

In view of our experiments and the analysis of autopsy material, we are led to believe that pericardial adhesions *per se*—especially adhesions to the diaphragm—do not play a rôle in the production of cardiac hypertrophy.

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Further Studies on the Thermostable Antigen in Beef Cells.

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A thermostable alcohol-insoluble (B.T.) antigen has been demonstrated in beef- and sheep-erythrocytes.^{1, 2} In order to study further the nature of this antigen the production of specific antibodies in some experimental animal was desirable. Weil³ demonstrated the formation of beef- and sheep-lysins in guinea pigs immunized with boiled sheep-erythrocytes. Although Weil's work has been criticized on the ground that the thermolabile sheep-beef antigen was incompletely destroyed it now seems probable that the beef-lysins were engendered by the B.T. antigen in the boiled sheep-cells. In view of Weil's experiment, and since no B.T. antigen has been demonstrated in the guinea pig this species was selected for further study of the thermostable beef-antigen.

Guinea pigs were immunized by intraabdominal injections of 1.5 to 2.5 ml. of washed raw, boiled, or autoclaved beef-erythrocytes at 5-day intervals. Boiled cells were prepared by boiling a 25% suspension for 20 minutes. Autoclaved cells consisted of boiled cells further treated by autoclaving at 125°C. for 15 minutes. The pigs were bled from the heart and serum tested for lysins and agglutinins for beef-, sheep- and horse-erythrocytes. In the lytic tests .25 ml. of 1:20 guinea pig's complement and .25 ml. of one percent cell-suspension were added to tubes containing .5 ml. of serial dilutions of the immune serum. The degree of hemolysis was recorded after 30 minutes at 37°C. Agglutinative titers were determined by adding .5 ml. of .5% cell-suspension to .5 ml. of the serum-dilution and reading after 2 hours at 37°C.

¹ Stuart, Griffin, Fulton and Anderson, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 209.

² Stuart, Griffin, Wheeler and Battey, *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 212.

³ Weil, *Bioch. Z.*, 1914, **58**, 257.

TABLE I.
Immunization of Guinea Pigs with Raw, Boiled and Autoclaved Beef-Erythrocytes.

Guinea pig No.	Immunized with	No. of injections	Titer for					
			Beef		Sheep		Horse	
			lysins	agglutinins	lysins	agglutinins	lysins	agglutinins
1	Raw beef cells	0	20	0				
		5	320	0	1280	0	0	0
		7	2560	0	640	80	0	80
2	" " "	0	0	0				
		4	640	0	320	0	0	0
		6	640	0	320	320		
		8	1280	80	320	80	0	320
11	Boiled beef cells	0	20	0				
		4	10240	0	320	320	0	
		6	5120	0	320	640	0	640
21	Autoclaved beef cells	0	0	0	0	0		
		2	0	0	0	0		
		5	40	0	0	0		
22	" " "	0	0	0	20	0	0	
		2	1280	0	40	0	0	
		4	5120	0	160	80	80	640
27	" " "	0	40	0	0			
		4	5120	0	80	40	40	40

Table I shows the titers produced by the injection of beef-cells. Guinea pigs 1, 2 and 3 received raw cells, 11 and 12 boiled cells and 21 to 28 autoclaved cells. Normal titers for beef-lysins were 1:20 or lower except in animals 26 and 27. There was considerable variation in the height of the immune beef-lysin titer; boiled and autoclaved cells produced the highest titers.* Only animal 21 failed to produce any significant amount of beef-lysins. Agglutinins for beef-cells were present only in guinea pig 2 in a low titer and then not until after the 8th injection. In contrast to the strong lysins for beef-cells, with the agglutinins low or absent, antibodies for horse-cells were principally agglutinins. Five of the 7 animals forming horse-cell antibodies had agglutinins only while 2 animals immunized with autoclaved beef-erythrocytes produced both lysins and agglutinins. A majority of the animals produced antibodies for sheep-cells. The sheep-lysin titers were considerably lower than the beef except in the serum of animal 1 tested after the 5th injection. Unlike the beef, titers for sheep-cells were higher in animals which received raw cells.

* Heated cells produced severe peritonitis resulting in the death of 8 of the 12 animals injected. This irritation may have served as an added stimulus to antibody formation.

To determine the specificity of the antibodies the immune serums were adsorbed with raw and autoclaved beef- and sheep-erythrocytes and raw horse-erythrocytes. A 1:5 dilution of the serum was adsorbed 3 successive times for one hour each with .25 ml. of packed raw or .5 ml. of packed autoclaved cells for each milliliter of serum-dilution. The titers before adsorption and after the third adsorption are listed in Table II.

TABLE II.
Adsorption of Immune Guinea Pig Serums.

Adsorbed with	Titer for			
	Beef lysins	Sheep lysins	Sheep agglutinins	Horse agglutinins
Anti-raw beef cell serum (aver. 2 serums)				
Control	1280	640	120	320
Raw beef cells	0	0	0	0
Autoclaved beef cells	320	160	0	0
Raw sheep cells	640	0	0	0
Autoclaved sheep cells	480	240	0	0
Raw horse cells	640	640	0	0
Anti-autoclaved beef cell serum (aver. 2 serums)				
Control	7680	160	60	320
Raw beef cells	0	0	0	0
Autoclaved beef cells	0	0	0	0
Raw sheep cells	2560	0	0	0
Autoclaved sheep cells	1920	0	0	0
Raw horse cells	3840	80	0	0

Adsorption with raw beef-cells of serums produced by immunization with raw beef-cells removed all antibodies for beef-, sheep- and horse-erythrocytes.† Raw sheep-cells adsorbed sheep-lysins and sheep- and horse-agglutinins but did not adsorb the beef-lysins. These remaining lysins were presumably antibodies produced by the species-specific isophile beef-cell antigen. Autoclaved beef-, autoclaved sheep- and raw horse-cells removed agglutinins for sheep- and horse-cells but not the sheep- or beef-lysins. The failure of autoclaved sheep-cells to adsorb the sheep-lysins indicated the presence of antibodies for the thermolabile sheep-beef antigen. The removal of sheep-agglutinins with no significant decrease in the lysins appears analogous to the adsorption of serum-sickness serums by beef- and rabbit-erythrocytes. The sheep-cell agglutinins in these serums were completely adsorbed with only a slight reduction in the lytic titer.⁴ These adsorptions indicate that the ag-

† There was one unexplained exception; 5 adsorptions of the serum from animal 2 with raw beef-cells reduced the sheep lysin titer only 75%.

⁴ Stuart, Fulton, Ash and Gregory, in press.

glutinins and lysins were produced by different antigenic constituents of the bovine cell.

Raw and autoclaved beef-cells removed all beef-, sheep- and horse-antibodies from serums produced by immunization with autoclaved beef-erythrocytes. Both raw and autoclaved sheep-cells adsorbed the sheep-lysins. Since the antibodies were produced by injection of autoclaved beef-cells in which no Forssman-antigen has been demonstrated the sheep-lysins in these serums must have been produced by the B.T. antigen. Sheep-cells did not remove the beef-lysins. If these lysins had been produced entirely by the B.T. antigen they should have been adsorbed by sheep-cells and the fact that they were not indicated a second thermostable antigen in the beef-cell. Horse-cells removed the sheep- and horse-agglutinins but did not significantly affect the sheep- or beef-lysins. Furthermore, 3 adsorptions with sheep-cells followed by 3 adsorptions with horse-cells (not shown in the tables) did not remove the beef-lysins. The antigen in the horse-erythrocyte which adsorbed the sheep- and horse-agglutinins, therefore, could not be the same as the B.T. antigen nor identical with the second thermostable antigen in the beef-cell.

Normal rabbit-serum, which contains the B.T. antigen,² was used to inhibit lysis of beef-cells by the immune serums. The technique employed has been previously described.¹ With serums produced by immunization with raw beef-cells inhibition of the beef-lysins was complete in a 1:20 and partial in a 1:40 dilution of the rabbit-serum. Inhibition was somewhat stronger in serums produced by injection of autoclaved cells, being complete in a 1:40 and partial in a 1:80 dilution of the rabbit-serum.

Summary. Adsorptive tests with raw and autoclaved beef- and sheep-erythrocytes, and inhibitive experiments with normal rabbit-serum demonstrated the presence of antibodies for the B.T. antigen in guinea pigs immunized with beef-erythrocytes.