also because they have been considered in connection with the time of onset of paralysis. This has seemed particularly desirable since Haas and Armstrong found that the occurrence of antibody could be correlated more closely with age than with the clinical disease.

Summary. Serum protection tests in mice have been done and the results are significant as tested by a simple statistical method. The experiments are in favor of the view that the Lansing strain is immunologically related to human poliomyelitis.

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A Human Serum Containing Four Distinct Isoagglutinins.

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The serum to be described was studied because the patient from whom it was derived had had hemolytic reactions following transfusions of apparently compatible blood. In this report we shall present the serologic findings; the clinical data will be described elsewhere.[1]

The patient belonged to group 0, type N. The patient's serum agglutinated every one of 28 consecutive group 0 bloods. That we were not dealing with an autoagglutinin was proved by the absence of agglutination in mixtures of the patient's serum with her own cells. Evidently the serum contained isoagglutinins in addition to anti-A and anti-B.

Since the patient was Rh negative,[2, 3] it seemed possible that anti-Rh isoagglutinins might be present in her serum. Accordingly the serum was absorbed with pooled, washed cells A_1MNRh- and

* Aided by a grant from the Committee on Human Heredity of the National Research Council.

1 Wiener, A. S., Forer, S., and Crooks, P. E., in preparation.

2 Landsteiner, K., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 223.

3 Wiener, A. S., and Peters, H. R., *Ann. Int. Med.*, 1940, **13**, 2306.

BMNRh-. The absorbed serum was found to give reactions coinciding with those of standard Rh antisera, proving that the anti-Rh isoagglutinin was present. Since tests on Rh negative bloods with the unabsorbed serum showed that most of the bloods were agglutinated, it was evident that another irregular isoagglutinin was present. Difficulty was encountered in separating the unknown isoagglutinin from the anti-Rh isoagglutinin by absorption experiments. A separation between them was readily effected, however, when it was found that the anti-Rh isoagglutinin acted strongly at body temperature, and not at all at refrigerator temperature, while the unidentified isoagglutinin acted only in the cold.

In order to ascertain the nature of the fourth isoagglutinin, it was necessary to test a larger series of bloods. On account of the presence in the patient's serum of anti-A and anti-B isoagglutinins, bloods of groups A, B, and AB could not be tested directly. It was found that by the simple addition to the patient's serum of a solution of the group substances A and B (Table I), the action of the interfering α and β isoagglutinins could be completely neutralized without affecting the activity of the anti-Rh isoagglutinin or the other irregular isoagglutinin. Moreover, by making tests with serial dilutions of the patient's serum at body and refrigerator temperatures, the reactions of the anti-Rh isoagglutinin and the fourth isoagglutinin were readily separated as shown in Table I. It will be seen that every blood not clumped by the patient's serum at low temperature, belongs to type N, like that of the patient, suggesting that the fourth isoagglutinin is an anti-M isoagglutinin. That this is correct was established by tests on a series of bloods including 10 of type N.

From the serologic standpoint, these observations are of some interest. Firstly, this is apparently the first human serum to be described in which 2 different irregular isoagglutinins could be clearly identified. Secondly, this is one of the few human sera to be described with anti-M isoagglutinins in it, of which only three[4, 5, 6] have previously been encountered among many thousands of human sera examined. The serum is also unusual because of the high titer of the anti-Rh isoagglutinin, the titer being 64 at 37°C. It is interesting that despite the high titer, the anti-Rh isoagglutinins were not active at all at ice-box temperature, in contrast to the sera previously reported by Wiener and Peters.[3] Such anti-Rh isoagglutinins acting at body temperature have been described by Levine, Katzin and

[4] Wolff, E., and Jonsson, B., *Deutsch Z. f. d. ges. gerichtl. Med.*, 1933, **22**, 84.
[5] Friedenreich, V. *Z. f. Immunitäts.*, 1937, **91**, 485.
[6] Moureau, P., and Lambert, J., *Ann. de Med. Leg.*, 1940, **20**, 163.

TABLE I.

Reactions of Serum[1] of Patient on Series[2] of 17 Bloods.

Blood specimen No.	Group and/or subgroup	M,N type	Bloods tested[3] for Rh factor at 37°C with patient's serum dilution						Interpretation of reactions with patient's serum at body temp.	Results of tests with Anti-Rh Immune Serum[4]	Bloods tested[5] for M factor in refrigerator with patient's serum dilution		Patient's serum absorbed with bloods 1-17 and tested vs. blood 14[6]	Interpretation of reactions with patient's serum at 0°C
			1:4	1:8	1:16	1:32	1:64	1:128			1:1	1:2		
1	B	M	++	++	++	+±	tr	—	Pos.	Pos.	++	+±		Pos.
2	B	MN	++	++	++	++	+±	tr	,,	,,	+±	+		,,
3	A_2	MN	++	+±	+±	+±	tr	—	,,	,,	+±	+	—	,,
4	A_1	M	+±	+	+	±	tr	—	,,	,,	+±	+±	—	,,
5	B	MN	+±	+±	+	+	±	—	,,	,,	++	+±	—	,,
6	A_1B	MN	tr.	—	—	—	—	—	Neg.	Neg.	+±	+	—	,,
7	A_1	MN	++	++	+±	+	+	tr	Pos.	Pos.	+±	+	—	,,
8	A_1	MN	+±	+	+	±	tr	—	,,	,,	++	+±	—	,,
9	A_2	MN	Hem.	++	+±	+	tr	—	,,	,,	++	+±	—	,,
10	O	MN	Hem.	++	+±	+±	tr	—	,,	,,	++	+±	—	,,
11	A_1	N	+	±	±	tr	tr	—	,, ?	,,	—	—	+±	Neg.
12	O	MN	+	+	+±	+±	±	—	,,	,,	+±	+	—	Pos.
13	B	N	+±	+	+	+	±	—	,,	,,	—	—	+±	Neg.
14	O	M	—	—	—	—	—	—	Neg.	Neg.	+±	+	—	Pos.
15	A_1B	N	+±	+±	+	+	±	—	Pos.	Pos.	±	—	+±	Neg.
16	O	N	++	++	++	++	tr	—	,,	,,	—	—	+±	,,
17	A_1B	M	+	±	tr	—	—	—	Neg.?	Neg.	++	+±	—	Pos.
Donor	O	N	—	—	—	—	—	—	,,		—	—		Neg.

[1] The patient's serum before use was prepared as follows. It was first inactivated by heating at 56°C for 20 minutes, and then it was mixed with one-fifth its volume of a "purified" solution of group substances A and B. The latter was prepared from salivas of group A and group B, heated in boiling water for 10 minutes, then centrifuged and cleared by filtration through a half thickness Seitz pad, the filtrates being pooled in the proportion of 2 parts A saliva to 1 part B saliva.

[2] The series tabulated is random except that individuals were selected so as to include representatives of each of the 4 blood groups. For comparison, the reactions of the blood of one of the compatible donors used for subsequent transfusions are also included.

[3] One drop each of serum dilution, blood suspension (2% in terms of blood sediment) and saline were mixed in small test tubes. Preliminary readings were taken after 1 hour at 37°C; final readings after standing over night at room temperature.

[4] These tests were made with anti-rhesus immune guinea-pig sera (*cf.* Landsteiner, K., and Wiener, A. S., article in preparation).

[5] One drop each of serum, blood suspension and saline were mixed in small test tubes, the rack was placed in the refrigerator, preliminary readings taken in 1 hour, final readings the next day.

[6] One drop of the packed, washed cells of each blood tested was mixed with 3 drops of the patient's serum. After 1 hour in the refrigerator, a drop of the supernatant was removed, tested against blood suspension No. 14, the latter blood being selected since it belonged to group O and was Rh negative, so that any agglutination obtained had to be due to the M factor.

Burnham,[7] however. Incidentally, the fact that it was possible to neutralize the interfering anti-A and anti-B isoagglutinins in human sera without affecting the irregular isoagglutinins, by the simple expedient of adding solutions of group substances from saliva, is evidence that the properties Rh and M are absent from saliva, in contrast to A and B.[8]

From the clinical standpoint, the case illustrates the difficulties that may be encountered on rare occasions in finding compatible donors for blood transfusion. That three subsequent transfusions from two different selected donors of type ONRh- were successful indicates that such obstacles can be overcome.

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Adrenolytic Action of Cyclopropane.*

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Previous work[1, 2, 3] has shown that 30 minutes of cyclopropane anesthesia sensitizes the heart of the dog so that ventricular tachycardia follows the injection of 0.01 mg of adrenalin† per kilogram. The only ventricular effect of this dose in the unanesthetized animal is extrasystoles. In studying the mechanism of this sensitization it was observed that some dogs which showed 30 seconds or more of

7 Levine, P., Katzin, E. M., and Burnham, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 346.

8 Dr. Philip Levine (personal communication) has also found that the property Rh is probably restricted to the blood cells.

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1 Meek, W. J., Hathaway, H. R., and Orth, O. S., *J. Pharm. and Exp. Therap.*, 1937, **61**, 240.

2 Orth, O. S., Leigh, M. D., Mellish, C. H., and Stutzman, J. W., *J. Pharm. and Exp. Therap.*, 1939, **67**, 1.

3 Allen, C. R., Stutzman, J. W., and Meek, W. J., *Anesthesiology*, 1940, **1**, 158.

† Parke Davis adrenalin hydrochloride.