peritoneally into rats.* Using constant amounts of the solvent with varying amounts of the drug to be tested, it was observed that all the rats behaved in a very peculiar manner, regardless of drug dosage. The solvent alone was tried by intraperitoneal injection and it was found that the solvent accounted for all the reactions observed.

A series of 20 albino rats weighing from 200 to 250 g were injected with the *N. dibutyl* succinate in graded amounts from 0.5 cc to 10 cc per kg intraperitoneally. The first effect observed was a tendency to gnaw the hind toes, then the rats assumed a somewhat flat crawling position, tending to drag the hind limbs. In a few minutes all the rats became sedated, and dropped off to sleep, with sudden rousing, dragging themselves about the cage and relapsing again into sleep. Respiration was quite markedly decreased. The minimal doses produced this effect lasting about one hour while the effects persisted for nearly 3 hours with the larger doses. All the animals recovered.

In view of the desirability of checking these observations with material from a different source, we obtained a second sample of the solvent from the Eastman Kodak Company. Twenty albino rats of about 200 to 250 g were selected and 10 were injected with the Eastman product and 10 with the original sample in doses of 0.5 cc to 5 cc per kg intraperitoneally. No consistent difference could be observed in the behavior of the rats with the different samples.

The toxicity of the *N. dibutyl* succinate was then extended to rabbits. Eight albino rabbits weighing from 2.8 to 3.4 kilos were injected intramuscularly with doses ranging from 0.33 cc to 2 cc per kg and 6 rabbits were injected intravenously in pairs with 0.5 cc, 0.75 cc and 1 cc per kg. No effects other than marked dilatation of the ear vessels could be observed in the rabbits injected intramuscularly. In the series of intravenous injection, dosages of 1 cc per kg caused the animals to give a loud cry in about 1½ minutes, had a slight convulsion and died of respiratory failure in a few minutes. The 0.75 cc per kg dosage produced slight convulsions in a few minutes, then considerable sedation and respiratory depression and death in 9 and 12 minutes respectively. The 0.5 cc per kg dose did not produce any convulsions, but the animals were sedated and respiration markedly depressed. They were returned to the stock cages after 2 hours in what appeared to be normal condition, but both were dead the next morning.

It is apparent that *N. dibutyl* succinate when rapidly absorbed is a toxic agent, and is not suitable as a parenteral solvent. When injected intramuscularly it shows little toxicity.

* The original sample of N dibutyl succinate was supplied by E. Bilhuber, Inc.

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A New Test (Blocking Test) for Rh Sensitization.*

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As was first shown by Wiener and Peters,[1] sensitization of Rh-negative individuals against the Rh factor can often be detected by *in vitro* tests for anti-Rh agglutinins in the individual's plasma. However, it was soon found[2,3] that there are many Rh-negative patients who are strongly sensitized to the

* Aided by a grant from the United Hospital Fund of N. Y. C.

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

2 Wiener, A. S., *Arch. Path.*, 1941, **32**, 227.

3 Levine, P., Burnham, L., Katzin, E. M., and Vogel, P., *Am. J. Obst. and Gyn.*, 1941, **42**, 925.

TABLE I.
Tests on a Series of Human Sera for Anti-Rh Blocking Antibodies.

Experiment	Test cells* (Group O)	Tests with sera from Rh-positive individuals							Tests with sera from Rh negative mothers of erythroblastotic babies					
		1	2	3	4	5	6	7	8	9	10	11	12	13
1	Rh_1	++±	++	++	++±	++±	++	+±	—	++	++	—	+±	—
2	Rh_2	++±	++	++	++	++	++	++	—	++	++	—	++	—

One drop of the serum being tested was mixed with a drop of test cells (2% suspension) in a small, narrow test-tube and the mixture allowed to interact in a water-bath at 38°C until sedimentation was complete (30 to 60 minutes). In experiment 1, the supernatant fluid was then removed, and a drop of diluted (1:5) anti-Rh_0 serum (original titer 60) was added to each tube. In experiment 2, the anti-Rh_0 serum was added directly without removing the supernatant. The tubes were shaken and then reincubated until sedimentation was complete, and the reactions were read grossly by inspecting the sediment in each tube, and microscopically after gentle shaking. Sera 8, 11, and 13 contain blocking antibodies.

* For nomenclature of the Rh blood types and Rh antisera, see Wiener, A. S., *Science*, 1944, **99**, 532.

Rh factor, as proved by the occurrence of an intragroup hemolytic transfusion reaction or a baby with erythroblastosis (hemolytic disease of the fetus and newborn), yet the plasma does not contain demonstrable anti-Rh agglutinins. The purpose of this paper is to describe a new *in vitro* test, the "blocking test," with the aid of which Rh sensitization can be detected in many of these problem cases.

The first blocking experiments were tried in 1941, when retests of some of the stored post-transfusion sera from the patients described by Wiener and Peters the year before showed these sera to be no longer active. It occurred to the writer that the antibodies might still be present and capable of combining with the test cells but incapable of agglutinating the cells. When the mixture of test cells and apparently inactive serum was first allowed to combine and subsequently active (capable of agglutinating Rh+ cells in control experiments), anti-Rh serum was added, it was found that the test cells were not agglutinated, apparently because the action of the active agglutinin had been blocked. However, the results obtained were irregular, and therefore experiments on this "blocking test" for Rh antibodies, which is a counterpart of the inhibition test for haptens and group-specific substances, were temporarily abandoned. Recently, when more satisfactory anti-Rh testing sera became available, the experiments were resumed. Tests have been carried out on a number of patients with erythroblastotic babies where the usual tests for anti-Rh agglutinins were unsuccessful, and in most of the cases clean-cut blocking reactions have been obtained, proving the presence of a special sort of anti-Rh isoantibody.

Table I presents the results of some blocking tests on a series of Rh-negative patients who have had erythroblastotic babies, as well as a control series of Rh-positive individuals. The technic of the test is simple: First, one drop of a 2% suspension of Rh-positive cells and a drop of the patient's serum are mixed in a small test tube and allowed to react in a water-bath at 38°C for 30 to 60 minutes. Then a drop of a suitable dilution of an active anti-Rh serum is added, and after an additional incubation period of 30 to 60 minutes, the reactions are read. If blocking antibodies are present, no agglutination will occur, or the clumping will be markedly weakened. That the reaction is specific follows from the fact that it has thus far been obtained only with sera from Rh-negative individuals sensitive to the Rh factor, such as mothers of erythroblastotic babies, and not with sera from Rh-positive patients or normal Rh-negative indi-

TABLE II.
Titration of Agglutinating and Blocking Anti-Rh Isoantibodies in the Serum of a Patient with an Erythroblastotic Infant.

Date of tests	Nature of tests	Test cells (Group O)	Dilution of patient's serum in test									
			Undil.	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
6 days after delivery	Direct Titration	Rh_1	—	—	tr.	+	+±	+±	tr.	—	—	
		Rh_2	±	+±	++	++	++	++	++	+±	tr.	—
1 month after delivery	Direct Titration	Rh_1	—	—	—	tr.	+	—	—	—		
		Rh_2	—	—	—	—	—	—	—	—		
	Blocking	Rh_1	—	—	tr.	+±	++	++±	++±	++±		
		Rh_2	—	—	—	++	++±	++±	++±	++±		

The direct titrations were carried out in the usual manner, the readings being taken after 45 minutes in the water-bath at 38°C.

The blocking tests were carried out as described in Table I; in the tests with Rh_2 cells the supernatants were removed before adding the anti-Rh_0 serum, while in the tests with Rh_1 cells, the supernatants were not removed.

viduals not sensitized to the Rh factor. That the reaction is due to an antibody that becomes fixed to the test cells follows from experiment 1, Table I, in which the supernatant fluid was removed before the anti-Rh test-serum was added.

A number of human anti-Rh sera have been obtained which exhibit a marked prozone effect.[4,5] Taylor *et al.*[4] have attempted to explain the behavior of such sera on the basis of optimal proportions of antigen and antibody, but if this explanation were correct, it would be difficult to explain why the phenomenon does not occur more frequently. The present author's experiments described above suggest that such prozone phenomena may be due to the presence in such sera of a mixture of blocking and agglutinating antibodies, the latter being of higher titer. In support of this idea may be cited some observations recently made on a patient after she had given birth to an erythroblastotic baby (*cf.* Table II). Tests 6 days after delivery showed her serum to contain strong anti-Rh agglutinins exhibiting a distinct prozone effect. Direct tests performed only 3 weeks later were almost entirely negative for anti-Rh agglutinins, but quantitative blocking tests showed the presence of blocking antibodies of a titer 2 to 4. The most reasonable explanation of these findings is that immediately after delivery, this patient's serum contained a mixture of blocking and agglutinating antibodies, and that the latter diminished in titer more rapidly than the former. To test this hypothesis, the serum obtained one month after delivery was treated with Rh-positive cells in order to attempt to remove the blocking antibodies in case these masked the presence of low-titered anti-Rh agglutinins. It was indeed found that the absorbed serum then gave distinct and specific clumping, even though the original unabsorbed serum gave no or only faint reactions. Similarly, the serum obtained 6 days after delivery was improved instead of weakened by absorption with Rh-positive cells. Repetition of these absorption experiments gave irregular results, probably on account of the competition between the blocking and agglutinating antibodies.

If, as there is some reason to believe, of the two sorts of Rh antibodies, the blocking antibodies prove to be of greater clinical significance in the causation of erythroblastosis, this would serve to explain the puzzling lack of correlation between the titer of anti-Rh agglutinins in the maternal serum and the severity of the disease in the infant.

Recently, studies have been conducted on the titer and specificity of Rh blocking antibodies in various human sera. To date, the highest titer encountered was 64, and all the blocking antibodies had specificities corresponding to anti-Rh_0. Thus, type Rh_1 blood suspensions treated with such blocking sera gave reactions indistinguishable from type Rh′ blood with the three sorts of Rh

[4] Taylor, G. L., Race, R. R., Prior, A. M., and Ikin, E. W., *Brit. Med. J.*, 1942, **2**, 572.

[5] Levine, P., *Arch. Path.*, 1944, **37**, 83.

antisera, and in the same way type Rh_2 blood suspensions could be "converted" into type Rh", etc.

The observations on blocking antibodies are of interest in connection with the problem of the nature of agglutination and precipitation reactions in general. According to Marrack's "framework" hypothesis,[6] the second stage as well as the first stage of these reactions is assumed to be specific, in contrast to the classic theory which postulates that the second stage is non-specific. Some observations on mixed agglutination reactions were previously reported which were more readily explained under Marrack's hypothesis than the classic theory.[7] The present observations can also be more easily explained under the framework hypothesis, if one postulates that the blocking antibodies are monovalent antibodies, in contrast to the usual agglutinating and precipitating antibodies which are assumed to be bivalent.[8]

In conclusion, it seems highly improbable that blocking antibodies are peculiar to the Rh factor. Doubtless, study of other antigen-antibody systems will reveal the existence of analogous phenomena.[9]

[6] Marrack, J. R., Report No. 230, Medical Research Council, His Majesty's Stationery Office, London, 1934; second edition, 1938.

[7] Wiener, A. S., and Herman, M., *J. Immunol.*, 1939, **36**, 255.

[8] Pauling, L., Campbell, D. H., and Pressman, D., *Physiol. Rev.*, 1943, **23**, 203.

[9] *Cf.* Jones, F. S., and Orcutt, M., *J. Immunol.*, 1934, **27**, 215; Kleckowski, A., *Brit. J. Exp. Path.*, 1941, **22**, 192; Hooker, S. B., and Boyd, W. C., *Ann. N. Y. Acad. Sci.*, 1942, **43**, 107.

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Effect of Picrotoxin on Electrical Excitability of the Respiratory Center.

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The inspiratory portion of the respiratory center has been accurately located in the ventral reticular formation of the medulla of the cat by means of the Horsley-Clarke stereotaxic instrument.[1] Electrical stimulation of this region results in a marked inspiratory response involving both thorax and diaphragm with fixation of the chest in inspiration. Since there is very little direct evidence as to the precise effect of respiratory stimulating drugs on the respiratory center itself, we have thought it worth while to study the influence of one of these drugs on the electrical excitability of this region.

Cats were anesthetized with sodium phenobarbital, 150 mg per kilo intraperitoneally. A tracheal cannula was inserted and the animal was allowed to breathe through a tube containing soda-lime into a small (400 cc) Krogh-type spirometer calibrated to 5 cc. The Horsley-Clarke stereotaxic instrument was placed on the head, and through a small burr hole in the skull a fine double pole electrode was inserted 12-13 mm posterior to the interaural plane and 1-2 mm lateral to the midline. By means of repeated stimulation as the electrode was lowered, the most sensitive region of the center was located.

The stimulus was applied to the center at the end of a normal expiration by means of a Goodwin stimulator[2] set to deliver a peak voltage of 1-5 volts at a frequency of 100 cycles per sec. Little spread of the current from the tips of the electrode appeared to exist since the response was markedly diminished by moving the electrode 0.5 mm up or

[1] Pitts, R. F., Magoun, H. W., and Ranson, S. W., *Am. J. Physiol.*, 1939, **126**, 673.

[2] Dusser de Barenne, J. G., Garol, H. W., and McCulloch, W. S., *J. Neurophysiol.*, 1941, **4**, 287.