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Nature of the Antibodies for Sheep-Cells in Infectious Mononucleosis.

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The sheep-cell antibodies in infectious mononucleosis serums have been called heterophile or Forssman antibodies by several investigators but since, as previously pointed out,[1, 2, 3, 4] the Forssman character of these antibodies is questionable, further studies on their nature are necessary. To this end the relationship of alcoholic extracts of beef- and sheep-cells to the sheep-cell antibodies in infectious mononucleosis and in several Forssman heterophile serums has been determined.

Extracts were prepared by treating one volume of packed cells with 5 volumes of alcohol for 12 hours at room-temperature followed by 8 hours in a mechanical shaker, filtering, and concentrating the filtrate to one-half its volume at 37°C. Equal volumes of this extract and saline were mixed and the alcohol removed by evaporation at 50°C. The turbid suspensions prepared in this manner were used to inhibit the hemolysis of sheep-cells by the various normal and immune serums noted in Table I.

In the inhibition-tests 0.25 ml. of the alcoholic extract in dilutions ranging from 1 :1.25 to 1 :320 was placed in a series of tubes; 0.25 ml. of the normal or immune serum containing sufficient hemolysin to hemolyse completely the dose of sheep-cells in 10 minutes, and 0.25 ml. of guinea pig-complement diluted 1 :20 was added to each tube in the series. Triplicate controls with 0.25 ml. of saline substituted for the extract were used in each test. After 15 minutes' incubation at 37°C. in a water-bath, 0.25 ml. of a 1.0% sheep-cell suspension was added to each tube. When all 3 control tubes showed complete hemolysis the degree of hemolysis in the tubes containing the extract was noted. Ten minutes later a second reading was taken. The anticomplementary action of the extracts themselves was controlled by using pure isophile immune sheep-serum (prepared by absorbing rabbit antisheep-cell serum

1 Stuart, Burgess, Lawson and Wellman, *Arch. Int. Med.*, 1935, **54**, 199.

2 Bailey and Raffel, *J. Clin. Invest.*, 1935, **14**, 228.

3 Stuart, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 861.

4 Davidson and Walker, *Am. J. Clin. Path.*, 1935, **5**, 455.

TABLE I.
Inhibition of Sheep Lysins in Various Serums by Alcoholic Extracts of Sheep- and Beef-Cells.

Serums tested	Number tested	Dilutions of Sheep extract 5	20	80	320	1280	Beef extract 5	10	20
Rabbit antisheep	2	C	C	C	P	—	—	—	—
" antiguinea pig-kidney	2	C	C	C	P	—	—	—	—
" antihuman A	2	C	C	C	P	—	—	—	—
*Normal human	6	C	C	C	P	—	—	—	—
Serum-sickness	5	C	C	C	P	P	—	—	—
Infectious mononucleosis	8	P	—	—	—	—	—	—	—
Pure isophile sheep	1	—	—	—	—	—	—	—	—

C = complete inhibition. P = partial inhibition. — = no inhibition.
*Selected for high sheep-cell lysin-titers.

TABLE II.
Adsorption of Sheep-Cell Antibodies from Infectious Mononucleosis Serum (55) with Alcohol-Extracted Sheep- and Beef-Cells.

	Sheep-agglutinins 20	40	80	160	320	640	1280	2560	Sheep-lysins 20	40	80	160	320	640	1280	2560	5120
Adsorbed with alcohol-extracted sheep-cells																	
Titer before adsorption	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4	4	2
" after first adsorption	4	4	4	4	4	2	—	—	4	4	4	4	4	3	1	—	—
" " second "	4	4	4	4	2	—	—	—	4	4	4	3	1	—	—	—	—
" " third "	4	4	2	—	—	—	—	—	4	3	1	—	—	—	—	—	—
" " fourth "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Adsorbed with alcohol-extracted beef-cells																	
Titer after first adsorption	4	2	—	—	—	—	—	—	4	4	3	—	—	—	—	—	—
" " second "	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

with boiled sheep- and raw beef-cells) as the hemolysing agent. In Table I is shown the inhibitory action of the alcoholic extracts of beef- and sheep-cells on the various serums. Though the second reading, taken 10 minutes after the controls were completely hemolysed, showed slightly less inhibitory action on the part of the extract it is a more rigid test and only this reading is recorded in the table.

It will be seen that the alcoholic extract of sheep-cells possessed marked inhibitory action on the 5 kinds of serums containing known heterophile lysins for sheep-cells, i. e., rabbit antisheep-cell, rabbit antiguinea pig-kidney, rabbit antihuman A, normal human and serum-sickness serums. Alcoholic extracts of beef-cells on the other hand showed no inhibitory action on any of the serums. Neither the beef- nor sheep-cell extracts inhibited the sheep-cell lysins of infectious mononucleosis. The partial inhibition in the infectious mononucleosis serums in the 1:5 dilution of the sheep-extract is doubtless accounted for by the presence of normal heterophile antibodies in these serums. Obviously, then, that fraction of the beef- and sheep-cell which adsorbs the sheep-cell antibodies of infectious mononucleosis is not alcohol-soluble, in contradistinction to the Forssman heterophile antigen.

The relationship of the sheep-cell antibodies in infectious mononucleosis to "alcohol-precipitated" and "alcohol-extracted" sheep- and beef-cells was also determined. The former were prepared by adding the cells to alcohol. When coagulation was complete the alcohol was immediately decanted and the cells dried in a current of warm air. Cells were extracted with 3 changes of alcohol for 7 days with frequent shaking. The alcohol was decanted and the residue after drying at 37°C. constituted the extracted cells.

Neither the alcohol-precipitated nor the alcohol-extracted sheep- or beef-cells adsorbed any appreciable amount of either agglutinins or lysins from isophile sheep-serum. Both the alcohol-precipitated and extracted sheep- and beef-cells, however, completely removed agglutinins and lysins for sheep-cells from infectious mononucleosis serums. Due, doubtless, to the physical characteristics of alcohol-treated cells the antibodies were adsorbed more slowly than by the equivalent raw or boiled cells. Table II shows the adsorption of the serum of case 55 with alcohol-extracted cells. The serum was adsorbed 4 times for one hour each at 37°C. and tested after each adsorption. It will be noted that while 4 adsorptions with the extracted sheep-cells were required to remove completely the sheep-

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.

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A Thermostable Antigen in Beef-Cells.

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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.