

# THE INHIBITING EFFECT OF NORMAL SERUM AND ITS GAMMA GLOBULIN FRACTION UPON THE VARIATION OF STAPHYLOCOCCUS AUREUS

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Received for publication January 26, 1948

The modifying or selective effect of environmental factors upon the variation of microorganisms, especially upon the phenomenon frequently termed dissociation, has been widely studied (Braun, 1947a). Among the agents used, those which originate from the host environment have created special interest; studies on the *in vitro* effect of such agents may permit a better understanding of the conditions which affect the bacterial population within the host environment. Investigations on the variational aspects of *Staphylococcus aureus* by Nutini and Lynch (1946) have previously demonstrated significant *in vitro* as well as *in vivo* effects with tissue extracts of beef brain, spleen, heart, and kidney. The presence of such extracts was reported to enhance the establishment of white R variants. In recent *in vitro* studies at this laboratory, the selective effect of beef brain extract upon variants of *Staphylococcus aureus* was reinvestigated and the extract's enhancing effect upon the establishment of nonpigmented variants was confirmed.

In contrast to this selective effect of brain extract favoring the establishment of nonsmooth variants, a specific selective effect in favor of the smooth types has been reported when variants of various species were grown in the presence of normal serum (Braun, 1946, 1947b). In work with *Brucella abortus* it has recently been possible to demonstrate that this selective factor is associated with the normal gamma globulin fraction (Braun, 1947b). Therefore, the effect of normal bovine serum and its gamma globulin fraction was tested on *Staphylococcus aureus*. Various strains of hemolytic and coagulase-positive *Staphylococcus aureus*, isolated from the bovine udder, were grown in plain beef extract broth or in beef extract broth containing normal bovine serum, bovine gamma globulin, beef brain extract, as well as a combination of these supplements. Samples from these cultures were plated after various intervals of growth, and the percentage of variant colonies was estimated by counting at least 100 colonies on each test plate.

A summary of the results presented in table 1 demonstrates that (1) the presence of beef brain extract significantly enhances the establishment of non-S variants, (2) the presence of 2 per cent normal beef serum or as little as 25 mg of bovine gamma globulin per 5 ml of broth inhibits the establishment of non-S variants, and (3) the presence of 2 per cent normal serum appears to counteract the enhancing effect of beef brain extract. No differences in growth rates between control cultures and those containing supplements were noted.

The observation on the specific selective effect of the gamma globulin fraction of normal serum upon *Staphylococcus aureus* variants demonstrates that this

activity is not restricted to *Brucella abortus* variants, but may be of a rather general nature.

TABLE 1  
*Staphylococcus aureus* variation in beef extract broth and various added agents

CULTURE MEDIA	AVERAGE % OF VARIANT COLONIES ON PLATES FROM 48-HOUR BROTH CULTURES	AVERAGE % OF VARIANT COLONIES ON PLATES FROM 7-DAY-OLD BROTH CULTURES	TOTAL NUMBER OF TESTS
Beef extract broth . . . . .	4.08	14.00	86
Beef extract broth plus 0.8-2.0% brain extract . . . . .	12.75	36.70	108
Beef extract broth plus 2% normal bovine serum . . . . .	0	0	54
Beef extract broth plus 0.8-2.0% brain extract, 2% normal bovine serum . . . . .	0.57	2.31	60
Beef extract broth plus normal bovine gamma globulin 25-50 mg . . . . .	0	1.78	32

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