LEAD STUDIES.

III. The Effects of Lead on Red Blood Cells.

Part 2. Surface Phenomena and Their Physiological Explanation.

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Plate 5.

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The first paper of this series describes the effects of lead upon the hemolysis of red blood cells. These were so striking that the possibility of demonstrating other changes in red corpuscles was sufficient to justify further experimentation. This paper outlines the positive results of such work. Since in all these studies the mechanisms underlying the phenomena are of great interest we are also recording experiments which help to explain physiologically some of the facts observed.

Agglutination.—The effect of lead on the agglutination of blood corpuscles was studied first. The technique used was as follows:

Red cells from human blood belonging to either Group II or Group III were washed three times with Ringer solution (pH 6.5). Equal parts of this cell suspension were then exposed to varying concentrations of lead in Ringer solution and to Ringer solution alone, and, after 1 hour, were again washed with Ringer solution. The cells were then mixed with Group IV serum in a white counter as Ashby describes (1). In individual experiments the concentration of corpuscles in all tubes was maintained approximately the same.

Six such experiments consistently showed that, after exposure to lead, agglutination was slow and slight if it occurred at all, whereas, in the control tubes, exposed merely to Ringer solution, strong agglutina-

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tion followed the addition of serum. Table I shows the number of unagglutinated cells in several experiments. The difficulty of accurately counting unagglutinated cells increases progressively as agglutination decreases, for no cells which touch even one other cell may be considered free. Such contact, of course, although it may be purely accidental, lowers the count markedly.

The photomicrographs demonstrate even more strikingly the degree to which these small quantities of lead inhibit agglutination.

Stickiness of Red Blood Corpuscles.—The discovery that lead inhibits agglutination of red blood cells in heterologous serum naturally makes one question whether it affects the natural stickiness of the cell

TABLE I.

Effect of Lead on the Agglutination of Red Blood Cells.
Group II Blood Cells Exposed to Group IV Serum.

Subject.	No. of cells per c. mm. remaining unagglutinated after exposure to serum.				
	Normal control cells.	Cells treated with lead before exposure to serum. Concentrations of lead per cc. of red blood cells.			
		0.01 mg.	0.03 mg.	0.06 mg.	0.1 mg.
P. D.	26,400	61,100	121,000	124,000	105,000
	44,000	101,000	103,000	108,000	257,000
L. F.	12,700	123,000	210,000		293,000
M. O.	69,300	163,000	196,000	294,000	
G. O.	112,000	212,000		424,000	

surface or whether the addition of agglutinating serum is the cause of such a change. This problem was studied by means of Fenn's method (2) which was followed in every detail.

In brief this method consists of placing a suspension of cells in a glass chamber which is later rotated 180°. The number of cells which remains on the ceiling of the chamber, as well as the number which falls off, is then determined under the microscope. In the preliminary experiments all cells, whether normal or treated with lead, stuck to the ceiling; but later it was found that a clear differentiation could be made by adding to the Ringer solution a small amount of blood serum (0.6 cc. per 100 cc. of Ringer solution). A hydrogen ion concentration of 6.4 was maintained in all solutions. Before rotation, all cells except the controls were exposed to lead chloride (concentrations of 0.03 and 0.05 mg. of Pb per cc.)

for 1 hour, and were then washed once with an excess of Ringer solution. All manipulations of cells treated with lead were, of course, repeated on the control cells with Ringer solution alone.

In the five experiments performed, all results agreed. Practically all the normal control cells remained on the ceiling of the chamber after rotation; whereas a large proportion of "leaded" cells fell (see Table II). From this it may be concluded that, after exposure to lead, the surface of red corpuscles is less sticky than normal. There are two explanations for this; the charge of the cell surface may have changed; or, because of alterations in its chemical composition, the surface may have changed its physical characteristics (Part 3).

To study further this latter suggestion the action of lead on polymorphonuclear leucocytes was tested because their surface is physiologically different from that of red cells.

TABLE II.

Effect of Lead on Normal Stickiness of Red Blood Cells. Adhesion of Cells to Ceiling of Glass Container.

Normal cells.			"Leaded" cells.*		
No. adherent.	No. detached.	Per cent detached.	No. adherent.	No. detached.	Per cent detached.
355	19	5	105	200	66
210	15	7	70	158	69
335	24	7	120	77	39
All.	0	0	34	45	57
"	0	0	175	202	54

* 0.05 mg. of Pb per cc. of red blood cells.

They were obtained from the peritoneal cavity of rats after injection of aleuronat. These were then washed in Ringer solution containing 0.1 per cent of sodium citrate—a procedure which Fenn advised—and then in Ringer solution alone. The concentration of lead in the solutions to which they were then exposed varied from 0.0 to 0.15 mg. per cc., and the concentration of serum in the solutions surrounding leucocytes in the glass chamber was graduated from 0 to 20 per cent of the mixture.

Eight experiments showed that practically all the cells stuck to the ceiling, and that no definite difference between the stickiness of "leaded" white cells and that of normal controls could be observed.

It therefore seems clear that lead affects white and red cells differently. This fact was also suggested by the work of Fine (3), which, in connection with observations reported in Part 1, indicates that lead is less toxic for white cells than for red, particularly when administered in relatively large quantities.

The following experiments were performed in the hope of finding a physiological explanation of these various phenomena, particularly the increased resistance of "leaded" red cells to hypotonic saline (Part 1).

Permeability of the Cell Surface.—In the salt hemolysis test the mechanism studied was the permeability of the cell to water, and the elasticity of its surface. When osmotic tensions of surrounding fluids vary, the cell swells or shrinks because of changes in water content. Only the elasticity of the cell will determine the degree of change of osmotic pressure which the cell can withstand without hemolysis. The permeability to water of normal cells and those exposed to lead was therefore studied in order to determine whether any change in elasticity occurred.

Two methods were employed:

1. The specific gravity of red blood cells was determined. With a definite content of solids, variations in the specific gravity should represent largely a transportation of water in or out of the cell, which should be largely dependent upon the permeability of the surface, or upon the elasticity of the membrane. Clearly, if the membrane were inelastic the entrance of water into the cell would be prevented in spite of normal permeability. Of course, an increased permeability accompanied by an abnormal escape of the solids of "leaded" cells into the bathing solutions would introduce a possible error in such experiments, but the occurrence of this is improbable because the specific gravity of "leaded" cells is higher than normal (Table III). If solid constituents diffused from the cells their specific gravity should be reduced. Determinations made by the second more direct method also show that this error is improbable.

2. The volume of a unit number of cells was determined by means of simultaneous hematocrit readings and cell counts.

These two methods, when used upon cells exposed to different

osmotic tensions, have allowed us to determine with some degree of precision the relative volume of a unit number of cells and the ability of normal and "leaded" cells to shrink and swell.

1. The Effect of Lead on the Specific Gravity of Blood Cells.—The specific gravity of red blood corpuscles was determined by the suspension method described by Reznikoff (4).

Three types of experiments were performed by this method in order to determine whether the specific gravity of "leaded" as well as of

TABLE III.

The Specific Gravity of Red Blood Cells.

Normal cells.	"Leaded" cells.
1.094	1.104
1.088	1.113
1.089	1.111
1.089	1.106
1.096	1.107
Average..........1.091	1.108

TABLE IV.

The Specific Gravity of Red Blood Cells after Exposure to Lead for Varying Lengths of Time.

Time.	Specific gravity.
15 min.	1.089
30 "	1.098
1 hr.	1.115
2 hrs.	1.113
3 "	1.112

normal cells changes when the osmotic tension of the medium surrounding them is varied. From such observations we may discover whether or not water and, perhaps, salts can pass normally through the membrane of "leaded" cells.

Five experiments showed that after treatment with lead the specific gravity of washed red blood cells in normal Ringer solution is always distinctly higher than that of the normal cells (Table III). This difference is so great as to be well outside the limits of experimental

error. The change, as would be expected, develops slowly and reaches its maximum only after an hour (Table IV). Since this change may be observed after the addition of as small a quantity of lead as 0.01 mg. per cc. it cannot be explained by the mere chemical effect of adding the heavy metal; but must be due to the fact that corpuscles decrease in volume after exposure to lead, although otherwise the osmotic tension of the surrounding fluid is not altered.

In two experiments in which the osmotic tension of the suspension fluid was varied, it was found that the specific gravity of both normal and "leaded" cells also changed. The technique used for preparing

TABLE V.

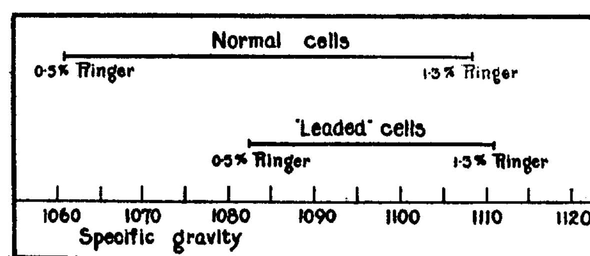
Effect of Osmotic Changes on Specific Gravity.

Concentration of Ringer solution.		Specific gravity of		Change of specific gravity from cells in normal Ringer solution.		Total change of specific gravity.	
Before and during "leading."	After "leading."	Normal cells.	"Leaded" cells.	Normal cells.	"Leaded" cells.	Normal cells.	"Leaded" cells.
0.9	0.9	1.089	1.111				
0.9	0.5	1.065	1.103	−24	−8		
0.9	1.35	1.109	1.115	+20	+4		
0.5	0.9	1.092	1.113	+3	+2		
1.35	0.9	1.090	1.114	+1	+3		
0.9	0.9	1.089	1.106				
0.5	0.5	1.069	1.097	−20	−9		
1.35	1.35	1.104	1.110	+15	+4		
0.5	1.35	1.107	1.115	+18	+9	+38	+18
1.35	0.5	1.073	1.095	−16	−11	−31	−15

the blood cells was similar in every way to that of the previous group except that in one experiment 0.04 mg. of Pb per cc. was added to whole blood, but the results in this experiment agreed with those obtained with washed red cells. After exposure to lead for 3 hours at 30°C. in normal Ringer solution, the suspension of corpuscles was thoroughly centrifuged. Equal parts of the cellular sediment were then added to equal volumes of Ringer solution of varying concentration (0.5, 0.9, and 1.35 per cent). At the end of 20 minutes the specific gravity of the corpuscles in the different tubes was determined (first experiment in Table V). Results showed that after treatment

with lead, red blood cells respond to osmotic changes in the media surrounding them with far less shrinking and swelling than do the normal cells used as control.

The third group of experiments (four in number) demonstrates the degree to which "leaded" and normal cells may shrink and swell if the concentration of the medium surrounding them is varied before the addition of lead. In some cases the cells were first washed with 0.5 per cent Ringer solution, which caused swelling. They were then exposed to lead, in the form of lead chloride, in 0.5 per cent Ringer solution, for 3 hours. The change in specific gravity which occurred after washing with 0.9 or 1.3 per cent Ringer solution was



TEXT-FIG. 1. Experiment demonstrating total changes of specific gravity of cells in varying concentrations of Ringer solution.

then compared with that found with normal control cells. In other tubes lead was added in 1.3 per cent of Ringer solution, and washing took place in concentrations of 0.9 or 0.5 per cent. Text-fig. 1 and the second experiment of Table V show typical results obtained in two such experiments. "Leaded" cells proved to be heavier in every case and their range of swelling and shrinking far smaller than normal. But they are not impervious to water for variations in weight within these restricted limits may be observed; and their usual specific gravity is regained in 0.9 per cent Ringer solution even after exposure to lead in solutions of other concentrations. The difference observed is apparently due to the fact that the exchange of substances through the cellular membrane is restricted—the permeability to water and possibly also to salts is apparently present, but quantitatively decreased. This is best explained by assuming that probably the cell

shrinks and becomes less elastic, or that possibly there is a change in the charge of the cell surface which would repel a definite quantity of water (page 203).

This diminished ability of the corpuscles to swell is probably important in explaining their greater resistance to hypotonic salt solution after exposure to lead (Part 1); for since the cell surface is not so stretched by swelling it does not break so readily. Within the normal physiological range of swelling in the body, however, the water exchange of "leaded" cells may well be normal, for it would not approach the degree of variation studied in these experiments.

2. Determination of the Volume of a Unit Number of Corpuscles.— The second method used to differentiate between normal and "leaded" cells was based on the assumption that the number of cells in a unit volume would vary with the size of the cells.

Whole blood was centrifuged, the plasma discarded, and similar amounts of Ringer solution, with and without lead chloride (0.04 mg. of Pb per cc.) were added to the cells, and allowed to stand for 3 hours. The concentration of the saline solution around both "leaded" and control cells was then changed from 0.9 to 0.6 per cent by mixing one volume of corpuscles with two volumes of 0.45 per cent Ringer solution. In no case did hemolysis follow this procedure. The red cell count of both normal and "leaded" cells was determined in both the 0.9 and 0.6 per cent solutions, and hematocrit determinations were made. The same hemocytometer squares and the same pipette were used throughout. The hematocrit ran for exactly 10 minutes at a speed of 1,860 revolutions per minute.

From data obtained in this way the number of cells packed into a unit volume and, therefore, the change of cell volume should be determined.

A considerable error in making the blood counts was introduced by dilution. In undiluted blood the maximum deviation from the mean was 5 per cent; in 0.6 per cent Ringer solution duplicate counts differed as much as 20 per cent. It was, therefore, important to compare the actual count obtained with the theoretical (one third of the number of cells found before dilution). Of seventeen experiments only two (Table VI) gave results in which this error did not vitiate the significance of the data. In the first of these, in which each figure is the average of five excellent determinations, the normal cell count in 0.6 per cent Ringer solution varies 7.7 per

TABLE VI.

The Effect of Change in the Osmotic Pressure upon Red Blood Cell Volume.

Experiment No.	Normal red blood cells.						"Leaded" red blood cells.					
	0.9% Ringer solution.			0.6% Ringer solution.			0.9% Ringer solution.			0.6% Ringer solution.		
	Hematocrit.	Red blood cell count.	Red blood cell count per unit volume.	Hematocrit.	Red blood cell count.	Red blood cell count per unit volume.	Hematocrit.	Red blood cell count.	Red blood cell count per unit volume.	Hematocrit.	Red blood cell count.	Red blood cell count per unit volume.
1	37.8	4,649,000	123,000	16.2	1,670,000	103,000	35.9	4,375,000	122,000	12.4	1,495,000	121,000
	Swelling 19%.						Swelling 1.0%					
2	37.5	4,810,000	128,000	15	1,606,000	107,000	33.5	4,756,000	142,000	11.75	1,562,000	133,000.
	Swelling 19.8%.						Swelling 6.6%.					

Hematocrit and red blood counts were first made on the bloods in normal Ringer solution. 1 cc. of this blood was then thoroughly mixed with 2 cc. of 0.45 per cent Ringer solution, and hematocrit readings and blood counts again determined. The unit count is the ratio, red blood cells: hematocrit.

cent from the theoretical count. If, in this case, the theoretical figure is substituted for the actual determination it will appear that normal cells swell 28.4 instead of 19 per cent, whereas "leaded" cells swell only 0.06 per cent. In the second experiment where all figures given are averages of two determinations, both of which agree very closely with the theoretical, normal cells swell 19.8 per cent, and "leaded" cells, 6.6 per cent.

Although the inability of cells to swell, except within narrow limits, after exposure to lead is quite apparent, the inaccuracy of this method makes the result of interest only as a check on similar data obtained by the other method.

The Effect of Swelling and Shrinking of Normal and "Leaded" Red Blood Corpuscles upon Hypotonic Salt Hemolysis.

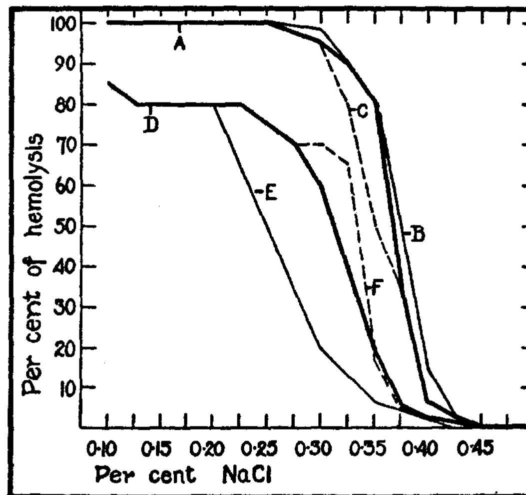
To differentiate more clearly between the swelling and shrinking of normal and "leaded" cells, corpuscles were washed in 0.5, 0.9, and 1.3 per cent solutions of sodium chloride. They were then "leaded" with 0.01 mg. of Pb per cc. of cells in salt solutions which maintained their original osmotic tension. After standing for 1 hour, the control and "leaded" corpuscles were washed once with 0.9 per cent saline solution to establish uniform conditions in all tubes, and the usual hypotonic salt tests were then made.

Four such experiments gave similar results. The data obtained in a typical one of these are graphically recorded in Text-fig. 2. The fragility of the control cells varied only slightly—the greatest hemolysis occurring in those washed originally with 0.5 per cent saline, the least in those washed in 1.3 per cent. The "leaded" cells were more markedly affected and not in the same way, for the cells washed in 0.5 per cent hemolyzed least.

It is hazardous to interpret these facts, but their explanation is possibly to be found in a relative inelasticity and fixation of the cell membrane[1] after exposure to lead. If fixation occurs while the membrane is distended (*i.e.*, in 0.5 per cent Ringer solution), then immersion

[1] "Membrane" as here used means merely a chemical phase, and not necessarily a membranous type of tissue. By changing physical-chemical relationships in such a phase, however, the physiological properties of the cell may be strikingly altered.

in 0.9 per cent Ringer solution and resulting equalization of osmotic tensions should allow the cell to reassume its usual size. The membrane might still, however, be relatively stretched. This should allow the cell to withstand a greater decrease of external tension than when its surface is shrunken, because the first influx of water would cause less internal pressure in the cell of larger surface area, and there-



TEXT-FIG. 2. The effect of swelling and shrinking of normal and "leaded" red blood corpuscles upon hypotonic salt hemolysis.

A, red blood cells in 0.9 per cent saline solution.

B, red blood cells in 0.5 per cent saline and then washed once with 0.9 per cent saline solution.

C, red blood cells in 1.3 per cent saline and then washed once with 0.9 per cent saline solution.

D, red blood cells and Pb 0.01 mg. per cc. in 0.9 per cent saline solution.

E, red blood cells and Pb 0.01 mg. per cc. in 0.5 per cent saline solution and then washed once with 0.9 per cent saline solution.

F, red blood cells and Pb 0.01 mg. per cc. in 1.3 per cent saline and then washed once with 0.9 per cent saline solution.

fore be less apt to cause hemolysis. As endosmosis continues, however, all cells would be exposed to tensions adequate to destroy them. Strength is given to such an hypothesis by the experimental data which show increased resistance of the "swollen" fixed cells only in hypotonic salt dilutions down to 0.225 per cent.

The Appearance of the Cell.—It is to be expected that such changes in the size of corpuscles as are suggested by the specific gravity experiments might be observed under the microscope. By examining large numbers of cells under an oil immersion lens and measuring them with a micrometer eyepiece, definite changes may be observed. If exposed to lead the cell margin first becomes pale and then swelling occurs. Within an hour this is followed by shrinking and definite crenation of the surface; then gradually the irregularity of these shrunk cells is lost and their outline becomes smooth and quite refractile to light. These phenomena, as well as others which also vary with time, suggest that the reaction of the corpuscles with lead is slow and, under the temperature conditions of our experiments, reaches equilibrium within 2 to 5 hours. Meneghetti (5) also found definite change in the appearance of cells exposed to lead salts.

That lead acts upon the surface of red blood cells is suggested by all the preceding data which include (*a*) increased resistance to hypotonic salt solution; (*b*) increased hemolysis on exposure to serum of a rabbit immunized to human blood (Part 1); (*c*) shrinking and accompanying increase in specific gravity; (*d*) inability of the cells to swell sufficiently to equalize large changes in osmotic tension; (*e*) increased rate of hemolysis without manipulation, as observed both *in vitro* and *in vivo* (Part 1); (*f*) disappearance of agglutination; (*g*) loss of normal surface stickiness (observed in red cells and not in white, probably because of their different surface). All these phenomena are good evidence of the surface action of lead. It is, therefore, of interest to determine whether corresponding physiological changes also occur in the interior of red blood corpuscles after exposure to lead. These would be practically dependent upon an interaction between lead and hemoglobin.

The Physiological Effect of Lead on Hemoglobin.—Two types of experiments were employed in studying this question. In the first group lead was mixed with an excess of pure hemoglobin (see Part 3) and the mixture allowed to stand so that any chemical combination might reach equilibrium. If this mixture is then added to suspensions of red blood corpuscles close chemical combinations which may have formed will be demonstrated by the occurrence of a less intense reaction between the lead solution mixture and red cells. By thus using

red blood corpuscles as a quantitative indicator of chemical reactions, it was found that lead, in the concentrations used, does not react with hemoglobin; for the resistance of blood cells to hypotonic salt solution is exactly the same after the addition of the lead-hemoglobin mixture as with the solutions of lead alone. These experiments are more fully described in Part 3.

In the second group of experiments physiological reactions of the red blood cells were observed by carefully studying the effect of lead upon their gas exchange.[2] The CO_2 exchange in reduced and oxygenated blood was found by determining the oxygen and carbon dioxide content of the corpuscles and of the air to which they had been exposed. Except that slightly smaller tonometers were used, the technique was similar to that described by Peters, Barr, and Rule (6). The gas in the tonometers, after the withdrawal of 1 cc. of blood, was analyzed by the Haldane method; blood analyses were made according to Van Slyke's method (7).

The standard procedure was as follows: The serum of approximately 80 cc. of defibrinated normal human blood was separated from the red corpuscles. These cells were then washed; half were exposed to lead in the usual way, and half saved for controls. The number of cells per unit volume was made the same in both suspensions; equal amounts of the serum were then added to the "leaded" and normal cells, thus synthesizing the blood with equal amounts of serum and equal numbers of cells. The carbon dioxide dissociation curves of both "leaded" and control samples were then determined at CO_2 tensions of 20, 40, and 60 mm. of Hg. This experiment was repeated three times with both reduced and oxidized bloods.

The curves thus obtained, demonstrating the reactions of the two bloods, have a similar slope, and those representing the difference of volumes per cent between the oxidized and reduced blood were also similar with both "leaded" and control cells. The oxygen capacity of the "leaded" blood was similar to that of its control; and also at very reduced pressures (6 and 7 mm. O_2) the two blood samples contain almost the same volume per cent of oxygen. No experiments were carried out with intermediate tensions of O_2, but it is reasonable to assume that there is probably no difference between the capacity of normal and "leaded" blood to combine with oxygen because of the

[2] We wish to take this opportunity to thank Dr. Arlie V. Bock for his great help and advice in performing these experiments.

normal behavior of such blood with respect to CO_2, since the close relationship between CO_2 and O_2 is a well established fact. The data are so convincingly negative that no graphic records of the experiments are shown.

These two series of experiments thus demonstrate (*a*) that lead does not react chemically with hemoglobin which is the chief constituent of the interior of the red blood cell and (*b*) that, when studied by present methods, exposure to lead does not alter the normal gas exchange of these cells in spite of the distinct limits of the fluid exchange. From these facts we may readily conclude that the interior of red blood corpuscles is not chemically or physiologically affected by lead.

CONCLUSIONS.

The physiological changes following the reaction of lead upon red blood cells are numerous and show the marked effects of a change in the cell surface. In experiments here reported 0.01 to 0.05 mg. of lead acting upon 5 billion red cells caused such marked variations from normal as:

1. Partial loss of the normal stickiness of red corpuscles, which is demonstrated by their falling from a clean glass surface.
2. Loss of the agglutination reaction which normally follows mixture with serum of a different isoagglutinating group.
3. Decrease in volume even in isotonic solutions.
4. Loss of normal elasticity and, therefore, reduced changes in volume upon exposure to marked variations in osmotic tension.
5. Increase in resistance to large changes in external osmotic pressure because of this inelasticity, and therefore decreased hemolysis in hypotonic salt solution (Part 1).
6. Increase in the speed of disintegration in spite of this increased resistance to external osmotic pressure. "Leaded" cells break up more readily upon standing than do normal cells, and are easily fractured by rotation or shaking (Part 1).

All these phenomena seem to be associated largely with surface changes in the corpuscles. Evidence is cited that there is no chemical reaction between lead and hemoglobin. The gas exchange is identical in normal and "leaded" cells. The function of the interior of the red cells, therefore, appears to be unaffected by lead.

The effects of lead upon red blood cells are thus manifested by shrinkage, inability to expand, increased brittleness, and loss of the normal consistency which makes their surface sticky. After exposure to lead, red blood corpuscles are more like hard inelastic brittle rubber balls, than like the soft, elastic, resilient cells characteristic of normal blood.

BIBLIOGRAPHY.

1. Ashby, W., The determination of the length of life of transfused blood corpuscles in man, *J. Exp. Med.*, 1919, xxix, 267.
2. Fenn, W. O., The adhesiveness of leucocytes to solid surfaces, *J. Gen. Physiol.*, 1922–23, v, 143.
3. Fine, J., The phagocytosis of lead compounds and their influence on the activity of the leucocyte, *J. Ind. Hyg.*, 1923, v, 138.
4. Reznikoff, P., A method for the determination of the specific gravity of red blood cells, *J. Exp. Med.*, 1923, xxxviii, 441.
5. Meneghetti, E., Über hämolytische und koagulierende Wirkung der Metallionen, *Biochem. Z.*, 1922, cxxxi, 38.
6. Peters, J. P., Barr, D. P., and Rule, F. D., I. The carbon dioxide absorption curve and carbon dioxide tension of the blood of normal resting individuals, *J. Biol. Chem.*, 1921, xlv, 489.
7. Van Slyke, D. D., and Cullen, G. E., Studies of acidosis. I. The bicarbonate concentration of the blood plasma; its significance, and its determination as a measure of acidosis, *J. Biol. Chem.*, 1917, xxx, 289.

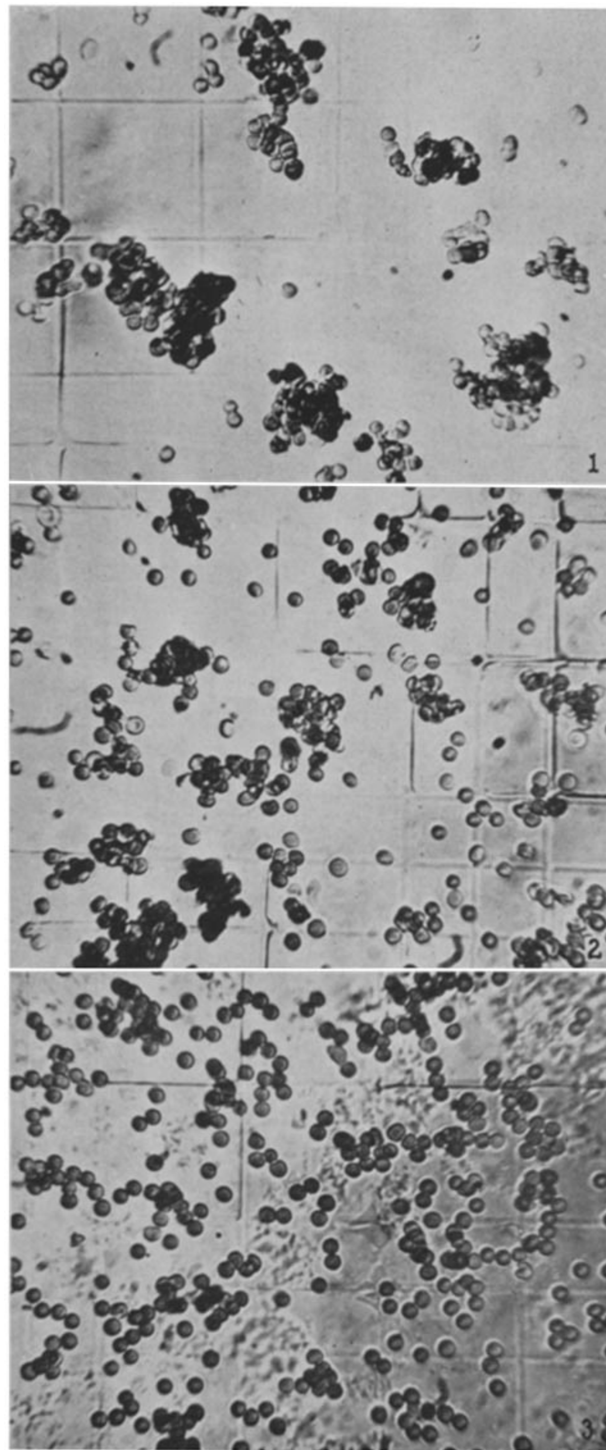
EXPLANATION OF PLATE 5.

Effect of lead on the agglutination of Group II cells by Group IV serum.

FIG. 1. Normal cells exposed only to Ringer solution.

FIG. 2. Cells exposed for 1 hour to 0.015 mg. of Pb per cc. of cells.

FIG. 3. Cells exposed for 1 hour to 0.05 mg. of Pb per cc. of cells.



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