XXX. *On the Cutaneous Pigmentary System of the Frog.* *By* JOSEPH LISTER, *Esq., F.R.C.S. Eng. and Edin., Assistant Surgeon to the Royal Infirmary, Edinburgh, Communicated by Dr.* SHARPEY, *Sec. R.S.*

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THE fact that the skin of the frog is capable of varying in colour, has been for some years known to German naturalists. The first account of the mechanism by which these changes are effected, appears to have been given by Professor BRÜCKE, of Vienna, in 1852†, and the subject has since been very carefully investigated by Dr. VON WITTICH of Königsberg‡, and Dr. E. HARLESS of Munich§. All these observers describe the dark pigment as contained in stellate cells, each composed of a central part or body and several tubular offsets, which, subdividing minutely and anastomosing freely with one another and also with those of neighbouring cells, constitute a delicate network in the substance of the true skin. They describe the dark contents as sometimes concentrated in the bodies of the cells, at other times diffused throughout the branching processes, the skin of the creature being pale in the former case and dark in the latter. In the tree-frog the change from a dark to a pale state of the body generally was induced by bringing the creature into a bright light, by psychical excitement (as was supposed¶), or by galvanizing the spinal cord; and a similar effect was produced on a particular portion of the surface by irritating it mechanically, or with oil of turpentine, or by galvanism applied either directly to the part, or through branches of nerves leading to it. After the source of irritation was removed, the skin returned somewhat slowly to its former colour; and VON WITTICH noticed that when the paleness produced by direct irritation had passed off, the tint became deeper in the irritated spot than elsewhere. The esculent frog exhibited similar phenomena, but was less sensitive. The concentrated state of the pigment is attributed by all the observers above named to contraction of the cells, while the diffused condition is supposed due to their relaxation. The con-

* During the time that has elapsed between the reading of this paper and its publication, several new observations have been made, which it has been thought best to introduce into the text, distinguished by date or foot-note from the matter of the original manuscript.

† Untersuchungen über den Farbenwechsel des Africanischen Chamaeleons, iv. Band der mathemat. naturwissenshaftl. Classe der Kaiserl. Acad. d. Wissenschft. Wien. This paper I have not yet had an opportunity of consulting.

‡ MÜLLER's Archiv, 1854.

§ Zeitschrift für Wissenschaftliche Zoologie, vol. v. 1854.

¶ This rests on the authority of VON WITTICH; but, for anything stated to the contrary in his paper, the effects ascribed to psychical excitement may have been connected with the efforts of the creature in struggling, independently of any emotional change.

tents of the cells are described as dark granules suspended in a fluid; and both VON WITTICH and HARLESS have distinctly seen the granules rolling along in the offsets during the process of concentration. All the authorities agree in the opinion that the fluid and granules move together from one part of the cell to another, the offsets being supposed empty of both when the pigment is accumulated in the body of the cell*.

In some respects the above description agrees with my own experience of the common frog of this country (*Rana temporaria*). I find that this well-known animal exhibits changes of hue almost as great as those of the chameleon, every specimen being capable of varying from a very pale to a very dark colour, the former being generally greenish yellow, but in some varieties reddish; and the latter brownish black, or sometimes coal black; while between these extremes any intermediate shade may be assumed. The depth of tint is generally proportioned to that of surrounding objects: thus a frog caught in a recess in a black rock was itself almost black; but after it had been kept for about an hour on white flagstones in the sun, was found to be dusky yellow, with dark spots here and there. It was then placed again in the hollow of the rock, and in a quarter of an hour had resumed its former darkness. These effects are independent of changes of temperature; for similar results may be obtained by placing a frog alternately in a vessel from which luminous rays are excluded, and in a white earthen jar covered with glass, in the same situation. Different examples, however, differ much in their sensitiveness to light. A violent struggle on the part of the animal is often followed by a speedy alteration from a dark to a pale state of the skin. It seems very doubtful whether psychical excitement has anything to do with this occurrence, any more than with the arterial contraction which invariably takes place under such circumstances. Neither oil of turpentine nor galvanism, when applied to the integument, produces, so far as I have seen, any effect upon its colour; our species being little influenced in this respect by direct irritation. I have however frequently observed, after forcibly pinching a dark web, that a pale ring, about $\frac{1}{16}$th of an inch in breadth, has formed around the area so treated; but this was very slow in appearing, being first noticed from half an hour to an hour after the pinch was given.

The webs of the hind feet, examined under a low power of the microscope, exhibit differences in the distribution of the dark pigment† according to the tint of the skin,

* From the way in which VON WITTICH alludes to BRÜCKE's description, it is clear that the latter supposed the cells to be contractile. VON WITTICH himself in his first paper speaks of the movement of the pigment induced by galvanism as "satisfactorily" showing "that the stellate pigment-cells are contractile." In his second paper (vide MÜLLER's Archiv, 1854, p. 263), he expresses some doubt regarding the contractility of the *cell-wall*, but clearly speaks of the contents (fluid and granules) as moving together. HARLESS, after describing "the rolling of the pigment-molecules towards the centre of the cell," goes on to say, "that this rolling may be possible, there must be a fluid in the cells and offsets, *to which* the molecules owe their movement." He takes it for granted that the movement of the fluid must be due to some contractile agency, and as he finds no apparatus of this nature around the cells, and as the unstriped muscular fibres of the skin have no special relation to them, he infers that the cell-wall is itself contractile.

† Other kinds of pigment are also present in the skin of the common frog, generally of yellow colour,

such as will be understood by referring to Plate XLVII., where fig. 1 is from a dark portion of web, and fig. 2 from a pale part in the same animal. In fig. 2 the colouring matter is seen to be collected in black spots of irregular angular shape. This, however, is not the state which exists when the colour is palest, for then the masses of pigment are in the form of round dots, as in the part to the right in fig. 1, Plate XLVIII. Neither does fig. 1 of Plate XLVII. give the condition met with when the skin is darkest, in which case all that meets the eye on superficial observation is a reticular appearance, such as is represented in the stripe down the middle of fig. 1, Plate XLVIII., and in the lower part of fig. 2 in the same Plate. When the colour of the integument is about medium, the pigment is disposed in a truly stellate manner, as on the left side of fig. 1, Plate XLVIII. It may be convenient for the purposes of description, to designate these various states as respectively the dotted, angular, stellate and reticular conditions of the pigment.

When a higher magnifying power is applied in an extremely dark state of the skin, the chromatophorous cells, for such they seem to be, appear as depicted in Plate XLVII. fig. 3, where two of them are given, along with an adjacent capillary distended with blood-corpuscles. Each cell consists of a somewhat flattened central part with several irregular offsets, of considerable diameter near the central part, but speedily breaking up into small branches. The ultimate ramifications, some of which are of extreme minuteness, anastomose freely with one another and with those of neighbouring cells, constituting a very delicate and close-meshed network, which pervades the whole thickness of the true skin, and especially follows the course of the blood-vessels, entering into the composition of the cellular coat of the arteries and veins, and twining about the capillaries in a very remarkable manner. The walls of these cells and of their tubular offsets appear to be extremely delicate, and some attempts which I have made to isolate them from surrounding tissues have barely served to demonstrate their existence. The cells vary considerably in dimensions according to the size of the animal; thus, those in figs. 8, 9 and 10, which are from young frogs, though magnified 500 diameters, show in the drawing even smaller than those in fig. 3, magnified only 250 times, the latter being from a full-grown specimen. In an average full-sized cell of a large frog, the middle portion was found to measure $\frac{1}{330}$th of an inch in length by $\frac{1}{670}$th of an inch in breadth, and $\frac{1}{1500}$th of an inch in thickness. The last-named dimension was obtained by carrying the focus of an object-glass of high power, from the most superficial to the deepest part by the screw for giving slow motion, and reading off on its graduated circle

but sometimes red. My attention has not been much directed to these, but I have noticed that they are contained in receptacles of the same general form and structure as those which hold dark pigment; and on one occasion, since the reading of the paper, I observed the colouring matter disposed in a stellate manner with complex ramifications in one part of a web, and in another part collected into round spots; implying that these cells possess the functions of concentration and diffusion of the pigment. They do not, however, always act in harmony with the dark cells; and it is probably through their agency that changes in tint, such as I have seen to occur in one and the same frog, independent of mere lightness and darkness of shade, are produced.

the number of divisions traversed, these having a known proportion to the depth measured. Opportunities for testing the correctness of this measurement were presented by other cells which lay edgewise, so that their thickness could be observed directly.

Perhaps the strongest argument in favour of the cellular nature of these receptacles of colouring matter is afforded by the universal presence of a nucleus in the central cavity of each. In large frogs it is often difficult or impossible to discover clear evidence of it, but in small ones, in which the web is much thinner and its constituent parts therefore capable of clearer definition with the microscope, it can be quite distinctly seen in the reticular condition of the pigment. Its form and relations may be gathered from figs 8, 9 and 10. In 8 and 10 the bodies of the cells are viewed on the flat, and the nucleus appears as an oval colourless body, about $\frac{1}{2500}$th of an inch long by $\frac{1}{3300}$th of an inch broad. In fig. 9 the body of the cell is seen edgewise applied to the wall of a capillary blood-vessel, which is embraced by its processes. The thickness of the nucleus is thus displayed, and is shown to be equal to that of the cell in which it lies, which in fact it causes to bulge slightly, and also nearly as great as the breadth of the nucleus in figs. 8 and 10. In the cell of fig. 10, the thickness of the nucleus, measured in the manner above described, was found about equal to its breadth. The nucleus in fig. 8 is not centrally placed in the body of the cell, and I have in some other cases seen it still more excentric*.

The contents of these cells are very minute dark granules or molecules suspended in a colourless fluid, in which I have often seen them moving freely: when in considerable mass they produce a jet-black appearance, but exhibit a brown tint when present only in small quantity.

When the skin of the animal is very pale, the colouring matter is all accumulated in the central parts of the cells. With regard to the method in which this change is effected, I am compelled to differ altogether from the before-mentioned authorities, who suppose that the granules and fluid are together forced by contraction from the processes into the bodies of the cells. They seem to take it for granted that the depth of tint of any one part of a cell depends simply upon the bulk of the contents situated there, and the consequent thickness of the coloured medium through which the light passes before reaching the eye. This, however, is by no means the case, as may be seen by referring again to Plate XLVII. fig. 3. The pigment is there represented fully diffused through the ramifications of the offsets, and some of the smallest of these are darker than the bodies of the cells and the adjoining broad parts of the processes; yet the former are far from being thicker than the latter: on the contrary, some of the branches, though conspicuous for their blackness, appear but as delicate lines which can be seen only at one focus when a glass of very high power is employed; while the bodies of the cells, as above mentioned, possess considerable thickness, and the processes are

* The precise relations and dimensions of the nucleus have been ascertained subsequently to the reading of the paper.

not flat, but subcylindrical. But the differences in tint are sufficiently accounted for by the circumstance that in the dark branches the colouring particles are closely packed together, whereas in the bodies of the cells and the paler parts of the offsets, the individual granules are separated from one another by considerable colourless intervals. Hence it is clear that the degree of darkness of any part of a cell does not depend so much on the bulk of its contents in the aggregate, as on the proportion which the pigment molecules in it bear to the fluid in which they are suspended.

If the whole contents of the processes were forced into the central parts during concentration of the pigment, and driven back again during diffusion, the bodies of the cells would be subject to great variations in capacity, becoming turgid in concentration and collapsed in diffusion; and the bulk of the central coloured mass would be great in the former case, but small in the latter. The very reverse, however, really takes place. Fig. 6 represents the appearance of the pigment in a concentrated condition, in one of the same cells which in fig. 3 show it in full diffusion. During the time in which this change took place, the adjacent capillary had shrunk to about half its former size, but it will be recognised by its general form, and will indicate which of the two cells is that under consideration. Both the figures were drawn on the same scale with the camera lucida*, so that accuracy of proportion is ensured. The circular black mass into which the colouring matter is now all collected, measures less across than either the length or breadth of the body of the cell in the diffused state of the pigment. Further, the mass is not spherical, but of flattened form, and its thickness is only about that of the central part of the cell in diffusion. This we know from the appearances presented by the spots of concentrated pigment in other cells seen edgewise, as is the case with some in fig. 7, which represents the outline of the wall of a large blood-vessel, and the pigment contained in its external coat in nearly complete concentration. Hence it appears that all the pigment-granules contained in the body of the cell and the minutely ramifying processes in the diffused state, have been brought together into a space considerably less than was then occupied by the pale contents of the body of the cell alone. The coloured particles have been concentrated into a dense disciform mass, but the fluid in which they were suspended has been left behind.

Fig. 4 shows the pigment in the same cells as fig. 3 in an intermediate stage, in which the process of concentration is about half accomplished; the upper one being in the condition which would appear stellate under a low magnifying power. The greater part of the pigment is collected in the bodies of the cells, especially towards their central parts: in the middle of each dark mass, however, is a pale spot, doubtless due to the circumstance of the granules not having yet insinuated themselves between the cell-wall and the nucleus, which, as shown above, probably lies in contact with it. This appearance of pale central points was very general in the web at the time when fig. 4 was drawn, but gradually disappeared as the aggregation of the pigment-molecules proceeded, and does

* All the drawings in the two Plates which accompany this paper were made with the assistance of this very valuable instrument.

not exist in fig. 5, which represents the lower of the two cells in a more advanced state of concentration. The remote branches of the processes were then for the most part invisible, and those which did appear were generally pale, instead of dark, as they had been during full diffusion. This difference does not depend on contraction of the branches, but on the granules being absent from them, or sparsely scattered instead of closely packed; and I have often ascertained from some granules remaining widely separated in a process, that it was of large calibre, though, without careful searching, it would have seemed invisible. Even in fig. 6 concentration is not represented absolutely perfect; for a few molecules are to be observed near the black mass in the more circumferential parts of the body of the cell. The extreme delicacy of the cell-wall makes it very difficult to trace it among the surrounding tissues, and I have not attempted to give it in these figures, which, it must be clearly borne in mind, represent only the colouring matter. The external parts of the body of the cell and the principal processes may, however, be sometimes discovered, though perfectly colourless in consequence of concentration: they are then found to be of the usual dimensions met with in full diffusion, showing that they are still full of fluid though destitute of granules. In fact the only change of form to which the cells appear liable, is a slight bulging of the central part at the seat of the black mass in the concentrated state, which I have detected in some cases by camera lucida sketching, and which is consistent with the separation of the cell-wall from the nucleus, implied by the ultimate disappearance of the central pale points of fig. 4.

The movement of the granules towards the centres of the cells may be seen without any great difficulty. The death of a healthy frog is always followed by complete concentration of the pigment for a time, however much diffused it may have previously been, and the process taking place gradually, its progress can be observed. If a frog with the skin dark, and the pigment therefore diffused, be killed and the web examined soon with a good glass of high power, the granules may be seen distinctly moving along the offsets of each cell to join the dark mass which is becoming accumulated in the central part. If the process is going on languidly, the individual molecules advance slowly with slightly dancing movements, indicating that they are free in the fluid and not confined in any way to the cell-wall. If concentration is taking place more speedily, the granules rush along so quickly that no time is allowed for observing their molecular movements, and often their motion is so rapid as to elude the eye altogether. In one instance a large-sized offset, which at first contained abundance of pigment, became gradually cleared in this way of its colouring matter without any change in its dimensions, till it was almost invisible on account of the very small number of molecules remaining in it.

It is thus a matter of direct observation, that the pigment-granules move along into the bodies of the cells during concentration, and leave colourless fluid behind them in the processes. It is clear that their motion cannot be explained by currents in the fluid; for streams proceeding towards the centre of a cell would necessarily be accompanied by a returning flow in the opposite direction, which would carry the pigment with it unless the molecules had a special tendency towards the centre. The circular form assumed by the mass of pigment when concentration is complete, is strongly suggestive of a central attractive force acting on the granules. The occurrence of the central pale points, which are represented in fig. 4, showing that the nucleus was there in the middle of the concentrating pigment, led me at first to suppose that this body was the attractive agent*. I afterwards took pains to ascertain whether the nucleus always has this relation to the mass, and found that such is not the case. On the 22nd of October, 1857, I watched three adjacent cells during the process of *post mortem* concentration; in two of them the nucleus ultimately projected by about a quarter of its length at one side from the black spot, while in the other cell the aggregated molecules covered only one-third of the nucleus, so that *no part* of that body lay in the middle of the mass. The point to which the granules appear to have a special tendency is the middle of the body of the cell, which seems always to correspond with the centre of the disc of molecules, whereas the nucleus is often excentrically placed in the cell.

The diffusion of the molecules is not merely a passive result of the cessation of concentration, as has been hitherto supposed. In watching closely the occurrence of the phenomenon, I have seen† the granules start off suddenly from the central mass, with a velocity which implied that they were under the influence of forces very different from those which cause molecular movements in them when shed from their containing cells. That the process requires the vital forces of the cells to be in full operation, is also proved by the fact, that any agency, such as a galvanic shock, which temporarily paralyses their functions, arrests diffusion as well as concentration; whereas, if the former were merely passive, it would take place as soon as the concentrating power was set at rest‡.

I have already pointed out the sparsely scattered state of the granules in the central receptacles, compared with their accumulation in the branches of the offsets, in the fully diffused state shown in fig. 3. This contrast is sometimes much more striking, so that the bodies of the cells are almost colourless, and require some experience with the tissue in order to detect them. This indicates a special tendency on the part of the granules to leave the middle of the cell. Yet to however great a degree diffusion be carried, there always remain some molecules in the body of the cell uniformly distributed throughout its thickness and not attached to the parietes, as they would have been had their dispersion been caused by attraction on the part of the cell-wall. This disposition of the granules, which obtains even in the immediate vicinity of the nucleus, appears also distinct evidence against the operation of a central repulsive force; for this would render the body and the adjoining parts of the processes as clear of pigment as the remote branches are made in concentration. The hypothesis which would seem most

* This was the view expressed in the paper as it was read.

† This observation was made after the reading of the paper.

‡ For further information regarding the effects of various agents on the functions of the pigment-cells, see the next paper in this volume.

consistent with the appearances described, is that of a mutual repulsion on the part of the pigment-granules, induced by some agency strongest at the centre of the cell and feeble in the remotest branches of the offsets.

On the 27th of October, 1857, I was observing a cell in which *post mortem* concentration had occurred, the pigment being in the angular condition. At one of the angles movements of the granules were going on, of which I will content myself with giving two examples. At one time a number of molecules started off together with great rapidity from the black mass, but stopped after having proceeded a certain distance; some of them remaining in their new position, while others returned at various rates towards the centre. At another time an individual granule moved slowly away for a little space, and then came back by a circuitous route to a different part of the mass from that which it had left. What I then saw has led me to believe that the movements of the pigment-molecules are of a complex character that will perhaps never be fully explained. In the mean time it is clear that concentration and diffusion are both active vital functions, and that both imply peculiar relations of the centre of each cell to the pigment-molecules, as distinguished from the fluid in which they are suspended.

These conclusions invest the pigmentary changes with deep physiological interest. In the movements of the granules towards and from the centres of their containing cells, we now have ocular demonstration that a particular kind of material may have impressed upon it by vital action, independently of muscular contraction or ciliary motion, tendencies to rush energetically to or from certain fixed points in the tissues, through distances equal to nearly twice the thickness of a villus of the human intestine, and several times greater than the average breadth of a human capillary interspace. Whether we be able to explain the means by which such results are accomplished or not, it is obvious that forces of similar powers and range of operation, if suitably modified according to the circumstances of each case, would be more than adequate to cause the passage of particles of fat from the cavity of the intestine into the central lacteals of the villi, or the transit of the material required for a particular secretion or act of nutrition out of a capillary into a neighbouring gland cell or other portion of tissue; and, again, for the discharge of an elaborated product of secretion into a duct, or the return of waste matter into the blood-vessels or lymphatics. We thus obtain a basis of fact for what has hitherto been merely conjectural, in the explanation of the processes of absorption, secretion and nutrition generally.

The functions of the pigment-cells are under the control of the nervous system *, as is evident from the effects produced on the colour of the skin by a struggle on the part of the animal.

Much attention has been devoted by VON WITTICH to the inquiry, by what ganglionic centres this control is exercised. He found that division of the sciatic nerve in the

* The part of the paper devoted to this branch of the subject has been entirely rewritten; and the dates in the text imply that most of the observations with reference to it have been made since the reading of the manuscript before the Society.

thigh, or of cutaneous branches in the dorsal region, did not prevent the parts of the skin supplied by them from varying in colour along with the rest of the body under the influence of light; and, supposing that in such operations all connexion was severed between the portions of integument concerned and the central organs of the nervous system, he inferred that the pigmentary changes induced by light were effected independently of either the cerebro-spinal axis or the usually recognised sympathetic ganglia. He nevertheless regarded such variations as probably reflex in their nature, and attributed them to a peripheral ganglionic apparatus in the skin itself; and this opinion appeared confirmed by the circumstance that direct irritation operated in the same manner upon the colour of a detached piece of integument as upon that of the living animal. At the same time, as he observed paleness of tint to result from irritation of the cord, or of the nerves distributed to a particular part of the surface, he concluded that the spinal system was also capable of acting on the pigment-cells, and so accounted for the supposed influence of psychical excitement upon the tint of the skin. Thus, according to his view, the cutaneous pigmentary system was circumstanced like the heart or intestines, which, though possessing the faculty of independent action by virtue of their intrinsic ganglia, may also have their movements affected by mental emotion*.

In the course of some experiments performed in April 1857, with reference to the influence exerted by the cord upon the calibre of the arteries, I noticed on two occasions that partial division of the roots of the nerves for one of the hind legs within the spinal canal was immediately followed by increased paleness of the limb, of transient character, after which the leg assumed precisely the same colour as the other, this result being in accordance with VON WITTICH'S description. But I further observed in two cases in which such operations had been performed, that when a considerable time had elapsed, viz. nine hours in one instance and two days in the other, the limb whose nerves had been cut was decidedly darker than the rest of the body. Similar results were once obtained from the division of the sciatic nerve in the thigh. When the operation was performed, viz. at 4^h 30^m P.M. on the 4th of April, 1857, the pigment was in the stellate condition in the webs, the tint of the skin being moderately dark; and this state of things continued unchanged in both limbs for the next six hours. On the following day, however, the leg operated on was seen to be very dark, and the pigment in its webs was reticular; while in the rest of the body the colour remained as before, and the pigment was still stellate. This striking contrast continued unaltered for two days, when it was destroyed by the body generally assuming the darkest possible tint.

The diffusion of the pigment in consequence of division of nerves appeared to be the counterpart of the concentration produced by their irritation, and it seemed probable that the want of constancy in the results in the former case was caused, like the variable amount and duration of arterial dilatation after such operations†, by the place of the divided trunks being supplied by other branches; and that, if the nerves of a limb were all completely severed, diffusion would necessarily take place. With the view of testing

* Vide MÜLLER'S Archiv, *loc. cit.* p. 56.

† See page 610.

the truth of this idea, the following experiment was performed. In the afternoon of the 10th of October 1857 I divided in a pale frog all the soft parts in the middle of the right thigh, except the femoral artery and vein and the sciatic nerve; and late in the evening, having ascertained that the circulation was going on freely in the webs, I cut the nerve also, no effect having been hitherto produced upon the colour of the limb. Next morning the body generally was still pale, but the right leg was black from the wound downwards. The same remarkable appearance continued till the evening, when circulation ceased in the limb. On the 13th I performed the same experiment upon both thighs of another large pale frog, leaving the sciatic trunks entire in the first instance, until I had ascertained that the circulation in the feet had not been interfered with. Three hours after this had been done I divided the nerve in the left thigh, and in about forty minutes observed that the leg was decidedly darker below the seat of operation. After another hour I found the pigment stellate in the left webs, whereas it was in the dotted condition in the right foot. I then cut the nerve in the right limb, and within a quarter of an hour the leg was already considerably darker below the wound, and the pigment in the webs had become stellate. Next morning the body was still pale, but the legs were very dark, and they continued to deepen in tint, although the animal was kept in a white earthen jar covered with glass in a bright light, till at about 3 P.M. they were almost absolutely black, while the pigment was diffused in the webs to the extremest degree, the body meanwhile and the upper parts of the thighs retaining their former light colour. The tint of the legs remained unaltered till the death of the animal, which took place several hours later.

The natural interpretation of these results appeared to be, that there exists a constant tendency to diffusion of the pigment in a limb so soon as it is liberated from the influence of the usually recognised nervous centres. It afterwards occurred to me, that if this were really true, diffusion of the pigment might, by proper management, be observed in an amputated limb before the supervention of the tendency to *post mortem* concentration: for I knew, from reasons to be mentioned hereafter, that this effect of death depended on the cessation of the flow of blood through the vessels, and, from what I had seen of arterial contractions in the frog's web, and vermicular movements of the mammalian intestine from a similar cause, I felt sure that, if the blood were retained within the vessels, the arrest of the circulation could not be instantaneous in its effects upon the pigment, but that some minutes would probably be required to develope them; during which time the diffusion resulting from liberation of the pigment-cells from the influence of the ganglia in the trunk would proceed unchecked. Accordingly, on the 3rd of September, 1858, having tied a string tightly round the ankle of a pale frog, I immediately amputated above the ligature, and, avoiding the loss of time involved in tying out the toes, placed the foot at once on a plate of glass with a drop of water, two adjacent toes being kept apart by morsels of moistened lint. Within a minute and a half of the application of the string, the pigment in the web was observed to be in the angular condition, with short simple projecting processes, *i. e.* approaching stellate, and two minutes later two contiguous cells were sketched in that state. About a minute after this it was evident that diffusion was taking place, and it continued to develope itself during the next ten minutes, at the end of which time the rays of the stellate pigment had shot out complicated offsets. Within the following five minutes, however, it was arrested by *post mortem* concentration, which gradually carried the pigment back to the angular state. This experiment, therefore, furnished confirmation of the view, that, in the ordinary circumstances of the animal, the influence of the central organs of the nervous system is required for the maintenance as well as the development of concentration of the pigment in the limbs.

Supposing this to be established, it would follow that the accommodation of the tint of the skin to that of surrounding objects is certainly not the result of direct action of the rays of light upon the pigment-cells, but a reflex phenomenon; and it was an interesting question whether the afferent nerves concerned were the optic pair, or branches in the skin sensitive to luminous impressions. With a view to determining this point, I completely removed the eyes of a pale frog on the 13th of September, 1858, at 1 P.M., and then placed it in a dark cupboard. During the first hour after the operation it became even paler than before, no doubt in consequence of the injury which had been inflicted *, assuming apparently the lightest possible shade; and this continued with very little change till night, although the animal was still kept in the dark. Next morning it was decidedly darker, and the tint was still deeper at 2^h 25^m P.M. The glass containing the frog was now placed in a bright light, and surrounded on all sides by white objects; but this change produced no difference in the colour of the skin, which continued till 7^h 30^m P.M. of a peculiar dingy hue. It was then put back into the dark place, and at 11^h 40^m P.M. was still exactly the same. On the following day, at 8 A.M., the animal seemed a little paler, and was even lighter at 10 A.M., though still in the dark; so that it was evident that no difference whatever was produced upon its colour by admission or exclusion of light. But that the nervous system generally was in a state quite disposed for acting upon the pigment-cells when subjected to appropriate irritation, was shown by the following circumstances. At the hour last mentioned, the animal, having escaped from the vessel in which it was contained, struggled violently during my attempts to secure it, and in the short time thus occupied changed to almost the palest possible tint. It was then placed at once in the bright light, as before, but, in spite of this, was within ten minutes already decidedly darker, and, half an hour later, was almost coal black, though still subject to the full influence of white light. Just after this observation was made, the frog again escaped, and having again struggled considerably before it was replaced in the glass, it was seen to be within four minutes as pale as when first observed in the morning, but after the lapse of another half hour it was again almost as dark as ever, and continued so till 2^h 30^m P.M., though all the while exposed to the same light. The observations were continued for two more days, during which period the same complete indifference to the brightness or obscurity of surrounding objects was still evinced.

* Probably from the irritation of the optic nerves.

These facts indicated pretty clearly that the eyes are the only channels through which the rays of light gain access to the nervous system so as to induce changes of colour in the skin. But for the sake of confirmation I thought it worth while to perform the following experiment. Two very dark frogs having been obtained, I put a hood of black cloth on the head of one of them, leaving the body and limbs uncovered, an aperture being made in the cloth below the throat for the purposes of respiration, and then placed them both in the same glass vessel exposed to white light. The struggles of the animal while the covering was being adapted and secured, had the effect of making it grow much paler, so that it was of about medium tint when introduced into the glass; while the other, which was from the first the darker of the two, still retained its original coal-black appearance. Half an hour after this had been done, the contrast between them was much diminished, partly in consequence of the dark one having become slightly paler, but much more from the paler having grown darker. After another half hour they were of precisely the same colour, and when another similar period had elapsed, that which was the darker to begin with was distinctly the paler of the two, being much lighter than at first, though still considerably darker than medium. A hood was now placed upon this animal, and that upon the other was removed, and both were replaced in the same light as before. This procedure occupied about ten minutes, and within seven minutes of its completion the creature which had the head uncovered was already the paler of the two, having grown decidedly lighter in colour; while that on which the cap had been last placed seemed somewhat darker; and after another hour, while the latter was still of much the same dark shade, the former, with the head exposed, was very much paler, being about midway between the medium and the palest possible tint. An experiment of the same kind was performed upon another pair of frogs with very similar results, the details of which it is not necessary to mention. I afterwards found that the presence of the hood tends to check diffusion, or even in some cases to give rise to concentration of the pigment, probably by making the animal struggle to throw it off; so that in one instance a frog which was put in a perfectly dark place, immediately after the cap had been put on, grew much paler in the course of two hours. This circumstance prevents the skin from becoming as dark on the application of the hood as it would do if the head could be covered without exciting the animal. This, however, only renders the facts above mentioned more striking, so that they afford of themselves sufficient proof that the direct action of light upon the integument is incapable of affecting the pigmentary functions; and thus the conclusion before arrived at receives complete confirmation from these experiments.

There is of course nothing new in the fact that other functions besides vision may be excited in a reflex manner through the optic nerves; the contraction of the pupil, and the sneezing experienced by many persons on coming suddenly into bright sunshine, being well-known examples of such phenomena. On the other hand, the view that the cutaneous nerves are sensitive to luminous impressions was destitute of any support from analogy *.

* In the chameleon, a part exposed to the sun becomes dark, while the rest of the body remains unaffected.

From the part taken by the second pair of nerves in bringing about the changes in the tint of the skin under the influence of light, and also from the darkening of the hind legs observed to occur after dividing within the canal the roots of the branches which supply them *, we learn that the cerebro-spinal axis is chiefly, if not exclusively, concerned in regulating the functions of the pigment-cells. Considering that those functions have probably a close affinity with the processes of secretion and nutrition, it is interesting to find that they are thus subject to the control of the spinal system.

The circumstance before alluded to, that a dark frog always becomes pale after death, is mentioned both by VON WITTICH and HARLESS, but without any discussion of its cause. This *post mortem* concentration takes place in a limb in spite of amputation, and therefore cannot be due to the agency of any ganglia contained in the head or trunk. Neither can it be the result of failure in action on the part of such ganglia; for if the circulation be artificially arrested in a part of a living frog without interfering with the nerves leading to it, a similar change in the pigment to that which results from death comes on before the nerves have become, so far as we can judge, at all impaired in their functions. This was proved by the following experiment:—On the 7th of June, 1858, having tied the right femoral artery of a moderately dark frog in the middle of its course, I divided it below the ligature, and also cut through, in the same situation, all the soft parts of the thigh except the sciatic nerve with a little adherent muscle. The operation was completed at noon, when the animal was put into a dark place; and at 1^h 40^m P.M. the body generally was darker, but the right leg from the wound downwards was decidedly paler than before; the animal, however, still moved it freely. At 6^h 20^m P.M. the general surface was as dark as ever, but the right foot presented the extreme degree of pallor; yet the creature still moved the leg both spontaneously and when the toes were pinched, showing that the motor and sensor nerves retained their functions. Sensation, however, was not so acute as in the left foot; in the latter a touch sufficed to induce movements in the body generally, whereas in the former a pinch was necessary to produce the same effect. At 10^h 15^m P.M. the same contrast in colour continued, but no movement could be induced in any manner in the pale limb, although obscure indications of a certain amount of sensibility remaining in it were still elicited by forcible pinching.

In this case, concentration of the pigment came on in the limb in consequence of arrest of the circulation through it, several hours before its nerves concerned in sensation and motion had lost their powers, and therefore at a time when we cannot doubt that the ganglia in the trunk had full opportunity for acting on the pigment-cells, which, as we know from experiments before mentioned, are capable of being influenced through the

I have little doubt, however, that this is due to the calorific, not the luminous rays. That heat does produce such an effect, was lately demonstrated to me by Professor GOODSIR upon a living chameleon, which, when held in broad daylight before a dull-red fire for a short time, grew much darker on the side that was warmed, but retained elsewhere its former pale green colour.

* See p. 635

sciatic trunk. Hence it appears that *post mortem* concentration is the result of the cessation of the flow of the blood through the vessels, and that it is a purely local phenomenon developed in some manner quite independent of the central organs of the nervous system.

The period at which it occurs varies a good deal in different cases. This seems to depend partly upon whether the blood is retained in the vessels or not. Thus in one instance in which a piece of web was cut out, so as to ensure complete escape of the vital fluid, the process was already considerably advanced within nine minutes; whereas in the case above related, in which the blood was retained in the limb by a ligature, concentration did not commence till full a quarter of an hour after amputation. The season of the year also seems to have a great effect. In a cold room, in the depth of winter, I have known some hours elapse before the pigment began to change in an amputated limb: this is probably owing to greater languor in all the vital processes during the period in which the creature naturally hibernates.

The dead frog, if previously healthy, assumes after a while a nearly uniform pale colour, concentration being carried to the extreme degree in all parts. It does not, however, remain in this condition; for when a variable time has passed, the skin becomes again somewhat darker, and on microscopic examination the pigment is found pretty uniformly angular or stellate. Nor are these the only changes to which the pigment is liable after death, as I first became aware in April 1858, when examining an amputated limb with reference to the *post mortem* contractions of the arteries, the blood being retained in the vessels. In that case, after complete concentration followed by slight diffusion had taken place, irregular changes began to appear; some tracts of the web under observation becoming affected with more or less full diffusion of the pigment, while in others it became more concentrated. Then after the lapse of some hours its state was found reversed, being concentrated in parts where it had been diffused, and *vice versâ*. These curious variations continued till so late as the tenth day after amputation, becoming more frequent after the first few days; so that sometimes a considerable alteration was observable within half an hour.

These facts appeared to me of great importance, as proving the continuance of vital actions for a much longer time than had been previously supposed possible in a severed portion of the body. They seem also valuable with reference to the influence of the nervous system over the pigmentary functions; for the circumstance that considerable patches of the web usually had the pigment in the same condition throughout at one time implies that a large number of pigment-cells were acting in concert, and therefore probably under the control of the nervous system, although, as the leg had been amputated, they were of course freed from the influence of the central ganglia. Hence we are led to suspect the existence in the limb of an apparatus, probably ganglionic in structure, coordinating the actions of the pigment-cells, just as we know that the muscular contractions in the mammalian intestine are harmonized by a local mechanism of that nature, while we have reason to think that the same thing holds regarding the arteries in the frog's web*. Such a view is in accordance with the results of recent anatomical discovery, which has revealed nerve-cells in many parts where their occurrence had not previously been conjectured. But in the absence of more positive evidence, we must be careful not to trust too much to analogy on such a point; for it by no means necessarily follows, that, even if muscular fibre-cells are incapable of acting in mutual harmony without the aid of the nervous system, the same must be the case with pigment-cells, which, it is to be remarked, resemble ganglion corpuscles in being connected together by anastomosing offsets. The nerve-cells, if such be really the means by which the harmonious actions of the pigment-cells in an amputated limb are induced, must be disseminated among the tissues of the web itself; for both *post mortem* concentration and secondary diffusion occur in a piece of a web cut out and placed in a drop of water on a plate of glass. This was ascertained on the 4th of September, 1858, in the case already alluded to as an instance of rapid occurrence of concentration. About half an hour after removal from the body, the pigment, previously reticular, was in the dotted state, and three hours later it was found to be again stellate.

The case of the pigment-cells is analogous to that of the arteries in this respect, that, so long as circulation is going on, they are generally in entire subjection to the central ganglia, and act only when stimulated by their influence. But as, in the arteries, it appears to be by the independent action of the local nerves that a contraction caused by direct irritation spreads to a considerable distance from the part operated on, so it is probably by local means that the pallor induced by pinching the web affects a circle of surrounding tissue. If this be true, the case of direct irritation will be an exception to the general rule, that, while circulation continues healthy, concentration always implies the operation of the central organs of the nervous system.

Comparing the changes in the pigment in an amputated limb with those which take place under similar circumstances in the arteries†, it appears that the first effect of removal from the influence of the nervous centres in the head and trunk is arterial relaxation and pigmentary diffusion, followed in a variable time by contraction of the vessels and concentration of the dark molecules, giving place again to relaxation and diffusion, after which succeed irregular alternations of contraction and dilatation in the one case, and of concentration and diffusion in the other. Here, though the vascular and pigmentary changes do not at all correspond with one another in point of time, yet there is an evident parallel between them; and, admitting that in each case the variations are the result of alternate action and inaction of the appropriate local nervous system, it is evident that concentration of the pigment corresponds to contraction of the muscular fibres of the arteries; these being both the results of nervous action, while diffusion of the pigment, like arterial relaxation, takes place when the nerves cease to operate. It

* See the preceding paper on the parts of the nervous system which regulate the contractions of the arteries, page 619.

† See the preceding paper before referred to, page 618.

will be remembered that a similar conclusion was derived from the study of the influence exerted upon the pigment-cells by the central ganglia. Hence it appears that the tendency to diffusion of the pigment-molecules is in constant operation in the cells, but kept in check by an antagonistic concentrating agency varying in obedience to nervous influence.

It is an interesting circumstance, that two functions seemingly so totally distinct as muscular contraction and pigmentary concentration, should both be thrown into a state of activity in consequence of arrest of the circulation. It is to be remembered, however, that there is no evidence that either the involuntary muscular fibre or the pigmentary tissue is directly influenced by the cessation of the flow of blood, the effect being apparently produced through the medium of the local nervous system. This we know with certainty in the case of the *post mortem* movements of the intestines; and we have seen reason to think it likely that the same is true regarding the contractions of the arteries after death, and the concentration of the pigment under similar circumstances. It is a curious question how the arrest of the circulation causes these actions of the local nerves. The idea suggested by the facts is that the tissues begin to suffer from the want of fresh supplies of the vital fluid, and resent the injury, as it were, by a struggle.

Rich in results of general physiological interest as the study of the pigmentary system of the frog has proved, it has yielded fruit of not less value in a pathological point of view. Indeed, what induced me to investigate the functions of concentration and diffusion, was the unexpected light thrown upon the nature of inflammation by the effects produced by irritants upon those processes. For information on this subject I beg to refer the reader to the next paper in these Transactions.

The pigmentary system also promises to render good service in toxological inquiry. Hitherto, in experiments performed upon animals with that object, attention has been directed chiefly, if not exclusively, to the effects produced upon the actions of the nervous centres, the nerves and the muscles. In the pigment-cells we have a form of tissue with entirely new functions, which, though apparently allied to the most recondite processes of the animal economy, yet produce very obvious effects, and thus afford great facilities for ascertaining whether or not they have been destroyed by any poison that may have been administered.

An experiment of this kind which I once performed, though with a different object, may be mentioned by way of example. Being desirous of confirming the conclusion to which I had been led by experiments above related, viz. that diffusion always tends to take place when the influence of the nerves is withdrawn from the pigment-cells, it occurred to me that the Urari poison might be brought into requisition for that purpose: for it has been shown by Professor KÖLLIKER of Würzburg, that this substance paralyses in the first instance the extremities of the motor nerves without affecting the contractility of the muscular tissue; and supposing the nerves concerned in regulating the pigmentary changes to be similarly deprived of their powers, while the pigment-cells themselves remained intact, diffusion should take place after exhibition of the drug, provided my view were correct. Accordingly, at 2^h 10^m P.M. on the 21st of December, 1857, I introduced beneath the skin of the back of a pale frog a portion of Urari extract, for which I was indebted to the kindness of Dr. CHRISTISON. At 2^h 25^m reflex action was entirely abolished, the creature being to all appearance dead, so far as could be judged by the naked eye, although the microscope showed that circulation continued in the webs. The pigment meanwhile had become stellate, but did not continue in that condition, being, half an hour later, found fully concentrated. Soon after this, however, diffusion again commenced, and continued to advance steadily till circulation ceased early the following morning, at which time the integument was almost black. In the course of a few hours, however, it was brought again back to the palest possible tint by *post mortem* concentration.

The diffusion which ultimately took place in this case was no doubt due to loss of function on the part of the central ganglia or the nerves connecting them with the pigment-cells. But from the occurrence of concentration half an hour after the faculty for reflex action had ceased, we learn that these nerves, like the intrinsic motor nerves of the heart and intestines, remain unaffected by the Urari poison for a considerably longer time than those which excite the contractions of the voluntary muscles. We further learn from the fact that *post mortem* concentration came on as usual, that the pigment-cells retain their powers, and also their capability of acting in mutual harmony after the rhythmical contractions of the heart have been abolished by this poison.

Such experiments are so readily performed, and the effects produced upon the pigment-cells or the nerves which govern them are so obviously indicated by the changes of colour in the integument, that I venture to recommend this method of investigation to those who are occupied in studying the action of poisons.