the agglutination test.(6) The present study indicates that this also applies to cerebrospinal fluid. In their review on neurobrucellosis, Roger and Poursines(7) point out instances of meningitis with *Brucella* agglutinins present in the cerebrospinal fluid in a low titer. In our own experience, the presence of *Brucella* antibodies in the cerebrospinal fluid has been associated with demonstrable organic changes such as encephalomeningitis or spondylitis. Perhaps further attention should be given to cultural studies of cerebrospinal fluid, especially in patients with chronic

brucellosis having psychosomatic disturbances. In the cases of encephalomeningitis (cases 2 and 4), *Br. abortus* was cultured from the cerebrospinal fluid with the aid of tryptose phosphate broth. Goodpasture and Anderson (8) first called attention to the marked susceptibility of viable embryonic chicken tissue to *Brucella*. This was subsequently confirmed by Hall and Spink.(9) In a limited number of cases of human brucellosis, Dr. Wendell Hall attempted to isolate *Brucella* from the cerebrospinal fluid of patients having a demonstrable bacteremia. He succeeded in growing *Br. abortus* from the tissues of 11 day old chick embryos after apparently normal cerebrospinal fluid from a patient with chronic brucellosis was injected into the allantoic sac. He repeated the results with another specimen of fluid from the same patient. Subsequently it was observed that this patient had *Brucella* spondylitis. Specimens of cerebrospinal fluid from this patient introduced simultaneously into tryptose phosphate broth showed sterile cultures.

**Summary.** Simultaneous tests for agglutinins and complement-fixing antibodies for *Brucella* were carried out on the serum and cerebrospinal fluid of 13 patients having brucellosis. Complement-fixing antibodies and agglutinins were demonstrated in the cerebrospinal fluid of one patient with chronic encephalomeningitis and in one patient with a paravertebral abscess associated with *Brucella* spondylitis. A third patient having chronic *Brucella* encephalomeningitis had agglutinins in the cerebrospinal fluid. In 13 cases these two serologic tests when performed with cerebrospinal fluid have not aided in resolving the problem of the incidence of organic brain damage in patients with brucellosis.

6. Wise, B., and Craig, H. W., *J. Infect. Dis.*, 1942, v70, 147.

7. Roger, H., and Poursines, Y., *Les Meningo Neurobrucelloses*, Paris, Masson et Cie, 1938.

8. Goodpasture, E. W., and Anderson, L., *Am. J. Path.*, 1937, v13, 149.

9. Hall, W. H., and Spink, W. W., *J. Immunol.*, 1948, v59, 379.

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