cell antibodies, extracted beef-cells removed them in 2 adsorptions. This difference in the action of sheep- and beef-cells on such antibodies has been noted before with raw and boiled cells[1] and it would seem that beef-cells possessed per unit-volume a greater amount of the antigen for the sheep-cell antibodies of infectious mononucleosis than did sheep-cells themselves.

The results of the inhibitory and adsorptive experiments agree in showing that the sheep-cell antibodies in infectious mononucleosis are not Forssman antibodies, for they react with alcohol-treated sheep- and beef-cells but not with the alcoholic extracts of these cells.

8562 C

A Thermostable Antigen in Beef-Cells.

C. A. STUART, A. M. GRIFFIN, K. M. WHEELER AND SHIRLEY BATTEY.

From Brown University and the Rhode Island Hospital, Providence, R. I.

Bailey and Raffel[1] showed that serums from 3 patients with infectious mononucleosis contained lysins for beef-cells in addition to agglutinins and lysins for sheep-cells.* Serums from 22 cases of infectious mononucleosis examined by us showed the presence of beef-cell lysins in dilutions from 1:1280 to 1:20480, much higher than the titer for sheep-cell lysins. Beef-cell agglutinins were not present in any of the 22 serums in a concentration exceeding that in normal serum. In this respect the antigen responsible for the beef-cell antibodies in infectious mononucleosis acts like a true Forssman antigen in that high lytic titers are produced with little or no corresponding agglutinins.

It has been shown that both raw and boiled beef- and sheep-cells adsorb the beef-lysins of infectious mononucleosis[1] and we have amply confirmed this fact. The presence of a thermostable antigen in the beef-cell, in which no heterophile antigen has yet been demonstrated, needs further investigation. Accordingly the relationship of the beef-cell lysins of infectious mononucleosis serum to the

[1] Bailey and Raffel, *J. Clin. Invest.*, 1935, **14**, 228.

* Agglutinins and lysins for horse cells are also present in infectious mononucleosis. In a group of 15 serums the titer of these antibodies ranged from 1:1280 to 1:40960.

alcoholic extracts and alcohol-treated sheep- and beef-cells previously described[2] was determined. The anticomplementary action of the extracts was controlled with pure isophile beef-serum prepared by adsorbing rabbit antibeef-cell serum with raw sheep-cells. The possibility of hemolysis by the complement must not be overlooked in testing for beef lysins. The complement from 25 of 43 guinea pigs had to be discarded because of the presence of normal beef-lysins in dilutions of 1:10 to 1:100. Sheep-lysins were also present in 1:40 dilution in the serum of 2 animals. These antibodies were not adsorbed by boiled beef-cells, which indicates their isophile nature.

Alcoholic extracts of sheep- and beef-cells failed to inhibit the beef-lysins of infectious mononucleosis serums. Both the alcohol-precipitated and extracted sheep- and beef-cells, however, adsorbed the beef-lysins from such serums; the alcohol-treated sheep-cells were much less efficient in this respect. Beef-cells extracted with alcohol in a Soxhlet apparatus for 24 hours retained their ability to adsorb the beef-lysins. Neither the alcohol-precipitated nor extracted cells adsorbed any of the antibodies from the isophile beef-serum.

From the present and previous investigations[2] it is obvious that the antigen of the beef-cell and sheep-cell which adsorbs the antibodies of infectious mononucleosis is not the well-known isophile fraction common to these cells since it is thermostable and "alcohol-resistant." Nor is it a Forssman heterophile antigen since it is not alcohol-soluble. This antigen like the Forssman antigen appears to have widespread distribution since it has been found in horse tissue,[1, 3] *Clostridium welchii*[1] and, as we shall show presently, in the rabbit. For convenience this antigen will be termed the B. T. (beef-thermostable) antigen.

While this antigen is not present in rabbit cells[4] nor rabbit-tissue[3] the immunization of rabbits with raw beef-cells produces no antibodies that can be removed by boiled beef-cells.[5] These facts led us to investigate the serum of the rabbit for the presence of the B. T. antigen. The serums from 6 normal rabbits were used to inhibit the beef-lysins in 5 infectious mononucleosis serums. Table 1 shows that the rabbit-serums markedly inhibited the beef-lysins. Similar experiments with 8 infectious mononucleosis serums

2 Stuart, Griffin, Fulton and Anderson. In press.

3 Davidsohn and Walker, *Am. J. Clin. Path.*, 1935, **5**, 455.

4 Stuart, Tallman and Brintzenhoff, *J. Immunol.*, 1935, **28**, 85.

5 Hyde, *Am. J. Hyg.*, 1925, **5**, 217.

TABLE I.

Inhibition of Beef-Lysins in Infectious Mononucleosis Serums by Normal Rabbit- and Horse-Serums.

Normal Rabbit-Serums*	Serum-dilutions 20	40	80	160	320	640
4382	C	C	C	P	P	—
4638	C	C	P	P	—	—
4204	C	C	C	P	P	—
4414	C	C	P	P	—	—
4381	C	C	P	P	—	—
4248	C	C	P	P	—	—
‡Iso. beef-serum	—	—	—	—	—	—

Normal Horse-Serum†	Serum-dilutions 5	10	20	40	80
1471	C	P	P	—	—
1472	C	P	P	—	—
1888	C	C	P	P	—
1892	C	C	P	P	—
‡Iso. beef serum	—	—	—	—	—

*Each rabbit-serum tested with 5 infectious mononucleosis serums.
†Each horse-serum tested with 8 infectious mononucleosis serums.
‡All rabbit- and horse-serums negative in anticomplementary control.
C = complete, P = partial inhibition.

demonstrated the presence of the B. T. antigen in 4 horse serums though in lower concentration.

The presence of the B. T. antigen in horse-serum is particularly important because it may account for the antibodies for beef- and other cells that are often found in the serums of humans treated with horse-serum. Serums from 5 cases of horse-serum sickness containing beef-agglutinins and lysins as well as sheep-agglutinins were adsorbed with boiled beef-cells. The agglutinins and lysins for beef- and agglutinins for sheep-cells were completely removed by a single adsorption. Despite the fact that the boiled beef- and sheep-cells completely adsorb the sheep-agglutinins from both infectious mononucleosis and serum-sickness, these agglutinins in the 2 serums are not identical since boiled rabbit-cells will adsorb the sheep-agglutinins of serum-sickness but not infectious mononucleosis.[6] There must consequently be a second thermostable antigen in the beef-cell which is present also in the rabbit-cell, the sheep-cell and horse-serum. This second antigen is probably related to but cannot be identical with the B. T. antigen.

A thorough investigation of these thermostable antigens can only be made when animals are found which lack these antigens and therefore possess the ability to produce antibodies. Man appears to lack them because antibodies of the type which they would

[6] Stuart, Fulton, Ash and Gregory. In press.

produce are found in cases of infectious mononucleosis and serum-sickness. Moreover in 3 normal human serums containing beef-lysins in low concentration these antibodies were completely adsorbed by boiled beef-cells. It might seem from the work of Bailey and Raffel[1] that animals lacking the B. T. antigen were rather common. But it should be pointed out that a test of the erythrocytes, the tissue or the serum alone is quite inadequate for the proper classification of an animal with respect to this antigen. Table II shows the presence and location of the B. T. antigen in

TABLE II.
Distribution of the B. T. (beef-thermostable) Antigen in Certain Animals.

	Erythrocyte	Serum	Tissue
Beef	+	+	—
Sheep	+	+	—
Horse	*+	+	+
Rabbit	—	+	—
Guinea pig	—	—	—
Human	—	—	

*Horse-cells did not completely adsorb the beef-lysins from infectious mononucleosis serums. A single adsorption reduced the beef-lysin titer about 90%, but no further decrease occurred on subsequent adsorptions.

those animals that have been studied most extensively. The absence of the B. T. antigen in the guinea pig strongly suggests that the sheep- and beef-cell antibodies produced in these animals by immunization with boiled sheep-cells[7] were actually antibodies from the B. T. antigen in these cells.

The experiments herein described indicated the existence of a class of antigens which are thermostable, insoluble in alcohol, but not activated by exposure to that reagent. The properties of the type-specific antigens, K_3 and K_5, found in rabbits[8] would seem to place them in this class. The properties of these thermostable antigens are partly those of the Forssman antigens and partly those of the isophile antigens.

7 Weil, *Bioch. Z.*, 1914, **58**, 257.

8 Fischer, *J. Immunol.*, 1935, **86**, 97.