spleen to 48 hours, and from lung to 32 hours following infusion. In fixed sections of normal livers or lungs bacterial colonies were not seen. On the other hand, colonies of Gram negative bacteria were frequently seen in fixed sections of lungs and in portal areas of fixed sections of liver of cirrhotic rats. Occasionally bacteria formed micro-abscesses. Cultures were sometimes negative when colonies were observed in the sections. In both normal and cirrhotic rats all urine cultures were negative.

II. *Distribution of radioactivity in blood and tissues following injections of I^{131} labelled E. coli into normal and cirrhotic rats.* In *normal* rats, injected bacteria were promptly cleared from the circulation by the liver (Figs. 1 and 2) and lysed since the injected bound radioactivity was rapidly converted to ionic or dialyzable radioactivity; and the latter was promptly excreted in urine (Fig. 3). This rapid disappearance of bacteria from blood, and their uptake by liver, with lysis and release of I^{131} confirmed the bacterial culture data which indicated prompt sterilization of blood and liver.

In *cirrhotic* rats, on the other hand, though uptake of radioactivity by the liver was equal to that in normal rats, the bound or non-dialyzable radioactivity persisted at higher levels than in normal rats for 48 hours (Fig. 3). Only 25% of total radioactivity injected was recovered from urine after 36 hours (Fig. 2). Furthermore, this impairment of bacterial lysis coincided with persistence of positive cultures in blood and liver.

Comment. These data show that the cirrhotic rat clears bacteria from the circulation as well as the normal rat. Persistence of viable bacteria in liver, lung, and spleen in cirrhotic rats suggests that the bactericidal defense in the R.E. system of lung and spleen as well as in liver is damaged. The persistence of viable bacteria in blood suggests that the blood is being reseeded from the persisting foci in the R.E. system.

Summary. Labelled (I^{131}) bacteria infused into portal vein of normal rats were promptly cleared and destroyed with release of ionic I^{131} and its prompt excretion into the urine. In cirrhotic rats the clearance process was normal, but capacity to destroy bacteria was impaired. This resulted in persistence of ingested bacteria and of "bacteria bound" radioactivity in liver, lung, and spleen, and continued reseeding of the blood stream from such foci.

1. Rutenburg, A. M., Sonnenblick, E., Koven, I., Aprahamian, H. A., Reiner, L., Fine, J., *J. Exp. Med.*, 1957, v106, 1.

2. Schweinburg, F. B., Seligman, A. M., Fine, J., *N.E.J. Med.*, 1950, v242, 747.

Received April 6, 1959. P.S.E.B.M., 1959, v101.

"Complete" and "Incomplete" Hemagglutin Formation in the Mouse.* (24911)

ARTHUR W. FRISCH AND GORDON H. DAVIES
Dept. of Microbiology, University of Oregon Medical School, Portland

The presence of antibodies other than those active in a saline environment has been well documented with human serums following isoimmunization in pregnancy and transfusions. These varieties have usually been categorized as "univalent" or "incomplete" and are demonstrable in solutions of increased viscosity provided by adding serum, albumin, or polyvinylpyrrolidone. Other methods of detecting such antibodies include use of trypsinized erythrocytes and the antiglobulin or Coombs' test. "Incomplete" types of antibodies have also been observed in experimental animals(1, 2,3,4,5,6) but have not been studied in detail. The purpose of this paper is to report the appearance of high titred, "incomplete" hemag-

* Supported by grant from Univ. of Oregon Med. School Research Fn. and Dorsey Endowment Fund.

TABLE I. Stability of "Complete" and "Incomplete" Antibodies in Anti-Human-Erythrocyte Mouse Serum.

Serum titrations→		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Saline	Original	++++	++++	++	—	—	—	—
	Refrig. 10 mo	"	"	+	—	—	—	—
Albumin (6%)	Original	"	"	++++	++++	+++	++	—
	Refrig. 10 mo	"	"	"	"	++++	+++	—
Trypsin	Refrig. 10 mo	"	"	"	"	+++	—	—
Coombs'	*Idem*	"	"	"	"	++++	+++	—

glutinins for human erythrocytes in response to immunization of the Webster strain of mice. They were demonstrated in 6% human albumin solutions, with trypsinized cells, and by means of a Coombs' reagent made by injecting whole mouse serum into a rabbit.

Materials and methods. To a 5% suspension of washed erythrocytes, was added 0.1 the volume of 1% trypsin followed by incubation at 37°C for 10 minutes. The cells were washed 3 more times in 0.85% saline and a 2% suspension prepared. Three sets of serial 2-fold dilutions of serum were made in 0.2 ml of saline and one in 6% human albumin. One drop from a 1.0 ml pipette (approximately 0.03 ml) of the proper 2% erythrocyte suspension was added to each series. For the saline, albumin and trypsin titrations, the tubes were incubated for 1 hour at room temperature, spun in an angle centrifuge at 1000 rpm for 1 minute and the button examined with a 10X hand lens. Reactions were graded as to intensity. The end-point of each titration was a 2+ agglutination which consisted of readily visible clumps of cells; lesser degrees of clumping were recorded as 1+ but were not included in determining final titre. For the Coombs' titration, approximately 0.03 ml of a 1:100 dilution of the anti-mouse Coombs' reagent was added after the titrations had been incubated for 1 hour and the cells had been washed thoroughly with saline. The tubes were incubated an additional 15 minutes; then centrifuged and examined as above.

Results. In Table I, serum was obtained following intravenous injection of 0.5 ml of packed human group O DCe cells on days 1, 2, 3, 15 and 16 into six 20 g Webster mice. On day 44, the mice received a final injection of 0.5 ml of cells subcutaneously and were bled from the heart 7 days later. When originally tested, this serum showed a saline titre of 1:10,000 and an albumin titre of 1:10,000,000. After 10 months in the refrigerator, it was retested with the results shown below. The remarkable stability of the antibody and the unusually high albumin, trypsin and Coombs' titres following multiple injections of antigen may be noted. Similar responses have also been obtained with sheep erythrocytes.

The result in saline as compared with albumin for a single intraperitoneal dose of varying amounts of human erythrocytes is shown in Table II. With 0.05 ml of cells, both varieties of antibody were produced on the second day after immunization and increased in amount thereafter. The albumin titre was always a few tubes higher than the saline titre. However, if the antigen dose was decreased to 0.005 ml or 0.0005 ml, only the albumin variety of antibody appeared in titres as high as two to the fourteenth power (1:16,384). In other experiments, the "incomplete" titres have varied from 1:512 to 1:4096. Subcutaneous injections of antigen gave similar results.

Discussion. The term "incomplete" anti-

TABLE II. Titer of "Complete" and "Incomplete" Mouse Hemagglutinins after Varying Amounts of Antigen.

	Intraper. dose*					
	.05		.005		.0005	
Day tested	Sal.	Alb.	Sal.	Alb.	Sal.	Alb.
0	0	0	0	0	0	0
2	6†	9	0	4	0	2
4	6	12	0	0	0	0
8	14	16	0	9	0	13
12	10	17	0	9	0	14

* ml packed erythrocytes.

† The number represents a dilution of 2 to the 6th power or 1:64.

body as used in this paper does not necessarily imply that each hemagglutin is physically and chemically separable into different components. It is entirely possible that the albumin, trypsin and Coombs' antibodies are the same as the saline variety but demonstrable only under special conditions. We have used the term in the latter sense until more specific data are available.

The dextran-active mouse hemagglutinin described by Gorer(1,2) in contrast to the one reported herein, was induced by tumor transplants. It was also quite labile on storage and of relatively low titre. Chattergee *et al.*(6) described a mouse, anti-sheep, and anti-rabbit hemagglutinin which was detected in tissue culture by the Coombs' test. Their data, however, were not entirely convincing. Owen(4) discussed a "blocking" and a Coombs' hemagglutinin in the serum of chickens immunized with human erythrocytes; his experimental data were not presented. An unsuccessful attempt to induce "incomplete" antibody in rabbits immunized with human erythrocytes was reported by Foster(7) together with qualitative differences in hemagglutinating versus hemolytic activity during the primary and secondary immune responses. With single injections of human erythrocytes into mice, we have not obtained antibodies with hemolytic properties. However, with sheep erythrocytes as antigen, a hemolysin is regularly induced.

Demonstration of a marked hemagglutin response to primary immunization which is not detected in saline will come as no surprise to those experienced with isoimmunization in pregnancy. One often receives human serums without saline antibodies which, on testing with trypsinized cells, with 20% albumin, or with an anti-globulin serum, are found to contain an Rh agglutinin at a titre of 1:1024 or greater(8). The presence of a similar situation in the mouse suggests that studies in which only the saline or hemolytic variety of antibody has been measured may require reexamination. Thus preliminary experiments with X-radiation of mice indicate that saline and trypsin hemagglutinin formation are inhibited to a greater degree than the albumin or the Coombs' response (Frisch and Davies, unpublished).

Summary. Injection of human, sheep and rabbit erythrocytes into Webster mice results in the appearance of "incomplete" varieties of hemagglutinins detectable in albumin, with trypsinized cells, and by means of a Coombs' reagent. In some animals given small doses of antigen, the saline agglutinin does not appear. At the same time, the "incomplete" ones may be present in high titre and escape notice unless tested for specifically.

1. Gorer, P. A., *Cancer Res.*, 1947, v7, 634.
2. Gorer, P. A., Mikulska, Z. B., *ibid.*, 1954, v14, 651.
3. Kaliss, N., Hoecker, G. T., Bryant, B. F., *J. Immun.*, 1956, v76, 83.
4. Owen, R. D., *Proc. R. Soc. M., Lond.*, 1956, v146, 8.
5. Foley, E. J., Morgan, W. A., Greco, G., *Antibiot.*, 1957, v7, 70.
6. Chatterjee, J., Chatterjee, B. R., Chadhuri, S. N., *Ann. Bioch. and Exp. Med.*, 1957, v17, 2.
7. Foster, W. D., *J. Immun.*, 1957, v78, 291.
8. Frisch, A. W., Jackets, V., *Am. J. Clin. Path.*, 1949, v19, 435.

Received April 8 1959. P.S.E.B.M., 1959, v101.

Distribution of Ions in Intestinal Smooth Muscle.* (24912)

LLOYD M. BARR†‡ (Introduced by H. W. Davenport)
Dept. of Physiology, University of Illinois, Urbana

The electrochemical properties of smooth muscle cells are so poorly understood that it is a question whether the relations between ions' distributions and transmembrane potentials is the same as, or merely similar to, the

* Part of thesis in partial fulfillment of requirements of Ph.D. degree.

† Predoctoral Research Fellow P.H.S.

‡ Present address: Dept. of Physiology, Univ. of Michigan, Ann Arbor.