A Fraction from Liver which Agglutinates Autologous Red Cells.* (23314)

ROBERT R. KOHN AND EDWARD R. ARQUILLA (Introduced by Alan R. Moritz)
Institute of Pathology, Western Reserve University, School of Medicine, Cleveland, Ohio

This investigation was initiated by observation that a homogenate of perfused rat liver would agglutinate red blood cells from the same animal *in vitro*. The substance, or substances, responsible for agglutination was partially purified and its distribution in various species and organs as well as some factors influencing its activity *in vitro* were studied. Evidence is presented suggesting that the agglutinin is a phospholipid.

Methods. Sprague-Dawley rats weighing between 300 and 400 g were used. Rats were anesthetized and bled from the carotid artery into Alsever's solution(3). Erythrocytes were washed several times with isotonic saline solution and diluted to a 2% suspension with isotonic veronal saline buffer (VBS)(3). Organs from the same animal were perfused free of blood with isotonic saline and fractionated as described below. Agglutinating titers of the liver fractions were determined by use of serial dilutions of fractions in VBS containing 0.1% bovine serum albumin. These dilutions were made to final volume of 0.5 ml. to each of which was added 0.05 ml of 2% autologous erythrocyte suspension. The red cells were evenly suspended by shaking and agglutination was determined by observing the pattern of erythrocytes on the bottom of tubes after 3 hours.

Results. After perfusion, rat liver was homogenized in cold acetone and filtered. The insoluble material was dried and extracted with VBS. The extract was dialyzed against distilled water in the cold for 48 hours and the precipitate was separated by centrifugation. Agglutinating activity is precipitated by acetone, extractable by VBS, and precipitates almost quantitatively when dialyzed against water. The titer of the precipitate after dialysis was 1:5120, while that of the supernate was 1:10. On the basis of nitrogen content, dialysis yielded a 500-fold purification.

Effect of heat on activity. Agglutinating activity of the VBS extract of rat liver acetone powder was completely destroyed by heating at 56°C for 30 minutes. However, the precipitate after dialysis did not lose a significant amount of activity after heating at 56°C for 2 hrs. or immersion in boiling water for 5 minutes. Agglutination took place to the same extent when autologous red cells were incubated with liver agglutinin at 6°C, room temperature and 37°C.

Effect of rat serum. High dilutions of both rat plasma and serum completely inhibited agglutination of autologous cells by rat liver fractions. An experiment was carried out in which each tube contained rat liver precipitate after dialysis with N content of 85 μg (0.2 μg enough for complete agglutination), plus various dilutions of rat serum. Agglutinations did not occur until serum was diluted 1:200.

Effect of complement. Incubation of the precipitate after dialysis with autologous cells at 37°C in the presence of fresh guinea pig serum resulted in agglutination, but no lysis occurred.

Action on cells of other species. The precipitate after dialysis of rat liver was tested for agglutination of red blood cells of several species. Table I shows that rat liver fraction strongly agglutinates rat, rabbit, and mouse cells, and has a small amount of activity against cells of the dog. Erythrocytes of guinea pig, sheep and human were not agglutinated.

Activity of other organs. Rat kidney, spleen, striated muscle and lung were fractionated as described above for liver and the precipitates after dialysis were tested for agglutinating activity against autologous red cells. Table II shows liver has the most activity, kidney has a slight amount of activity, while spleen, lung and muscle have only traces. Rat spleen could not be perfused free of blood, and it is possible that agglutinating

* Part of this material was presented before the Am. Assn. of Immunologists, April, 1956.

TABLE I. Agglutination of Red Cells of Various Species by Rat Liver Precipitate after Dialysis.

Species of RBC's	Titer of rat liver precipitate
Rat	1/ 5120
Rabbit	>1/20480
Mouse	1/10240
Dog	1/ 160

activity present in spleen was inhibited by serum.

Agglutinating activity of liver from other species. Guinea pig liver was fractionated and the precipitate after dialysis was tested against autologous red cells. Two samples of human liver were obtained at operation and similarly fractionated and tested. Guinea pig liver fraction had a titer of 1:32, while the fractions from human livers had titers of 1:32 and 1:512.

Further identification of the agglutinin. Since various lipid agglutinins have been described, a lipid extraction of rat liver precipitate after dialysis was carried out according to the procedure of Horsfall and Goodner(2). The starting material had a titer of 1:1280, the lipid extract a titer of 1:2560, and the non-lipid residue a titer of 1:10. Thus, the lipid fraction contained essentially all of the agglutinating activity. Four ml of the lipid fraction were evaporated to dryness and analyzed for cholesterol by the method of Kingsley and Schaffert(4). There was no detectable cholesterol in the fraction.

Discussion. Various tissue agglutinins have been described by other workers. Salaman (5) extracted an autohemagglutinin from mouse neoplasms and normal tissues. This agglutinin differed from the one described in this report by not being found in liver and not being inhibited by serum. Burnet and Stone (1) found various lipid extracts of tissues would agglutinate erythrocytes and that the agglutinating activity was inhibited by serum. However, the extracts would agglutinate only red cells susceptible to agglutination by the vaccinia virus hemagglutinin. Stone(6) in studying agglutinating activity of known lipids found that various lipids agglutinated only red cells susceptible to virus hemagglutination, with the exception of cholesterol ergosterol, and cardiolipin, which also agglutinated non susceptible cells. The agglutinin we described contains no cholesterol, and presumably no ergosterol but it may be related to cardiolipin.

TABLE II. Agglutinating Activity of Various Rat Organs against Autologous Red Cells. Reciprocals of titers were calculated/mg N wet tissue and expressed in arbitrary units.

Organ	Agglutinating activity
Liver	1000
Kidney	6.1
Spleen	3.4×10^{-1}
Lung	8.6×10^{-2}
Skeletal muscle	3.7×10^{-3}

Summary. Homogenates of perfused rat, human and guinea pig livers agglutinate washed autologous red cells. The factor responsible for agglutination was partially purified and it appears to be a heat-stable phospholipid. Agglutination occurred in the cold, at room temperature and at 37°C. Lysis did not occur in the presence of complement. High dilutions of autologous serum completely inhibited hemagglutination. Agglutinating activity was high in liver, present in kidney and practically absent in spleen, lung and skeletal muscle. Rat liver preparations strongly agglutinated red cells of rat, rabbit and mouse, but not cells of dog, sheep, guinea pig or human. Human and guinea pig liver had much less autohemagglutinating activity than rat liver.

1. Burnet, F. M., and Stone, J. D., *Aus. J. Exp. Biol. and Med. Sci.*, 1946, v24, 1.

2. Horsfall, F. L., and Goodner, K., *J. Exp. Med.*, 1935, v65, 485.

3. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, Springfield, Charles C. Thomas, 1948, pp. 107.

4. Kingsley, G. R., and Schaffert, R. R., *J. Biol. Chem.*, 1949, v180, 315.

5. Salaman, M. H., *Br. J. Canc.*, 1948, v2, 253.

6. Stone, J. D., *Aus. J. Exp. Biol. and Med. Sci.*, 1946, v24, 197.

Received April 24, 1957. P.S.E.B.M., 1957, v95.