"On the Action of certain Reagents upon the Coloured Blood-Corpuscles. Part I. The Coloured Blood-Corpuscles of the Newt and Frog."

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[Plate 1.]

The histological and chemical constitution of the coloured blood-corpuscles of man and other animals has formed a fertile source of investigation for a large number of observers, and it might seem that further investigations on this subject were unnecessary. We have, however, devoted considerable time to a systematic study of the effects of certain reagents upon the blood of the newt and frog which have yielded results, some of them of not a little interest and importance.

The literature of the subject, chiefly of the German papers, is given somewhat fully in Rollett's article, "Blood," in Stricker's "Histology," and a careful *résumé* of most of the more recent and some of the older observations will be found in a short paper by G. F. Dowdeswell, in the "Quarterly Journal of Microscopical Science" for 1881.* Reference will be made to the literature of each reagent under its appropriate heading.

The *method* of conducting the investigation was as follows:—A newt was pithed; its heart exposed; the auricle snipped through and the blood collected.† A drop of this blood was then placed on a slide, and covered with a cover-glass, a hair having first been placed between the cover-glass and the slide to admit of the corpuscles rolling freely over and over so as to be seen on edge as well as on the flat, and also to allow the corpuscles to expand freely under the influence of reagents. The blood was then irrigated with a solution of the reagent to be investigated and examined with a magnifying power of 300 diameters, or a higher magnifying power when this was deemed desirable. Similar experiments were made with frog's blood, but newt's blood was preferred on account of the corpuscles being larger.

Pyrogallic Acid.

On irrigating a drop of the blood with a 2 per cent. solution of

* An excellent digest up to date is given by Professor Lankester in his exhaustive paper "Observations and Experiments on the Red Blood-Corpuscle," in the same Journal for the year 1871.

† Results were obtained equally with defibrinated and ordinary blood.

pyrogallic acid—which had become yellow through having been kept a day or two after it was made—the following appearances were observed:—The biconvex oval red corpuscles soon became globular, and in a very short time were observed to recoil or give a sudden jerk, a small portion of the contents being at the same time extruded in a direction opposite to that in which the recoil took place. In fact, the recoil seemed to be due to the sudden extrusion of a small portion of the contents of the corpuscle at one place, or in rare cases at two places, at the margin of the corpuscle. These small extrusions resemble, but are not identical with the "pullulations" described by Dr. Roberts as the effect of the action of a solution of tannic acid.*

It is very interesting to watch the sudden effect. The globular corpuscle suddenly jerks in one direction, and simultaneously with this, one observes a small mass of the contents of the corpuscle pouring out of a very fine aperture or crack at one side of the corpuscle. (Pl. 1, fig. 1, *b*, *c*, *d*.) The motion or jerk seems to be caused by the sudden ejection of material, and so the corpuscle moves in an opposite direction. The opening is very small, and the extruded mass remains adherent to the corpuscle. It is much less than a twentieth the bulk of the corpuscle, and it is rarely larger than one-third the diameter of the corpuscle. It is slightly coloured and slightly granular, remains adherent to the corpuscle, and does not show any of the "hooded" character described by Dr. Roberts as the effect of tannic acid. The rent or crack in the envelope or at least in the now thickened rind of the corpuscle, through which the little mass is forced or ejected can often be clearly seen. If such a preparation be sealed up, such buds or projections may be kept for a considerable time. If a sufficient amount of the acid be added other effects follow. The corpuscles begin to assume a coarsely granular appearance, and the nucleus, until now unaffected, begins to assume a granular appearance, but still remains pale as at first. Owing to the granular condition of the hull (Dowdeswell) of the corpuscle, the nucleus cannot be seen until the centre of the corpuscle is focussed. Some of the corpuscles appeared to become granular without any extrusion of a part of their contents. In those corpuscles which had given way at one side the granular contents soon began to pass out through the rent, and often the nucleus also was extruded. In many of the corpuscles the nucleus was observed to be half within and half without the body of the corpuscle. A distinct, highly refractive envelope was traceable around the body of the corpuscle as far as the break in its side, when it suddenly ceased. This envelope was often traceable on one or both sides of the protruded part of the corpuscle for some distance, but never completely around it. The protruded mass generally remained attached to the side of

* "Proc. Roy. Soc.," 1863.

the corpuscle, even when the whole of its granular contents had passed out. The nucleus generally remained within the envelope, and became tinged of a yellow colour. The intranuclear plexus of fibrils was also revealed. (Pl. 1, fig 1, *g*.) The envelope of a corpuscle devoid of its granular contents was observed to be tinged of a yellow colour, and to show a double contour. A similar appearance was observed around the nuclei. The remaining corpuscles ultimately became completely disintegrated, and the field of the microscope became covered with free nuclei, granular colouring matter, and fragments of envelopes sometimes containing nuclei. (Pl. 1, fig. 1, *i*.) Sometimes an envelope was seen entire but with a rent in its side extending half through it, and through which its contents had escaped. (Fig. 1, *h*.) Sometimes a mass of granules was observed with a dark line partially enveloping it. The dark line represented a highly refractive envelope now distinctly shown—perhaps produced—by the action of the pyrogallic acid.

It is an interesting question to determine why and how this *sudden* protrusion of a portion of the coloured corpuscles is produced at one part of the circumference. That a process of endosmosis goes on is shown by the corpuscles assuming a globular form, but that of itself is not enough to account for the sudden protrusion already referred to, for water and many other fluids also produce endosmosis, but give rise to no such ejection of the contents of the corpuscles. The pyrogallic acid obviously has some chemical effect on the corpuscular contents, and it is just probable that the envelope of the corpuscle is not perfectly uniform in its resistance all round. We are inclined to view the above described phenomenon in connexion with the spots or thickenings described by Dr. Roberts as occurring in the wall of blood-corpuscles under the action of magenta solution, and also with the curious spots observed by us in the corpuscular wall after the action of gallic acid. It is to be noted that no contraction or diminution of bulk of the corpuscle is observable as coincident with the extrusion of the mass, which, however, is relatively so small that it would scarcely visibly affect the diameter of the corpuscle. The various effects are shown in Pl. 1, fig. 1.

Seeing that tannic acid has already yielded such remarkable results in the hands of Dr. Roberts, and that pyrogallic acid gave rise to equally peculiar phenomena, it occurred to us to ascertain the effect of a substance closely related to both, viz., gallic acid. [Wedel* also recognised that pyrogallic acid causes a separation of the hæmoglobin from the stroma.]

Gallic Acid.

A saturated solution of gallic acid causes the corpuscle to become

* Hermann's "Handbuch der Physiologie," vol. 4, p. 18, 1880.

spherical and the nucleus to become more distinct and tinged yellow. Then a sudden recoil or jerk is observed, the corpuscle at the same time elongating and swelling up. No rupture of the envelope was seen. The contents of the corpuscle appeared gradually to pass out, or to be dissolved out, and the field of the microscope became covered with granular *débris.* The nucleus was left of a bright yellow colour and quite smooth and homogeneous in appearance. As all the hæmoglobin is dissolved out of the corpuscle, the deeply stained homogeneous nucleus is seen in the interior of the globular corpuscle, which is surrounded by a highly refractive envelope, in which one, two, three, or more slight thickenings, or highly refractive elongated bodies, are to be seen. (Pl. 1, fig. 2, *b*, *c*.) Whether these bodies are at all comparable to the thickenings, or "maculæ," already described by Roberts, it would be difficult to say. Perhaps they are merely the *débris* of the intracellular stroma. Slight remains of this stroma may sometimes be seen in the perinuclear portion of the corpuscle, as after a time it becomes slightly tinged yellow and is stained by fuchsin or magenta. Within the nucleus one, two, or more highly refractive dots are always to be seen. When single the dot presents the appearance of a nucleolus, but it occupies very variable positions and is larger than the nucleolus revealed by the action of dilute alcohol, already described by Ranvier and one of us.* These dots are perhaps the remains of the intranuclear plexus. (Fig. 2.)

Hydrochloric Acid.

1 *per cent. Solution.*—One of us has already described the sudden enlargement and as sudden collapse of the corpuscles, with a simultaneous discharge of the hæmoglobin, which results from irrigation with a 1 per cent. solution of this acid.†

2 *per cent. Solution.*—On irrigating a drop of blood with a 2 per cent. solution of hydrochloric acid the nucleus began apparently to shrink away from its surroundings, becoming at the same time tinged of a yellow colour, and showing an intranuclear plexus of fibrils. In some of the corpuscles the nucleus had taken up an excentric situation, being placed at one end of the corpuscle or nearer one end than another, showing how plastic the perinuclear portion of the corpuscle is. (Fig. 3, *b*.) Its long axis was sometimes found to lie across that of the corpuscle. (Fig. 3, *h*.)

In some corpuscles the apparent shrinking of the nucleus was not observed and in some it was very slight. In a corpuscle seen on edge this change in the nucleus is very striking (fig. 3, *e*, *g*), and is perhaps due to the acid fluid passing by endosmosis through the body

* W. Stirling, "Journal of Anat. and Physiol.," vol. x, p. 778.

† W. Stirling, "Text-book of Practical Histology," 1882, p. 2.

of the corpuscle into the nucleus, and so affecting the latter as to cause it to shrivel up and thus to retreat from the mass of hæmoglobin in which it lies embedded.

In its passage through the body of the corpuscle the fluid had dissolved out a portion of the colouring matter of the corpuscle and carried it into the nucleus, so that the latter had become tinged with it. The nucleus generally retained its original position in the corpuscle with its long axis parallel with that of the corpuscle. Although apparently free in the hæmoglobin, the nucleus was not observed to change its position within its cavity. This may be accounted for on the supposition that some of the intranuclear fibrils pass through the envelope of the nucleus, and are in direct continuity with some of the fibrils which form the stroma of the hull of the corpuscle, and that these fibrils support the nucleus in the fluid by which it is surrounded. The size and coloration of the corpuscles were slightly diminished.

To appreciate the full effect of the acid it is necessary to have two views of the corpuscles, one on the flat and the other on edge. When viewed on the flat the shrunken nucleus can be seen lying within the corpuscle, with a clear wide space, probably filled with fluid, lying between it and the hæmoglobin-charged stroma. On causing the corpuscles to roll over, however, one observes that there is not only a shrinking of the nucleus, but also a simultaneous expansion of that portion of the corpuscle which lies immediately outside the nucleus, so that on edge the corpuscles, instead of presenting the usual graceful biconvex curves, suddenly bulge out in the centre, as represented in fig. 3, *f*.

A somewhat similar effect is described by Rollett* :—" The nucleus appears to be not very sharply defined, but frequently shrivelled and surrounded by an empty space, as though lying in a cavity of the substance of the blood-corpuscle (chromic acid, hydrochloric acid, nitric acid, picric acid, tannic acid, and concentrated tincture of iodine)." No figures are given, but we find most certainly that these acids yield more characteristic results than is conveyed in the above description. Corpuscles exhibiting this peculiar change can be kept for a considerable time. In some cases, just when the acid begins to act, a slight shrinking of the hæmoglobin from the envelope of the corpuscle can be seen.

It is curious to note the very different effects produced by solutions of the same acid—a 1 per cent. solution producing a *general* expansion of the whole corpuscle, whilst the stronger solution causes only a *partial* swelling up of one portion of the corpuscle, and a simultaneous shrinking of the nucleus.

* *Op. cit.*, p. 399.

Benzoic Acid.

This acid is soluble to the extent of 1 in 200 parts of water. A saturated solution causes the nuclei to become distinct and many of the corpuscles to become spherical. The contents of the stroma of the corpuscle gradually pass out through the membrane and the field of the microscope becomes covered with granular *débris*, which is in greatest abundance close to the corpuscles. The latter at length become quite clear, except for a few granules here and there in the stroma. The nuclei are distinct and of a bright yellow colour and are generally oval and more or less irregular in outline, but sometimes spherical. A double contour-line can nearly always be made out both around the nucleus and body of the corpuscle. Some of the nuclei show an intranuclear plexus, others are smooth and homogeneous.

Salicylic Acid.

A saturated watery solution of salicylic acid caused the corpuscles to swell up rapidly and become globular, the nuclei at the same time becoming tinged yellow.

In a very short time the corpuscles begin to elongate one after another, with a sudden jerk. No visible break could be observed in the side of the corpuscle, although the nucleus at the moment of recoil was often observed to pass out through the side of the corpuscle. The field of the microscope quickly became covered with yellowish *debris*—the contents of the stroma of the corpuscles—while the perinuclear part of the corpuscles became decolorised. Some of the nuclei were smooth in appearance, and bright yellow in colour; others showed beautifully the intranuclear plexus of fibrils, with narrow meshes; and still others had become swollen up to about twice their normal dimensions, and exhibited a wide-meshed plexus in their interior, due no doubt to the separation of the fibrils by the swelling up of the interfibrillar substance. Those nuclei which showed a plexus in their interior were observed to be bounded by a double contour-line, tinged of a yellow colour. A similar line indicated the position of the envelope of the corpuscle. Many free nuclei were observed, and some with the collapsed envelope and stroma of the corpuscle still clinging to them.

Other nuclei were noticed half within and half without the corpuscle. When the effect of the reagent had been more gradual, the perinuclear part of the corpuscle was granular and darkened in colour. The nucleus was pale—slightly tinged yellow—and showed an intranuclear plexus.

With this acid, as with many others, we frequently observed indications in the nuclei as if they were dividing, and we recall the observation of Preyer, that in the breeding season, he observed that

partially divided nuclei were frequently to be seen in the coloured blood-corpuscles of the frog.

Tartaric Acid.

On irrigation with a 12 per cent. solution of this acid, the first effect observed was an unequal shrinking of the corpuscles, so as to produce a very peculiar effect. Each corpuscle appeared with a series of bars across it, so as to present a series of alternate dark and light coloured areas. These areas not unfrequently resemble a series of folds or creases in the corpuscles. Sometimes these areas were arranged with considerable regularity across the corpuscle, whilst at other times they were irregular, and sometimes radiated outwards. The lighter areas seemed to be produced by the corpuscle becoming thinner at these parts, as if it were the result of osmosis taking place unequally and irregularly. This effect, however, soon gives place to another, wherein the corpuscles *suddenly* swell up and burst. Coincident with this swelling up, there is a great commotion in the material elements of the corpuscles, the nucleus is not unfrequently liberated, and can be seen to pass out of the disintegrated hull of the corpuscle, becoming at the same time completely decolorised. Immediately before bursting, the barred arrangement of the hæmoglobin just described disappears, and the nucleus, which until then had been pale and indistinct, becomes more distinct, yellowish in colour, and more granular in appearance. The swelling and decoloration could often be observed to commence at one end of the corpuscle and pass towards the other end. After the corpuscles had burst, the nuclei were left stained of a deep yellow colour, and showing a beautiful intranuclear plexus of fibrils. Many nuclei were seen with the collapsed envelope and stroma of the corpuscle still adherent to them. After a time the collapsed envelope and stroma often became slightly stained of a yellow colour.

Citric Acid.

The action of a 12 per cent. solution of citric acid was in every respect the same as that of tartaric acid.

Formic Acid.

On irrigation of a drop of blood with a 12 per cent. solution of this acid, the nuclei became distinct, and many of the corpuscles very soon became globular, gave way at one side, and became decolorised. The giving way was accompanied by a recoil or jerk. The nucleus sometimes escaped, but was generally surrounded by vestiges of the collapsed envelope, and of the stroma of the corpuscle. These effects were only seen in those corpuscles which first came under the influence of the reagent. In those corpuscles which were least exposed to the

reagent, the effect was more gradual, and somewhat different. The nucleus became brighter as with the other corpuscles, but after a short time the corpuscle suddenly expanded to several times its former size. No bursting was observed, nor did the corpuscle become globular before expansion. The nuclei became bright yellow and granular after the expansion of the corpuscle. The outline of the expanded hull, though faint, could be distinctly seen, as also could indications of a fibrillar stroma in the hull.

Lactic Acid.

A solution of lactic acid (24 per cent.) was found to cause at first an irregular crenation of the hæmoglobin *within* its envelope, so that the latter could be seen as a glass-clear structure, separated from its contents at different parts. The nucleus gradually became more distinct and "granular." The corpuscle soon expanded suddenly to several times its original size, the nucleus being well defined, and showing beautifully the fibrillar plexus in its interior. Some of the corpuscles doubtless burst, as free nuclei are found in many parts of the field. Nuclei are also seen with the remains of the hull and envelope of the corpuscle attached to one side.

Oxalic Acid.

A 2 per cent. solution of oxalic acid caused the corpuscles and their nuclei to swell up and become globular. The nuclei became tinged yellow. Very soon the corpuscles gave a sudden recoil or jerk, and at the same time elongated. The nucleus was often extruded at the moment of recoil, but in the case of other corpuscles it simply shifted its situation within the corpuscle. Not unfrequently we could watch the nucleus being extruded, and when it was half out and half in the corpuscle it was constricted, but still no envelope was visible in the corpuscle. No actual break was visible on the side of the corpuscle, but the contents of the latter was scattered over the field of the microscope in small yellowish granules. The corpuscles gradually lost their colouring matter, but the nuclei became stained of a bright yellow colour, and showed beautiful plexuses of fibrils in their interior. In many of the corpuscles also many fine fibrils, stained yellow, were observed in the perinuclear part of the corpuscles, mostly passing in a radial manner from the nucleus to the *envelope*. (Fig. 4, *e*, *h*.)

These fibrils and the nucleus are well stained by fuchsin or magenta.

If the action of the acid is gradual both nucleus and corpuscle may be completely decolorised.

Carbolic Acid.

The changes induced in the corpuscles by a saturated (1 in 20) watery solution of carbolic acid were peculiar, and somewhat difficult to describe on account of the rapidity with which the final stage was reached. The appearances seen were as follows:—On irrigating the blood with the solution of the reagent it was found that the corpuscles first affected had lost a great part—or the whole—of their hæmoglobin, while the nucleus had become much swollen and of a globular form. (Fig. 5, *a.*) The nucleus in this condition often showed well the plexus in its interior, but at other times it was seen to contain a number of dark yellow globules—derived no doubt from the perinuclear part of the corpuscle—but did not show the plexus. The corpuscle itself consisted of the swollen nucleus with only a narrow rim of the perinuclear part around it. In some corpuscles hæmoglobin was collected into a semi-globular mass attached to one side of the nucleus. (Fig. 5, *e*, *f*.) In other corpuscles the nucleus had a crescent-shaped mass of hæmoglobin on either side of it. The hæmoglobin still in connexion with the nucleus, either in its interior or attached to its sides, was smooth in appearance and darkened in colour; over the part of the slide first invaded by the reagent many long dark streaks of granular colouring matter were seen. It appeared as if the corpuscles attacked by the acid had had their envelope dissolved or had burst at one side, allowing of the escape of the colouring matter.

On selecting some unaltered corpuscles in the centre of the slide, and watching the gradual action of the acid upon them as it passed under the cover-glass, the following appearances were observed:—The corpuscles seen on the flat first showed the barred arrangement of the perinuclear part already described as occurring under the action of several other reagents, and which is due to the varying thickness of the corpuscles at different parts. (This variation was seen in a corpuscle on edge, which has then a crenated appearance.) Very soon the corpuscles became granular in appearance, the hæmoglobin becoming at the same time much darkened. The nucleus became more distinct, was pale in colour, and had a granular appearance. Many of the corpuscles were observed to have given way at one side, and their contents to have been partly extruded. The nucleus soon became much swollen, and often exhibited an intranuclear plexus. The contents of the corpuscles were scattered about the field of the microscope in the form of small dark granules. Many of the darkly granular corpuscles above mentioned appear to become gradually decolorised, while in others the granules appeared to melt down into dark yellow homogeneous semi-fluid particles, which were seen adhering to the greatly distended nucleus.*

* The liberation of the hæmoglobin is of importance in connexion with poisoning

Ammonium Hydrate.

On irrigation of a drop of blood with a 12 per cent. solution of ammonium hydrate the corpuscles became spherical, and at the same time darkened in colour. The nuclei could not be seen distinctly, but appeared to have undergone the same change of shape as the corpuscles. Very soon the corpuscles *suddenly* collapsed, and they and their nuclei disappeared from view entirely. Sometimes, instead of collapsing, the corpuscles suddenly expanded, appearing to burst, and then melted away. The expansion was accompanied with a slight recoil or jerk. The solution of the corpuscles was sometimes more gradual, this depending, however, on the strength of the solution used. A 2 per cent. solution causes a more gradual solution of the corpuscles.

The effects of ammonium hydrate and the alkalies generally have been investigated very frequently, and solutions much weaker than we have employed have been used. Dr. William Addison* describes and figures what he called the acid and alkaline forms of human blood, while Kneuttinger† has shown that "alkalies as a general rule, when in a state of moderate concentration, exert a solvent action on all the constituents of the blood-corpuscles, including the nuclei." A solution of ·1 grm. in 100 cub. centims. is quite sufficient for this purpose. There is, therefore, nothing remarkable in a much stronger solution rapidly producing the same effect, but what we have found is that some time after complete solution of the corpuscles has occurred, small microscopic crystals are to be found scattered over the field of view. If such a preparation be sealed up the crystals gradually grow and assume a considerable, although still microscopic, size. In some cases these crystals are coloured of a slightly yellowish tint. Some are prismatic, whilst others are like two triangles placed with their obtuse angles towards each other. They resemble very closely in shape some of the forms of triple phosphate which are found in urine after decomposition of the urea has set in. At present we are unacquainted with their exact nature.

A very careful description of the action of the *vapour* of ammonia is given by Professor Lankester in his paper already cited, and it is curious to note that Professor Lankester saw particles separate from the hæmoglobin of frog's corpuscles, and "in these it was quite easy

by carbolic acid, when the urine has a dark smoky tint from the presence of altered hæmoglobin; Huls, under Landois' direction, also found that carbolic acid caused a separation of the hæmoglobin from the stroma. "Lehrbuch der Physiologie," 3rd edition, by Landois. January 10th, 1883.

* "Quart. Journ. of Mic. Sc.," N.S., vol. i, p. 20, and "Proc. Roy. Soc.," vol. 10, p. 186.

† "Zur Histologie des Blutes." Würzburg, 1865. (Stricker's "Histology," vol. i, p. 398.)

to recognize the well-known double rhomboid form of hæmoglobin crystals." The crystals which we found, however, were deposited in the fluid after the solution of the corpuscles under the action of ammonium hydrate.

Sulpho-Cyanide of Ammonium.

The action of this reagent on the coloured blood-corpuscle is, perhaps, one of the most interesting which we have examined—not only as regards its immediate action, but also as regards the ultimate effect it produces upon the nucleus in which it reveals an intranuclear plexus of fibrils with the greatest distinctness. (Fig. 8, *a*, *b*.)

On irrigating blood with a 10 per cent. solution of this salt, the corpuscles first became somewhat larger, and clear bands appeared in the perinuclear part. (Fig. 6, *a*.) The direction of these bands was generally across the long axis of the corpuscles. The corpuscle looked as if there were a series of folds or creases in it. The effect was thus similar to the early effect produced by citric and tartaric acids upon the corpuscles, except that the clear bands were more numerous in the case of the salt. The nucleus at the same time was brought out more distinctly, though still remaining pale, and the outline of its intranuclear plexus was faintly seen.

On selecting a corpuscle and observing the effect of the reagent closely, it was seen after a minute or two to lose its barred appearance, and then small, highly refractive, coloured globules began to form near the edge of the corpuscle. These small globules were soon seen on the outside of the corpuscle, to which they remained attached for a few seconds by a tailed process of their own substance. The droplets began to exhibit active molecular or Brownian movements. The processes connecting the globules to the outside of the corpuscle soon gave way, and then the spherical masses of coloured protoplasm began to dance about over the field of the microscope, in active molecular movement. (Fig. 6, *c*, *d*, *e*, *f*.) The margin of the corpuscle was left crenated, or rather dentated, and from the dentations other small globules began to come off and dance about like their predecessors. After some of these small globules were cast off, or at least after the threads which fixed them to the corpuscle were severed, which one can see taking place in the field of the microscope, the corpuscle often assumes a variety of shapes, giving out a lobose process, which may also change its shape and dimensions. (Fig. 6, *n*, *p*, *q*.) It is most interesting to watch the liberation of the droplets, and the variety of shapes assumed by the corpuscle. The detached droplets gradually become decolorised. The corpuscle begins to shrink visibly in size as the droplets extrude from it, and at the same time becomes darker in colour. The dentations disappear with the shrinking of the corpuscles, which commenced about five or six seconds after the first

globules were seen on the surface of the corpuscle. The outline of the corpuscle while shrinking was more or less irregular, and the droplets continued to form on the margins of the corpuscles. The corpuscles were obviously in a very *plastic* condition, if one may judge from the ease with which they changed their shape. Ultimately the corpuscle —or rather what remained of it—became condensed into a small globular mass of a dark yellow colour, usually with the pale nucleus in its centre. In a short time the nucleus which had hitherto been but slightly affected, suddenly expanded to a considerable extent, sometimes breaking up in the process.

A beautiful intranuclear plexus of fibrils was then seen to exist in the interior of the nucleus. (Fig. 8, *a*, *b*.) With the swelling up of the nucleus, the rest of the corpuscles underwent complete decolorisation. Traces of a stroma were detectable in the colourless hull of the corpuscle.

Ultimately the microscopic field contained a large mumber of nuclei, now considerably enlarged, and each one containing a beautiful view of its intranuclear plexus of fibrils. It was obvious that the nucleus had become enlarged through the swelling up of the material—whatever its nature—which lies within the meshes of the plexus. The fibrils themselves are also enlarged, and they bound meshes which in some cases are polygonal, in others hexagonal in shape. This reagent shows the intranuclear plexus quite as distinctly as ammonium chromate. On subsequently staining the distended nuclei with magenta or fuchsin, the plexus becomes stained, and they present a singularly fine demonstration of the arrangement of the fibrils. They may be kept for a considerable time.

One cannot fail to notice how closely the phenomena above described agree with the action of certain other reagents upon the blood-corpuscles—notably a 5 per cent. solution of ammonium chromate which shows the separation of particles of the hæmoglobin in the form of droplets of the most bizarre forms, and the changes of shape with the utmost distinctness. A strong solution of urea exerts an almost similar action upon the coloured corpuscles—and so does heat, as was described by Max Schulze.

Somewhat similar phenomena were observed by G. F. Dowdeswell,* in the blood of man and the dog when acted upon by septic matter, such as an aqueous extract of putrid muscle. These phenomena closely resemble the results obtained on human blood by Dr. Wm. Addison,† F.R.S., with pale sherry, either alone or in combination with various substances.

Coloured corpuscles of amphibian blood have been observed by Rindfleisch and Beale to undergo remarkable changes in shape.

* "Quart. Journ. of Mic. Sc.," 1881, p. 154.

† "Proc. Roy. Soc.," vol. 10, p. 186.

Rindfleisch is inclined to believe that small portions of the "hæmatin-containing contents must pass through pores in the cell membrane, or through holes produced in some other way in the same." But Preyer remarks* that such forms of coloured corpuscle have no membrane. Beale† found similar forms with long thread-like processes which became separated from the parent corpuscle when a drop of blood was warmed.

Sometimes another series of phenomena is obtained. Large globular or flask-shaped processes are given off from the margins of the corpuscles. (Fig. 6, *p*, *q*.) It may be at one side or on both. Some of them become detached, and coalesce to form globular or semiglobular large masses, which float off into the surrounding fluid, and are ultimately dissolved. Ultimately the nucleus undergoes the same changes as have already been described.

Sulpho-Cyanide of Potassium.

A 10 per cent. solution of this salt, gave exactly the same results as the corresponding salt of ammonium.

Ammonium Chromate.

When a drop of frog's blood was mixed in the cold with a drop of a 5 per cent. solution of this salt, the corpuscles were first observed to take on the barred appearance of the perinuclear part, described as part of the effect of citric, tartaric, and other acids, &c. The nucleus was more distinctly seen and was pale and slightly granular. Small coloured droplets were soon observed to form at the margin or periphery of the corpuscles. The corpuscles then usually became more or less regularly crenated, and the small yellow coloured droplets began to assume a beaded appearance on the surface or edges of the corpuscles. They then began to exhibit active Brownian movements. The corpuscles had by this time again become homogeneous in appearance, and appeared to be very mobile or plastic, as they changed their shape with great facility. The corpuscles diminished in size as the coloured droplets passed out, while at the same time the crenation disappeared, and the outline of the corpuscles became more or less uneven. The coloured part of the corpuscles often became aggregated into two or three or more rounded masses, causing a projection at those points. The corpuscle thus often had a dumb-bell, triradiate, or stellate form with rounded angles. Very often one or more of these knobs would become pedunculated, and at length break off from the corpuscle. Sometimes a corpuscle would be seen floating about with several of these coloured globular masses attached to it by long processes, while but a very small amount of colouring matter remained

* Preyer, "Virchow's Archiv," vol. xxx, p. 432.

† "Trans. Mic. Soc.," vol. xii, N.S., p. 32, and "Quart. Journ. Mic. Sc.," vol. iv, N.S., 1864.

around the nuclei. The processes attaching the masses of coloured matter to the nucleus often appeared to be membranous in character. Many of the corpuscles ultimately became perfectly spherical, the nucleus being indistinctly seen in the centre or at one side. These spherical corpuscles were often seen to contain several globular masses grouped usually around the nucleus. Very often the ultimate effect was to leave the nucleus with a dark yellow rounded knob of the coloured part of the corpuscle at either end of it. Sometimes the whole of the coloured part of the corpuscle had disappeared, leaving the nucleus pale and swollen, and with indications of a plexus in its interior. (These peculiar forms which the corpuscles assume may be preserved some time by sealing up the preparation.)

From some of the corpuscles long delicate processes were observed to pass. Some of these processes appeared to be made up of minute globules of coloured material which had coalesced to form a continuous bead-like string. They resembled very much the processes seen passing from the blood-corpuscles of the frog after treatment with a 20 per cent. solution of urea. Other processes appeared to be of a membranous character, and were tipped at their free extremities by a minute coloured globule.

All these processes were remarkable for their length, which was sometimes several times that of the corpuscle itself. They were more easily induced in the corpuscles by gently heating the slide over a spirit-lamp.

If some of the blood of a newt be kept for forty-eight hours in a 5 per cent. solution of ammonium chromate, it will be found on examining it that in most of the corpuscles the perinuclear part has entirely disappeared, leaving the nucleus much swollen and of a globular form. An intranuclear plexus with wide meshes is seen. The nucleus stains readily—though not very deeply—with picro-carmine, the interior of the nucleus becoming reddish in colour and the envelope yellowish. (Various forms assumed by the corpuscles are shown in Fig. 7.)

The action of this substance on the coloured blood-corpuscles of the frog is accurately described by Mr. Dowdeswell as far as regards the extension, retraction, and detaching of the protuberances, and he remarks that no rupture of a membrane, or anything of the kind, was to be seen, even with a power of 1,000 diameters. Dr. Klein* has also shown the immense importance of this substance for a variety of purposes, but especially for revealing the fibrillar plexuses within cells and nuclei, *e.g.*, in non-striped muscle, &c.

Urea.

A 20 per cent. solution of urea first caused the corpuscles to become

* "Quart. Journ. Mic. Sc.," 1878, 1879.

crenated and then to assume a globular form. This change to a globular form occurred for the most part suddenly with a recoil or jerk. At the same time processes were usually thrown out from the corpuscles, sometimes to a considerable distance. Usually the corpuscle itself underwent considerable changes in shape. The nucleus could not be seen. Small coloured droplets became extruded from the corpuscle and danced over the field of the microscope with active molecular movements. Sometimes the action of the reagent was less vigorous—due to less being present—and then the corpuscles gradually became globular, the sudden recoil not being observed. The crenation was observed as before, and then the corpuscle began to diminish in size, owing to the formation and detaching of its plastic coloured substance. All the corpuscles ultimately become spherical and very much lessened in bulk, and then gradually become decolorised. From some of those which had been of a globular form for some time long delicate beaded processes were observed to pass.

Kölliker* found that solutions of urea of 15 per cent. and upwards produced similar changes in frog's blood. "The coloured blood-corpuscles became gradually more jagged, and some became transformed into the most beautiful stellate cells with at most three to six tolerably long and more flask-like processes. The latter began as it were to dissolve and disappear, partly by their margins being dissolved, and partly by small coloured particles being detached from their surface. These particles at once became pale and gradually disappeared. At last there remained only the nucleus-containing portion of the cell, as a small, round, dark red, refractive globule, which eventually became pale, and which, even to the nucleus, disappeared without leaving a trace behind."

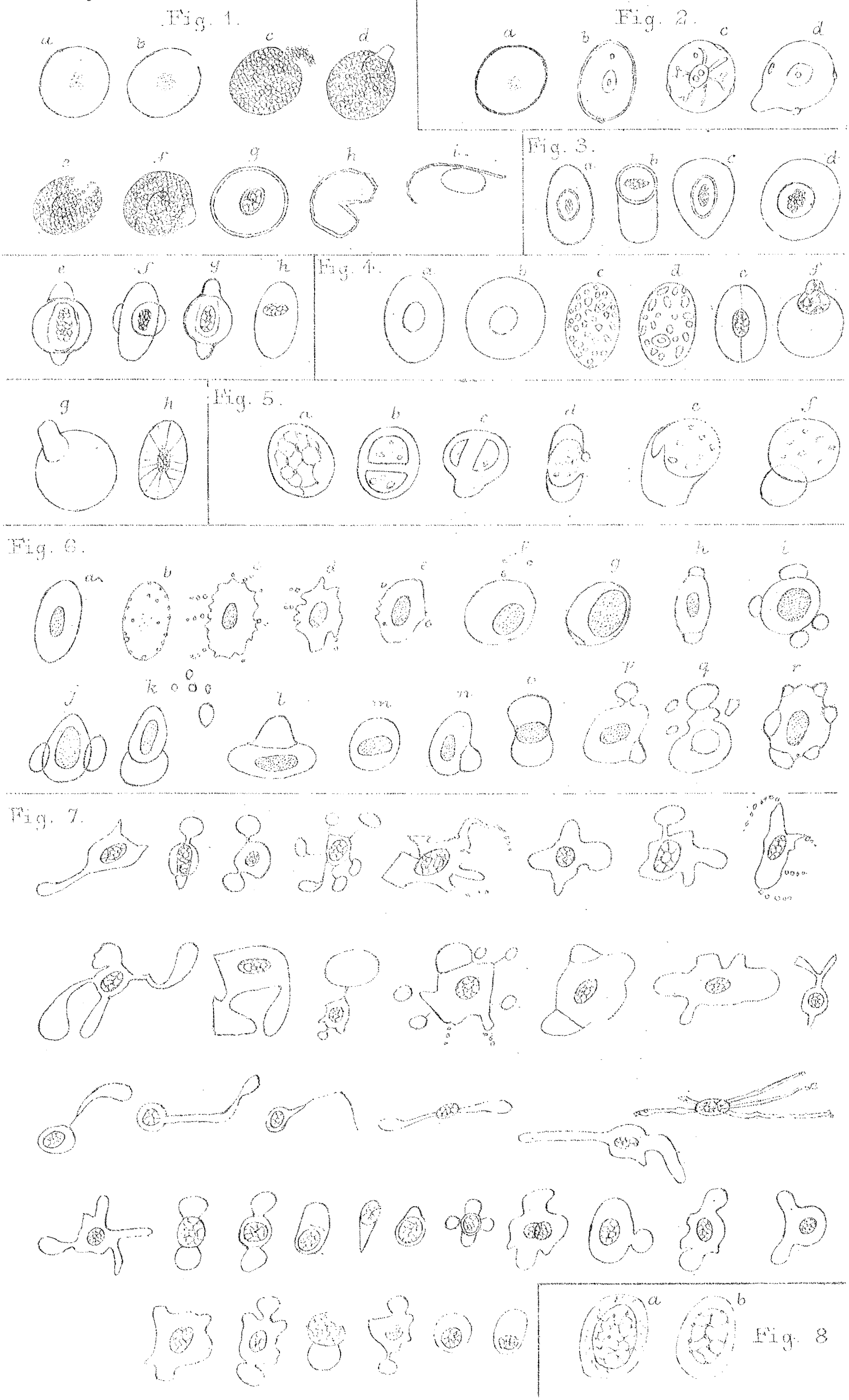
Preyer agrees that the above description is accurate, with the exception of the part which refers to their becoming pale, which Preyer ascribes to the action of water. Indeed, Preyer† obtained similar results by allowing a drop of solution of urea to evaporate on a slide and then placing a drop of blood upon the thin crystalline layer of urea thus formed. The results he obtained are carefully figured, and they agree exactly with the results we have obtained.

The interest which attaches to the solvent action of urea is considerable, but the remarkable variety of shapes and the detaching of droplets from the corpuscles are also interesting, more especially as urea is only one of a number of reagents which cause a similar reaction.

We propose to continue our observations upon the effects of the foregoing and other reagents upon human blood or mammalian blood generally, which will form Part II.

* V. Siebold u. Kölliker's "Zeitsch. f. Wiss. Zoolog.," vol. vii, 1855, p. 183.

† "Virchow's Archiv f. Path. Anat.," vol. xxx, p. 432.



West Newman & C Lith

DESCRIPTION OF PLATE 1.

Fig. 1. Effect of pyrogallic acid solution upon the red blood-corpuscles of the newt.
,, 2. Effect of gallic acid.
,, 3. Effect of hydrochloric acid.
,, 4. Effect of oxalic acid.
,, 5. Effect of carbolic acid.
,, 6. Various forms assumed by the corpuscles when acted upon by ammonium sulphocyanide or potassic sulphocyanide.
,, 7. Various forms produced by the action of ammonium chromate.
,, 8. Shows final effect of ammonium sulphocyanide on the nucleus, viz., to reveal an intranuclear plexus.

April 5, 1883.

THE PRESIDENT in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read:—

I. "On a hitherto unobserved Resemblance between Carbonic Acid and Bisulphide of Carbon." By JOHN TYNDALL, F.R.S. Received March 15, 1883.

Chemists are ever on the alert to notice analogies and resemblances in the atomic structure of different bodies. They long ago indicated points of resemblance between bisulphide of carbon and carbonic acid. In the case of the latter we have one atom of carbon united to two of oxygen, in the case of the former one atom of carbon united to two of sulphur. Attempts have been made to push the analogy still further by the discovery of a compound of carbon and sulphur analogous to carbonic oxide, but hitherto, I believe, without success. I have now to note a resemblance of some interest to the physicist, and of a more subtle character than any hitherto observed.

When, by means of an electric current, a metal is volatilized and subjected to spectrum analysis, the "reversal" of the bright band of the incandescent vapour is commonly observed. This is known to be due to the absorption of the rays emitted by the hot vapour in the partially cooled envelope of its own substance which surrounds it. The effect is the same in kind as the absorption by cold carbonic acid of the heat emitted by a carbonic oxide flame. For most sources of

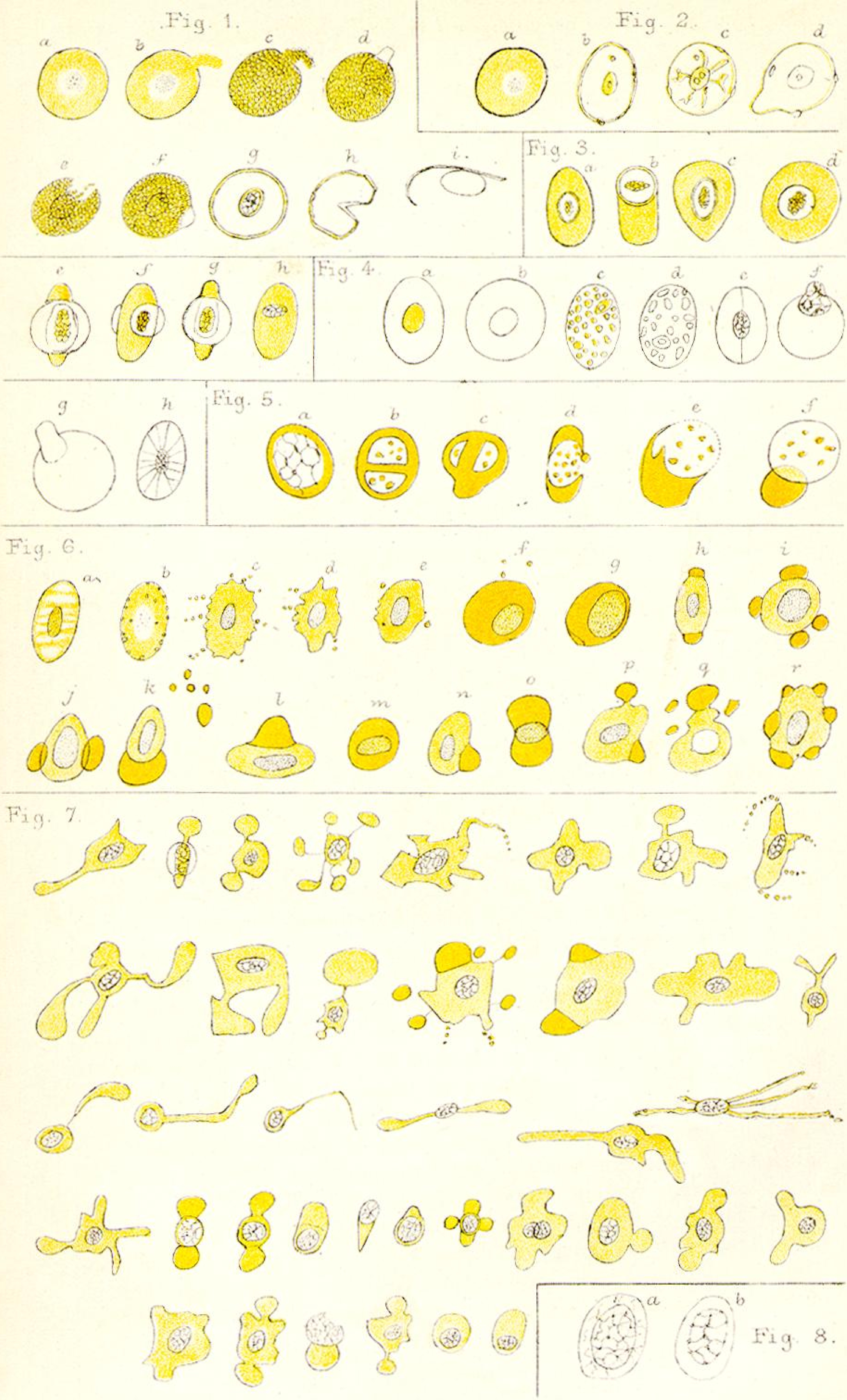


Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.
Fig. 8.