Aphidirhodeine in ether . . .	$3\frac{1}{2}$ --	$4\frac{3}{8}$ … 5 –	6 ..	$7\frac{5}{8}$ ..	$9\frac{1}{4}$ –
„ suspended in dilute alcohol with ammonia				$2\frac{3}{4}$ --	$4\frac{1}{8}$ ..

3. As dissolved in bisulphide of carbon :

Aphidiluteine						$8\frac{1}{2}$ --	$10\frac{1}{4}$ ..
Aphidiluteoleine						$7\frac{1}{8}$ --	$8\frac{3}{4}$ ..
Aphidirhodeine . . .	$3\frac{1}{4}$ --	$4\frac{1}{8}$ … $4\frac{3}{4}$ –		$5\frac{3}{4}$ ..	$7\frac{3}{8}$ ..	9 –

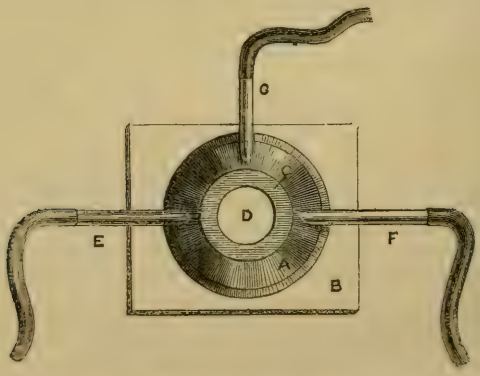
Observations *and* Experiments *on the* Red Blood-corpuscle, chiefly *with* regard *to the* action *of* Gases *and* Vapours. By E. Ray Lankester, Radcliff Travelling Fellow, University of Oxford.

Preliminary.—1. The uses of gases and vapours as a means of micro-chemical research.—2. Opinions and doubts concerning the red blood-corpuscle (bibliography).—3. The normal appearance of the frog's red blood-corpuscle.—4. The normal appearance of the human red blood-corpuscle.—5. Means of studying the changes of the blood-corpuscles in disease.—6. Effect of pressure on the red blood-corpuscle.—7. Effect of isolation: *a*, by adhesion; *b*, by oil.—8. Effect of water in minute quantities gradually added.—9. Effect of CO_2 gas.—10. Effect of osmic acid (vapour).—11. Effect of acetic acid (vapour and liquid). 12. Effect of alcohol.—13. Effect of ammonia gas.—14. Effect of chloroform (vapour and liquid).—15. Effect of bisulphide of carbon.—16. Effect of benzine.—17. Effect of turpentine oil.—18. Effect of solution of acetate of rosanilin and of tannin (Robert's experiments).—19. Effect of carbonic oxide.—20. Effect of cyanogen gas.—21. Effect of sulphuretted hydrogen.—General conclusions and summary.

The object of the disconnected observations which are here recorded was threefold: firstly, to ascertain whether certain vapours and gases having marked physiological influence on animals exert any direct action on the red blood-corpuscles, and to determine whether those known, by investigation with the spectroscope, to affect the hæmoglobin produce visible changes in the corpuscle; secondly, to examine into the chemical and formal structure of the red corpuscle; thirdly, to obtain, by a detailed examination of the influence of reagents, and especially gaseous reagents, on a typical histological element, a starting-point for further micro-chemical studies. I cannot consider, as far as relates to the chemical

side of the inquiry, that I have by any means carried out my object, for the subject has yet to be approached by the methods of analysis, with the view of recognising in this or that separable portion of the corpuscle this or that proximate chemical substance, in place of the tentative method of introducing reagents in order to observe how they may possibly act. At the same time, whilst we are waiting for chemists to give us some precise tests or series of reactions by which such highly complex bodies as those forming the blood-corpuscles can be recognised under such restricted conditions as microscopic inquiry involves, my results may not be uninteresting as bearing on the physical structure of the corpuscles, and as indicating a method of applying reagents in micro-chemical research, which has hitherto been but little used.

1. *The use of gases and vapours as a means of micro-chemical research.*—By means of the gas-chamber, Kühne, von Recklinghausen, Boettscher, Stricker, and Schweigger-Seidel have studied the action of oxygen, and more especially carbonic acid, on cilia, leucocytes, and the red blood-corpuscles. I have found a modification of one used by Schweigger-Seidel the most cleanly and convenient for the employment of a variety of gases. A is a watch-glass-shaped



piece of glass, with its edges ground flat and cemented (by paraffin, Canada balsam, putty, or whatever may best suit the case) to a flat piece of glass (B). D is an aperture in the dome or convex portion of the glass, with its edges also ground flat, and of considerable breadth (C). On to this an ordinary thin cover glass is placed, with the blood or other object to be examined on its lower surface, the rim C having been first smeared with oil, so as to render the closing of the aperture

air-tight. Three glass tubes (E, F, G) form part of the glass dome A, being blown in one piece with it. To these caoutchouc tubes are attached, and the desired gases drawn into the chamber by their means. When the third tube is not in use it is simply closed by a pinch-cock. The tube coming from E is placed in the mouth or attached to an aspirator, whilst that from F is connected with the reservoir of gas or vapour to be used. In the case of vapours the tube from F is attached to the shorter tube of a Wolff's bottle containing the evaporating liquid, and by suction at the tube E a stream of air charged with the vapour can be obtained, varying in its intensity at the pleasure of the observer. Heat applied to the generating flask will of course furnish an increased strength of vapour. In some cases it is desirable to pass the stream of vapour through water to prevent a too rapid desiccation of the drop of blood under observation. This is readily effected by means of a second Wolff's bottle containing water a little warm. Gases which are passed from a gas-holder of course do not require the use of the suction tube at E. The chief use of the third tube (G), which in experimenting with a single gas or vapour is closed, is to introduce a second gaseous reagent immediately after, or simultaneously with, the introduction of another. It also may be used for the insertion of a stout copper wire, which is bent round after its introduction into the chamber, the other end remaining projecting from the apparatus. The projecting portion of the wire being heated as in Stricker's hot plate, a considerable temperature may be obtained within the chamber, and vapour may by this means be generated from a few drops of water placed in the chamber in contact with the copper wire. In the same way other vapours, for the production of which a high temperature is required, may be evolved from liquids introduced into the chamber. Further, in place of the copper wire, a platinum wire in connection with the poles of a galvanic battery may be used, and a very much higher temperature obtained, if desired. Since the cement which fastens the glass plate and dome together is easily removed and replaced, the pieces can continually be separated for thorough cleaning, and thus any contamination of the reagents used prevented.

As to the reagents which may be used in this way they are sufficiently numerous, and though used in combination with liquid reagents, still have great advantages. The advantage which I claim for gaseous reagents—apart from the fact that some bodies are necessarily only to be used in the gaseous state—are, firstly, that in this manner the re-

agents are applied to the microscopic particle under observation without a deluging stream being produced so as to carry the particle right out of the field of the microscope. Such a stream is produced when a liquid is allowed to pass under the thin glass cover as ordinarily used, but with the gas chamber the reagent acts quietly, and without the least inconvenience to the observer, so that he is able to retain one individual particle under observation throughout the process. A second advantage in the gaseous method over that of solutions is, that the action of the diluents, water, or spirit is avoided. A third, and perhaps the most striking, is, that exceedingly minute traces of a reagent can thus be brought to bear and very gradually increased in strength whilst the observer is watching the object submitted to the reagent; at any moment the action may be stopped, and with the greatest facility and rapidity a second counteracting or other reagent introduced by the use of the second tube of the chamber.

Among the reagents which may thus be used and of which I have made some trial are water, hydrochloric acid gas (by current of air drawn through its solution), carbonic acid gas, acetic acid, osmic acid, nitrogen tetroxide, hydrogen sulphide, chlorine, iodine, bromine, ammonia, alcohol, ether, chloroform, carbon bisulphide, carbolic acid, and other gases and vapours of volatile liquids. Without now entering into further detail, I will merely express my belief that it will be found of great importance to apply all reagents used in chemical histology in the gaseous form where possible, though, of course, it is necessary also to have recourse to liquid bodies.

2. *Opinions and doubts concerning the red blood corpuscle.*—The literature on such a subject as the red blood-corpuscle is so extensive that it would be quite out of the question to attempt to give here a summary of it. The excellent though somewhat partial article of Rollett in 'Stricker's Handbuch' contains a general statement of what has been done and thought in the matter. I was led to make the observations described below from repeating with Stricker, in the spring of last year, his experiments on the action of alternating carbonic acid and atmospheric air on the red blood-corpuscle. I thought it would be desirable to ascertain whether if the carbonic acid were alternated with any other inactive gas a similar result could be obtained, and accordingly examined the action of a variety of gases and vapours, a consideration of which necessarily throws some light on the still unsettled points concerning the structure of the corpuscle. The problems (belonging

to the second category alluded to in the first paragraph of this paper, viz. the chemical and formal structure of the red blood-corpuscle) which present themselves are,—Can any of the chemical constituents of the stroma of the red blood-corpuscle be distinguished and identified by means of reagents under the microscope, and what is their condition in the living corpuscle? Does the red corpuscle of either mammal or other vertebrate possess a differentiated envelope or wall? Is the nucleus of the frog's red corpuscle merely a post-mortem product? Is there any trace of a nucleus in the mammalian red corpuscle? These questions are suggested by well-known researches. Another important question, viz. What is the nature of the Robertsian macula? is suggested by certainly the most remarkable contribution to the histology of the blood published of late years, that of Dr. Roberts of Manchester. I must confess that I am at a loss to understand how it is that his most important observation, demonstrating a well-marked point (either normal or cadaveric) capable of taking strongly the aniline dye—in the walls of the red corpuscle of all vertebrata, both mammals and ovipara—have been so largely ignored. Rindfleisch has, I believe, made a similar observation, but in the writings of Stricker, Schweigger-Seidel, and others, specially dealing with similar phenomena, and in the article of Rollett, I find no reference to Roberts' paper. Boettscher, it is true, discusses Roberts' views and observations at length. In the following list I have given references to the more recent papers relating to the structure of the red blood-corpuscle, and have briefly indicated the contents of the paper in each case. This list and the works cited in Rollett's article, as well as the standard hand-books will furnish the reader with a nearly complete catalogue of the literature of this subject.

Gulliver, in Hewson's works, *ibique citata*, Sydenham Society, 1846-47. (References to the older writers and to Mr. Gulliver's other papers are here to be found, as well as observations on the properties of the corpuscles and the most extensive series of measurements of the vertebrate red blood-corpuscle.)

Rollett, in Stricker's 'Handbuch der Histologie,' or the Sydenham Society's translation, *ibique citata*. (References to numerous papers by Rollett himself and the Vienna school are given, and a discussion of most of the important points relating to the red corpuscle.)

Brücke, "Ueber den Bau der rothen Blut-körper," lvi Bd.

of the 'Sitzungsb. der Wiener Akad.,' ii Abthl., 1867. (Describes the action of boracic acid, distinguishes the "œcoid" or sac of the corpuscle and the "zooid" or coloured contents.)

Hensen, "Untersuchungen zur Physiologie der Blut-körperchen sowie über die Zellennatur derselben," 'Zeitschrift für wissensch. Zoologie,' vol. xi, 1862, p. 253. (Describes peculiar appearances in corpuscles of a frog in which the blood was nearly devoid of these bodies, also a method of producing this condition of Acythæmia. Distinguishes a layer of fluid protoplasm surrounding the colouring matter by cadaveric alteration of which he believes the supposed membrane of the corpuscle to be formed.

Boettscher, "Untersuchungen über die rothen Blut-körperchen der Wirbelthiere," 'Virchow's Archiv,' vol. 36, p. 342, 1866. (Discusses at great length the various views and observations on the red blood-corpuscle, gives some account of the effect of chloroform vapour and oxygen, advances the opinion that the mammalian red corpuscle is nucleated, also replies to objections in a later volume, vol. 39, and describes decolouration and formation of a Robertsian pullulation by placing red corpuscles in humor aqueus or serum of another species of animal.)

Schmidt and Schweigger-Seidel, 'Ludwig's Arbeiten,' 1867. (Describe action of aqueous vapour Co_2 and Chloroform, oppose Böttscher's notion of a nucleus.)

Klebs ('Virchow's Archiv,' Bd. 38) opposes Böttscher's view as to a nucleus in normal mammalian blood, and describes the nucleus present in the red corpuscles of leukæmic subjects.

Busk ('Quarterly Journ. Micros. Science,' 1852) describes the nucleation of the red corpuscle in pregnant women, first observed by Nasse ('Wagner's Handwörterbuch,' i, 90).

Rolleston (ibidem, 1867, p. 127) describes an appearance of nucleation in dried blood-corpuscles of the sloth, also in somewhat stale but liquid blood of the elephant, cites various authors.

Beale ('Quart. Journ. Micros. Science,' 1864, two papers) describes effect of heat on red corpuscles, also spontaneous changes in frog's red corpuscle, effects of pressure, &c., molecular matter of the blood, small red corpuscles, &c., opposes the notion of a wall to the corpuscles.

Preyer ('Virchow's Archiv,' vol. 30, 1864) describes amœboid movements and processes exhibited by red corpuscles of the frog extravasated into the lymph sacs, also spontaneous and regular fission of the same.

Max Schultze ('Archiv f. Mikrosk. Anatomie,' vol. 1) de-

scribes effect of heat, and denies contractility to the red blood-corpuscle, describes small-sized corpuscles from human blood.

Stricker ('Pfluger's Archiv,' vol. i, 1868) describes the effect of alternate Co_2 and atmospheric air on red corpuscles first acted on by aqueous vapour; distinguishes the "body" and the "nucleus" of the corpuscle, without prejudging the question of the existence of an œcoid, discusses the structure and varieties of form which the corpuscle exhibits.

Addison ('Quart. Journ. Micros. Science,' 1861) describes changes in form produced by acidity and alkalinity.

Roberts ('Proceedings of Royal Society,' vol. xii; and 'Quart. Journ. Micros. Science,' 1863) describes a "macula" produced by nitrate of aniline dye in mammalian and other red blood-corpuscles; also a corresponding pullulation caused by tannin.

Savory ('Proc. Roy. Soc.,' xvii, 1868-69) denies the existence of a nucleus in living corpuscles of oviparous vertebrata.

Richardson ('Trans. American Medic. Assoc.,' Philadelphia, 1870) argues from the red contents separating from the contour-line as crystals within the corpuscles of Menobranchus, that the latter have a distinct wall. The observation is not new, Owsjannikow ('Bull. de l'Acad. St. Petersburg,' vol. viii, p. 561) having figured such, as well as other writers.

Norris, on the laws and principles concerned in the aggregation of blood-corpuscles, both within and without the vessels ('Proceedings of the Royal Society,' vol. xvii, p. 429, 1868-69).

Brunton, "On the Chemical Constitution of the Nuclei of Red Blood-corpuscles" ('Journal of Anatomy and Physiology,' November, 1869), gives reasons for supposing mucin to be present; this is one of the first attempts at methodical microchemical investigation of the red corpuscle.

The separately published works or essays of Kneuttinger and of Rindfleisch on the histology of the blood I have not seen, and cannot therefore speak of their contents.

3. *The normal appearance of the Frog's red corpuscle.* In fig. 1, plate XV, I have represented the blood discs of the frog. It is certainly quite impossible to detect anything like a membrane to these corpuscles in their fresh untouched condition; what *might* be taken for such proving, when the highest powers are used, to be a refraction illusion. It has also been denied that in the perfectly fresh corpuscle a nucleus can be detected (Savory, loc. cit.). This, I think, is an error. It sometimes happens that no nucleus can be at first

made out in freshly drawn corpuscles, that is to say, no outline marking off the central portion of the corpuscle, fig. 1*a*; but this appears to be a matter of degree. In perfectly fresh red corpuscles of the frog, and even in such corpuscles whilst still within the vessels of the mesentery, I have been able to distinguish the faint outline of the nucleus; and it seems to me that its greater or less distinctness depends on the condition of the blood in the frog, that is to say, on the frog's physiological condition. There is no doubt that, after the corpuscles are drawn and placed on a glass slide, the nucleus becomes more and more distinct in outline until it reaches a state of sharp definition. This *delimitation* of the nucleus must, however, be carefully distinguished from the *granulation* of it caused by acids. Whilst both may, no doubt, be considered as depending on the coagulation of albuminoid substances, the first may be compared to that form of muscle-coagulation or rigidity which is resolvable, and does not indicate death; whilst the granular condition is like the second or final form of cadaveric rigidity, and indicates an irremediable change. The mere delimitation, then, of the nucleus is not to be considered as always of *post-mortem* origin, but may occur more or less during life.

Although, as remarked above, no trace of a wall to the blood-disc can be made out with the highest powers on normal specimens, yet the elasticity and preciseness with which they recover their form and their sharp outline decidedly suggest something like a limiting outer coat or pellicle.

Amongst the normal blood-corpuscles in some perfectly fresh drawn, I observed the two copied in fig. 2*a*, *a*, in which the red-coloured portion of the cell or "zooid," as it is termed by Brücke, was shrunk and separated somewhat from the oval outline (œcoid) of the corpuscle, thus resembling, in some degree, corpuscles which have been acted on by certain reagents (boracic acid, Brücke; sugar, Hensen). I am not aware that such exceptional forms have previously been observed in normal unmanipulated blood. They are of importance as tending to show that the separation into zooid and œcoid is not an entirely artificial phenomenon, since it is thus seen to occur in otherwise healthy corpuscles whilst circulating in the blood. The two corpuscles figured had in every other respect the usual form of the frog's blood-disc.

It is necessary to observe that they were obtained in the early spring from one of a number of "winter frogs," that is, frogs preserved in a cage through the winter. From another frog were obtained the remaining corpuscles figured in fig. 2,

the blood being perfectly fresh, and placed directly from the animal's finger on to the microscope-slide. It seems highly probable that the red blood-corpuscles of the frog present, at different seasons of the year, somewhat different properties, as I shall have again to remark in speaking of the action of ammonia upon them; and it is likely that these curious forms are extreme examples of this variability. The corpuscles drawn in fig. 2 are remarkable for their irregular shapes; they were quite exceptional in the blood from which they came, by far the majority being of the usual oval contour; but in this same blood nearly all the corpuscles presented that curious frilled appearance of the margin which can be brought about by the action of many reagents in ordinary corpuscles. That this appearance was not due to any change after separation from the body I am tolerably certain, and I have observed such a condition of the corpuscles in quite fresh blood from other frogs. It is comparable to the so-called "thorn-apple form" of the human blood-corpuscle (fig. 18 *e*). The frilled appearance seen in these corpuscles is due to the commencing radial cleavage of its substance, successive stages of which phenomenon are seen in fig. 7 *a, b, c.*

4. *The normal appearance of the human red blood-corpuscle.*—The human red blood-corpuscle is a circular biconcave plate. It is, however, erroneous to regard this as the only normal form. In the blood of perfectly healthy persons I have frequently noticed the "thorn-apple form" (fig. 18 *e*), so immediately after the shedding of the blood, that I do not doubt that these forms existed in the circulating fluid. My own blood almost invariably presents these thorn-apple forms in large number, and I have not yet been able to connect their presence, or greater or less quantity, with any particular condition of health or nutrition. In addition to the thorn-apple form, my own blood frequently presents what I will term the "single" and "double watch-glass forms." In these corpuscles, in place of a concavity on each face of the disc, we have a very large convexity, of delicate appearance, and paler than the rim or margin of the corpuscle. Sometimes only on one face of the corpuscle is there this swelling out, and then the appearance is that of an old-fashioned watch seen from its side, the darker coloured rim of the corpuscle representing the metal watch, and the swelling representing the convex glass. Often these convexities appear on both sides of the corpuscle (fig. 18 *c*) I do not see any reason for attributing these forms to changes occurring in the corpuscle after it has been shed. I have observed them (with Hartnack's No. 10 à immersion), with the greatest

rapidity possible, after being shed from the finger, and do not doubt that they exist in their peculiar form whilst within the body.

5. *Means of studying the changes of the blood-corpuscles in disease.*—The study of pathological changes in the form-elements of the blood, as to their numbers, absolute and relative, size, shape, and properties, has hardly been yet attempted. Yet it can hardly be doubted that the physician should receive as important information from the careful examination of the blood in many cases of disease, as he does at present from the study of the urine. The reason why the blood is almost or totally neglected is, firstly, that the microscopes in the hands of most hospital students and practitioners are quite inadequate (a power of 200 diameters being their highest, whereas 450 is required) ; and, secondly, that hitherto the examination of the blood has been really a very difficult matter, requiring great haste and the exercise of some skill in drawing. A drop of blood taken from a patient must be immediately examined, drawings made, and notes written, and then it rapidly begins to change, dries up, and is lost. The observer too is never sure that what he may have seen is not due to the progressive changes accompanying the death of the drop of blood before him, and as he can never re-examine anything remarkable which he may have observed, can have but little confidence in his impressions concerning it. Drying has been used as a means of preserving blood, but is of very little or no use, since it necessarily causes distortion and changes in the corpuscles. All the reagents commonly used in microscopy affect, more or less, the form of the corpuscles. If the medical man possessed a reagent which would enable him instantaneously, on removing a drop of blood from his patient, to preserve all its form-elements *absolutely* unchanged, and in such a condition that he could place the specimen aside, and compare it with other cases and with specimens from the same individual from day to day, it is likely that our knowledge of pathological changes in the blood would advance, and that the condition of the blood would be made the subject of study in the wards of hospitals. Such a reagent exists in the so-called hyper-osmic or osmic acid[1] introduced as a preservative agent by Professor Max Schultze. It is sufficient to expose a thin film of blood on a glass cover to the vapour arising from a bottle containing the two per cent. solution of osmic acid, during three minutes, to ensure its complete preservation.[2]

[1] To be bought of Messrs. Hopkin and Williams.

[2] This method is due to Professor Schweigger Seidel, of Leipzig.

Every corpuscle thus becomes "set," as it were, in its living form; there is no coagulation, no shrinking, no dissolution; but, as the corpuscle was at the moment of exposure to the vapour, so it remains. The white corpuscles even exhibit their pseudopodial processes arrested in the act of movement. It is as though the osmic acid bottle contained a Gorgon's head, which freezes the corpuscles, as they face it, into stone. Having been thus acted on by the osmic acid, the cover glass, with the blood on it, is placed on an ordinary glass slide, on which is a drop of a nearly saturated solution of acetate of potash, as recently recommended by Max Schultz (see the last number of this Journal), and there it may remain unchanged for as long as the physician wishes. The whole process is so simple that, in less time than it takes to examine the chest, a drop of blood may be taken, thus prepared, and placed on one side, for examination at a later moment. One may have perfect confidence, from careful comparative observations (see below), that the osmic acid does not change the form of the corpuscles *at all*, and thus all the advantages are obtained for a leisurely and deliberate study, which otherwise are only to be obtained by most inconvenient haste and precipitation. At the same time the indispensable opportunity is provided of retaining the corpuscles in their living form for *comparison* from day to day and from case to case.

6. *The effect of pressure on the red blood-corpuscle of the frog and of man.*—In fig. 3 *a, a,* corpuscles of the frog are drawn which were subjected to an oblique pressure, caused by squeezing the covering-glass under which they lay. A wrinkling of the surface has been produced, indicating the existence of a differentiated pellicle forming the outer wall. Such a wrinkling could not be produced were the corpuscle of homogeneous consistency.

The same result, due to pressure, is observabl ein the human corpuscles drawn in fig. 18*f*.

In connection with the effect of pressure, I may refer to the observations which have been made by various microscopists on the tearing or cutting of the red corpuscle of the frog. It has been found that, by drawing a needle sharply across the slide on which a drop of blood is placed, the red corpuscles may be cut or torn in halves, and that, under these circumstances, no escape of the viscid matter from the corpuscle takes place, as would be expected were they membranous sacs containing a semi-fluid substance, but that the cut edges collapse, and each piece retains a rounded form. This, though negativing the view that the corpuscles possess a membrane sharply limited on both sides, is not in antagonism

with the existence of a pellicle having no definite *inner* boundary, and similar to such a scum or pellicle as forms on the surface of a cooling mass of jelly.

7. *Effect of isolation from the plasma* (*a*) *by adhesion to a foreign body* (*b*) *by mixture with salad oil.*—A very strange phenomenon (not hitherto described) is seen when some of the corpuscles of the frog's blood become separated from the plasma through adhesion to the glass cover on which they are placed, and the drainage away from them of the liquid in which they usually float.

Such corpuscles may often be observed in using the gas-chamber, since the drop of blood is in contact with only one surface, not between two, as in the case of an ordinary slide, and they may be seen thus before any appreciable desiccation has taken place. They entirely lose their oval form, and have a tendency to run together, forming polygonal mosaic works.

By pressing a drop of blood into a small drop of salad oil, numbers of the corpuscles may also be obtained isolated from the plasma, and floating freely in the oil. The ready way in which the corpuscles float into the oil, whilst the plasma, of course, does not mix with it, seems to indicate a condition of the outer wall of the blood-corpuscle, which is *not* that of a membrane simply moistened with water. Both human and frog's blood-corpuscles, when thus passed into oil, lose their normal shape. Those of the frog run together, and become very closely adpressed in small groups, and some lose their hæmoglobin, which seems to be taken up by the oil (see fig. 4). The human corpuscles lose their biconcave character, and become more nearly spherical or polygonal when adhering together in masses (see fig. 21 *a*). By tapping the covering-glass I have seen the frog's corpuscles pass readily from the oil back into plasma, and *vice versâ;* but I am not sure whether, after they have once lost their oval form in the oil, they can resume it on re-entering the plasma.

The effect of isolation from the plasma by means of oil is interesting in connection with the views of Dr. Norris[1] on the cause of the formation of the rouleaux of red blood-corpuscles, and deserves further investigation.

8. *Effect of water in minute quantities gradually added* (*by vapour*).—By means of his hot plate, consisting of a small glass-bottomed well, surrounded by a copper ring connected with a copper wire, which can be heated, Stricker was able to study the effect of minute quantities of water on the cor-

[1] 'Proceedings Royal Society,' vol. xvii.

puscles. A drop of water being placed in the well, and the blood on the under surface of the covering-glass closing it in above, the temperature is gradually raised by means of a spirit-lamp applied to the copper wire, and the aqueous vapour thus produced condenses on the glass. Thus the observer can watch the gradual addition of water to the sides of the drop of blood. The effect on the frog's blood-corpuscle is very remarkable, and has been described by Stricker, who does not, however, give figures of the corpuscles. As the plasma gradually becomes diluted, some of the corpuscles are seen to float about, and at first become oat-shaped, assuming sharper extremities than the normal form (fig. 5 *a*); then they gradually become spherical (fig. 5 *b*, *c*). If the addition of water is continued the corpuscles discharge their hæmoglobin, and, finally, with excess, appear to break up more or less, becoming irregular stromata, in which a large clear nucleus can generally be seen.

Human red corpuscles, treated in the same manner, become globular, *not swelling*, as has been asserted, of the action of water, by some writers, but simply changing their proportions. They finally with excess discharge their colour, and become irregular stromata, retaining a definite spherical outline if the action has not been rapid.

9. *Effect of carbonic acid gas.*—Stricker, who states that the object of his experiments was to ascertain the effect of the alternate action of carbonic acid and atmospheric oxygen on the red blood-corpuscles, found that on fresh normal corpuscles a stream of CO_2 introduced into the gas-chamber has no effect. But when the corpuscles have been previously acted on by aqueous vapour, as described above, to the extent that they have assumed the spherical form, a remarkable action is obtained.

In the case of the frog the nucleus immediately becomes sharply granulated (see fig. 6 *a*). Stricker then proceeds to pass atmospheric air into the gas-chamber in place of the CO_2, and the granulation of the nucleus at once disappeared (fig. 6 *b*), and the corpuscle assumed once again an elongated shape. This change of shape is very strange, and not readily accounted for; the granulation of the nucleus is due, as pointed out by Schweigger-Seydel and Schmidt, to precipitation of paraglobulin, which is redissolved on removal of the carbonic acid. The nucleus can thus be made to come and go by alternate steams of CO_2 and atmospheric air several times; but after the first change of form from the spherical condition they retain the elongated shape. After a time the "starred" condition of the body of the corpuscle is

brought about (fig. 6 *d*), and this will sometimes disappear by the action of the CO_2, and reappear with the atmospheric air.

If aqueous vapour be again allowed to act, the corpuscles again assume a rounded form, and, after the experiment has been carried on some time, lose their colouring matter. They do not so readily resume the elongate form after the second action of water, and the final stage of the experiment is obtained when they have become colourless, spherical, and the "body"[1] granulated as well as the nucleus. The granulation of the body, which is not obtained until water has acted two or three times and the carbonic acid for a considerable period, disappears and reappears at first with alternation of the CO_2 and atmospheric air. The nucleus finally becomes permanent (fig. 6 *e*, *f*). A small pullulation is sometimes obtained in the wall of the corpuscle at a late stage of the experiment, as drawn in fig. 6 *e*, *f*).

Having witnessed and repeated these experiments in Stricker's laboratory, I was anxious to ascertain whether the alternation of any neutral gas with CO_2 would produce the same results as the alternation of atmospheric air. I found that by passing a mixed current of CO_2 and hydrogen the granulation was obtained, and that on stopping the CO_2, and allowing the hydrogen to continue, it disappeared just as when atmospheric air was used, the change in the forms of the corpuscles also occurring. The same result was obtained when using CO in alternation with CO_2; and further, by simply creating a minus pressure in the gas chamber by means of suction, exactly the same effects were obtained as when the stream of atmospheric air or inactive gas was used. Thus it was sufficiently demonstrated (what, indeed, was tolerably certain *à priori*) that the disappearance of the granulation of the nucleus, on passing the stream of atmospheric air, is due simply to the diffusion of the carbonic acid gas, and not to any specific action of the atmospheric oxygen.

[1] Brucke distinguishes the envelope of the frog's red corpuscle as "œcoid" —its contents as "zooid." Stricker divides Brüke's zooid into a "body" and a nucleus. Rollett distinguishes colouring matter and stroma. We thus get the tabular statement:

Red blood-corpuscle of ovipara, divisible into . . .	Stroma. Colouring matter. Œcoid = or outer part of stroma. Zooid = rest of stroma plus hœmoglobin. Membrane = œcoid. Body = zooid minus nucleus. Nucleus = zooid minus body.

Though a small point, this was left uncertain from Stricker's experiments.

When human corpuscles are used in place of those of the frog, the spherical condition having been first obtained by the action of aqueous vapour, Stricker showed that the normal biconcave form is obtained by the action of CO_2, whilst the substitution of atmospheric air causes a more spheroidal condition again, and in many cases the "thorn-apple form," which again yields to the normal biconcave form on renewal of the CO_2 (see figs. 19 *a*, *b*, *c*, *d*). The alternation can be obtained several times.

10. *Effect of osmic acid vapour.*—I have above spoken of the use of the vapour of osmic acid. To observe its gradual working a drop of the concentrated solution may be placed in the gas-chamber, or air drawn into the chamber which has been allowed to bubble through a solution. On the human corpuscles it has absolutely no visible action, excepting that it changes their tint, by acting on the hæmoglobin. Although the blood becomes set by its action into a jelly-like film, no alteration of the form or inner aspect of the corpuscles is produced. This was determined by comparison of fresh blood and blood acted on by the OsO_4. In the frog's blood-corpuscles the nucleus becomes rather sharply defined under the influence of the osmic acid vapour, but it is not coarsely granulated as by most acids.

11. *Effect of acetic acid, vapour, and in solution.*—If air be drawn into the gas-chamber, which has bubbled through acetic acid and the corpuscles of the frog exposed to it, the following effects are observed. If the solution of the acid be weak, so that but very little acts on the corpuscles, the first result noted is a "starring" of the body of the corpuscle and the sharp definition of the nucleus (fig. 8 *a*). The strength being increased, the nucleus becomes coarsely granulated (fig. 8 *b*). The strength being still further increased, so as to reach its maximum, the body of the corpuscles becomes granulated here and there, and its outline finely irregular and thickened; the plasma at this stage also exhibits a very fine molecular precipitate (fig. 8 *c*). When a solution of acetic acid is added directly to the blood, the extreme action is obtained at once, unless the solution is very dilute (2 per cent. of the glacial acid in water) when the earlier conditions are obtained.

12. *Effect of alcohol.*—Although the effect of alcohol is to produce a precipitation in the corpuscle, yet this is different from that produced by acetic acid. Weak vapours of alcohol have little effect; stronger cause an irregularity in the out-

line of the corpuscle of the frog, and a precipitation in the body, whilst the nucleus remains clear. If corpuscles of the frog be submitted suddenly to the action of absolute alcohol, many become distorted in shape; the outline of all assumes some irregularity, and the wall seems thickened (fig. 8 *d*). A coarse flocculent precipitate is visible in the body of the corpuscle, whilst the nucleus is not affected, except in so far as it is rendered less distinct by the turbidity of the body. Mammalian corpuscles become granulated by the action of alcohol.

We thus see in the action of weak acetic acid, or Co_2, on the one hand, and of alcohol on the other, an important distinction between the chemical nature of the body and the nucleus of the oviparous corpuscle, and an agreement of the *entire* mammalian corpuscle with the body only of the ovivarous corpuscle.

13. *Effect of ammonia gas.*—The action of ammonia on the red blood-corpuscles has not hitherto been studied. It is very curious, and I have examined it carefully. First, as to its effect on the frog's corpuscle. The first experiments I made on this point were in the summer of 1870.[1] I then found that on drawing strong ammonia gas into the chamber I instantly obtained the forms drawn in figs. 14 *a* and 5 *d*, and the corpuscles became very soon entirely broken up. But on diluting the source from which the ammoniacal fumes were given off, so as to admit but the merest trace of ammonia, just perceptible by the nose, I obtained curious changes in the form of the corpuscles, as seen in fig. 14 *a*, these being restored to the normal form (or to an oval form very nearly identical with their original one) by the substitution of a current of Co_2 for the ammonia. If the ammonia had been allowed to act a certain time, and Co_2 were then substituted, the nuclei became granulated as after the action of water. Atmospheric air then drawn in caused the radiate condition of the body. When the ammonia was slowly increased in strength, the corpuscles were caused gradually to assume a spherical form, then the sphere became smaller and smaller, and suddenly all the colouring matter, and probably other constituents, passed out of it, and left a pale, irregular stroma, with a large clear nucleus swollen up beyond its normal size. Further increase in the strength of the ammonia completely dissolved this. When liquid ammonia of considerable strength (I cannot give the exact strength at which such phenomena will occur) is allowed to run under the covering-glass where frog's blood already is,

[1] I have to thank Dr. Burdon Sanderson for allowing me to make some of these observations in his laboratory.

the corpuscles almost instantly assume the form of small spheres. Then it may be seen that the nucleus breaks up into bits which float about in the sphere (fig. 14 *a*), swimming round and round as the action goes on, then suddenly the sphere collapses, the colouring matter is diffused all round, and no trace of the corpuscle, except a few scattered specks, remain. The complete disappearance of the corpuscle, or the survival of a ghost-like stroma, depends on whether a strong or a moderately intense solution of the ammonia be used. As I set to work to repeat the observations on the effect of weak ammoniacal gas, in the early spring, I was surprised at not at once getting the change in the shape of the corpuscle which I have seen in the summer. The results which follow are explicable on the supposition that the outer portion or wall of the corpuscle is denser and more resisting in the early spring than later. It has often been remarked that one cannot at will reproduce changes in form caused by reagents acting on blood-corpuscles, and this, it is most probable, is due to some slight variation in their constitution from season to season, and from individual to individual. The elongated, pointed, and triangular shapes drawn in fig. 9 *a*. I did again get this summer with very weak vapour. In the spring, however (as also in some cases this summer), the action of very weak ammoniacal gas on the frog's blood gave three different types of action, which did not occur simultaneously in the same drop, nor lead one into the other, but seemed to depend on very slight differences in the rate at which the ammonia was allowed to act, its strength, and the condition of the corpuscles themselves.

The first condition which was obtained most frequently, and which I heve again obtained during the summer, is drawn in fig. 9 *b*. As soon as a very small amount of ammonia is drawn into the gas chamber, a very weak solution of the gas being used as the source, and air being allowed to bubble through it and thence into the gas chamber, the corpuscles become irregular in shape, and assume lobular forms. The lobules tend to constrict themselves in various ways, and send out long irregular processes as represented in the figure (fig. 12), whilst the nucleus-remaining pellucid enlarges somewhat. Acetic acid vapour of maximum strength now substituted for the ammonia produces a remarkable result. The processes are withdrawn, and the corpuscles remain of an irregular form, but instead of the nucleus becoming granulated it remains perfectly clear and pellucid and much swollen, whilst the body of the corpuscle is granulated (fig. 12 A′). The matter which is precipitable by acetic acid passes under the

influence of the ammonia from the nucleus into the body. In some corpuscles I have found that the action had not proceeded to this extent, and the nucleus though much swollen granulated very sharply (fig. 13 *b*). In many cases the nucleus assumed the colouring matter or hæmoglobin, and remained pellucid whilst the body became colourless and granulated under the influence of the acetic acid (fig. 14 *c*). Similar results without much change of shape in the corpuscle were obtained when a weak solution of ammonia was allowed to act directly on the corpuscles (fig. 14 *c*). When strong ammonia gas or solution was allowed to act on the corpuscles so as to produce the small spherical form, the addition of acetic acid caused these small spherical corpuscles to burst, and then granulated spherical masses were obtained in various parts of the field, due to the fragments of the burst corpusles. In one case (fig. 12 A) a corpuscle had thrown out two pseudopodial-like processes under the influence of the ammonia gas. I watched then the gradual working of acetic acid vapour, causing the processes to be slowly retracted just as in protoplasmic movement, and after a nearly circular outline had been assumed (fig. 12 A′), the granulation of the body came on, the nucleus remaining clear. The behaviour of these corpuscles under alternate weak ammoniacal and acid vapours furnished a very curious parallel to the movements of amœboid protoplasm, and a careful consideration of the phenomena may throw some light on the nature of protoplasmic contractility.

The second type of form assumed by the frog's red blood-corpuscles under the influence of weak ammoniacal vapour is seen in fig. 10 *a*. The red content of the corpuscles, the zooid of Brücke, contracts vigorously, and separates itself from the œcoid or dense superficial membrane, giving the various appearances depicted. This resembles the action of boracic acid described by Brücke, except in this, that the red matter, the zooid, is in no way granulated, but remains perfectly clear and homogeneous. Acetic acid vapour caused to act on the corpuscles in this state gives a general granulation of the whole of the red mass (fig. 10 *b*, *b*), but no delimitation or granulation of a nucleus, which seems to be lost or dissolved. In one case, from my notes, I find that weak ammoniacal vapour having been allowed to act, some of the corpuscles had assumed the form represented in fig. 10 *a*, with the "zooid" contracted away from the "œcoid," others had not reached this condition, and contributed but little change of form. Acetic acid vapour was now drawn in, the corpuscles with the contracted zooid became granulated in that part,

whilst no nucleus could be detected in them, the others exhibited then a granulated body and a *clear pellucid nucleus which was coloured red by hæmoglobin* as in fig. 14 *c*.

The third type of ammonia-action was seen in some corpuscles which at first exhibited a tendency towards the second type, the zooid partially contracting; instead, however, of remaining in this state, the edges of the corpuscles and of their contracted zooids began to break up, as seen in fig. 11 *a*, and particles separated from them and floated about exhibiting Brunonian movements. Larger particles separated in many cases, and in these it was quite easy to recognise the well-known double rhomboid form of hæmoglobin crystals. This observation is exceedingly curious, since it demonstrates a readiness of the material of the blood-corpuscle to assume the crystalline form which was only known previously in some mammalia. Beale has figured (loc. cit.) from the guinea-pig corpuscles disintegrating into small crystals, just as I have here seen them in the frog; but in that case the phenomenon was independent of ammonia or other reagents; also the crystals so formed were the so-called tetrahedral or sphenoidal forms characteristic of the guinea-pig's blood.[1] I have not been able to reproduce this crystalline disintegration of the corpuscles at pleasure, though I obtained it in several successive experiments from the blood of a frog in the spring of this year, at Leipzig.

Whilst the corpuscles were undergoing this disintegration, in one case I passed acetic acid vapour into the chamber, with the result depicted in fig. 11 *b*. The body of the corpuscle appeared to retain its hæmoglobin, and was coloured red; the nucleus became round and was pellucid; round it, however, was a very intensely marked ring, due to coagulation by the acid, and in its middle one two or three sharply cut coagula were seen.

These various effects of the action of ammonia in small quantities require explanation, in view of the chemical constitution of the various parts of the oviparous red blood-corpuscle. They appear to demonstrate that the wall of the corpuscle is readily soluble in ammonia, and more so in some physiological conditions than others, the amœboid figures produced under the action of ammonia being due to the

[1] I may take this opportunity of mentioning that in a specimen of the annelid *Tubifex rivulorum*, mounted in glycerine jelly, I obtained crystals of Hæmoglobin in the drops of the red vascular fluid of the worm expressed in the preparation. The crystals in one and the same preparation exhibited three of the forms seen in different mammalia, viz. rhomboid prisms (dog, man), sphenoids (guinea-pig) and hexahedral plates.

removal of the containing wall of the semifluid zooid. Secondly, they seem to demonstrate that there are at least two constituents of the nucleus, the one precipitable by acids, probably paraglobulin, which passes out into the body of the corpuscle under the influence of the alkaline gas, whilst the second (possibly the mucin which Dr. Brunton has shown reason to believe is a constituent of the nuclei) remains, and is not precipitable by acetic acid. I am conscious that the action of ammonia, which I have described, deserves to be investigated in a more methodical manner, and I draw attention to it on that account.

On the human blood-corpuscle ammonia has the same action as on that of the frog, excepting such phenomena as concern the nucleus. A fluidity (due as it seems to me to a solution of the wall or pellicle of the corpuscle) is produced by very weak ammoniacal vapour, resulting in the production of long threads or processes from the corpuscles, and the separation of minute particles from them, as in the case of the frog (fig. 20).

Acetic acid vapour admitted to human corpuscles after the action of weak ammoniacal vapour gave no granulation of the corpuscles, but the fine thread-like processes were rendered more distinct, and some corpuscles exhibited pullulations like those produced by tannin in Dr. Roberts' experiments (fig. 21 *b*, *c*).

In speaking of the action of magenta dye below I shall refer to its action after the corpuscles have been acted upon by ammonia.

14. *Effect of chloroform.*—Chloroform or ether being so generally used for separating the hæmoglobin of the red corpuscles from their stroma, it seemed to me to be interesting to examine carefully the steps of its action. Böttscher, Schweigger-Seidel, and Schmidt have noticed in their papers the fact of the removal of the colouring matter and the survival of a colourless stroma having the form of the original corpuscle. By the use of the gas chamber and the method of suction I have watched the process more closely.

When frog's red-corpuscles are submitted in the gas chamber to an increasing quantity of chloroform vapour, I have observed the following series of phenomena.

The first change noticeable is a very fine plication or wrinkling of the whole surface of the corpuscle (fig. 15 *a*), as though a delicate membrane were being caused to contract. Then an angular form is assumed by the corpuscle (figs. 16 *a*, 15 *b*), generally hexagonal, but sometimes diamond-shaped. I cannot refrain from pointing out the resemblance of these

angular forms to the crystalline form of hæmoglobin, and suggest that they are possibly due to modified crystallisation. Then, thirdly, the corpuscle resumes an oval shape (fig. 16 *b*, *b*), but not the original oval shape. It is now shorter and broader, and very distinct from the normal ellipsoid. Soon after this oval form has been assumed, numbers of the corpuscles are observed to become colourless (fig. 16 *c*). There is no collapse, no movement on their part, but simply their colouring matter passes from them, and they remain as oval "ghosts" or stromata of very definite shape with pellucid nucleus and well-marked outline. All this is under the influence of chloroform vapour drawn into the gas chamber, as described. Acetic acid vapour now drawn in granulates the nucleus very sharply (fig. 16 *d*). If instead of acetic acid vapour more chloroform vapour is drawn, no further result is obtained, the "ghosts" remain unchanged. But if, instead of the vapour, the observer now proceeds to make use of the liquid chloroform, vigorous action is obtained. A drop of chloroform placed on the already decolorised corpuscles causes an intense action, which looks like effervescence, around each ghostly stroma (fig. 17 *b*, *b*). Hundreds of minute globules, the character of which is not gaseous, form along the sides of the stromata and along the edge of the nuclei, and then float off and disappear in the plasma. This action goes on with repeated additions of the chloroform until the outer portions of the stromata are dissolved, so that only the nuclei remain. These seem to be more resistant, but finally are broken up also with formation of the evanescent globules (fig. 17 *e*, *e*, *f*).

When chloroform liquid is added to fresh red blood-corpuscles of the frog, they almost instantly give up their hæmoglobin, becoming nearly spherical, and the stromata have a collapsed and less regular outline than when chloroform vapour is gradually allowed to act.

Human corpuscles, subjected to the gradual action of chloroform vapour or to the action of chloroform liquid, exhibit identical appearances.

15. *Effect of bisulphide of carbon.*—With carbon bisulphide vapour I have obtained in the frog's corpuscles nearly identical results with those given by chloroform—the assumption of an angular form (fig. 15 *b*), followed by a short oval or spherical form, the diffusion of the colouring matter, and the production of colourless stromata. It appears to take longer to discharge the colouring matter than chloroform, though the change of form is rapidly produced. The direct addition of the liquid to the corpuscles caused the solution of the stro-

mata, with the formation of numbers of minute evanescent globules, just as with chloroform.

16. *Effect of benzine.*—Essentially the same as that of chloroform and carbon disulphide.

17. *Effect of turpentine-oil.*—Essentially the same as, but less vigorous than, chloroform on carbon disulphide. When the turpentine oil was added in the liquid form to the corpuscles the formation of the numerous fine globules was not obtained, and the stromata were not dissolved. Turpentine spirit may have a more vigorous action.

18. *Effect of solution of acetate of rosanilin and of tannin (Roberts' experiments).*—As discovered by Dr. Roberts, of Manchester (loc. cit.), magenta dye, when allowed to act on either human or frog's red blood-corpuscles (also those of other vertebrates), causes a discharge of the hæmoglobin, and (colouring the nucleus deep red in the latter case) produces a well-marked red "macula," more or less oblong on the wall of the corpuscle. Tannin, on the other hand, causes a sharp little pullulation in the wall of the corpuscle, at the same time granulating the nucleus. In the human corpuscle Roberts found that there was rarely more than one such macula or pullulation; in the frog often two, three, or more. I have repeated Roberts' experiments, using a nearly saturated solution of the reagent in each case. In figs. 22, 23, 24, the results are depicted. The macula and the pullulation are due to something entirely distinct from what we have had evidence of in the action of the reagents hitherto considered. When the red corpuscles of the frog are mixed on a glass slide with a little of the magenta solution, and examined, they are found to have become small and spherical, as under the action of ammonia. The nucleus is rounded and sometimes granulated, sometimes not so, whilst the body is faintly stained or colourless. The outline or wall of the corpuscle looks thicker than under other circumstances, and one, or as many as four, points are seen in the circumference of the corpuscle deeply stained, like the nucleus (fig. 23). Besides this, very generally round each corpuscle, and more especially near the stained points or "maculæ," deeply stained granular matter is seen in the plasma. By allowing the magenta slowly to come in contact with the blood, whilst the slide was on the microscope stage, I was able, after many trials, to watch one corpuscle through its changes. The corpuscle first shortened and became nearly spherical (fig. 23 *a*, *b*), and then discharged its colouring matter. It then took a very slight pink tint from the magenta fluid (fig. 23 *c*), and next, suddenly the nucleus, which had previously been indistinct, assumed

a deep red colour, and burst into view. Almost at the same moment a point in the wall of the corpuscle gave way, and a very finely granular matter issued, which was stained red by the magenta, whilst, at the same time, a red "macula" formed internally at the point whence this had escaped. A second macula, accompanied with the escape of matter which took up the red colour, formed very shortly afterwards at another point of the corpuscle (fig. 23 *d*). I thus saw that the molecular matter, stained red in the plasma, was due to the escape of something from the corpuscles themselves, identical with the maculæ. The action of tannin (fig. 24) appeared to me to be very closely similar to that of magenta, excepting that the matter which escapes and forms a coloured molecular mass in the plasma when magenta is used, in the case of tannin is arrested and "set" as it escapes, thus forming the pullulation. That it is not always set, and that much of it escapes, may be seen from Roberts' figures, as also I observed in both human and frog's corpuscles.

I merely point to the sketches of human corpuscles treated with magenta and with tannin in order to confirm Roberts' description of them (fig. 22 *a*, *b*). The phenomenon is much better studied on account of size in the corpuscles of the frog. The magenta 'macula' in the human corpuscle almost invariably appeared to stand out a little from the surface, to be a very little raised, as it were, which is not the case in the frog. The reason I believe to be the greater delicacy of the pellicle or membrane of the corpuscle in Mammalia than in Ovipara.

There can, I think, be little doubt as to the identity of the magenta macula and the tannin pullulation. The questions which occur are: Is the Robertsian macula a physiological or a cadaveric differentiation? and what is the nature of this substance which thus takes up the magenta dye and is coagulated by tannin? As to the first question, there appears to be no ground for supposing that this differentiated 'macula' exists during the life of the corpuscles, since, as is obvious by their form, and the steps of the process as described above, the whole corpuscle is very much altered before the macula or pullulation makes its appearance. Moreover, they occur with great irregularity in the frog's corpuscle as to number and position, and, as observed by Dr. Roberts in the oval corpuscle of the camel, the usually single macula appears to have no definite position. It seems to me, then, likely that this macula is due to the separation or segregration of a constituent of the blood-corpuscles, which occurs as a cadaveric change under the influence of some reagents. The material, whatever it is, collects at the most yielding point or points

beneath the œcoid or pellicle of the corpuscle, and there, forced by increased contraction of the more solid parts of the corpuscle, bursts out through the pellicle, escaping to a large extent in the case of magenta, and becoming diffused, but becoming fixed as a pullulation when tannin is used. It seems probable that the separation and escape of this constituent of the corpuscle goes on under the influence of other reagents, but that being neither stained nor precipitated by them we do not see it. Thus, after weak ammonia has been allowed to act, I found that neither human nor frog's corpuscles exhibit the macula with magenta. In the frog's red corpuscle so treated, the nucleus remained unstained in many cases, whilst the body took up the colour (fig. 23 *f*)—a similar result to that described above as to acetic acid and as to ammonia. Frogs' red corpuscles acted on with chloroform to the third stage did not give the magenta macula, though the nuclei stained well. Frogs' red corpuscles treated with aqueous vapour till they assumed the rounded form did not give the macula with magenta. Frogs' red corpuscles dried, as also human ones dried, did not, on treatment with magenta, give the macula, though the nuclei of the former stain most readily and brilliantly (fig. 23 *g*).

The reason that the ammoniacal solution of carmine does not bring out the macula as does magenta is probably due to the ammonia causing solution of the substance, whatever it may be, which is thus segregated or squeezed out from the rest of the corpuscle. The absence of the macula in dried corpuscles is easily understood as due to a prevention of the contraction which causes the separation of it under certain reagents from the other constituents of the corpuscle.

By further microchemical research, no doubt, the nature of the substance which forms the macula and pullulation may be more definitely ascertained. I am inclined to believe it to be a more fluid constituent of the body of the corpuscle.

It is especially necessary to remark that neither magenta nor tannin act on one part only of the red blood-corpuscle. They both affect it through and through. The connection of the molecular matter, supposed to belong to the plasma by Roberts with the maculæ, as seen by me, is of especial significance as to the mode of origin of the latter, and its connection with the tannin pullulation.

Experiments with certain gases remarkable for their combinations with the hæmoglobin of the corpuscles.

19. *Effect of carbonic oxide.*—CO, as is well known, forms a compound with hæmoglobin, having a characteristic ab-

sorption spectrum. I submitted frog's red corpuscles to the action of CO in the gas-chamber, but obtained no change of form whatever, though the carbonic oxide had acted on the hæmoglobin within the corpuscles, as demonstrated by the microspectroscope. On passing carbonic oxide into the chamber where frog's corpuscles were subjected to it, which had already begun to assume the stellate or radiate arrangement of the "body," I found that the radiate condition disappeared, this being apparently due to the action of the carbonic oxide.

20. *Effect of cyanogen gas.*—Cyanogen gas, as I have elsewhere pointed out ('Journal of Anatomy,' November, 1869, and Pflüger's 'Archiv,' 1869), acts upon hæmoglobin, first, by combining with it, without destroying its complex character, probably forming, first, hydrocyanic acid, which Hoppe Seyler showed could thus combine with Hæmoglobin.[1] Then, after a time, it breaks up the hæmoglobin forming the cyanhæmatin of Hoppe Seyler, having its own very definite broad absorption-band. When the red blood-corpuscles of the frog are submitted to the action of a stream of cyanogen gas in the gas-chamber, the nuclei at first become distinct; there is then a visible contraction and plication of the surface of the corpuscle and the colouring matter, in the form of cyanhæmatin, as proved by the microspectroscope, is suddenly discharged. A clear stroma, similar to that produced by chloroform, remains.[1] With human blood-corpuscles, clear, somewhat irregular stromata are left.

21. *Effect of sulphuretted hydrogen.*—Hydrogen sulphide produces a definitely characteristic absorption-band in hæmoglobin, as also do alkaline sulphides. This was first pointed out by Nawroscki. I have proposed to call the substance thus indicated sulphhæmoglobin, a name which has been accepted by Professor Preyer in his work 'Die Blutkrystalle,' wherein a detailed study of the action of sulphides on hæmoglobin is given.

When the red blood-corpuscles of the frog are submitted to a stream of H_2S the radiate form of the body was rapidly assumed, similar to that drawn in fig. 7 *b*, whilst at the same time the corpuscles changed colour, though they did not discharge it at all. An examination with the microspectroscope proved that the hæmoglobin had been completely acted

[1] I was led to believe that the absorption spectrum of blood treated with cyanogen gas was at first identical with that of CO hæmoglobin. Preyer however, whilst confirming me on other points, and working with a large spectroscope, states (Die Blutkrystalle) that the spectrum is that of OHb.

upon, the corpuscles now containing sulphæmoglobin, as indicated by the persistence of two lines of oxyhæmoglobin, together with a new line in the red having a definite position, which was duly compared and recognised. It is interesting to find that sulphæmoglobin, like hæmoglobin, does not diffuse from the corpuscles under normal conditions.

If we compare now the reagents which affect the hæmoglobin of the blood-corpuscles, we find them acting very differently.

Causing its discharge:

Unchanged.—Chloroform, CS_2, benzine, &c. Ammonia (in very small quantity). Water (requires time).
Changed.—Cyanogen (Cyanhæmatin), Ammonia (alkaline hæmatin).

Not causing its discharge:

Not changing.—Alcohol (or only to methæmoglobin) but precipitating.
Changing.—Carbonic oxide (CO hæmoglobin). Sulphuretted hydrogen (sulphæmoglobin). Acetic acid (acid hæmatin).

General conclusions and summary.—The red blood-corpuscle of the vertebrata is a viscid and at the same time elastic disc, oval, or round in outline, its surface being differentiated somewhat from the underlying material, and forming a pellicle or membrane of great tenuity, not distinguishable with the highest powers (whilst the corpuscle is normal and living), and having no pronounced inner limitation. The viscid mass consists of (or rather *yields,* since the state of combination of the components is not known) a variety of albuminoid and other bodies, the most easily separable of which is hæmoglobin; secondly, the matter which segregates to form Roberts's macula; and thirdly, a residuary stroma, apparently homogeneous in the mammalia (excepting so far as the outer surface or pellicle may be of a different chemical nature), but containing in the other vertebrata a sharply definable nucleus, this nucleus being already differentiated, but not sharply delineated during life, and consisting of (or separable into) at least two components, one (paraglobulin) precipitable by Co_2, and removable by the action of weak NH_3; the other pellucid and not granulated by acids.

The chemical differentiation of the outer pellicle is rendered probable by the behaviour of the corpuscles under weak NH_3, which appears to dissolve this pellicle, and so loose the viscid matter from that which restrained it to its oval shape; also from the inability of CO_2 to act on the corpuscle until it has

been subjected to the influence of aqueous vapour, which may be supposed to remove or render permeable this pellicle; also from the action of chloroform, oil, and cyanogen, which cause the discharge or diffusion of the hæmoglobin from the corpuscle, perhaps by first removing or rendering permeable —at any rate modifying—this outer pellicle.

Steam, chloroform, benzine, bisulphide of carbon, ammonia and cyanogen, act on the red blood-corpuscle so as to cause the escape of the hæmoglobin.

The further action of these reagents causes the elimination of what may be called Roberts's constituent, that which is stained by magenta and set by tannin.

A still further action of chloroform, of water, or of ammonia, dissolves first the stroma, lastly the nucleus.

The details of these actions are given in the paper.

Carbonic oxide and sulphuretted hydrogen produce their respective changes on the hæmoglobin, as demonstrated spectroscopically, without altering the form of the corpuscle, merely effecting the radiation of its body.

On Undulina, *the type of a* New Group *of* Infusoria.

By E. Ray Lankester.

In making the numerous examinations of the blood of frogs above recorded, I have now and then met with the interesting little parasite drawn in the woodcut. When I first saw it, in some blood from a frog last summer, I took it for a very active white blood-corpuscle, since it is a very little smaller than one of the red corpuscles of the frog's blood. On using, however, a higher power (No. 10 à immersion of Hartnack) I made out its infusorial nature, though, on account of the great activity of its movements, I was long uncertain as to the nature of its locomotive organs. Numerous specimens occurred in the blood of a frog (*Rana esculenta*) examined at Leipzig, in March last, and by the use of a small quantity of acetic acid vapour, I was able to kill the little creature without injuring it, and then to make out its structure. It was seen to be a minute pyriform sac, with the narrower end bent round on itself somewhat spirally, and the broader end spread out into a thin membrane, which exhibited four or five folds, and was produced on one side into a very long flagellum. The wall of the sac was striated coarsely, as in Opalina; and the direction of the striæ on the