With few exceptions daily reticulocyte counts were made on the 4 splenectomized animals throughout the entire periods following the first treatment. These periods covered approximately 7 weeks. Splenectomized and normal controls were followed with practically daily reticulocyte counts over a period of 3 weeks from the beginning of the experiment. The wet technic for counting reticulocytes was employed using brilliant cresyl blue dye. One thousand cells were counted on each determination. The same 3 observers made all counts. The liver extract used was known to be potent in the treatment of pernicious anemia.

Results. No significant increase in reticulocytes was noted in any of the animals which has been splenectomized and adequately treated with either liver extract or PGA. The normal controls showed reticulocyte ranges from .6 to 5.3%; the splenectomized controls showed ranges of 1.3 to 4.8%; the animals treated showed ranges of .7 to 5.2%, the values bearing no relation to material administered. Chart 1 shows the reticulocyte range of a normal control animal. Chart 2 shows the range of a splenectomized control. No charts are shown for rabbits treated since the reticulocyte ranges of these animals differed in no respects from those shown. The reticulocyte ranges given were experienced

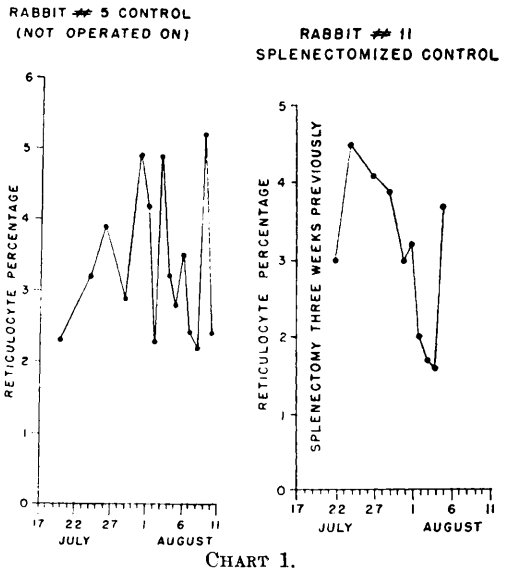

CHART 1.

by practically all of the animals used, regardless of group. No trend in any direction was noted in the case of a single animal.

Conclusions. Splenectomized white rabbits showed no significant change in percentage of reticulocytes following oral and parenteral administration of either pteroylglutamic acid or of liver extract effective in pernicious anemia. Greater variations were found in daily reticulocyte counts, both in controls and in splenectomized animals, than were reported by Jacobson and Williams.

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Observations on Isosensitization to the M Agglutinogen in Man.

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It is generally considered that the agglutinogens M and N can be disregarded when selecting donors for blood transfusion therapy. While this is true for patients requiring only a single transfusion, or multiple transfusions within a short space of time, in cases where several transfusions spaced over a long period of time (months or years) are required, one must take into account the possibility of isosensitization to the M agglutinogen as well as the Rh factor.

Only a relatively small number of cases have been reported of type N individuals with natural anti-M agglutinins in their sera.[1,2] In addition, a few cases have been encoun-

1 Wiener, A. S., *Blood Groups and Transfusion*, 3rd ed., p. 219, C. C. Thomas, Springfield, Ill., 1945.

2 Unger, L. J., Wiener, A. S., and Sonn, E. B., *Am. J. Clin. Path.*, 1946, **16**, 45.

tered with anti-M agglutinins produced apparently as a result of transfusions of type M blood into type N individuals. Wiener and Forer[3] reported a case in which a woman had hemolytic reactions following blood transfusions, and difficulty was encountered in finding a completely compatible blood in cross-matching tests. On testing the blood of the patient, these investigators found her blood to belong to group O, type N, Rh-negative, while her serum contained anti-M and anti-Rh agglutinins. Broman[4] has reported a hemolytic transfusion reaction caused by M sensitization.

In analyzing our cases of Rh sensitization, we found that the spacing of injections was far more important in bringing about isosensitization than the quantity of antigen introduced into the body.[5] In fact, we have applied this principle successfully in devising a simple method of producing anti-Rh serum in normal male type rh donors.[6] While these investigations on the preparation of anti-Rh sera were being carried out, some observations were made on the isosensitization of normal male individuals to the M agglutinogen. The purpose of this paper is to describe these observations.

Case I. This group AB, type rh volunteer was given repeated intravenous injections of 2 cc each of whole Rh-positive blood diluted to 5 cc with sodium citrate solution. The first (priming) injection was given in October 1946. The second injection, or first "stimulating" injection, was given in the middle of February 1947. Ten days later the donor's serum was found to contain univalent Rh_0 antibodies (Rh_0 glutinins) of 50 units titer by the plasma conglutination method, together with weak anti-rh″ agglutinins. Since the titer was not high enough to yield a usable serum, the donor was not bled at that time, and a second stimulating injection was given 2 months later. After this injection the Rh_0 glutinin titer was found to be 85 units by the plasma conglutination method, while the anti-rh″ agglutinin titer rose to 20 units. At this time, we also detected in the volunteer's serum a weak agglutinin reacting with Rh-negative blood but not with the donor's own blood cells. The donor was given a fourth injection of 2 months later, after which the anti-Rh_0 glutinin titer was 512 units by the albumin-plasma method, while the anti-rh″ agglutinin titer proved to be 40 units. Again the presence of a third antibody reacting with type rh blood was noted, and it was decided to investigate the nature of the third antibody.

Titrations showed the third antibody to be an agglutinin of 3 units titer, approximately equally active at body, room and refrigerator temperatures. Tests were set up against a series of 28 blood samples of type rh and type Rh_1, but otherwise unselected, by the agglutination method at room temperature.* Of the 28 bloods tested, only 2 gave negative reactions, besides the volunteer's own blood. One of the negatively reacting blood samples belonged to type $ONRh_1rh$ and the other to type BNrh. Since these 2 blood samples were the only ones in the series besides the volunteer's which belonged to type N, this suggested that we were dealing with an anti-M immune agglutinin. In fact, further tests proved that all type N bloods of types Rh_1 and rh were not agglutinated by the serum, while all other bloods were agglutinated, confirming the diagnosis of anti-M as the specificity of the antibody.

For subsequent stimulating injectings only type N, Rh-positive blood was used, and as shown in Table I, later bleedings obtained from this volunteer contained no demonstrable M antibodies.

Case II. The second volunteer, also group AB and type rh, has not been studied as intensively as the first one. Unlike the first volunteer, this individual produced a potent

[3] Wiener, A. S., and Forer, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 215.

[4] Broman, B., *Acta Paediat.*, 1944, **31**, supp. 2.

[5] Unger, L. J., and Wiener, A. S., *Am. J. Clin. Path.*, 1945, **15**, 280.

[6] Wiener, A. S., and Sonn-Gordon, E. B., *Am. J. Clin. Path.*, 1947, **17**, 67.

* The Rh_0 glutinin in the serum did not clump type Rh_1 bloods under these conditions. Bloods of types Rh_2 and Rh_1Rh_2 could not be used in these tests because of the anti-rh″ agglutinin in the serum.

TABLE I. Results of Immunization Experiment on Two Type N, Rh-Negative Individuals.

Case history	Date*	Rh_0 antibodies (titer in units†)				Anti-rh" agglutinins (titration in saline media)	Anti-M agglutinins (titrations in saline media§)
		Agglutinins (titration in saline media)	Univalent antibodies as determined by — Blocking test	Conglutination test in — Plasma	Albumin-Plasma mixture		
Volunteer No. 1, A_1BNrh	2/26/47	0		50		5	0
Blood used for injection $OMRh_2Rh_2$	3/ 5/47	0	4	85		2	0
	4/29/47	0	4	85		20	2
	6/18/47	0	4	104	214	16	3
	6/25/47	0	6		512	40	3
	8/27/47	0	12		500	12	0
	12/ 5/47	0			300	40	0
	1/30/48	0	8			160	0
	2/ 9/48	0			192	64	0
Volunteer No. 2, A_2BNrh	8/28/47	640				0	0
Blood used for injection $OMRh_0$	3/ 1/48	70‡			192	0	8

* These tests were usually made 10 days after a stimulating injection.
† The number of units equals the reciprocal of the highest serum dilution giving a one-plus reaction.
‡ Clumping weak throughout. § Tests for anti-M done at refrigerator, room, and body temperatures (no difference in titer).

anti-Rh_0 agglutinin of 640 units after the first stimulating injection. Following a second stimulating injection about 6 months later, the Rh_0 agglutinin titer was 70 units but there were also Rh_0 glutinins of a titer of 192 units by the albumin-plasma method, as well as an additional agglutinin of 8 units titer which reacted with type rh blood cells. Specificity tests proved the third antibody to be an anti-M immune agglutinin.

Comment. The observations just described demonstrate the necessity of administering only type N blood to type N patients requiring a series of transfusions at widely spaced intervals. When injections are spaced at sufficiently wide intervals, the agglutinogen M seems to be a fairly good antigen for individuals of type N. This was well illustrated by a case concerning which we were consulted several years ago, because of difficulty encountered in the cross-matching tests. This we found to be due to an anti-M immune agglutinin which was active in dilutions as high as 1:256 at body temperature. On the other hand, the N agglutinogen appears to be a weak antigen for individuals to type M. Only a single convincing case of isosensitization to the N agglutinogen has been reported[7] in the literature and this involved a patient with an extraordinary capacity to produce antibodies, who in response to a series of transfusions formed in succession antibodies specific for factor hr′, N, and 3 previously undescribed agglutinogens.

With regard to the previous report by Singer[8] claiming isosensitization to the N agglutinogen by transfusion, only a few bloods were tested with the patient's serum and this is not adequate to establish the specificity of an abnormal antibody. Natural anti-N agglutinins were found by the late G. L. Taylor in one case (cited by Callender and Race[7]) and another such example was reported by Allott and Holman.[9] The case reported by

[7] Callender, A. T., and Race, R. R., *Ann. Eug.*, 1946, **13**, 102.

[8] Singer, E., *Med. J. Aust.*, 1943, **2**, 29.

[9] Allott, E. N., and Holman, C. A., *Lancet*, 1947, **2**, 130.

TABLE II.
Analogies Among the A-B-O, M-N, and Rh-Hr Systems of Blood Factors.*

System*	Major agglutinogens (More antigenic)	Minor agglutinogens (Less antigenic)
A-B-O	A, B, A_1, C†	O(A_2)‡
M-N	M	N
Rh-Hr	Rh_0, rh′, rh″	Hr_0, hr′, hr″

* For simplicity the rare agglutinogens A_3, N_2, rh‴, etc., are not included in this table.
† The factor C is shared by bloods containing agglutinogens A_1, B, or both.
‡ The factor O(A_2) is shared by agglutinogens O and A_2, but absent from agglutinogens A_1 and B.

TABLE III.
Allelic Relationships Among the Landsteiner Blood Groups and Subgroups.

C-O genotypes	Reaction with serums: Anti-A_1,B (Anti-C)	Reaction with serums: Anti-A_2,O (Anti-O)	Blood groups: Phenotypes	Blood groups: Genotypes
CC	+	—	A_1, B, A_1B	A_1A_1, BB, and A_1B
CO	+	+	A_1, B, and A_2B	A_1O, A_1A_2, BO, and A_2B
OO	—	+	O, A_2	OO, A_2A_2, and A_2O

DeKromme and Van der Spek[10] also seems to be an example of natural anti-N rather immune anti-N, as claimed by these workers, considering that their abnormal antibody had the properties of a cold agglutinin.

As is well known, in the Rh-Hr system, the Rh and Hr agglutinogens are related to one another serologically and genetically like the M and N agglutinogens.[1] Of the Rh-Hr agglutinogens, the Rh factors are the more potent antigens and thus correspond to the agglutinogens M in the M-N system, while the Hr factors are the less potent antigens and correspond to the agglutinogen N (cf. table II). That similar relationships also exist in the A-B-O groups is demonstrated in Table II and Table III.

Summary. Two cases are described in which type N individuals produced anti-M agglutinins following injections at widely spaced intervals of blood containing the M agglutinogen. On the basis of these and other observations, it is pointed out that type N patients requiring a series of transfusions at wide intervals should be transfused only with type N blood, if isosensitization and consequent transfusion reactions are to be avoided. Genetic and serologic analogies among the A-B-O, M-N and Rh-Hr systems of blood factors are pointed out. Evidence is cited to show that the factors in each system fall naturally into two major subdivisions.

[10] DeKromme, L., and Van der Spek, L. A. M., *Brit. Med. J.*, 1948, **1**, 643.

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Cure of Ulcerative Cecitis of Rats by Streptomycin.

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Ulcerative cecitis of rats is a disease of considerable importance from two standpoints. First of all, when prevalent in a rat colony, experimental work carried out with the affected animals becomes confused, and incorrect conclusions may be drawn.