TABLE I.
Results of Five Perfusions Withdrawing Blood from the Vena Cava and Returning It Through a Femoral Artery.

Length of perfusion (min.)	Blood flow (cc/min.)	Film vol. (cc)	No. of cylinders filmed	$cc O_2$ introd. per cc film per min.	$cc O_2$ introd. per min.	Survival time	Plasma hemoglobin (g%)	
							Before	After
10½	720	300	4	.272	81.7	6 wk*		
12	680	270	5	.272	73.4	1½ hr	.57	.54
11	840	210	5	.284	59.6	10 min.	.25	.23
21	760	200	4	.266	53.2	1½ hr	.70	.71
22	1250	265	6	.295	78.3	3 hr	.80	.94

* Alive.

introduced into the blood per cc of film per minute) is seen to compare favorably with that in earlier experiments (0.247 cc). There was one other survival. The remainder expired at periods of 30 minutes to 36 hours after perfusion. Hemolysis was found to increase very little during perfusion.

Conclusions. 1. An oxygenator consisting of 8 concentric revolving cylinders which utilizes a funnel to deliver the blood into a small cup is described.

2. Animal perfusions demonstrate that its efficiency as an oxygenator is high enough to make it possible to perfuse the entire animal body without using an impractically large amount of blood in the extra-corporeal circulation.

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17135. Hemagglutination by Normal and Poliomyelitis Stool Suspensions.*

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The phenomenon of agglutination of erythrocytes by certain viruses is well known.[1] Viruses known to produce hemagglutination include influenza A & B, vaccinia, ectromelia, mumps, mouse pneumonitis, fowl plague and Newcastle disease. These agglutination reactions can be differentiated from each other by inhibition with specific immune sera. Certain bacteria and pleuropneumonia organisms also have the property of agglutinating red blood cells. Recently, the presence of a hemagglutinin in bacteria-free amniotic fluid of normal embryonated hens eggs has been described by the Army Commission on Acute Respiratory Diseases.[2] This substance was shown to be associated with the globulin fraction of egg albumin and gains access to amniotic fluid when the contents of the albumin sac ruptures into the amniotic cavity between the 11th and 13th day of incubation. Hemagglutination can also be induced by ricin, abrin, crotin and certain inorganic colloidal acids or bases such as salicylic acid and basic proteins as protamine.

In studies on the detection of possible hemagglutination by the poliomyelitis virus, it was found that both normal and infected saline stool suspensions agglutinated the erythrocytes of a wide variety of animal species. This paper is a report describing the agglutination of group "O" human, chick, rabbit, guinea pig and sheep erythrocytes by stool suspensions and some of the properties of the stool hemagglutinin.

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1 Rivers, T. M., Viral and Rickettsial Infections of Man, J. B. Lippincott Co., 1948, p. 77.

2 Commission on Acute Respiratory Diseases, PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 118.

TABLE I.
Hemagglutination of Different Species by Normal and Poliomyelitis Stool Suspensions.

Stool No.	Final agglutination titer				
	Human group O	Chick	Rabbit	Guinea pig	Sheep
	Poliomyelitis stools.				
7	1:30	1:30	1:20	1:20	1:20
16	1:20	1:40	1:160+	1:20	1:20
17	1:320+	1:320+	1:320+	1:60	1:320+
18					
	Normal stools.				
3	1:20	1:30	1:320+	1:40	1:20
4	1:40	1:40	1:160	1:20	1:20
5	1:20	1:20	1:320+	1:20	1:20
6	1:120	1:120	1:320+	1:40	1:80

TABLE II.
Inhibition of Stool Hemagglutination by Serum and Bovine Albumin Using Chick Cells.

Exp.	Serum	Serum dilution								Titer
		1:6	1:12	1:24	1:48	1:96	1:192	1:384	1:768	
1	Human	0	0	0	0	±	+	+	+	1:48+
2	Rabbit	0	0	0	0	+	+	+	+	1:48
3	Monkey	0	0	0	0	0	0	0	±	1:384+
4	20% bovine albumin	0	0	0	+	+	—	—	—	1:24

0 = No agglutination.
+ = Agglutination.

Methods. 10% stool suspensions were prepared by grinding the stools in a mortar and pestle with a small amount of physiological saline, diluting the suspensions to a final concentration of 10% and centrifuging in an angle centrifuge at 5000 r.p.m. for 20 minutes. The supernatant was then removed and cultured for the presence of bacteria. Titrations were carried out by making serial 2-fold dilutions of stool suspension in saline. 0.5 cc of material was added to each test tube. Next equal parts of 0.75% of the various erythrocyte suspensions which had been previously washed 3 times prior to use were added. The tubes were mixed and allowed to stand at room temperature for 1 hour prior to reading. The titer was read as the highest dilution showing a hemagglutination pattern on the bottom of the test tube. This pattern closely resembled that produced by influenza and other viruses.

Results. Titrations obtained comparing poliomyelitis and normal stool suspensions with group "O" human, chick, rabbit, guinea pig and sheep erythrocytes are shown in Table I. Highest titers were obtained with rabbit erythrocytes while titers with the erythrocytes of other species did not differ significantly. It was found that rabbit cells in saline occasionally showed spontaneous agglutination and such experiments were discarded. The results summarized in Table I indicate that there was no significant difference in hemagglutination titers between normal and poliomyelitis stool suspensions. In other experiments it was found that a 10% mouse brain and cord suspension infected with the Lansing strain of poliomyelitis virus failed to agglutinate chick, human group "O" and rabbit cells.

Table II shows the inhibition of hemagglutination of chick erythrocytes by human, rabbit, and monkey serum and 20% bovine albumin in relatively high dilutions. Salk's technic[3] of the hemagglutination-inhibition test for antibodies against the influenza virus was employed. Similar results were obtained in hemagglutination-inhibition tests with the erythrocytes of other species. The effect of heat on hemagglutination of chick cells by

[3] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE III.
Effect of Heat on Hemagglutination by Stool Suspensions of Chick Cells.

Stool No.	Temperature °C	Time	Titer
3	Unheated	—	1:30
	65	15 min.	0
7	Unheated	—	1:400
	56	30 min.	1:60
	65	15 min.	0
17	Unheated	—	1:120
	65	15 min.	0
18	Unheated	—	1:40
	65	15 min.	0

TABLE IV.
Hemagglutination of a Stool Suspension after Previous Absorption with Human Group O, Chick and Rabbit Erythrocytes.

	Final agglutination titer			
Absorbing cells	Human group O	Chick	Guinea pig	Rabbit
Group O	1:10	1:20	1:20	1:240?
Rabbit cells	1:60	1:20	1:20	1:20
Chick cells	1:20	1:20	—	—
None	1:240	1:240	1:60	1:320+

stool suspensions is shown in Table III. Incubation in a water bath at 65°C for 15 minutes completely destroyed the stool hemagglutinin in the experiments listed in Table III, while heating at 56°C for 30 minutes also reduced the titer significantly. In other experiments, the hemagglutination titer was approximately the same when carried out at 3-5°C, room temperature and 37°C. Storage for 2 weeks in the icebox resulted in a marked drop in titer. Stool suspension Seitz filtrates failed to show hemagglutination, but titers could be partially restored (¼ to ½) the original) by methanol concentration at low temperatures.[4]

The hemagglutinin could be absorbed in differential erythrocyte absorption experiments by group "O" human, chick and rabbit erythrocytes (Table IV). The absorption was carried out by adding equal parts of 50% packed cells to the stool suspension, incubating for 15 minutes at room temperature and centrifuging at 1500 r.p.m. for 15 minutes. The supernatant was then drawn off and tested for the presence of hemagglutinins against homologous and heterologous erythrocytes. Usually a single absorption was adequate for almost complete removal of the hemagglutinins.

Discussion. The relationship of the stool hemagglutinins to those produced by viruses, bacteria and normal amniotic fluid is unknown. The stool hemagglutinins resemble those produced by bacteria more closely because both are thermolabile, decrease in titer on storage and are inhibited by normal serum. On the other hand, the virus hemagglutinins are more heat stable and are inhibited only by specific sera.

Occasionally the stool suspensions were bacteriologically sterile but generally *Escherichia coli, Staphylococcus,* yeasts or diphtheroids were isolated. Turbid saline suspensions of the various isolated organisms (containing approximately 1 billion per cc) were tested for agglutination with chick cells, group "O" human, rabbit and guinea pig cells. No hemagglutination was observed with any of the cells tested except the rabbit erythrocytes which agglutinated in relatively low titers (1:2 to 1:6 dilutions of the bacterial suspensions). These results are in accord with those recently

[4] Cox, H. R., *et al., J. Immunol.,* 1947, **56**, 149.

reported by Griffitts.[5] This worker found that *E. coli, Aerobacter aerogenes, Alcaligenes faecalis, Bacillus anthracis, Brucella abortus, Corynebacterium diphtheriae, Eberthella typhosa, Proteus vulgaris, Salmonella, Shigella, Staphylococcus aureus* and a few other bacterial species failed to agglutinate human erythrocytes. On the other hand, 23 strains of *Shigella alkalescens,* some strains of *Hemophilus pertussis, Vibrio comma,* and *Streptococcus pyogenes* showed hemagglutination of human erythrocytes. None of the species of bacteria which Griffitts reported to produce hemagglutination were isolated from stool suspensions cultured in the present study.

Summary. Saline suspensions of stools obtained from both normal and poliomyelitis patients showed hemagglutination of group "O" human, chick, rabbit, guinea pig and sheep erythrocytes. The rabbit cells were agglutinated in highest titers. No significant difference in hemagglutination titer was noted with the normal and poliomyelitis stools. Normal human, rabbit and monkey serum and 20% bovine albumin inhibited hemagglutination. The hemagglutinin was thermolabile and absorbed by group "O" human, chick and rabbit erythrocytes. Stool suspension Seitz filtrates failed to show hemagglutination, but the titer could be partially restored by methanol precipitation. Various species of bacteria isolated from stool suspensions failed to agglutinate chick, group "O" human and guinea pig erythrocytes but showed some agglutination of rabbit cells in low titer. Mouse brain and cord suspensions infected with the Lansing strain of poliomyelitis virus also failed to agglutinate chick, human group "O" and rabbit erythrocytes.

[5] Griffitts, J. J., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 358.

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17136. An Antithyrotoxic Factor for the Rat Not Identical with Vitamin B_{12}.*

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It has been demonstrated that whole liver powder will prolong survival and counteract growth retardation of immature rats fed massive doses of desiccated thyroid.[1-3] Some confusion exists, however, regarding the nature of the factor or factors responsible for the above effects. On rations containing soybean meal the retardation in growth caused by massive doses of desiccated thyroid or iodinated casein has been counteracted both in the rat and chick by liver fractions with vitamin B_{12} activity.[4-6] In subsequent work similar findings were obtained with crystalline vitamin

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[1] Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 500.

[2] Ershoff, B. H., *Arch. Biochem.*, 1947, **15**, 365.

[3] Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition,* 1947, **34**, 431.

[4] Bosshardt, D. K., Paul, W. J., O'Doherty, K., Huff, J. W., and Barnes, R. H., *J. Nutrition,* 1949, **37**, 21.

[5] Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 129.

[6] Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 631.