

THE RESISTANCE TO A SPECIFIC HEMOLYSIN OF
HUMAN ERYTHROCYTES IN HEALTH
AND DISEASE.¹

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It is the rule in hemolytic experiments to take for granted that the erythrocytes of all healthy animals of one species possess the same general resistance to the hemolysin employed. Titer-figures are based on this assumption. And inasmuch as the results from their use are consistent, the point would seem to be well taken. Yet there have been few direct observations to prove it.

With regard to the resistance of pathological red cells to specific hemolysins we have only meager data. The studies in vogue at present of the behavior of human erythrocytes when placed in serum from another individual hardly bear on the matter because of the many complex and unregulated forces involved. N. Chkliarevitch² and G. Bielonovsky³ are quoted⁴ as having tested a series of pathological bloods with a specific hemolysin; but their papers are not accessible. Observations of the sort have more than an academic interest, in view of the rôle played by hemolysins in disease. Despite Ehrlich's⁵ demonstration that the red cells possess different receptors for each serum-hemolysin, there are not a few to insist with Nolf,⁶ that the mechanism of hemolysis by specific serums, and by agents of simpler chemical constitution, is identical in principle with that by distilled water. Some have based large inferences as regards the red cell and hypothetical disease-hemolysins on the behavior of these cells in hypotonic salt solution. True,

¹ Received for publication Aug. 20, 1909.

² N. Chkliarevitch, *Ann. de l'Acad. mèd. milit. de St. Petersburg*, 1901.

³ G. Bielonovsky, *ibid.*, 1902.

⁴ P. Ribierre, *Folia Hæmatolog.*, 1905, ii, 154.

⁵ P. Ehrlich, *Collected Studies on Immunity*, London, 1906, p. 395.

⁶ P. Nolf, *Ann. de l'Inst. Pasteur*, 1900, xiv, 656.

Morawitz and Pratt⁷ have shown that injections of phenylhydrazin increase the resistance of the corpuscles of the rabbit to all types of hemolysins. On the other hand Rywosch,⁸ studying the normal erythrocytes of ten species, found their relative behavior to vary much, according as water, saponin, chloroform, acetone, sulphuric acid or potassium hydroxide was used as the laking agent. In general, the more resistant corpuscles were to saponin, the less resistant were they to water. Weil⁹ asserts that erythrocytes rendered resistant to eel serum or to saponin by injections of the agent into the animal furnishing the erythrocytes are "almost invariably more easily destroyed than normal control cells, by all other hemolytic agencies including anisotonic salt solution." So much there is to point against wide deductions from the test of corpuscle resistance to salt solution.

But as has been said, there are available no data which illustrate the resistance of human red cells, normal and pathological, to a hemolysin specific for them. Such data would certainly bear on the value of clinical determinations of corpuscle resistance, and further, might furnish some insight on those diseases characterized by blood destruction,—though in view of the considerations just cited, this seems doubtful.

In the following pages are presented results from the test with a specific hemolysin of 107 blood specimens,—26 from normal human beings and 81 from persons suffering from disease.

METHOD.

In order to simulate in some degree the condition of affairs when a specific hemolysin (amboceptor) is introduced into the circulation, human serum was used as complement in the tests.

For the hemolytic amboceptor rabbits were repeatedly injected with washed human corpuscles, collected from the placental end of the cord at normal labor. Immunization of the rabbits proved difficult, necessitating ten to twelve intraperitoneal injections (2.5 to 5 cubic centimeters of washed cells at intervals of four to seven days) before the serum acquired a working strength. Even then at least 0.1 cubic centimeter of it (inactivated at 56° for thirty minutes) was required to give complete hemolysis of 1.0 cubic centimeter 5 per cent. red corpuscles, in the presence of abundant human complement. With guinea-pigs' serum as complement the hemolysin is much more active.

⁷ P. Morawitz and J. Pratt, *Münchener. med. Woch.*, 1908, lv, 1817.

⁸ D. Rywosch, *Arch. f. d. ges. Physiol.*, 1907, cxvi, 220.

⁹ R. Weil, *Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vi, 49.

To obtain human serum for use as complement, fifteen to twenty cubic centimeters of blood were aspirated from the brachio-cephalic vein of a normal adult, or of a well-nourished convalescent from a non-infectious malady. That normal serum will hemolyze the corpuscles of some pathological states has long been known. Accordingly controls were made, that this source of error, when present, might be noted. Fortunately the hemolysis manifested itself only occasionally and very faintly in the dilutions employed in this work. Again it must be remembered, as affecting hemolysis, that certain normal sera will agglutinate the red cells of certain other individuals. But the rabbit serum used as amboceptor, being polyvalent, strongly agglutinated all the corpuscles tested; and thus a second source of error was disposed of. There remains to be considered the protective influence of the small quantity of human serum present. That this might interfere with the tests by a selective action is possible, and the controls do not rule it out. But the results suggest no such influence. To complement one unit of rabbit amboceptor that it may hemolyze completely 1.0 cubic centimeter 5 per cent. red corpuscles at least 0.13 to 0.2 cubic centimeter of human serum is required. The serum was taken from the clot and used after it had stood 3 to 5 hours in contact with it.

For all the tests that follow 0.1 cubic centimeter of complement-serum, made to 0.5 cubic centimeter with 0.85 per cent. salt solution, was mixed with a second 0.5 cubic centimeter containing the amboceptor-serum, and 0.5 cubic centimeter of the 5 per cent. emulsion of red corpuscles added. Thus in each tube there was a uniform volume of 1.5 cubic centimeters. Incubation was for two hours at 37°C.

The following experiment was performed to determine whether normal human sera, obtained under identical conditions and used as complement, differ sufficiently to interfere with the comparison of hemolytic series of which they are a part.

EXPERIMENT I.—A small quantity of blood was taken from the ear of each of six healthy adult males, and each serum, after three hours on the clot, was used to complement a hemolytic series. In all six of these the same emulsion of washed human corpuscles and the same dilutions of amboceptor were employed.

Hemolysis.

Amboceptor:—	$\frac{1}{10}$ c.c.	$\frac{1}{20}$ c.c.	$\frac{1}{40}$ c.c.	$\frac{1}{80}$ c.c.	$\frac{1}{160}$ c.c.	$\frac{1}{320}$ c.c.	Hemolysis with complement-serum alone.
Complement-sera Nos. 1, 2, 3, 4, 5.	Strong	Mod.	Slight	Trace	Faint trace	o	o
Complement-serum No. 6.	Strong	Mod.	Slight	Trace	Faint trace	Faintest trace	Faintest trace ¹⁰

¹⁰ Complement-serum is itself blood-stained, which accounts for the apparent hemolysis here observed.

Here the normal sera used as complement gave identical results. In the later work similar findings were, in general, obtained. But W. L. Moss¹¹ has recently discovered that some normal human sera in full strength will hemolyze other normal human corpuscles. With the dilutions that I have employed, this was several times noted, though only as the faintest trace of hemolysis. The error so introduced has been duly recognized in casting up results.

Since many negro patients were available it became important to know whether the erythrocytes from white and colored persons show the same degree of resistance to the specific hemolysin. Accordingly, two sets of rabbits were immunized, the one with blood from negro deliveries, the other with that from whites, and comparative tests conducted. The following is a typical one.

EXPERIMENT II.—The corpuscles and complement-sera were obtained from normal adults.

Hemolysis.

Complement.	Amboceptor.	R. b. c.	$\frac{1}{2}$ c.c.	$\frac{1}{4}$ c.c.	$\frac{1}{16}$ c.c. Amboceptor.
Negro A	Rabbit-Negro	Negro B	Complete	Slight	Faintest Tr.
"	"	White X	"	"	"
"	Rabbit-White	Negro B	"	"	"
"	"	White X	"	Mod.	O
White X	Rabbit-Negro	Negro B	Complete	Mod.	Tr.
"	"	White X	"	"	Tr.
"	Rabbit-White	Negro B	Almost	Slight	O
"	"	White X	"	"	O
Negro C	Rabbit-Negro	Negro B	Complete	Mod.	Tr.
"	"	White X	"	"	Tr.
"	Rabbit-White	Negro B	"	Slight	O
"	"	White X	"	"	O

The findings correspond to those of Marshall and Teague¹² with specific precipitins. The immune sera differed in general strength, but no difference in their action on the corpuscles of the two races could be made out. So the majority of the rabbits were immunized with blood from whites and blacks indiscriminately. The serum for use as complement was always taken from a white person.

The erythrocytes to be examined were caught in a sodium citrate

¹¹ Personal communication.

¹² H. T. Marshall and O. Teague, *Philippine Jour. of Science*, 1908, iii, 357.

solution (1 per cent. or 1.5 per cent. in 0.85 per cent. salt solution), washed twice in 0.9 per cent. salt solution, and made to a uniform 5 per cent. emulsion with it. The influence of these solutions, and of the washing, to alter corpuscle resistance could not be avoided. But the specimens in each set of tests were treated alike, even to length and speed of centrifugalization, and the results compared, not with an alien standard, but with normals included in the same set; so this influence is, as far as may be, distributed equally. Mixing and incubation were carried out immediately after the specimens had been washed. The tubes were allowed to stand over-night in the ice-chest before readings were made.

That the erythrocyte-emulsions should be comparable, it was needful for them to be, not 5 per cent. emulsions of cells, or 5 per cent. emulsions of hemoglobin, but 5 per cent. emulsions of that special element involved in hemolysis. Most authors agree that this is the stroma.¹³ In attempt to measure this graduated centrifuge tubes were used for the last washing, centrifugalization kept at high speed until the sediment had reached a constant bulk, the amount read off, and an accurate 5 per cent. emulsion by volume made from it. The general behavior of the red cells when packed together,—as observed, for example, during the process of thrombosis in the frog's tongue,—makes it seem certain that in a sediment got by the above method the individual erythrocytes are not rigidly tangent to one another, like apples in a barrel, but are very closely apposed. Thus an error in the measurement of stroma, due to variation of the average cell-size in pathological states, is ruled out.

Far more important should be the error through variation in the quantitative relationship of stroma and those other elements which go to make up the corpuscular mass. Yet that the method as outlined yields an accurate standard emulsion for hemolytic work is made clear by the demonstration with it that the resistance of most bloods, even those very pathological, is the same. Results with a 5 per cent. emulsion made in the usual way, by pipetting the semi-solid mass of corpuscles from the bottom of a

¹³ Bordet, *Ann. de l'Inst. Pasteur*, 1900, xiv, 257; P. Nolf, *ibid.*, 656. Ford and Halsey, *Jour. of Med. Research*, 1904, xi, 403.

centrifuge tube, and adding to it nineteen times its volume of salt solution, had previously led me to suppose that the resistance varied within wide limits.

To reduce the sediment of red cells to a constant volume, centrifugalization must be maintained at the high speed of an electric machine for from twenty to forty-five minutes. When the sediment is once well packed, further centrifugalization, even though much more forcible, fails to disturb its volume. This was well proven by several tests. The following is a characteristic result :

Five minutes centrifugalization sedimented the corpuscles to an initial bulk of 2.1 cubic centimeters. With a continuance of the same speed of the machine the sediment had contracted to 1.8 cubic centimeters after 24 minutes, 1.75 cubic centimeters after 34 minutes, 1.72 cubic centimeters after 40 minutes, and after 49 minutes to 1.70 cubic centimeters. Prolonged centrifugalization at twice the former speed failed now to change the reading.

It is best to employ enough blood to yield a considerable bulk of cells, so that the error of reading may be minimized. For most of the tests sufficient whole blood was taken to yield at least 0.5 cubic centimeter of sediment. This involved as much as 5 cubic centimeters of whole blood from those cases of pernicious anemia that showed a red count of less than one million cells. All of the specimens were obtained from the lobe of the ear.

It may be urged that, while the 5 per cent. emulsion is made up in terms of stroma, its destruction is read off in terms of hemoglobin, and that the two do not necessarily vary together. This would seem an important objection to the method. According to Capps¹⁴ the quantitative relation of hemoglobin to cell-volume varies considerably in both primary and secondary anemias. This does not mean that the quantitative relation of hemoglobin to stroma necessarily is altered. As has been said, the vast majority of blood-specimens, normal and pathological, dealt with by the method exhibit a similar resistance to the specific hemolysin; and this although the emulsions for test were made up in terms of cell-bulk and read in those of hemoglobin liberated. So, apparently, the quantitative relation of hemoglobin to stroma, or, to put it differently, of hemoglobin liberation to stroma destruction by the specific hemolysin, cannot be in this connection an important variable.

¹⁴J. A. Capps, *Jour. of Med. Research*, 1903, x, 367.

The individual red cells of one blood differ much in their resistance to a laking agent;¹⁵ hence, the determination merely of the point of beginning hemolysis is not sufficient. Smith and Brown¹⁶ have accurately indicated with curves the per cent. of breaking down in salt solutions of different tonicity. Most authors have ascertained only the points of beginning and of complete hemolysis (thus of minimum and of maximum resistance), with such data concerning average resistance as could be incidentally noted. This was thought sufficient for the present work.

A considerable number of cells is necessary to bring out by sharp color change the limits of hemolysis, and for this reason a 5 per cent. emulsion was chosen. It proved satisfactory where minimum resistance was concerned, but gave so heavy a tint to the tubes indicating maximum resistance that the character of the sediment had also to be relied on for the determination. The Neisser-Wechsberg phenomenon interfered not infrequently. On the whole, the minimum resistance and resistance of the bulk of the cells were more accurately gauged.

RESISTANCE OF NORMAL RED CELLS.

EXPERIMENT III.—Corpuscles were obtained from eight healthy young men. The same complement and the same amboceptor were employed in testing each specimen.

Hemolysis.

R. b. c.	$\frac{1}{2}$ c.c.	$\frac{1}{3}$ c.c.	$\frac{1}{4}$ c.c.	$\frac{1}{5}$ c.c.	$\frac{1}{7}$ c.c.	$\frac{1}{12}$ c.c. Amboceptor.
Nos. 1, 2, 5, 7	Strong	Strong	Mod.	Slight	Faint trace	o
Nos. 3, 4	Strong	Strong	Mod.	Slight	o	o
No. 8	Strong	Strong	Mod.	Slight	Faintest trace	o
No. 6	Strong	Strong	Mod.	Slight	Trace	o

Unfortunately no comparison of the maximum resistances is yielded here. But the work on pathological cases gave opportunity for this, since it entailed the use of two normal specimens as control to each set of tests. In this way twenty-six normal specimens came

¹⁵ Hamburger, *Osmotischer Druck. u. Ionenlehre*, Wiesbaden, 1902, Vol. i. Theobald Smith, *Jour. of Med. Research*, 1904, xii, 385.

¹⁶ T. Smith and H. R. Brown, *Jour. of Med. Research*, 1906, xviii, 425.

under observation. Always six to eight dilutions of amboceptor were employed to define the range of resistance. It was found that the individuals of each of the thirteen couples differed in their minimum resistance only within the limits illustrated by the above table, and that they had always the same maximum resistance, and, above all, the same average resistance. Even the slight differences in minimum resistance were sometimes to be traced to a selective hemolytic action of the complement-serum.

Example.—Normals of Series XI.

Hemolysis.

	Amboceptor.								Hemolysis with complement-serum alone.
	$\frac{1}{2}$ c.c.	$\frac{1}{3}$ c.c.	$\frac{1}{4}$ c.c.	$\frac{1}{8}$ c.c.	$\frac{1}{16}$ c.c.	$\frac{1}{32}$ c.c.	$\frac{1}{64}$ c.c.	$\frac{1}{128}$ c.c.	
No. 1	Comp.	Almost +	Very strong	Moderate +	Moderate	Slight	Trace	o	o
No. 2	Comp.	Comp. —	Very strong	Strong +	Moderate +	Slight +	Trace	Faintest trace	Faintest trace

The generalization seems justified that the resistance of the erythrocytes of healthy men to a specific hemolysin, obtained by the injection into rabbits of human red cells, is practically a constant when human serum is used as complement.

At first an attempt was made to express all results in terms of a standard immune serum. But the very slight variations met with, even in pathological instances, as well as the many lots of serum employed rendered this both unnecessary and difficult. So each set of observations was made complete in itself by the inclusion of two normal blood-specimens as a standard.

To draw general conclusions from such entities one must be certain that the different rabbit sera act in relatively the same manner on all the bloods tested. As Ehrlich and Morgenroth¹⁷ have shown in their study of goat isolymins, it does not by any means follow, because the serum of individual A hemolyzes better the erythrocytes of B than those of C, that the serum of individual D will act in like manner. Yet it might reasonably be hoped that with polyvalent sera, such as those here employed, this would be found

¹⁷ Ehrlich and Morgenroth, *loc. cit.*

true in general. No direct investigation on the point was made, because suitable cases were rare and only a large range of them could even approximately bring out the fact for each new serum employed. But instances in the body of the work may be taken to indicate that the different sera give relatively the same result:—

Blood from a patient with pernicious anemia, which showed a lessened minimum resistance to the hemolysin, was tested with a second serum. The same finding was obtained. In a case of acute lymphatic leukemia the lessened minimum resistance to one serum existed also for another. So too the increased minimum resistance noted in the blood of a patient with hemophilia was evident with each of two amboceptor-sera. The sera for all of these parallel observations came from rabbits which had been immunized with different bloods. The variations in resistance dealt with were but slight.

RESISTANCE OF THE RED CELLS IN DISEASE.

The resistance of the erythrocytes from 68 hospital patients, most of them severely ill, was tested. The condition of the blood was accurately known in nearly all. All except three were adults. When a variation from the normal resistance was encountered a second test was usually made, after an interval of some days. Thus it was brought out that the slight variations observed were not due to error, and that they sometimes persisted over a period of weeks.

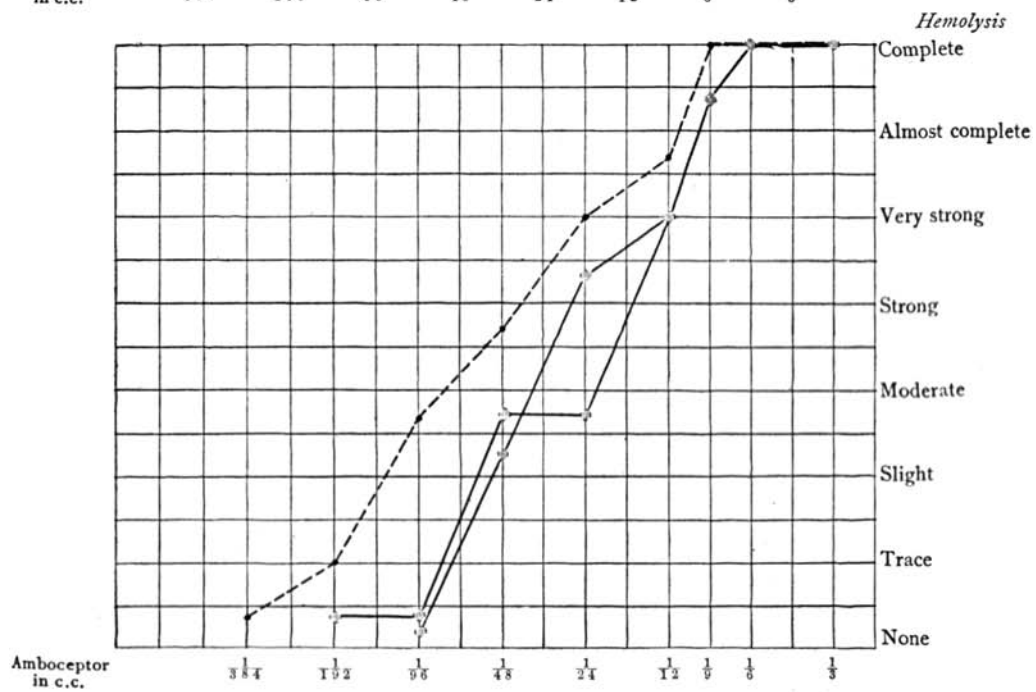
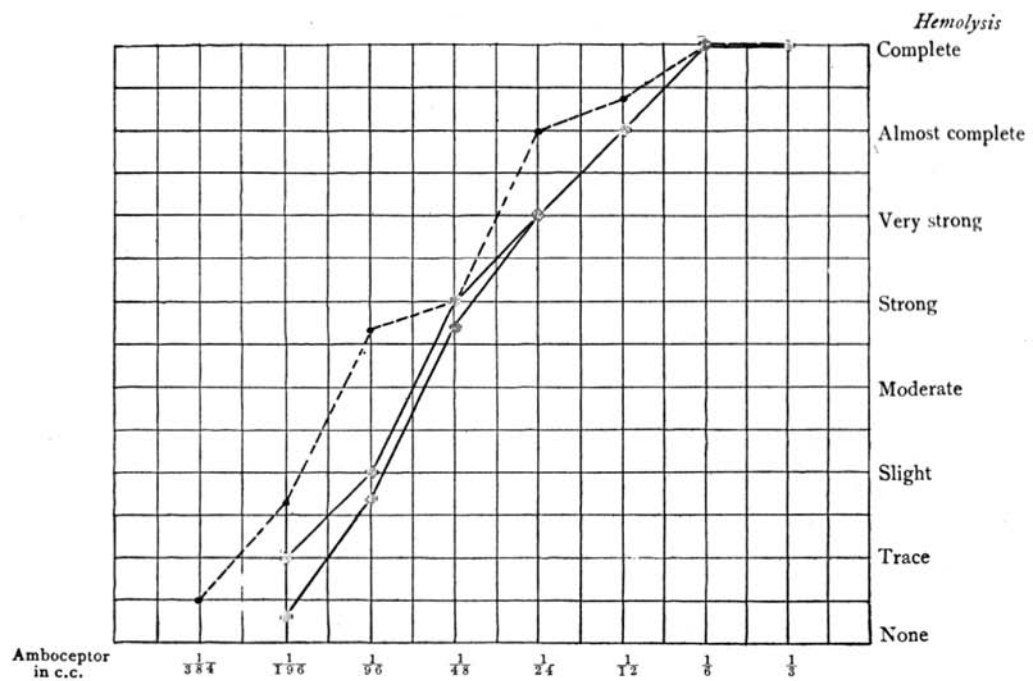
In 11 of the 68 cases, an abnormality of the resistance was found. Since a special attempt was made to include cases in which a variation was probable, these figures exaggerate greatly the relative proportion of abnormal bloods. Taking hospital patients as they come, the proportion would be found very much smaller, certainly not more than one or two per cent. of the total number. In comparatively few diseases, and most of these unusual, was the resistance abnormal: in the primary anemias (4 cases out of 5), hemophilia (2 cases), jaundice from various causes (4 cases out of 10), uncinariasis (1 case) and nephritis (1 case out of 5). On the other hand, it was unchanged in typhoid (6 cases), tuberculosis (6), carcinoma (5), gastric ulcer (4), chronic valvular endocarditis (3), simple anemia (3), empyema (2), croupous pneumonia, paratyphoid fever, pleurisy with effusion, arthritis deformans, portal ob-

struction, neurasthenia, septicemia, thoracic aneurism, rheumatic fever, tertiary syphilis, trichinosis, gas poisoning (stupor), and double tertian malaria (one case of all these). The patient with malaria was very anemic,—red cells 2,400,000 per cubic millimeter, hemoglobin 40 per cent. It is interesting, though not surprising, in view of the specificity of serum hemolysins, that the injury done by carbon monoxide, and by the malarial parasites was without effect on corpuscle resistance to the rabbit-amboceptor.

A severe grade of secondary anemia is compatible with quite normal resistance. To test cases in which the blood was in poor condition had been taken as one aim of the work; and thus it happened that of the whole number of patients (68) there were but thirteen in which a considerable anemia was absent. The red cells were frequently between 2,500,000 and 3,500,000 per cubic millimeter, the hemoglobin between 40 and 60 per cent. Yet in all except two of the 41 cases of secondary anemia unaccompanied by jaundice the resistance was normal. In one instance there were but 1,600,000 red cells per cubic millimeter, yet the resistance was unchanged. Summing up, it may be said that in the acute infections, in tuberculosis, in malignant disease, and in well-marked secondary anemia from other causes the resistance of the corpuscles to our specific hemolysin is rarely disturbed. This is of a piece with the behavior of tissue in general under the conditions of disease. It is the exception to find that a specific pathological process brings about pan-susceptibility.

Such abnormalities of the resistance as occurred were never very marked. Indeed in most instances they were so slight as to call for a repetition of the test. It may again be stated here that for the determination of corpuscle resistance six to eight dilutions of amboceptor were used, ranging from one that gave complete hemolysis of normal blood to one which produced no hemolysis whatever.

The deviation from the normal most often seen was a *lessening of the minimum resistance*. Hemolysis took place in the presence of an amount of amboceptor that produced no visible effect on normal blood-cells; and there was more of it than usual in those tubes arranged for weak and moderate hemolysis. Charts I and II illustrate well the type of change, and its persistence.



CHARTS I AND II. Resistance of erythrocytes from a case of lymphatic leukemia (broken line) as contrasted with the resistance of normal erythrocytes. Two normals were used as a standard in each test.

These charts represent the findings in a case of lymphatic leukemia (red cells 2,500,000, white cells 19,000 per cubic millimeter). Eight days, during which the patient's condition changed slightly for the worse, intervened between the tests. Another case of leukemia, this of spleno-myelogenous type, came to hand, and here the same abnormality of corpuscle resistance, of similar extent, was repeatedly noted. Unfortunately the blood contained such a large proportion of white cells (536,000 per cubic millimeter, as compared with 2,026,000 erythrocytes) that it was impossible to make an emulsion of the standard 5 per cent.; and so the results are inexact. No such source of error was present in the patient with lymphatic leukemia. Controls from which the amboceptor was omitted proved that the marked hemolysis could not be due in either instance to digestion with a leukocytic enzyme.

The blood from three cases of pernicious anemia was examined. Two of these were in serious condition, with the red cells at 944,000 and 1,048,000 per cubic millimeter respectively, and the hemoglobin at 25 and 20 per cent. The erythrocytes of both of these showed, in repeated tests at intervals of several days, a lessened minimum and average resistance, resembling that of Charts I and II, and approaching it in extent. The third patient was in good condition, her red cells 2,500,000 per cubic millimeter, and her corpuscle-resistance within normal limits.

Some may object that in these very pathological bloods the quantitative relation of hemoglobin to stroma is probably so changed that the amount liberated of the former but poorly indicates the destruction present, and thus the degree of resistance. The matter has already been partially discussed. Capps¹⁸ states that in pernicious anemia the cell-volume is more increased than the hemoglobin content. Granted that the volume of a cell and its amount of stroma vary in general together, then in pernicious anemia the hemoglobin as compared with stroma should be relatively less than normal. But it so happens that in these very cases liberation of coloring-matter by the specific hemolysin is unusually marked. The stroma destruction should be greater still, and, therefore, the erythrocyte-resistance even less than that indicated by "hemolysis" present.

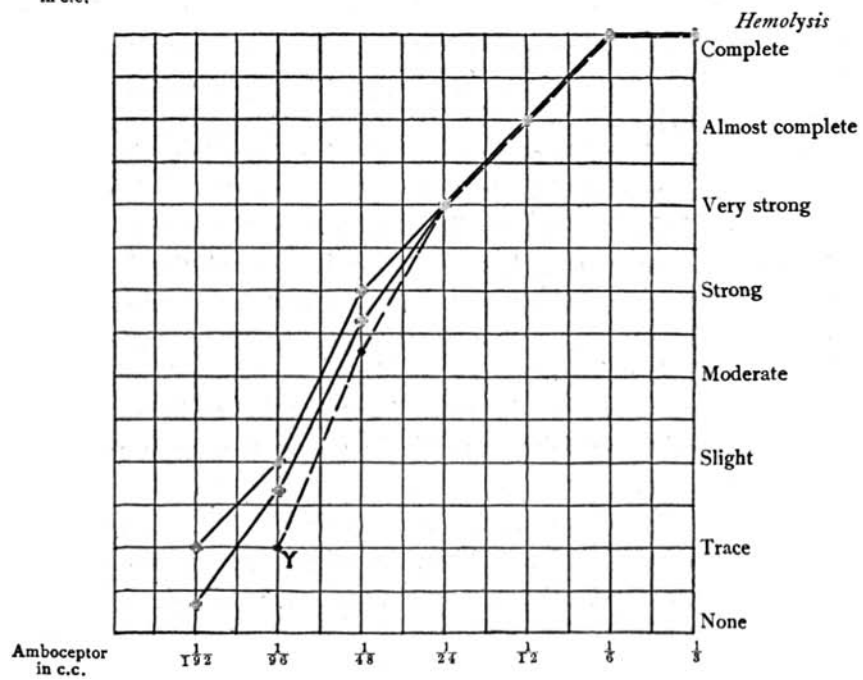
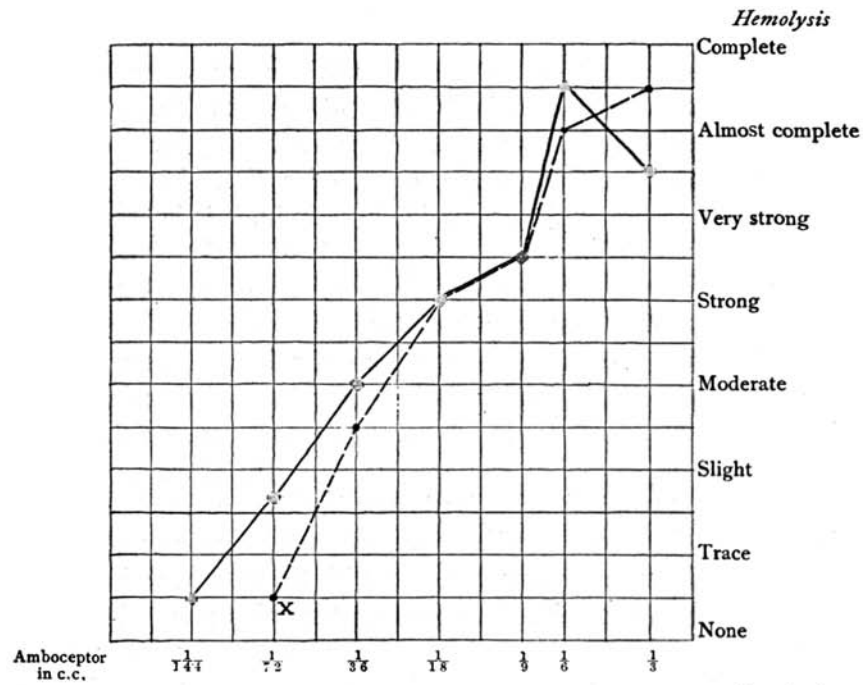
¹⁸ J. A. Capps, *loc. cit.*

Whether these inferences are warranted in our present uncertainty about the processes involved in the destruction of the red cell seems doubtful. But they are given for what they may be worth as affecting a point that inevitably comes up for discussion.

In view of results with the specific hemolysin, it is interesting to note that several observers have found in pernicious anemia an increased resistance of the erythrocytes to hypotonic salt solution. On this basis they have argued for the presence of an auto-hemolysin, with a consequent auto-immunization of the red cells. Not only do the few observations here recorded point against such inferences, but a direct comparison of the resistance of the same corpuscles to salt solution and to the hemolysin proves them unwarranted,—as will be shown further on in this paper.

The low resistance to the hemolysin observed in primary anemias gains in significance when contrasted with the resistance in secondary ones. Putting aside jaundice cases (which may with good reason be classed separately) there were, as has been said, 41 cases in which secondary anemia was well-marked. In only one of these was resistance to the hemolysin lowered. The patient was a negro with uncinariasis (red cells 4,400,000 per cubic millimeter, hemoglobin 47 per cent.). The variation from the normal was of the same type and as considerable as that charted for the primary anemias. No second test was made. In three other cases (two of tuberculosis, one of infective endocarditis) an apparent lessening of the resistance was ruled out, because controls showed the increased hemolysis to be due entirely to a selective hemolytic action of the complement-serum, dilute though it was. In many instances a numerical anemia existed as great as in the cases of leukemia above cited; but there were none in which the count fell so low as in the two patients with pernicious anemia in whom the corpuscle-resistance was affected.

A heightened minimum resistance was thrice seen. It was persistent in two cases of hemophilia. A slight anemia was present in one of these (red cells 4,400,000 per cubic millimeter, hemoglobin 70 per cent.), but the blood of the other was in excellent condition (red cells 5,200,000 per cubic millimeter, hemoglobin 100 per cent.). The third case with heightened minimum resistance



CHARTS III AND IV. Resistance of erythrocytes from two cases of hemophilia (X and Y) as contrasted with the resistance of normal erythrocytes. Two normals are represented in each chart.

was one of chronic parenchymatous nephritis (hemoglobin 60 per cent.). Charts III and IV illustrate the deviation and its extent. The change is just the reverse of that already dealt with.

An increase in maximum resistance was found only once,—in a case of the rare congenital icterus (Minkowski type). With it a discussion of the jaundice cases may well be taken up.

A decade ago it was observed that jaundice is almost always accompanied by an increased resistance of the red cells to hypotonic salt solution; and the point has now been well brought out by numerous studies. When the hepatic duct of a dog, or rabbit, is ligated, an increase in resistance of the erythrocytes occurs during the first twenty-four hours, coincident with appearance of the jaundice.¹⁹ The red cells of such an animal preserve when washed their character of increased resistance. The maximum resistance is especially affected, a large number of the erythrocytes remaining intact in a hypotonic solution that ordinarily would cause complete laking of them. Recently Chauffard,²⁰ studying the congenital icterus of the Minkowski type—"congenital icterus in the adult"—has come upon another and different phenomenon. In this disease the corpuscle resistance, especially at its minimum and average points, is startlingly lowered. Chauffard's statements have been confirmed by Widal, Abrami and Brule.²¹ The fragility of the red cells in the Minkowski icterus is an important finding in view of the anemia, and evidence of marked blood destruction, that characterize it. Recently H. P. Hawkins and L. S. Dudgeon²² have discussed under the title "Congenital Family Cholemia," several British cases; but there seems to have been as yet no report of the disease from this country.²³

Among ten cases of jaundice tested with the hemolysin was a well-marked example of the Minkowski icterus. The patient, a white female twenty years old, had been jaundiced from birth, and

¹⁹ Voquez and Ribierre, *Compt. rend. Soc. de. biol.*, 1902, liv, 1074; Brissaud and Bauer, *ibid.*, 1907, lix, 1068.

²⁰ *Semaine méd.*, 1907, xxvii, 25.

²¹ *Presse méd.*, 1907, xv, 641; also Widal and Abrami, *Bull. et mém. Soc. méd. d. hop. de Paris*, 1907, xxiv, 1127.

²² *Quart. Jour. of Med.*, 1909, ii, 165.

²³ P. Ribierre, *Folia Haematolog.*, 1905, ii, 153; A. Chauffard and H. Rendu, *Presse méd.*, 1907, xv, 345.

was moderately so when seen. With the icterus she had the characteristic association of anemia (red cells 3,800,000 per cubic millimeter, hemoglobin 77 per cent.), an enlarged spleen, and an amount of bile-derivatives in the stools that was normal. Her red cells were most sensitive to hypotonic salt solution. Normally hemolysis begins in 0.46 to 0.42 per cent. salt solution, and is complete only in 0.36 to 0.34 per cent., when whole blood is used; but the cells of this patient hemolyzed slightly in 0.75 per cent. salt solution and had broken down completely in 0.55 per cent. It was but natural to suppose that there would be some indication of this fragility on test with the specific hemolysin. Instead there was found a markedly increased maximum resistance and a minimum resistance slightly, if at all, below the normal bounds. With $\frac{1}{18}$ cubic centimeter of amboceptor-serum hemolysis was "strong" for both the patient's cells and the two normals; with $\frac{1}{9}$ cubic centimeter it was "almost complete" for the three; with $\frac{1}{6}$ cubic centimeter and again with twice that quantity it was "complete" for the two normals, whereas with neither had the patients' cells totally hemolyzed, and "almost complete" was still the reading. Unfortunately the observations with salt solution and those with the hemolysin were separated by an interval of three days, and there was opportunity for no further tests. But the difference in behavior to the two agents was sufficiently striking to suggest the general value of a series of comparative tests, and such were accordingly begun.

Cases of retention-jaundice seemed especially suited for the work because of that increase in maximum resistance to salt solution which is the rule in them. Nine of these (3 of catarrhal jaundice, 4 of gall-stones, 1 of carcinoma of the gall-ducts, and 1 of hypertrophic cirrhosis of the liver) were tested with the specific hemolysin, and five of the number were also tested with hypotonic salt solution. The five showed to this agent the very high maximum resistance expected; whereas to the hemolysin the maximum resistance was normal in the full nine.

For the tests that brought out this contrast the same emulsion of washed cells was submitted to both agents, and the mixtures with hypotonic salt solution were prepared while others with the specific hemolysin were incubating. One-half cubic centimeter of

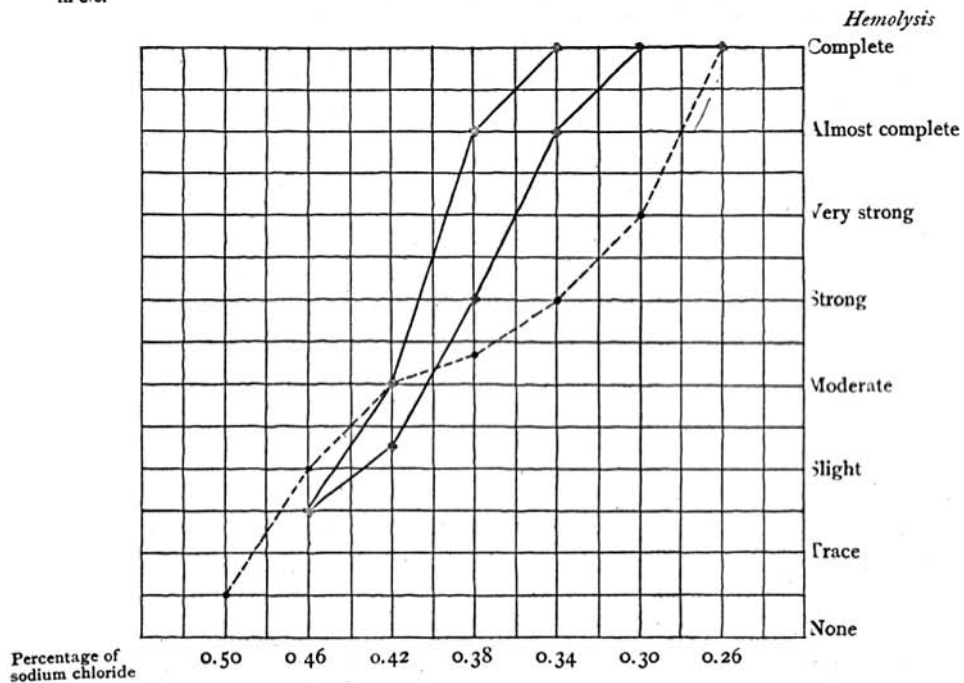
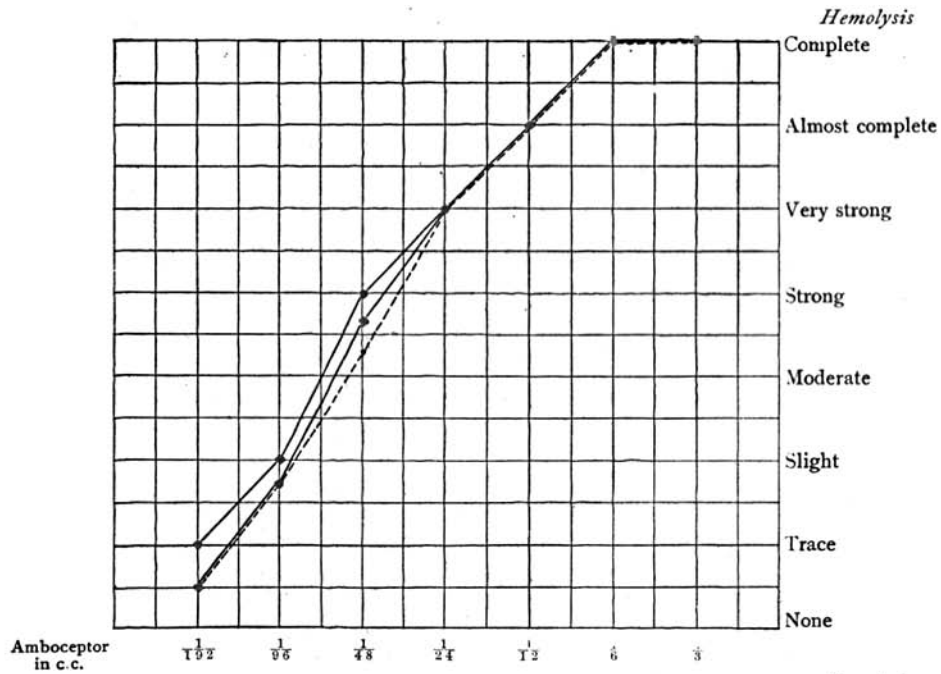


CHART. V. Resistance of the red cells of two normal individuals and of a case of jaundice due to hypertrophic cirrhosis.

Tests were made with the specific hemolysin and with hypotonic salt solution. The broken line represents hemolysis in the jaundice case. Note the great difference in results with the two agents.

the 5 per cent. cell emulsion (in 0.9 per cent. salt solution) was dropped into each of a number of tubes containing 2 cubic centimeters of salt solution so dilute that the resulting mixtures had a strength of 0.58 per cent., 0.54 per cent., 0.50 per cent., etc., respectively. In such a series the hemoglobin set free diffused itself through 2.5 cubic centimeters of fluid as compared with 1.5 cubic centimeters in the case of the hemolysin. For a direct comparison of results with the two agents this difference in concentration of coloring matter was made up by a careful quantitative addition of 0.9 per cent. salt solution to fluid pipetted from each tube of the series submitted to the hemolysin.

Chart V gives a typical finding. The increase of resistance to salt solution is that characteristic of retention-jaundice, and that which was found in all the cases tested; while the normal resistance to the specific hemolysin is well brought out.

In three instances (2 of catarrhal jaundice, 1 of gall-stones) there was to the hemolysin a definitely *lessened* minimum resistance, but with salt solution there was no such finding. This second lack of correspondence in behavior to the two agents is brought out in Chart VI.

The nature of these changes in resistance which accompany jaundice is unknown, and the problem thus presented is not unworthy of attention. But immediate interest centers in the fact that variations of the erythrocyte-resistance to salt solution form no basis for inferences regarding the behavior of the same erythrocytes to a specific hemolysin. This is true for other conditions as well as for jaundice. Twenty cases selected at random were tested, and a marked divergence in behavior to the two agents was noted throughout.

The statement seems warranted that resistance to salt solution and to a specific hemolysin are so far independent that results with one form in general no index to those with the other. Rywosch²⁴ quotes Hamburger as convinced that resistance determinations by his own method (the method with salt solution, adopted generally by clinicians) are nearly valueless because of complex factors involved—concentration of the intraglobular fluid, percentage volume

²⁴Rywosch, *loc. cit.*

of this fluid, and "protoplasmic scaffolding of the blood cell." Charts VII and VIII bear on this point. They depict the resistance of a number of blood emulsions tested at one time with salt solution and with the specific hemolysin.

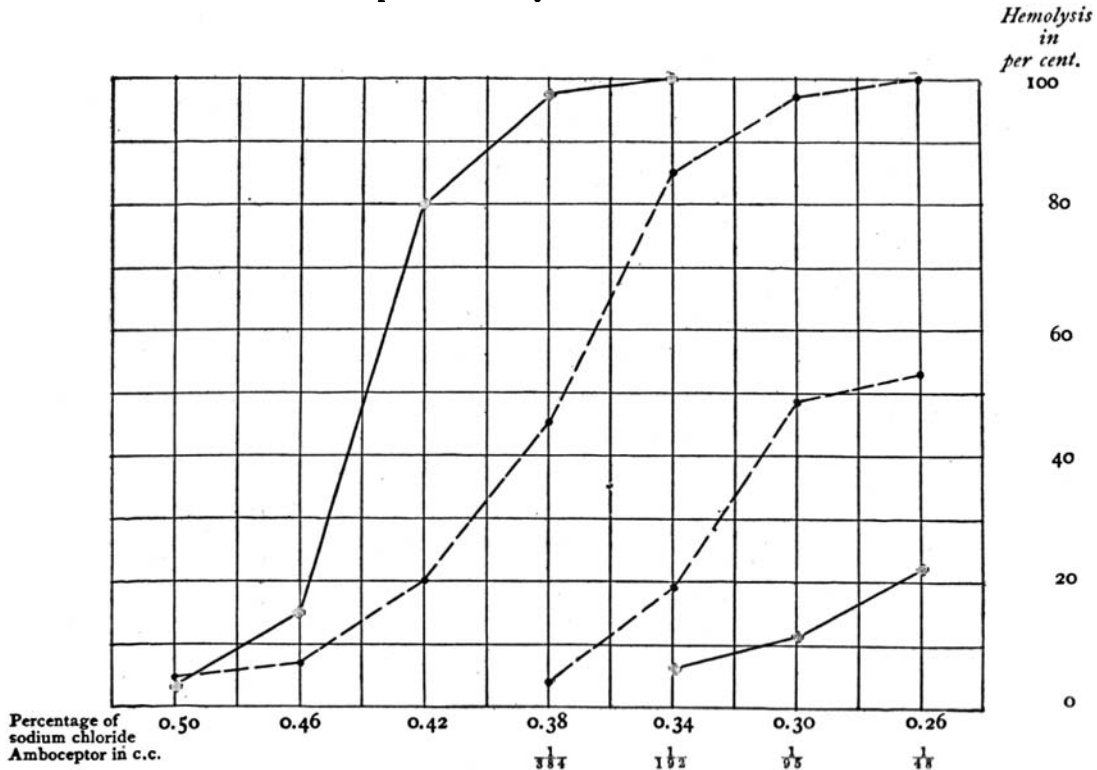


CHART VI. Resistance to the specific hemolysin and to salt solution of erythrocytes from a case of retention jaundice (cholelithiasis) and from a normal individual. The results are expressed in terms of a hemoglobin scale made with the laked normal erythrocytes. This was permissible since both five per cent. emulsions had the same color strength.

Note that the two hemolytic agents gave opposite results as regards minimum corpuscle resistance. Maximum resistance to the hemolysin is not charted. It was the same for both blood-specimens.

It will be seen that the range of variation is much wider with salt solution than with the hemolysin. Indeed the many published data, on resistance to salt solution in disease-states all go to prove that this resistance varies much, and without apparent rule, except where jaundice is concerned.²⁵ On the other hand resis-

²⁵ P. Ribierre, *loc. cit.*

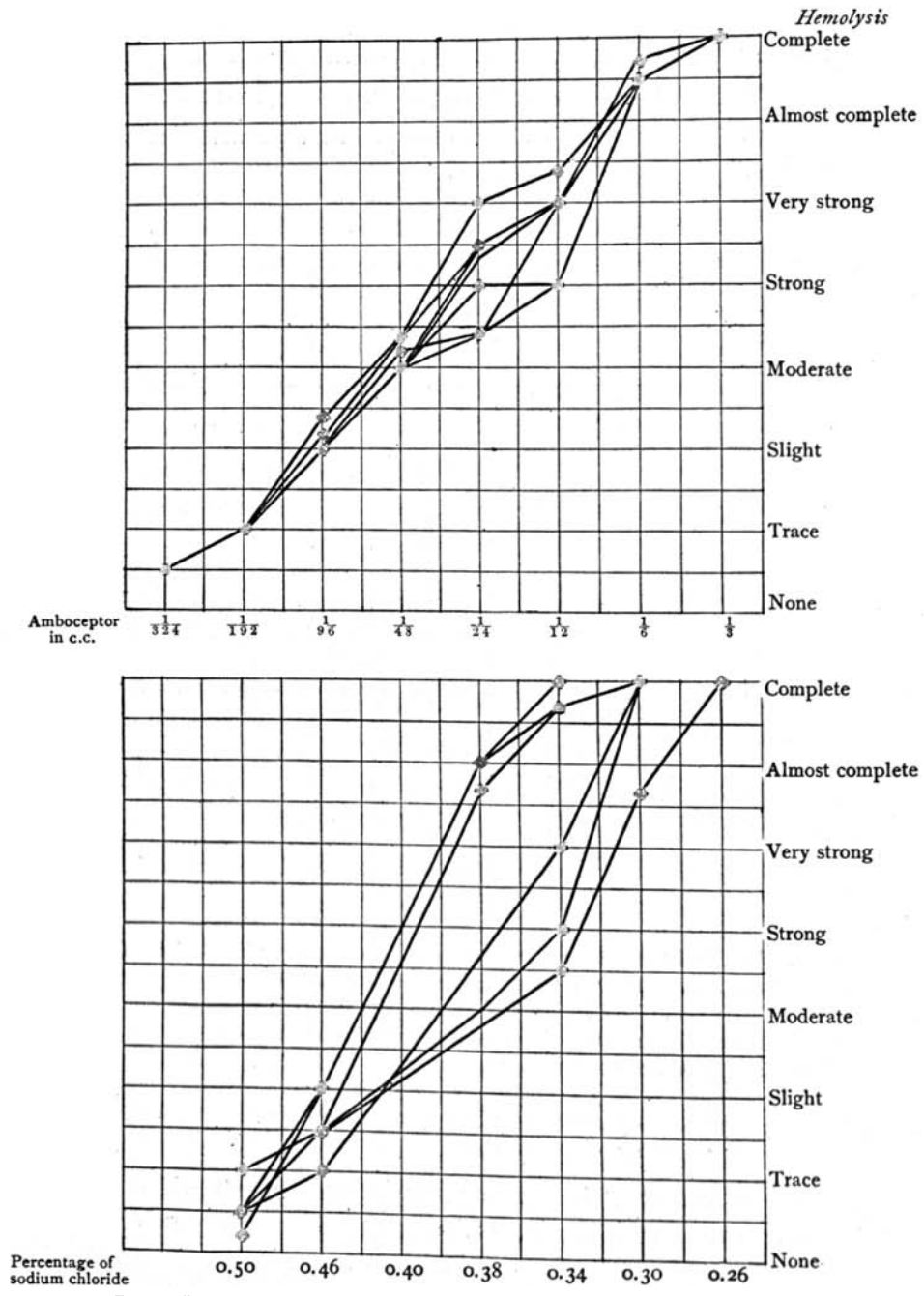


CHART VII. Resistance of the red cells from two normal individuals and five diseased. Tests were made with both the specific hemolysin and hypotonic salt solution. The chart is meant to bring out, not the variation in any individual instance, but the general difference in range of resistance to the two agents.

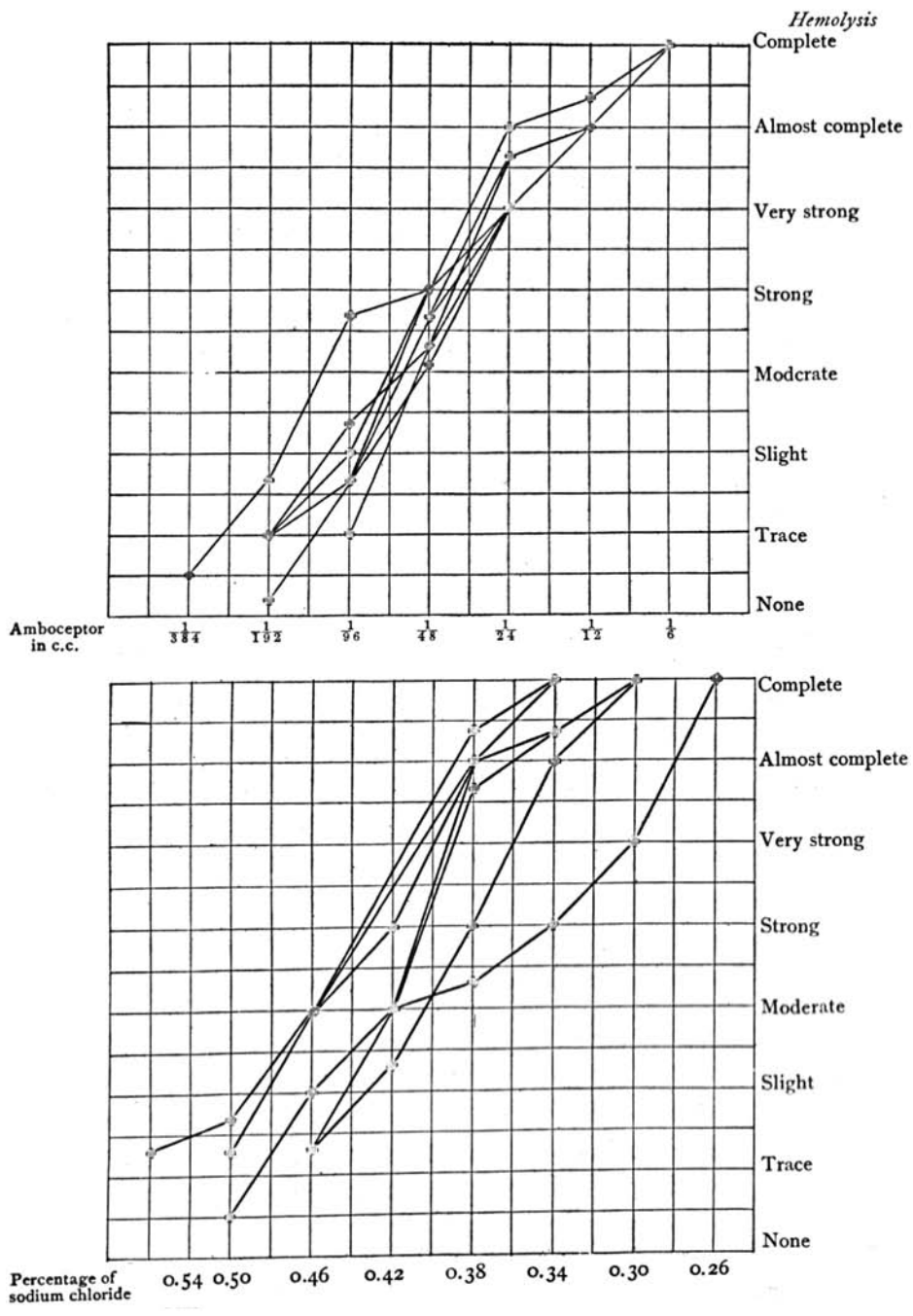


CHART VIII. Resistance of the red cells from five diseased individuals and two normal ones. The cases are not those that furnished Chart VII. Two of them (lymphatic leukemia, hemophilia) gave respectively the greatest lowering of resistance to the hemolysin, and nearly the greatest heightening of it, that were encountered. Tests were made at the same time with hypotonic salt solution. The chart shows the general differences in range of resistance to the two agents.

tance to the specific hemolysin which I have taken as a type is subject to relatively little change. Evidently, resistance to hypotonic salt solution must depend on more variables than does that to the specific hemolysin. In view of this, the clinical determination of "resistance" by means of hypotonic salt solution seems of very doubtful value, and inferences regarding the behavior of red cells to hypothetical disease-hemolysins, quite unwarranted.

CONCLUSIONS.

It is evident that this study might be extended with results perhaps of considerable value. As matters stand the following conclusions can be formulated:

1. The resistance of washed human erythrocytes to a specific hemolysin (immune-rabbit serum activated with human complement) is the same for all normal adults.

2. Disease alters this resistance of the red cells only in very special instances. Among 41 cases of moderate and severe secondary anemia from various causes, one only (if we exclude jaundice cases) showed a decrease in the resistance. But in two cases of pernicious anemia out of three the resistance was lowered, as well as in the only two instances of leukemia observed. Retention-jaundice was accompanied sometimes (3 cases out of 9) by a lowering of the resistance similar in type. In all of these instances it was the minimum, and to a less extent the mean resistance that was affected. An increase in them instead of a lowering was three times seen,—in two cases of hemophilia (where the finding was repeatedly obtained), and in one of chronic parenchymatous nephritis. Among the whole 68 patients, there was but one in whom the maximum resistance was abnormal: it was increased in an adult with the rare congenital icterus of Minkowski. These are all the variations encountered. They were extremely limited in extent.

3. The resistance to hypotonic salt solution and to a specific hemolysin are so far independent that results with one form in general absolutely no index to those with the other. In retention-jaundice, for example, the corpuscle-resistance to salt solution is usually much increased, while to the hemolysin it is either normal or slightly lowered.

4. A general comparison of the behavior of erythrocytes to hypotonic salt solution and to a specific hemolysin demonstrates that resistance to salt solution is much the more variable of the two. This is evidence in favor of Hamburger's view that the "resistance" of red cells to hypotonic salt solution has little clinical value because it depends on several factors within the cell, which fluctuate independently.

The work of which the results are here presented was begun at the Johns Hopkins Hospital in the Laboratory of Medical Biology, —then under the direction of Dr. Rufus Cole,—and was continued in the Rockefeller Institute for Medical Research. It is my pleasant duty to thank Dr. Cole and Dr. Lewellys F. Barker for many courtesies, as also Dr. Meakins of the Presbyterian Hospital in New York.