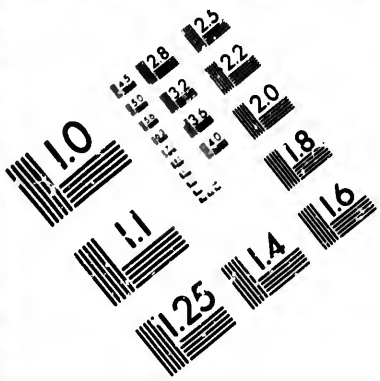

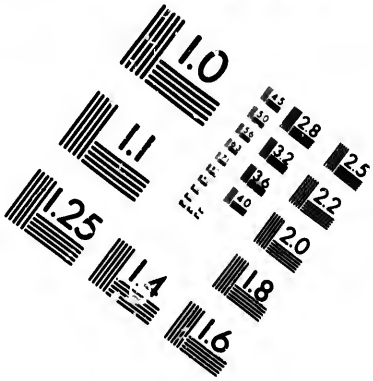

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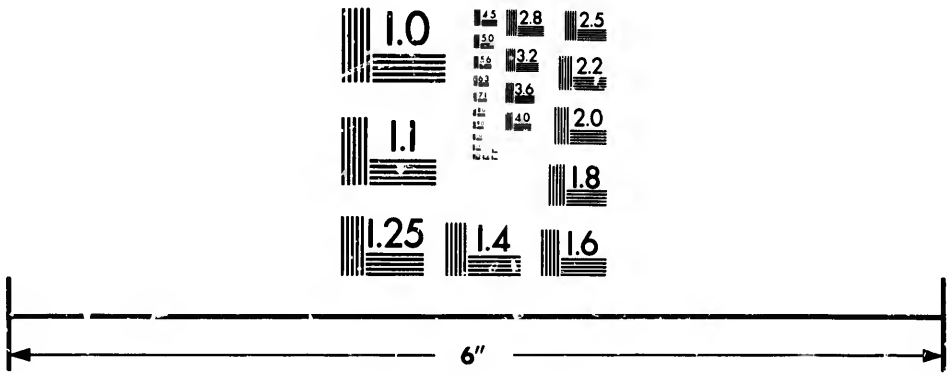

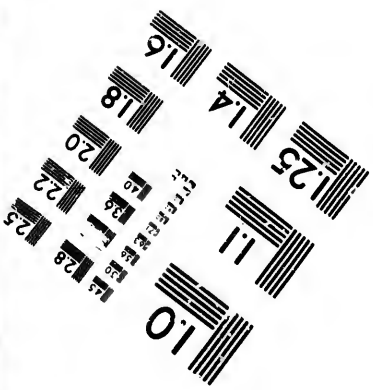

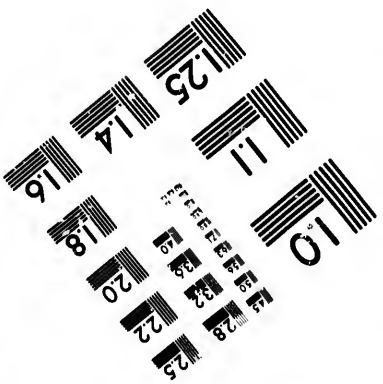

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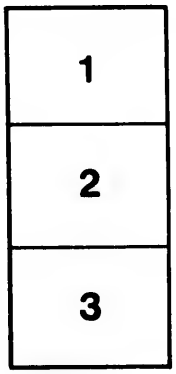

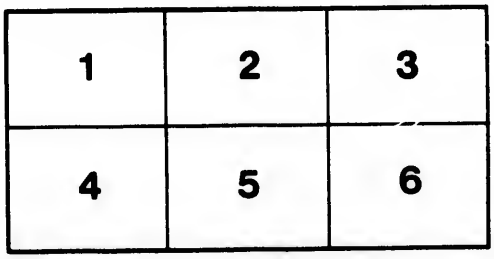

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No. III.—STUDIES ON THE BLOOD OF AMPHIBIA.

By A. B. MACALLUM, M.B., Ph.D.

(Reprinted from the Transactions of the Canadian Institute, Vol. II., Pt. 2.)

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[*Extract from Transactions of the Canadian Institute, 1890-91.*]

STUDIES ON THE BLOOD OF AMPHIBIA.

By A. B. Macallum, M.B., Ph.D.

Lecturer on Physiology, University of Toronto.

(Read 17th January, 1891.)

CONTENTS.

1. The Origin of Hæmoglobin.*

In the following pages are given the results of studies commenced five years ago and continued with short intermissions till last summer. The length of time taken up in this work was necessarily great because of the lack of previous studies in the same line and because of the want of definite and exact knowledge on the subject of the micro-chemical reactions of hæmoglobin. The difficulty of detecting, by chemical or microscopical methods, any antecedents of hæmoglobin appeared so formidable that, at one time early in the work, I was on the point of abandoning the line of investigation altogether.

I have used for this investigation our Lake Lizard, *Necturus lateralis*, and the larvæ of *Amblystoma punctatum* which are readily obtainable in large numbers in the immediate neighborhood of Toronto in April and May. The advantages which the tissues and structures in the *Necturus* present for cytological work far outweigh those which a comparative study of the blood in a larger number of Amphibian forms would have and there is, therefore, a justification for narrowing the investigation to the two named forms.

* The subject matter of this paper was included in a thesis presented for the degree of Doctor of Philosophy in the Johns Hopkins University, in April, 1888.

A. METHODS OF STUDY.

At the outset of an investigation like this, one has to answer the question: How far can we rely on the different effects in staining produced by a dye in determining the dissimilarity in composition of the objects stained? We can illustrate the question by a case in point: safranin stains the nuclei of the red blood cells of *Necturus* orange-red when they are fixed in a certain way, while the nuclei of ordinary cells under the same conditions take a red color. Does this indicate that the substance in the nuclei of the red corpuscles which is stained orange-red is different in its chemical composition from that in ordinary nuclei? An affirmative and a negative answer are equally consistent with what we know as yet of the relation between staining reagent and object stained. It is quite possible to imagine the molecules of the staining reagent in the object stained so placed relatively to the molecules of the latter that though no chemical union results, certain kinds of the light rays become absorbed in their passage through the object. It is further possible to conceive that variation of the distance of the molecules from each other in the object stained may result in a variation of the rays transmitted. Staining is in this sense a result of a physical condition, and as such many consider it. It is easy also to understand that if the molecules of one stained object should be different in structure from those of another, the interarrangement of these with those of the same staining reagent might affect the light transmitted in each of the two cases differently. In such the difference in color would depend on a difference in chemical composition while the stain in itself would be referable to a physical condition. In addition to these three possible modes in the production of staining reactions there are two others, viz., the action of the stained material in bringing about a change in the composition of the staining reagent and the definite chemical combination of the staining and stained material. The action of the stained object on the staining reagent is illustrated by the effect produced by the chromatin of the hæmatoblasts in the *Amblystoma* larvæ on alum-hæmatoxylin, the usual color given by the latter reagent to ordinary nuclear constituents being there turned to a slate tint. That chemical combination does occur in the case of some reagents is shown by Unna's experiments with several aniline dyes.*

There being, thus, probably several ways by which a stain in an object could be effected, it is manifestly impossible to prove in regard to any particular dye, whether, when it stains a series of objects, the same resulting colors in the latter are produced by the same or different

*Arch. für Mikr. Anat., Bd. XXX. p. 38.

interaction, physical or chemical, of dye and object. It is of course not even probable that the chromatin elements of all cells are chemically the same except in the main outlines of their structural formulæ, yet alum-hæmatoxylin or alum-cochineal gives usually the same color reaction in all Here the effect is the same but the interaction may or may not be the same in all cases. The subject belongs to borderland between physics and chemistry and we can conceive that the interaction may lie on one side or the other of any arbitrary line drawn to separate the two domains without resulting in any visible difference in color. If different colors should result when chromatin elements, for example, are stained by a dye, then it may be safely inferred that the groups of atoms in the variously stained elements are differently related to the groups of atoms in the staining reagent. It might be suspected in such a case that the difference in stain might depend on a difference in chemical composition and this suspicion would become certainty, if a second dye were found to act in a similar way towards the same chromatin elements.

The difficulties which surround the solution of questions of this sort are very numerous but they are multiplied when one multiplies the methods of hardening or fixing tissues. These methods greatly vary the effects of a single staining reagent on cellular structures. On this account no conclusion of any great value has been drawn as to chemical nature of any cellular substance from the employment of staining reagents alone. On the other hand the employment in cytological research of chemical reagents on objects under the microscope has not been, even to a limited extent, successful.

I have put forward all the difficulties which a research like this presents and they have all through this work been before my mind. I have resorted to the processes of staining, because the question of the origin of hæmoglobin is an all important one and because I can see no other means of settling it. It may be said that the means are insufficient. I can only say in answer that I have tried to do the best with them and the conclusions given in this paper are drawn from the results obtained by the employment not of a few but of a very large number of methods of hardening and staining. It is only by the employment of various staining reagents that one can avoid the errors resulting from an adherence to one or to a few microscopical methods and at the same time reach, usually, at least, measureably certain conclusions.

My first labors in this investigation were directed to finding a reagent which would show the presence not only of hæmoglobin, but of its

antecedent if such existed. I need hardly go over the list of wearisome experiments which I made for this purpose. Many, but not all, of these were resultless. Of the dyes at my disposal belonging to the aromatic group of organic compounds, Eosin is the only one which I found useful. As will be shown below it reacts with hæmoglobin and, in conjunction with alum-hæmatoxylin or alum-cochineal, it is a reagent for the antecedent of the pigment. Taken of course alone, without employing any other reagent for control purposes, it gives results far from satisfactory and it is also very misleading. Another reagent, the employment of which has been of great value to me, is the staining fluid of Shakespeare and Norris,* and which I shall name throughout this paper, for the sake of brevity, the Indigo-carmine Mixture or Fluid.

This fluid is made according to a formula which I have modified from that given by Bayerl, and consists of a mixture of equal volumes of the following solutions :—

A—Carmine, 2 grms ; Borax, 8 grms ; Distilled Water, 100 c.c.
B.—Sulphindigotate of Soda (Indigo-carmine), 8 grams ; Borax, 8 grms ; Distilled Water, 100 c.c.

In preparing each of these solutions, the borax is ground up in a mortar with the dye, the water poured on, and the whole allowed to stand for from five to seven hours before filtering. Owing to the fact that much of the Indigo-carmine in the market is impure, and consequently alters its composition in solution in a couple of weeks, it is not advisable to prepare more than 25—50 cc. of solution B at a time. I have obtained quantities of the reagent which retained in solution for three months its normal staining properties. As A, when kept for a year or more, readily shows undiminished staining power, a larger quantity may be prepared as "stock" solution.

The section to be stained is left in the fluid for fifteen minutes, then plunged in a saturated solution of oxalic acid for ten minutes, washed in distilled water, dehydrated with absolute alcohol, cleared in pure xyloi, and mounted in benzol balsam. Preparations made in this way two years ago still retain undiminished their original stain. When I first employed the fluid, four years ago, I used clove-oil for clearing, and found that my preparations faded, or contained a dirty precipitate after three or four weeks. The removal of the clove-oil after clearing with

* I have not seen the paper of Shakespeare and Norris describing the stain or its properties and capacities and my attention was first directed to it by Bayerl's work on the formation of blood corpuscles on the margins of ossifying zones in bones : *Arch. für Mikr. Anat.* Bd XXIII. p. 30.

xylol, postponed, but did not prevent, fading. It seems that the essential oils, even in small quantities, possess an oxidising power to which the sulphindigotate of soda is subject.

In order to get the best effects with this stain, the tissues are to be hardened with reagents which preserve the hæmoglobin and its normal distribution in the corpuscles. Some of the ordinary hardening reagents do not fix hæmoglobin (Müller's Fluid and solutions of potassic bichromate), others decompose it (weak solutions of chromic acid), while others again cause the hæmoglobin and the so-called stroma containing it to shrink irregularly in the corpuscles. The very fact that a reagent removes or decomposes the hæmoglobin does not prevent its employment in the study of the mode of formation of the pigment, but points to its usefulness in testing and controlling the results obtained by reagents which fix the hæmoglobin well. For instance, I have used chromic acid for the purpose of removing the hæmoglobin and fixing the antecedent. Even in the list given below the hæmoglobin-fixing property is not the same in all, and again the reagent which fixes the hæmoglobin in the red corpuscles in pieces of the spleen may not have the same property as regards cover-glass preparations of the blood. These facts should be borne in mind in every research on red blood corpuscles. The method which I adopted after a long series of experiments was as follows :—

Small portions of the spleen of *Necturus* were allowed to lie half-an-hour in a saturated solution of corrosive sublimate, or five days in Erlicki's Fluid, or twenty-four hours in a $\frac{1}{8}$—$\frac{1}{3}$% solution of chromic acid, five hours in a saturated solution of picric acid or two to five hours in 1% solution of osmic acid. They were afterwards washed in distilled water and put in alcohol of 50% strength for two hours and then in 70% for twenty-four hours and finally in 95%. The 70% alcohol was changed several times, each at an interval of twenty-four hours in the case of the chromic and picric preparations. The pieces were imbedded, either in mucilage and sectioned on the freezing microtome, or by the chloroform method in paraffin by which sections of about 5—10μ were made. The latter were freed from paraffin with turpentine and passed through absolute alcohol to water in the usual way. These, as well as those prepared with the freezing microtome. were transferred to the Indigo-carmine Fluid and treated in the manner described above.

The great value of these preparations consists in the fact that hæmoglobin is stained grass-green or greenish-blue while other proteid elements are colored red. This grass-green or greenish-blue is shown by a few other elements, but these are so well known and so easily recognised that no confusion can result. The number of structures other than hæmatogenic

to which the Indigo-carmine Fluid gives a grass green color are so few that they may be mentioned here: the yolk spherules, the degenerating, peripherally arranged, nucleolar bodies in the nuclei of maturing amphibian ova, the nuclei of some of the clavate cells in the skin of *Necturus*, also some of the nuclei of some of the cutaneous mucous glands of the same (in chromic acid preparations), the nuclear and cellular elements in the stratum granulosum and stratum lucidum of the epidermis, structures in the sheaths and cellular layers of hair follicles, yolk-like elements in the protoplasmic layer (syncytium) covering the chorionic villi in the cat, the substance of the dim band in striated muscle fibre, and finally, though not so distinctly, the lardaceous of amyloid degeneration. It will be seen from this list that except in the Amblystoma larvæ in which there is abundance of yolk spherules, there is no danger of mistaking any other compound for hæmoglobin. Where such a mistake was possible as in the case of the larvæ, I resorted to other staining reagents. From the list given it is to be inferred that the Indigo-carmine Fluid is a valuable reagent for certain processes of cellular degeneration. In connection with striated muscle fibre the reaction is significant, pointing to the derivation in the *Amblystoma* larvæ, of a portion at least of the dim band from the yolk spherules (the hæmatogen of Bunge?) or demonstrating in the dim bands in *Necturus* the presence of the red pigment described as hæmoglobin (Kühne, Ray-Lankester, Levy and Hoppe-Seyler) or as myohæmatin (MacMunn).

I stated that the reaction of the Indigo-carmine Fluid with hæmoglobin results in a grass-green or a greenish-blue color, but, strictly speaking, the greenish-blue color or stain should appear only when the hæmoglobin has been fixed with corrosive sublimate. I omitted to state, moreover, that the antecedent of hæmoglobin gives under certain conditions the grass green color with the staining reagent.

Bayerl* endeavoured in the following way to prove that the substance in the red corpuscles staining grass green with the Indigo-carmine Fluid is hæmoglobin: A quantity of dried amorphous hæmoglobin from dog's blood was dissolved in water, mixed with the indigo-carmine Fluid and the mixture treated with a saturated solution of oxalic acid. The color of the whole was grass-green. This experiment is not so decisive as it appears from the description, for I found that it is only once in a while that a green shade appears in the mixture. I found also on spectroscopic examination of the mixture, that the hæmoglobin was on the addition of oxalic acid more or less rapidly transformed into hæmatin. Even

* Loc cit.

when a quantity of solution B. (see p. 224) alone was mixed with a pure solution of hæmoglobin and the mixture treated with a saturated solution of oxalic acid there resulted only a dirty brownish precipitate from the decomposed hæmoglobin. This proves that soluble hæmoglobin cannot yield any reliable reactions with the Indigo-carmine Fluid.

Acting on the view that the hæmoglobin in my preparation is a fixed insoluble compound and therefore quite different from that obtained for example, by mere crystallization from dog's blood, I modified Bayerl's experiment. I took pure crystallized hæmoglobin from dog's blood, dissolved it in distilled water and mixed it with an equal volume of agar-agar solution* made liquid at 42°C. Stirred rapidly and then cooled by plunging the base of the containing vessel in pounded ice, the deep red agar-agar mixture becomes firm enough to cut with a knife. I made pieces about one-eighth of an inch in thickness which I put in various hardening fluids, as in the case of the spleen of the *Necturus*. When the fixation was complete the excess of the reagent was removed with alcohols 50%, 70% and 90% successively, sections of the pieces were made on the freezing microtome and stained with the Indigo-carmine Fluid. The preparations made with chromic acid or Erlicki's Fluid gave a grass-green reaction while those made with corrosive sublimate gave a greenish blue, practically the same results as in the case of the hæmoglobin in the red corpuscles. The fact that the corrosive sublimate preparations gave a greenish blue color with the Indigo-carmine Fluid, while the other preparations gave a grass-green, would lead one to suspect that there might be a difference in the chemical composition of the reagent when absorbed in the two kinds of preparations. If there is such a difference, it cannot be in the indigo portion of the staining molecule, for blue and grass green sections with the spectroscope, give alike the indigo absorption bands and no more.

I used also in staining sections of the spleen alum-hæmatoxylin solutions, in which ammonia alum is dissolved to saturation, and Czokor's alum-cochineal. These two reagents are of great value, especially the former, in connection with the studies on the hæmatoblasts in the *Amblystoma* larvæ, the latter having been in various stages of their development fixed in chromic acid (⅓%), Flemming's Fluid, corrosive sublimate, and Erlicki's Fluid. Though the other reagents have their uses, the second and third mentioned were the only ones to give good general results. My preference is decidedly for Flemming's Fluid for larval or embryonic tissues. Half an hour is long enough for this reagent to act, since with

*Of the strength and characters recommended by Biondi. *Arch. für Mikr. Anat.* Bd. XXXI., p. 105.

a longer stay in it the yolk-spherules blacken and the chromatin elements in the nuclei are stainable with more difficulty in alum-hæmatoxylin. After the employment of any of the hardening reagents the larvæ were washed for a couple of minutes in distilled water, for ten minutes in 50% alcohol, then in 70% alcohol, until all traces of the hardening reagent were removed, when they were put into and kept in 95% alcohol. The larvæ were, as a rule, and more advantageously, stained *in toto* in alum-hæmatoxylin or alum-cochineal. When the sections, obtained after imbedding by the chloroform-paraffin method, were fixed on the slide with clove oil-collodion, a second stain, eosin, was, when desired, employed. I used, also, the triple and quadruple stains of Gaule for the larvæ as well as for sections of the sp[illegible]en in *Necturus*, but I cannot say that I have derived any advantage fr[illegible] them.

Cover-glass preparations were made of the blood of the larvæ and of *Necturus*. These were fixed either in the fumes of osmic acid (1% solution for two hours), or by a saturated solution of corrosive sublimate, or picric acid, or by Erlicki's Fluid. These were the only reagents which I found serviceable. The method of operating was to decapitate the living specimen, to allow a small drop of the blood to fall on the cover-glass on which it was evenly spread, then to submerge the cover in corrosive sublimate solution for five minutes, in picric solution for five hours, or in Erlicki's fluid for two days. When osmic acid was used the cover was put, with the preparation surface downward, on the mouth of the unstoppered reagent bottle for two hours. The fixation was completed as usual with alcohol and the various dyes referred to above were used for staining the preparations.

Fresh cover-glass preparations of blood were also extensively studied both before and after the addition of coloring reagents, such as acetic methyl-green, acetic methyl-violet, picrocarmine, &c.

As regards the optical apparatus, I had for the finer work the $\frac{1}{12}$ in. hom. imm. of Leitz, the $\frac{1}{12}$ in. hom. imm. (1·43 N. A.) of Powell and Lealand, the $\frac{1}{8}$ in. hom. imm. and the L. (water imm. $\frac{1}{25}$ in.) of Zeiss. I used during the last summer the 3 mm. apochromatic of the last named maker when studying the blood of the larval *Amblystomata*.

B. STRUCTURE OF THE BLOOD CORPUSCLES IN NECTURUS.

The freshly drawn blood of *Necturus* contains the usual red corpuscles of known form, leucocytes and the so-called fusiform corpuscles. The first and last classes of elements merit a detailed description, owing

to their relation to each other and to the importance of the questions raised in these studies.

The red cells measure 50—53μ in length and 30—32μ in breadth. In the fresh and normal condition they present usually in nucleus and disc a uniform yellow red tint, and in the disc a completely homogeneous discoplasma. There are sometimes corpuscles possessing whitish nuclei which appear contrasted in this respect with the colored disc, but these are not numerous until the preparation has been kept under certain conditions, as in a moist stage, for some time. In such nuclei one can determine the presence of a coarse network. The membrane of the disc is very thin, so much so that when it is ruptured and freed of its contents it is rarely visible. I have frequently, by artificial means, ruptured a large number of the discs in a moist chamber and in only a very few cases was I able to see the resulting free membranes, although there were in such preparations an abundance of free nuclei. The contents of the ruptured corpuscles have different fates. That of the nucleus and of a portion of the protoplasm I shall describe fully when treating of the fusiform corpuscle. The hæmoglobin and the stroma containing it become dissolved in the serum, hardly leaving a trace visible. This points to the fluid character of the discoplasma and I now proceed to prove that view of its structure.

If a cover-glass preparation of the blood is fixed with a saturated solution of corrosive sublimate, stained with hæmatoxylin and eosin, mounted in balsam and studied with the best objectives at one's disposal, the protoplasm of the disc will appear perfectly homogeneous and will be seen stained uniformly and intensely by the eosin. Granules and vacuoles are absent, and if the nuclear membrane is shrunken away from the discoplasma, the edge of the latter next it will then appear regularly and evenly outlined. Vapor of osmic acid fixes the discoplasma in the same way that corrosive sublimate does. This brings out distinctly the fact that there can be no natural separation of stroma and haemoglobin in the discoplasma. In other words, we may say that the latter is not homologous with the cytoplasma and enchylema of ordinary cells, but that in the normal condition it is in the physiological sense a single element. It is true that in certain methods of fixation the protoplasm of the disc appears reticulated, and this may occur in a few of the cells fixed by corrosive sublimate (Fig. 1), but in every case the fineness and arrangement of the reticular trabeculæ depends on the method of fixation, and this shows that the reticulum is artificially produced. One has but to look at Figs. 1, 2, 3, and 4 to see how the artificial structure varies in character. The preparation of the blood

corpuscles of the *Amblystoma* larvæ illustrates this variation also (Figs. 5, and 7), the corpuscles treated with Flemming's fluid frequently presenting a coarse network; those made with acetic methyl-green showing a fine one, while those fixed with osmic acid showed none at all.

If there is a stroma or any network, it does not separate itself from the hæmoglobin, when the latter crystallizes, even in the corpuscle. I have often watched in the moist-stage chamber the crystallization of the hæmoglobin, especially when the instrument permits a slow evaporation of the water of the blood, and found on the border or edge of the drop that the hæmoglobin contents of a single blood corpuscle crystallized without exuding from or passing out of the cell membrane. In some cases the latter was seen to be more and more pushed out at certain points until it possessed a rhomboid form like that of the contained crystal. The membrane became invisible when evaporation passed a certain limit owing no doubt to the greater density of the medium (serum). These crystals are usually of the same length and breadth as the original corpuscle and they contain, moreover, a large central oval space, the cavity of the nucleus. Now these crystals differ in size, but not in form, from those obtained by rupturing the corpuscles and slow drying of the blood. In the latter the crystals are very long and narrow. If there is a stroma why does it not interfere, not only with the crystalline form, but with the power of crystallization in the hæmoglobin?

The nuclei measure 20—21μ by 12μ. With ordinary powers (Zeiss *D*), they appear homogeneous, less deeply shaded than the disc, the hæmoglobin tint which they may appear to have being merely due to that of the superposed portion of the disc, and they often are uncolored or whitish in contrast with the latter. With high powers, such as oil-immersion objectives, one can, in a perfectly normal and fresh corpuscle, determine the existence of a wide-meshed network. This is formed sometimes of thick, sometimes of thin trabeculæ, and it is often straw-yellow in color, in other words, it apparently contains hæmoglobin.

I now leave the description of the red corpuscles to take up the question of the origin of hæmoglobin in them.

C. THE ORIGIN OF THE HÆMOGLOBIN IN THE RED DISCS.

If cover-glass preparations of the blood of *Necturus* or portions of the spleen of the same animal be fixed in various ways it will be found that the hæmoglobin of the red cells in the different preparations is obtained in various degrees of preservation.

One of the most convenient fixative reagents for hæmoglobin in the

red discs, and especially when employed on the spleen, is Erlicki's Fluid. This, combined with the Indigo-carmine Fluid described in the foregoing pages, gives a remarkably sure means by which one can determine the presence of the pigment. The red cells of the spleen present with this fixative reagent and the staining fluid a uniformly grass-green disc in which no structural elements can be observed and a nucleus which may be either carmine red or grass-green, or of a shade in green. Sometimes the nucleus presents a network as deeply grass-green as the substance of the disc, while the substance in the meshes of the net-work is red. These different effects obtained on the nuclear structures are not due to artificial or physical conditions such as the early or late action of the fixative reagent, for all the described features can be found in the nuclei of cells placed side by side. Without raising the question at present whether there is any hæmoglobin in the nucleus, a question which might be prompted by an observation already made above, it may be concluded that the nuclei of the red cells are not all similar in their chemical relations towards sulphindigotate of sodium This conclusion may be also drawn from a study of cover-glass preparations of blood in which it is often easy to see a grass-green network in the carmine-red nuclei of the red cells.

In cover-glass preparations fixed with osmic acid vapor in which the layer of blood is very thin, the haemoglobin is also well preserved. Here the nuclei of the red cells have, after the employment of Indigo-carmine Fluid, a grass-green network in the meshes of which the substance is faint red. In similar cover-glass preparations in which the layer of blood is comparatively thick the discs of the red cells are grass-green, the nuclei distinctly red with a green net-work. In cover preparations on which the solution (1%), instead of the vapor of osmic acid, was used the same staining reagent gave red nuclei and grass-green discs to the red cells.

In cover preparations of the blood made with corrosive sublimate solutions the Indigo-carmine Fluid stained the discs and nuclear network deep blue green, while the substance in the meshes of the net-work is colored from a light to a deep red, oftener the former. Frequently, with an ordinary power such as a D of Zeiss, very many, or nearly all nuclei of the red cells appear homogeneously red, but with the employment of an oil immersion ($\frac{1}{12}$ in.) the presence of the blue green network can be distinctly determined.

Flemming's Fluid, Müller's Fluid and chromic acid dissolve the hæmoglobin out of the red discs in cover-glass preparations of the blood and

in such cases it is difficult or even impossible to get any reactions at all with the Indigo-carmine Fluid.

In preparations of the blood, therefore, made with corrosive sublimate, osmic vapor, and Erlicki's Fluid, and subsequently stained with the Indigo-carmine Fluid, the nuclei of the red cells are shown to contain two substances: one which stains grass-green or blue-green arranged as a network, the other colored red (light or deep), situated in the spaces formed by the network.

It is now pertinent to ask whether the nuclear network is formed of or contains hæmoglobin, or whether, as it may happen to be chromatin, it, as such, merely shows a special affinity for sodic sulphindigotate, without pointing to any relationship between it and hæmoglobin. I have already stated in the description of the fresh and living red cell that its nucleus frequently presents, under oil-immersion objectives, a straw-yellow network which is seen in contrast with the slight paleness of the rest of the nuclear substance. This would seem to indicate the existence of hæmoglobin in the nuclear network. That it is not hæmoglobin, though a substance allied to it—judging from its color in the fresh cell and its reactions with sodic sulphindigotate in the fixed cell—is shown by the employment of picric acid as a fixative reagent on cover preparations and the use of the Indigo-carmine Fluid. In such the discs of the red cells are somewhat vacuolated but they are colored grass-green while their nuclei are either light red with a deep red network, or, sometimes, light blue with a deep red network. If hæmoglobin is present in the nucleus it ought in picric acid preparations to be as readily detectable there as in the disc.

The question now advanced is: what is the composition of the substance forming the nuclear network and of that filling its meshes?

If a section of the spleen hardened in chromic acid is stained with the Indigo-carmine Fluid, the discs of the red cells appear faint red while their nuclei are colored a deep grass-green. In the latter there is not the slightest trace of a differentiation into network and mesh substance. Evidently then the employment of chromic acid has converted the whole of the nuclear substance into something which stains grass-green with the Indigo-carmine Fluid. The latter reagent is not the only one which shows this conversion for alum-hæmatoxylin, alum-cochineal and safranin stain homogeneously the nuclei of the red cells of such preparations. The whole of the nucleus, both network and mesh substance, must be regarded therefore, as modified chromatin or as a mixture of chromatin and achromatin, the latter being rendered capable by the chromic acid of absorbing staining matters. That we have nothing to

do here with achromatic substance is shown in sections of the spleen hardened with Flemming's fluid and stained with alum-hæmatoxylin. In such preparations the nuclei of the red cells take a homogeneous deep stain thus proving that there is no conversion of achromatin into chromatin or into a substance which reacts towards dyes like the latter. Hence we may conclude that the nuclear contents in the red cells are formed of chromatin more or less modified.

If the nuclei of the fully formed red cells in a larval *Amblystoma* hardened in Flemming's Fluid be put under observation, a condition is seen in them similar to that found in the nuclei of the red cells of the spleen hardened in chromic acid, that is, they stain in the majority of cases with alum-hæmatoxylin, alum-cochineal in the same way, taking a uniform homogeneous tint. There can be no doubt that here the nuclei are well preserved. In some larvæ again, there are found a few fully formed corpuscles in which the nuclear network alone is stained. There are also other nuclei in such larvæ which present different amounts of a stainable mesh substance and the inference gained from the study of such nuclei is that the stainable mesh substance takes its origin in the network and as the latter in the newly formed corpuscles contains the whole of the chromatin, the stainable mesh substance is modified chromatin. That it is modified and no longer fully functional may be seen by glancing at Figs. 13 and 14 which represent fully formed red corpuscles of the larva in division. Examples of the latter are not very numerous, not more than three or four occurring in a whole series of sections. In these one finds that there is a quantity of chromatin between the loops of the chromatin figure in the daughter nuclei and that this unorganised chromatin has only taken a passive share in the process of division. The latter species of chromatin was in a few cases so abundant as to obscure the regular chromatin loops.

The substance, then, in the spaces of the nuclear network is a derived chromatin which, fixed with chromic acid or Flemming's Fluid, gives with alum-cochineal or alum-hæmatoxylin a deep and homogeneous stain and which when fixed with chromic acid has the property of giving, as hæmoglobin does, a grass-green stain with the Indigo-carmine Fluid. I believe this modified chromatin is the parent substance of hæmoglobin, that is, it is a true hæmatogen.

That this modified chromatin is derived from the primitive chromatin of the hæmatoblast is also shown by a study of sections from the spleen of *Necturus* hardened in chromic acid and stained with the Indigo-carmine Fluid. Fig. 8 is an exact representation of a group of cells from one of the blood sinuses in such a section, in which a dividing hæmatoblast is

shown with the chromatin loops alone colored grass-green while the cytoplasma and, if present, the caryoplasma are colored, in contrast, light red. There is evidently no derived or modified chromatin here and the only substance related to it must be situated in the chromatin loops. I saw, indeed, in a number of other examples of dividing hæmatoblasts that there was a grass-green substance between the usual chromatin loops and this substance which was, evidently, modified chromatin, varied in quantity from that condition where it was scarcely detectable to that where it was so abundant as to obscure the outlines of the similarly-stained chromatin loops. The latter condition is, certainly, a later stage than that shown in Fig. 8 and the nucleus of the fully formed red cell, in all probability, represents the culmination phase of the conversion of chromatin into hæmatogen.

The chromatin of hæmatoblasts can be shown to be different in composition from that of an ordinary cell. In order to demonstrate this one must resort for material to those *Amblystoma* larvæ in which the majority of the blood corpuscles are more or less pigmented. The latter condition can be readily determined by putting the larva in water on a glass slide and examining its gills through the low power of the microscope. Indeed almost any larva, not very long after its escape from the envelope, will answer the purpose. It is fixed in Flemming's Fluid for half an hour, then put in 50% alcohol for fifteen minutes, afterwards in 70% for twenty-four hours and finally in 95% for four or five hours. If it is stained *in toto* with alum-hæmatoxylin, imbedded in paraffin, sectioned, and the sections mounted in series on the slide in benzol balsam, one can in the concave sides of the aortic arches and in the developing spleen find a large number of dividing hæmatoblasts which at once betray their presence by the dull slate, or slate-brown color which their chromatin possesses, while the chromatin of ordinary cells is stained a tint between purple and navy-blue. Figs. 9 *a* and *b* are contrast drawings made from specimens in the concave side of the same aortic arch and in the same section, the one representing an endothelial cell, the other a hæmatoblast. In the latter the slate-brown color of the cytoplasma was not very marked and this may frequently be found free from any color whatever. No more decisive proof could be given that the chromatin of hæmatoblasts differs chemically from that of ordinary cells. That which gives with the hæmatoxylin a slate-brown color is probably a hæmatogen or hæmatogenous chromatin.

Flemming* has noticed this reaction of the chromatin of the hæmatoblasts on the hæmatoxylin, and he states that dividing hæmatoblasts

* Arch. für Mikr. Anat. Bd. XVI., p. 396 and Taf. XVII., Figs. 19 and 20.

fixed in chromic acid have *in the unstained condition a greenish-brown or brownish-yellow color* which he considers due to hæmoglobin. This color is maintained in the hæmatoxylin staining fluid while all the nuclei of other cells become blue. I also have observed similarly colored hæmatoblasts in chromic acid preparations, and I attributed the color at first to the presence of hæmoglobin. In such preparations, however, there are examples in which the chromatin elements only are greenish-brown or brownish-yellow, and from this condition to that where the brownish-yellow substance is so abundant as to obscure the view of the internal structure of the cell there are all shades of transition. This substance is not hæmoglobin but rather an antecedent of it, that is hæmatogen, and is of the same nature and origin as the modified chromatin in the nuclei of the fully formed red cells which also show the same greenish, greenish-brown or greenish-yellow color when they have been treated with chromic acid. It differs from chromatin in its action on hæmatoxylin and from hæmoglobin in that it is more easily fixed with hardening reagents in the cell, and in that, as I will now show, it has a greater capacity for staining with eosin.

In the preparations of the hæmatoblasts of larval *Amblystomata* fixed with Flemming's Fluid and stained, as described, with hæmatoxylin and afterwards with eosin, one finds the modified chromatin or hæmatogen stained very deeply with the latter reagent. The dividing haematoblasts, according to this reaction, are separable into the following divisions: (1) those in which the cell body is only feebly stained while the chromatin elements are stained deep terra-cotta red (Fig. 10); (2) those in which the cell body is only little less deeply colored terra-cotta red than the chromatin loops (Fig. 11); (3) those in which the staining in the cell body presents conditions transitional between (1) and (2). There can be no doubt that in these forms the eosinophilous substance originates in the chromatin. The hæmatoblasts are the only cells in such preparations which show this decisive eosin reaction.

Now this modified chromatin or hæmatogen, as I prefer to call it, when once secreted into the cell of the hæmatoblast persists there through all the divisions of the latter. This certainly cannot be proved, and I believe it is impossible to prove, but it is a reasonable inference from facts gained by a careful study of the preparations. After a certain stage in larval life, nearly all the hæmatoblasts show it to be present and *it is converted into hæmoglobin when the cycle of divisions has been gone through.* After the formation of hæmatogen once commences it goes on, with the result that each of the numerous daughter or descendant hæmatoblasts possesses by inheritance and through secretion

a quantity of hæmatogen as definitely as it has unmodified chromatin. This hæmatogen plays no part at all in the division, and when the power of division is lost or greatly diminished the unmodified chromatin is confined in the nuclear membrane and the terra-cotta-red stain in the cell body gives place to that characteristic of hæmoglobin.

It has been already observed by Flemming* that chromatin is very abundant in dividing hæmatoblasts, and he compares this great volume with that of the same substance in the fully formed red cells.† He also speculates on the cause of the increase in the quantity of chromatin and mentions two possible explanations: either the stainable substance is taken fromthe protoplasm of the disc into the nucleus or the nuclei of the red cells contain chromatin in a greatly condensed, form so when that division commences it suffices to fill out the enlarged nuclear figure. He, apparently, inclines to the latter view because the nuclei of fully formed red cells stain more deeply than do those of other cells, yet expresses himself as not quite certain that a portion of the protoplasm of the disc does not go into the nuclear figure in division. Strasburger‡ adopts the second explanation. Flemming§ further states that the mitotic figure in the hæmatoblasts is 2—3 times greater than the nucleus of the resting or fully formed cell.

Flemming's observation as to the great amount of chromatin present in the hæmatoblast is correct, but he has used a wrong or incorrect standard when he selected the nucleus of the resting red cell. I have already pointed out that there are two kinds of chromatin in the latter. The network chromatin is never reinforced by that in the spaces of the network and it alone is a direct descendant of the mitotic chromatin of the hæmatoblast. This is very clearly shown by hæmatoblasts one of which is represented in Fig. 14. Now the original chromatin of the hæmatoblasts is from the time of their differentiation as such specially abundant. The quantity of this substance is from this time on to that of the formation of the red cells so great that the hæmatoblast seems hardly capable of containing much else, and, as a consequence, divisions appear so rapidly that I have never yet succeeded in observing the resting stage and the same has been the experience of other observers. There is in this, plainly, a reason for a degeneration of part of the chromatin into the eosinophilous substance already described.

* Zellsubstanz Kern-und Zelltheilung, p. 262-3.

† The two upper cells represented in his fig. T. p. 263, *op. cit.*, are fully developed blood cells.

‡ Zellbildung und Zelltheilung, 1880, p. 330.

§ Arch. für Mikr. Anat., Bd. XVI, p. 396.

When the amount of chromatin has become so much reduced by division and by degeneration of itself, then and not till then is reached the stage of the fully formed corpuscle. Even in this stage there may be just so much network chromatin left as to prompt a somewhat imperfect division (Figs. 12-14), but these forms are extremely rare and the fully formed red corpuscle is incapable of division henceforth, in other words, it has less than the usual quantity of unmodified chromatin that an ordinary cell has. It may be seen from this that Flemming's theory of the condensation of the chromatin of hæmatoblasts is not supported by the example which he brought forward. The chromatin exists in the hæmatoblasts from the first, there is no condensation of chromatin in the nuclei of red cells, but there is, for the greater part of it, degeneration.

Had Flemming, Pfitzner, and Strasburger studied fully the origin and development of the hæmatoblasts they would, I believe, not have been puzzled by the extraordinary abundance of the chromatin therein and Flemming would hardly have striven to account for this abundance in the way he did, either by derivation out of the cytoplasma, or by expansion of originally condensed chromatin.

From a study of my preparations there can be no doubt that the eosinophilous substance of the hæmatoblasts is, on the one hand, derived from the chromatin and on the other, transformed at the close of hæmatoblastic life into hæmoglobin. The transformation sometimes occurs before this epoch for in the freshly shed blood of larval *Amblystomata* I have seen mitotic haematoblasts in which a faint hæmoglobin coloration was present and in a few other, somewhat deeply pigmented cells the addition of weak acetic acid solution dissolved out the hæmoglobin and showed mitotic figures. This was the rare exception of course. I do not think the eosinophilous substance, although it also deserves to be called hæmatogen, is the same as the interfiiar or modified chromatin of the fully formed red cells, for the latter does not react so definitely towards eosin, and it does not as readily affect the hæmatoxylin in the same way. As I have shown, they both, however, are derived from the same source, and, apparently, the eosinophilous substance is farther on the road to the formation of hæmoglobin than the other.

There are a number of facts which also support the view that hæmoglobin is derived from chromatin. Bunge* has extracted from the yolk of hen's egg and from milk, nucleins which contain iron very firmly bound in the nuclein molecule. That found in the yolk Bunge especially calls hæmatogen, because he believes that it is the antecedent of the hæmoglobin of the chick, and he puts forward the view that all the iron

*Ueber die Assimilation des Eisens. Zeit. für Physiol. Chemie, Bd. IX., pp. 49-59.

which enters the animal body for assimilation does so in firm combination with complicated organic compounds, the elaboration of which occurs only in the vegetable kingdom. Such compounds, he contends, when absorbed and assimilated, yield hæmoglobin. Kossel* has corroborated Bunge's observations as to the occurrence of iron in the nucleins of yolk and milk.

Furthermore, Zaleski† found in the livers of various animals, washed out and thoroughly freed from hæmoglobin and inorganic iron salts, proteids which contained iron more or less firmly combined. These were albuminates of iron (Albuminat-verbindung des Eisens), and two, probably three, nucleins containing iron (Nucleo-verbindungen des Eisens). The latter vary in the power with which they hold the iron, and in one of the nucleins which he calls *hepatin* the iron is so firmly combined that the ordinary tests fail to show its presence, *it being only detected in the ash.* This hepatin differs from the hæmatogen of Bunge in that the latter yields up its iron more readily and has a greater amount of the metal, the hæmatogen containing 0·29%, the hepatin 0·011%. Zalesk , moreover, determined that his iron-holding nucleins are present in the nuclei of the hepatic cells.

These nucleins have all the characters of the ordinary nucleins isolated from pus, semen, etc., and as the latter are supposed to be present in, or to form the substance known as chromatin to the cytologist, it is possible that chromatin usually if not always contains iron as firmly bound as in the hæmatogen of Bunge and in the hepatin of Zaleski. It is true that the analyses of nucleins, as given generally, do not point to the occurrence of iron, but this can be explained by reference to the method employed in their preparation. The nucleins, or rather chromatins, are soluble in, and after a short time decomposed by, alkalies. Bunge has shown that his hæmatogen loses its iron in solutions of potassic hydrate after some days and contact with ammonic sulphide causes its decomposition with the separation of sulphide of iron. In the preparation of nucleins alkaline fluids have been used to dissolve the residue left by digesting tissues, pus, etc., with pepsin and weak hydrochloric acid, or with hydrochloric acid alone, and the alkaline fluid used contains the nucleins (soluble variety) which one would expect, from the results of Bunge's researches‡ to be free from iron (combined), if originally they contained it. In this way we may explain why the nucleins from various

*Weitere Beiträge zur Chemie des Zellkerns. Zeit. für Physiol. Chemie, Bd. X., p. 249.

†Studien über die Leber. I. Eisengehalt der Leber. Zeit. für Physiol. Chemie, Bd. X., pp. 452-502.

‡See on this subject specially the appendix.

sources analysed by different chemists present so many variations in composition as to lead some observers, Gamgee* amongst them, to deny a chemical individuality to these substances. The nucleins so extracted can hardly be considered as more than derivatives of the chromatin substances, for the latter in the living cell is undoubtedly the seat of the more important vital processes, and the changes resulting in these vital phenomena can hardly occur in a compound so comparatively simple as the nuclein, to which Miescher ascribed the formula $C_{29}H_{49}N_9P_3O_{22}$.

I have succeeded during the last summer in definitely demonstrating that the great part, if not the greater part of the yolk of the ovum of the frog and of *Necturus* is derived by diffusion from the chromatin of nucleus of the ovum†. Now this chromatin so diffused is the analogue in amphibian egg of the hæmatogen of the hen's egg. This taken in conjunction with the fact that the iron-holding nuclein of milk can apparently, and possibly, only be the chromatins which Nissen‡ has shown that the degenerating cells of the mammary gland throw out into the lumen of the secreting tubules, distinctly points to the presence of iron firmly combined in the chromatin of every cell.

All these points support and confirm the view that the hæmoglobin of the blood is derived from the chromatin§ of the hæmatoblasts. It may be asked, Why if chromatin contains iron, should not all cells contain hæmoglobin? All cells do not contain the excess which hæmatoblasts have, and therefore have none to spare for transformation into that compound. Why the hæmatoblasts have an excess of chromatin I shall endeavor to show when I come to speak of their origin further on. Enough has been said to show that the compounds which Bunge and Zaleski isolated and called respectively *hæmatogen* and *hepatin* do not merit these names, the hæmatogen not going directly, except probably in developing muscle fibre in larval amphibia, to form hæmoglobin, while Zaleski has not shown that every cell of the body does not contain a nuclein in which the iron is as firmly combined as in the so called *hepatin*.

As an additional proof that hæmoglobin is derived from chromatin, the occurrence of phosphorus in the hæmoglobin of the blood of the goose may be quoted. It is suspected by many that the phosphorus belongs

*Physiological Chemistry of the Animal Body, Vol. I., p. 243.

†The results of the research will be published shortly.

‡Arch. für Mikr. Anat., Bd. XXVI., p. 337.

§I am inclined to believe, from the results of my own observations, that the hæmoglobin of muscle fibre in Amphibia is derived directly from the yolk chromatin or, as Bunge calls it, hæmatogen.

to a compound which, in no way uniting with the hæmoglobin, yet in an admixture with it, is so difficult to separate that after many crystallizations of the hæmoglobin some will always adhere to the crystals. Recently, however, Jacquet* has isolated the hæmoglobin of hen's blood after recrystallization and has found that it contains 0·197% of phosphorus and 0·335% of iron. Hoppe-Seyler had previously found in the hæmoglobin of goose's blood 0·77% of phosphorus and 0·43% of iron. The anomaly of the presence of phosphorus in the hæmoglobin of Avian blood is readily explained away by the fact that the hæmoglobin is derived from a class of proteids which are peculiar in containing phosphorus.

It is, indeed, an important question whether the chromatin of all cells does not act as an oxygen-absorber like hæmoglobin. I made some experiments on this point. Methylene blue in living tissues in which the metabolic processes are vigorous becomes discolored owing to the abstraction of oxygen. This reagent has been recently much used on this account in the determination of the course of nerve fibres. Into solutions of this dye I put a number of free-swimming larval *Amblystomata* and examined them from time to time to determine the effect on the cells of the gills and in the tail. With weak solutions I found the free portions of the membranes only of the epithelial cells colored, while with gradually increasing strength of solution granules in the cytoplasma of the same cells become stained, especially those between the radicles of the cilia on the gills. Sometimes a red blood corpuscle presents in the disc in this case one or more blue granules. If one increases the strength of the reagent almost up to the limit of endurance on the part of the animal, other cytoplasmic elements are stained, but in no instance have I seen a single nuclear body stained. This was not due to slower penetration and, therefore, readier deoxidation, or reduction of the dye, for, in the few examples of epithelial cells in division which I found in that stage in which the nuclear membrane is absent, the chromatin elements were absolutely colorless. Indeed, it is only when the dividing cell is moribund or dead that the chromatin elements stain at all. The probable explanation of the phenomena described is that the chromatin has a marked capacity for storing up oxygen in itself and that it differs from hæmoglobin in that it gives up this element only to the products of its metabolism.

If chromatins and the iron-holding proteids derived from them, like the yolk nuclein of Bunge, have the capacity of storing up oxygen, then it is possible that part of the oxygen required for respiratory purposes in

*Zeit. für Physiol. Chemie., Bd. XIV., pp. 289-296.

the yolk-holding ova may be derived from this source. It is somewhat difficult, otherwise, to explain the process of respiration in larval *Amblystomata* which pass a week or more imbedded deeply in gelatinous masses floating in stagnant ponds.

I have seen, in a few cases, the straw-yellow crystal-like bodies in the immediate neighborhood of the nuclear membrane as Cuenot* has described. I have represented in Fig. 26 the arrangement of the bodies but they are not always as closely applied to the nucleus as there shown, for they, in the greater number of cells in which they were found, lie free in an apparently empty space between nuclear and cell membranes. I regard all these cells, as well as those described by Cuenot—who believes that they indicate the secretion of hæmoglobin from the nucleus—as the products of pathological conditions. I have not seen more than half a dozen of such cells and yet I have diligently examined the fresh blood of several hundred larvæ in various stages of development.

Research demonstrates more and more the influence which the nucleus exercises on the nutrition and function of the cell and among the observations put forward in this line those of Korschelt† may be mentioned, in which it is shown that the formation of chitin is directly dependent on the nucleus. Among the covering cells of the ova of *Nepa* and *Ranatra* the nuclei of two fused cellular elements approach each other and enclose between them a cavity in which chitin is deposited. Platner‡ also considers that the derivation of enzymes in gland cells takes place by the constriction and separation of a portion of the nucleus and the subsequent formation of zymogen granules at the same time that the chromatin of the separated nuclear portion is undergoing degeneration and absorption in the cytoplasma. He believes that there is a direct causal relation between this budding of the nucleus with the subsequent degeneration of the separated part and the formation of zymogen granules. I have failed to find that Platner's description is true so far as formation of zymogen in the pancreatic cells of amphibia is concerned, but I have found, nevertheless, that the nuclei of these cells play a very important part in the elaboration of the zymogen. It is, also, evident from the trend of researches in vegetable cytology that the nuclei of green cells are the important factors in the elaboration of carbohydrates and that the latter are converted into starch in the chlorophyll grains.§

*Comptes Rendus. 1888. p. 673.

†Ueber einige interessante Vorgänge bei der Bildung der Insekeneier. Zeit. für Wiss. Zool., Bd. 45.

‡ Arch. für Mikr. Anat., Bd. XXXIII. p. 180.

§ See on this point Strasburger's Histologische Beiträge. Heft I.: Ueber Kern und Zelltheilung im Pflanzenreiche, pp. 194-204.

II. THE FUSIFORM CORPUSCLES.

The fusiform corpuscles, which measure 26μ × 16μ, are quite numerous in the shed blood of *Necturus*. They, as their name implies, are elongated and oval, and with usually sharply truncated ends. They have no cell membrane, and their protoplasm, especially at one or both of the ends, is amœboid or protrusible in the form of fine straight rays, which, with careful observation, are sometimes seen to manifest a slow vibratory motion. Sometimes these cells are fixed with the processes extended (Fig. 22*b*). Often the protoplasmic periphery is formed of a series of granules which render the exact outline indistinct. The protoplasm is usually homogeneous, except for the presence of one or more vacuoles at either end of the oval nucleus and a few granules which seem to be of the same character as those of the periphery.

The nucleus is oval usually and measures 16μ × 14μ. It may in some cases be lobed, and the lobation may have gone so far as to originate several small spherical nuclei. It may be homogeneous or it may be coarsely reticulated. Kept in a moist chamber the reticulated as well as the homogeneous nuclei undergo a process of chromatolysis. In the case of the reticulated nuclei the first stage of degeneration is seen in the trabeculae of the network becoming elongated and parallel, the elongation occurring transversely to the long axis of the nucleus. At the same time the spaces in the network become larger and the nucleus apparently distended. This condition passes into that wherein the whole nuclear substance becomes homogeneous or in which its chromatin forms a thick zone next to the now spherical membrane. The history of the corpuscle terminates with the disintegration of the whole into globules more or less spherical and varying in size, suspended in the serum. Very little of the cytoplasma is found in connection with these globules, for, while the nucleus is passing through the conditions described, the cytoplasma granulates and becomes dissolved in the serum.

Such is the fate of the fusiform corpuscle when it lies by itself. When, however, it meets with another the two fuse, either by their ends, as is commonly the case, or by their sides, and this capacity for fusion may be exercised so much that small masses of them (white thrombi) exist here and there over the field of the preparation. The fusion is complete, all the lines of demarcation disappearing, even the granules which formed the protoplasmic periphery being dissolved.

These corpuscles are free from color and are like the leucocytes in many respects. From the latter they are distinguished by the absence of true amœboid movement and by their regular shape and size.

I have now to discuss the nature of these corpuscles and will first of all detail the various views which have been advanced concerning them in this respect.

It is probable that the first observation of these corpuscles was made by von Recklinghausen* in 1866, who described structures, which could have been no other than fusiform cells, in his preparations undergoing transformation into red cells. He found all the stages of transition between the spindles (fusiform cells) and the elliptical (red) corpuscles, while he saw under favorable conditions in some of the spindles a red shade like that in the ordinary red cells and he regarded these colored spindles as developing red cells. He refers to the fact that in his preparations there are at first small white points, afterwards becoming flat islands (white thrombi?) consisting of contractile cells which attain enormous sizes and possess contractile processes. In these large cells are developed homogeneous, refracting spheres, sometimes to the number of forty, which may, or may not, be considered as endogenously formed cells.

Ranvier† is the next to refer to these elements in frog's blood. He describes them as sometimes sharply pointed at both ends or with one end rounded, the other pointed, finely granular and uncolored. He considers them to be free endothelial cells.

Hayem‡ regards these, as well as the platelets of mammalian blood, as hæmatoblasts. He describes them, as they occur in frog's blood, as smooth, homogeneous, slightly clouded and with a tint less silvery than that of the white corpuscles. They present sometimes a central area lightly shaded, occupying the place of the nucleus, and inside this one or two refracting granules. The nucleus is in every respect like that of the red cell, oval, nucleolated and finely granulated. The disc which is small in volume is flattened, has an elongated, variable form and contains, like the red cells, two distinct constituents, a stroma and a specially organized substance. The stroma is very delicate and, therefore, more difficult to demonstrate than in red cells. The organized matter pervading the stroma differentiates the hæmatoblasts from the red cell, and it is uncolored or faintly tinted with a small quantity of hæmoglobin which it loses easily. This substance is extremely diffusible, and it is endowed with a particular kind of contractility. It is very easily injured, and to this property is due the formation of these corpuscles so readily into granular masses. Hayem subjected frogs to repeated bleedings and

*Ueber die Erzeugung von rothen Blutkörperchen. Arch. für Mikr. Anat., Bd. II., S. 137.

†Traitè technique d'histologie, 1875, p. 191 and 192.

‡Archives de Physiologie, Tome 5, 1878, Tome 6, 1879. Also a later publication: Du Sang et de ses alterations anatomiques. Paris, 1889, pp. 124-151.

found in the blood finally all the intermediate stages between the fusiform and the red cells.

Bizzozero and Torre* reject this view of the hæmatoblastic nature of the red cells and state that though they are like red cells in some respects they are smaller and unpigmented, while young blood cells are round in form and always contain hæmoglobin. These elements are also unlike the leucocytes in their simple oval nucleus and non-contractile protoplasm. These authors believe that the corpuscles in question are related, in spite of many points of dissimilarity, to the structures in mammalian blood known as platelets.

Hlava† considers the fusiform corpuscle to be a variety of the white cell brought about by the contractile capacity of the latter.

Löwit‡ describes the transformation of the spindles into spherical forms like that of the white cells with which he classes these elements. He maintains that all forms of white blood cells may appear in the spindle form, but he admits that certain stages of the developing red cell exist in this form from which hæmoglobin is absent. According to his view the fusiform cell is not a separate species of white blood cell but only a form of the latter which may appear under those conditions offered by the circulating blood, and it may in some cases have a hæmatoblastic nature.

Eberth§ describes the elements as being spindle, club, or almond-shaped, somewhat smaller than the red discs, probably slightly flattened, possessing a finely granulated nucleus and an almost homogeneous cell protoplasm which is chiefly gathered at the poles. Their contour does not change, they have no amœboid processes, and when they are collected into great masses they never present a trace even of a yellow or hæmoglobin tint. When they are kept for hours in their normal physiological condition, *e. g.*, inside the bloodvessels of an excised piece of mesentery, protected from evaporation, they have never been observed to change in shape, they exhibit no amœboid movement whatever and they do not fuse together. In the spindles fixed by osmic acid there is

*Virchow's Arch., Bd. 90.

†Die Beziehung der Blutplättchen Bizzozero's zur Blutgerinnung und Thrombose. Arch. für Experim. Pathologie, Bd. XVII., 1883.

‡Ueber Neubildung and Zerfall weisser Blutkörperchen. Sitzungsber. der Wiener Akad., Bd. XCII., Abth. III., 1885.

Also: Ueber den dritten Formbestandtheil des Blutes. "Lotos," Jahrbuch für Naturwissenschaft. Prag, 1885.

§Zur Kentniss der Blutkörperchen bei den niedern Wirbelthieren. Festschrift für Kölliker Leipzig, 1887, p. 37.

the longitudinal stripe, or folding, described by Hayem and Bizzozero and Torre and several refracting bodies in the nucleus, with one larger and rounder than the rest to represent a nucleolus.

The spindles undergo change quickly under the microscope with the ordinary conditions of observation. Their protoplasm swells up and disintegrates into a quantity of fine granules which partly dissolve and leave a faint, somewhat irregular body in which the nucleus still persists. The chromatin in the nucleus of the ordinary spindle is more irregular in its arrangement and more fully developed than in the white cells, and it does not form a network as in the latter or in red cells.

As salient points in their character, Eberth emphasizes their colorlessness and their lack of amœboid movement, both of which separate them from the white and red cells. They are not young red blood cells, for these even, in division, contain from their beginning hæmoglobin. That the fusiform cells do not contain even the slightest trace of hæmoglobin is shown by the fact that thick masses of them have not the faintest color, which would not have been the case if some of them contained hæmoglobin. Hayem regarded them as hæmatoblasts in his first paper, but the phenomena of Karyokinesis* in hæmoglobin-holding blood cells was then unknown, and it is probable that he mistook the true hæmoglobin-holding hæmatoblast for the forms intermediate between the fusiform and the red cells.

Eberth does not advance any view as to the origin or nature of the fusiform elements, simply contenting himself with pointing out the analogies between them and the platelets of mammalian blood.

It will be seen by a comparison of the above views that von Recklinghausen and Hayem postulate the presence of hæmoglobin in the fusiform elements while Bizzozero and Torre and Eberth deny this. Again, Hayem and Hlava state that it is contractile and this is expressly opposed by Eberth. Hayem considers them to be hæmatoblasts, with Hlava they are white corpuscles or a variety of the same, while with Bizzozero and Eberth they can only be compared to the platelets of mammalian blood. Such constitutes, in brief, the diversity of views as to their nature.

My own view is that these elements represent the remains of the destroyed or broken up red cells and the following are the facts on which the view is based:

1. Their nuclei are oval and nearly the same in size as those of the red cells ($16\mu \times 14\mu$ and $20\mu \times 12\mu$ respectively). The difference between

*In his more recent work (Du Sang &c.) all reference to these points is omitted.

the two in the latter respect is caused, I maintain, by the nucleus of the fusiform cell enlarging in its transverse diameter and diminishing consequently in its longitudinal diameter. If one keeps a specimen of blood under observation for a while, during which it is protected from evaporation, one finds that the nuclei of the fusiform elements actually undergo this enlargement in its transverse diameter, the transversely placed trabeculæ of its network elongate till the chromatin appears arranged in a number of parallel bars transversely placed. One can, moreover, by sudden pressure on the cover glass, rupture a number of red cells, set free their nuclei which undergo the same series of changes that the nuclei of the fusiform cells do, and shortly after the rupture the nuclei of the red cells measured exactly the same ($16\mu \times 13\mu$ and 14μ). In the free nuclei there is the same transverse enlargement, the chromatolysis and nuclear disintegration.

2. When a number of nuclei of red cells are set free by pressure there is the same tendency to adhere to each other that is so marked in the case of the fusiform element. To each of these free nuclei there is enough of cytoplasma adherent to constitute the cement necessary to agglutinate them together, and in the masses so formed there is nothing to distinguish them from the thrombi formed of fusiform cells. I have not yet succeeded in observing in them any pseudopodial movement, but it is not often that this is observed in the fusiform elements and it is possible that it is the result of a survival from a well nourished condition in the blood vessels, a condition not at all present under the cover glass.

3. The free nuclei and those of the fusiform elements have the same staining reactions. In a cover glass preparation fixed with corrosive sublimate or picric acid, in which free nuclei are abundant, the latter, as well as those of the fusiform cells, give with the Indigo-carmine Fluid a blue-black, sometimes an intense black, and with hæmatoxylin a black reaction. In fact there is the same, or nearly the same stain with all the dyes. There is one important difference so far as the cytoplasma of both is concerned: eosin takes intensely the cytoplasma of the fusiform cells while it stains lightly or not at all the slender protoplasm around the free nuclei. The explanation of this is that the interfilar chromatin (the hæmatogen) of the nucleus of the ruptured red cell gradually diffuses out from the nucleus into the cytoplasma without being converted into hæmoglobin, as it is in the normal corpuscle and that it is this altered chromatin which takes eosin deeply. In some of the fusiform cells there is the same differentiation of the nuclear substance into network and interfilar chromatin, the latter staining deeply with eosin, the former with hæmatoxylin. There can be no doubt about the fact that in such cells

the nuclear chromatin is arranged in the form of a network in every respect like that in the nucleus of the red cell. In such cases one rarely finds the Indigo-carmine Fluid to react as it does in the nuclei of the intact red cells, giving a light red stain to the interfilar chromatin and a green or a blue-green color to network. These are evidently cells which have had but a very short history as fusiform cells, that is, they have been but recently formed, while the other elements which do not show these peculiarities are more pathological by reason of their longer existence as fusiform cells.

4. The nuclei of these elements are admitted by Bizzozero, Hayem, to present resemblances to those of the red cells. These observers, however, took for study the blood of animals in which the red, white and fusiform cells are comparatively small, and consequently were unable to determine the more important points of resemblance.

We can, therefore, on the view that the fusiform elements are the remains of ruptured red cells, explain the absence of a membrane, the capacity for adhering to each other, the similarity in shape, size, structure and staining reactions between their nuclei and those of the red cells when freshly ruptured. We can, moreover, explain their occurrence thereby without referring in any way to the hæmatoblasts or to the leucocytes, and we have also explained to a certain extent the fate of the red cells—what was not done before.

One can readily determine the fate of these fusiform corpuscles even in cover-glass preparations of *Necturus*' blood fixed with osmic acid, picric and especially corrosive sublimate. Fig. 22 *a* represents a fusiform corpuscle in which there is a distinct and coarse chromatin network with a certain amount of interfilar chromatin. At a later stage the trabeculæ of this network become thinner and finally disappear, and when this happens the whole nucleus takes a uniform stain with various dyes. Sometimes the nodal points of this network alone persist and may appear as nucleoli. In the now homogeneous nucleus lobation may ensue (Fig. 22 *c*, *e*. *f*.), and the lobation may go so far, accompanied by a transformation of the shape into that of a more or less round mass, as to render them extremely like leucocytes. They possess now no amœboid properties whatever, and their cytoplasma, which is now comparatively abundant, begins to lose its eosinophilous character while the nuclear chromatin reacts less readily and more feebly to dyes. As such they are broken up, probably in the circulation and more especially in the vessels of the spleen.

As factors operating in the production of the fusiform cells, mechani-

cal conditions inside the blood vessels may be mentioned. It always appeared to me that my cover preparations were far richer in fusiform cells when the blood was obtained from the firmly pressed or squeezed tail of a specimen of *Necturus* than when the blood was simply allowed to drop on the cover glass from the tail tip. Of course there may be other circumstances which serve to increase or diminish the number of fusiform cells in the preparations, but it seems reasonable to suppose that the pressure which is employed between two cover glasses to rupture the red cells can be as effectually exercised in the blood vessels of the intact body. There is, however, another factor which may be less extensive in its effects. I refer to the giant cells in the spleen of the same animal. In a portion of the spleen of a freshly killed *Necturus* teased out, a few giant cells are always observable in which one finds one or more large spherules of hæmoglobin-holding substance imbedded in the cytoplasma. These giant cells are amœboid, and it is, presumably, reasonable to suppose that these masses of hæmoglobin have been removed from the discs of red cells by the invaginating power of the amœboid cells. There is in these same cells no evidence whatever of nuclei, either chromatolysed or intact, which could be considered as derived from the red discs, and the only inference possible is that the nuclei and the remainder of the disc cytoplasma have passed away into the general circulation as fusiform elements. What becomes of them finally after they have passed through the cycle of changes described, whether the leucocytes eat up their disintegrated remains, cannot be determined. I do not know why the nuclei of ruptured red cells do not possess the same amount of peripherally disposed cytoplasma as the fusiform corpuscles do, but it is supposable that either the cytoplasma is deposited from the nucleus or that fully formed fusiform cells are derived from red corpuscles only at a certain time in the life history of the latter, and that the conditions demanded by either of these hypotheses is assisted, in the formation and transformation of the fusiform cells, by the chemical and physiological equilibrium of the blood inside the blood vessels.

We can explain the fate of the leucocytes. No observation has hitherto been made as to the fate of the red cells. My view, I think, presents the easiest and best solution of the question. With it there is no necessity for considering the fusiform elements as hæmatoblasts; it is consistent, furthermore, with Stricker's observations on the transformation of spindles into globular "white" cells* and it specially explains why

* Quoted by Löwit, op. cit.

the fusiform elements are found only in the blood of those animals which contain nucleated red corpuscles.*

III. The Origin of the Hæmatoblasts in Amphibian Embryo.

There is probably no biological subject on which there is a greater diversity of view than that of the origin of the blood corpuscles in the embryo and adult vertebrate. The views on this point have multiplied greatly within the last five years and as they have not much in common, a resumé of them can hardly serve any useful purpose in a paper so limited in its scope as this one is. The observations, nevertheless, which have been already published as to the origin of the hæmatoblasts in Fishes and Amphibia have an important bearing on the facts which I am about to describe and I shall, therefore, give here an outline sketch of them before proceeding with the description of my own observations.

Goette† found the blood cells arise in the mass of the yolk cells. On the under and lateral edges of the yolk mass in Batrachian larvæ blood cells are formed by the breaking up of the large peripheral yolk cells into smaller ones, and at the same time there separates from the inner side of the visceral layer a number of cells forming a covering for the groove in the yolk in which the blood cells are developed. As the interstitial fluidity of the mass increases it extends over the yolk and affects the surrounding tissue just in the same manner as the interstitial fluid shapes the origin of the primary vessels, producing pouch-like diverticula connected with one another, from the yolk vessels. Goette regards the red and white cells of the spleen as direct descendants of the yolk cells.

Davidoff‡ reservedly expresses the view that the yolk spherules give origin by, possibly, protoplasmic transformation to parablastic elements and that the latter develop, in many cases, into blood cells. On this view the nucleus of the blood cell is but a yolk spherule imbedded in a protoplasmic basis, and Davidoff thinks that this is, in a sense, a confirmation of Brass' theory that the chromatin of the nucleus of every cell is secreted or stored up food material.

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* As the red corpuscle in mammalia is comparatively a fragile element its disintegration can scarcely involve the survival of any formed or structural element. If the fusiform element is the nucleus and a small portion of cytoplasma of the red cell in lower vertebrates, we may suppose since the platelets of mammalian blood are recognised generally as the homologues of the fusiform cells that the former are nuclei which have been extruded from hæmatoblasts, an extrusion which Rindfleisch and Howell observed.

† Entwicklungsgeschichte der Unke.

‡ Ueber die Entstehung der rothen Blut Körperchen und den Parablast von Salamandra maculosa. Zoologischer Anzeiger, 1884, s. 453.

Wenckebach* found that in Teleost embryos the blood cells originate from a mass of cells placed under the notochord and between it and the hypoblastic layer. The origin of this cell mass could not be determined, when he published his first paper, but afterwards he traced it to the mesoblast and was able, therefore, to corroborate Ziegler's† first observations on this point. This intermediate cell mass may arise, as in *Belone*, from an impaired organ but in the Salmon it is formed by the fusion of two separate columns of cells. The blood cells are thus, according to Wenckebach, of mesoblastic origin and are not derivable in any way from the hypoblast or from the periblastic cells.

Ziegler‡ confirms Wenckebach's observations on the development of the blood cells in the majority of Teleost embryos out of the cellular elements of the intermediate cell mass placed between the entoderm and chorda. This mass is of mesodermal origin and the cells constituting it wander away over the yolk and, in a measure, as they do this they make the cavities previously occupied by them larger and larger, the cavities forming, finally, the cardinal veins. Up to this time the blood which is free from cellular elements, flows in closed vessels represented at this stage by the heart, aorta, caudal vein and subintestinal veins. The latter empty on the yolk and the blood passes from the posterior surface of the yolk sack to the heart, not in a closed vessel, but free in the space between the yolk and the ectoderm. There arises in the yolk a corresponding furrow to which wandering cells pass to form a vascular wall. These wandering cells are in no way distinguishable from the blood corpuscles of the same stage which are abundant on the surface of the yolk and which arise, as already said, from the elements of the intermediate cell mass. Sometimes, as in the pike, a formation of blood cells, similar to that occurring in the intermediate cell mass, obtains in a portion of the aorta.

According to this view the blood cells are derived from the columns of cells which occupy the position of the developing cardinal and other veins and they are not, except accidentally, and through their amœboid movement, connected with the yolk.

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* The development of the blood corpuscles in the Embryo of Perca fluviatilis. Jour. of Anat. and Phys. Vol. XIX., 1885, p. 231. Also: Beiträge zur Entwicklungsgeschichte der Knochenfische. Arch. für Mikr. Anat., Bd. XXVIII, p. 225.

* Die Embryonale Entwicklung von Salmo Salar. (Inaugural Dissertation). Freiburg, 1882.

* Die Entstehung des Blutes bei Knochenfischembryonen. Arch. für Mikr. Anat., Bd. XXX, s. 596. Also: Die Entstehung des Blutes der Wirbelthiere. Berichte d. Naturforsch. Gesell. zu Freiburg i. B. Bd. IV. s. 171.

Rückert* gives a full description of the origin of the blood cells in Torpedo embryos. He found them to arise in the peripheral mesoblast where they constitute groups situated in cavities formed between the spindle-shaped mesoblastic cells. Where the outer and inner layers of the blastoderm are closely applied to the yolk these groups give off cells which constitute the blood islands of the posterior germinal area. At the latter point, according to Rückert, there can be no doubt about the origin of the blood cells out of the mesoblast. Laterally, and in front where the mesoblast is thin, the formation of the blood and of the vessels occurs through the accession to this part of freshly divided yolk cells (merocytes). Far anteriorly, the merocytes may be very large in size and appear then as megaspheres. The latter may, through unequal, indirect division, budding and fragmentation, give also origin to blood cells and mesoblast.

This brief sketch of the various theories as to the method of blood formation and the origin of blood cells shows how discordant they are. Goette believes that the peripheral yolk cells break up into hæmatoblasts, Davidoff thinks that yolk spherules become the nuclei of the red cells and that the discoplasma is derived from transformed protoplasm of the yolk, Wenckebach and Ziegler considered that the hæmatoblasts are of mesoblastic origin wholly, while Rückert is apparently disposed to believe that they are derived from the yolk cells on the one hand and from the mesoblast on the other.

As far as my observations on the *Amblystoma* larvæ go they are in accord with those of Wenckebach and Ziegler on Teleostean embryos, as to the derivation of the hæmatoblasts from the mesoblast alone.

The first blood corpuscles of the *Amblystoma* larvæ appear at about the twelfth or thirteenth day† after the deposition of the ova. At this date the heart is in the process of formation, the endothelial portions of it being derived from the entoblast in the manner described by Rabl‡ for *Salamandra* and *Triton*. The heart cavity, for thirty-six hours after this, even when fully formed, contains no cellular elements of any sort. The first blood vessels to be formed appear also at the twelfth day, constituting the subintestinal veins§ and it is in association with the formation of these that the hæmatoblasts make their appearance.

* Ueber die Anlage des mittleren Kiemblattes und die erste Blutbildung bei Torpedo. Anat. Anz., 1887, Nos. 4 and 6. Also: Weitere Beiträge zur Keimblattbildung bei Selachiern. Anat. Anz., 1889, No. 12.

† These dates are only approximate as there is a great variation in the development of the larvæ in the same mass of eggs.

‡ Morph. Jahrbuch, Bd. XII. p. 252.

§ The occurrence of two subintestinal veins instead of one in Selachii was first pointed out by Mayer (Mitth. aus des Zool. Stat. zu Neapel, Vol. VII., p. 340) and subsequently by Rückert (*loc. cit.*)

At about the eleventh day the ventral portion of the mesoblastic plate on each side consists of two layers of cells forming the visceral and parietal portion of the plate. These layers are closely applied to the entoblast and ectoblast respectively, but not at first to each other, for evidences of a slit-like space between them which represents a persistent part of the primitive body cavity, can be very well seen at this date. This slit quickly disappears through the growth of the adjacent parts and the consequent pressure exercised on the mesoblastic cells. The latter are, at first, more or less rounded in outline but the pressure exerted on them gives them a somewhat flattened appearance, except at the lower, extreme margin where the visceral and parietal layers become connected, the cells of the visceral layer here retaining, to a considerable extent, their original shape.

This part of the mesoblast seems to possess a greater capacity for proliferation than the more dorsally placed portions of the ventral half. The proliferation is limited chiefly to the cells at the extremity of the plate and to those immediately above this belonging to the visceral layer. The latter at the point in question is, about the twelfth day, formed of two or more series of cells, those constituting the most internal layer becoming very much flattened and like, in this respect, the cells of the single layer of the parietal portion. The cells placed between are obviously in the position occupied previously by the slit-like space, the more ventrally placed portion of the primary body cavity, and as they undergo division more frequently than the other cells, they cause a still greater flattening of the remaining cells of the visceral layer and of those of the parietal portion, with the result that these resemble fully formed endothelial cells. In a transverse section of the larva at about the thirteenth day, taken a short distance behind the developing heart, the cells first described lie in two large masses one on each of the ventro-lateral margins of the entoblast in which depressions exist to contain the masses of cells. The depressions are lined by the flattened endothelial elements derived from the visceral layer which are now recognisable with difficulty, and covered externally by similarly flattened endothelial cells derived from the parietal layer. The visceral and parietal layers above this are still at this time formed each of only one layer of cells more or less flattened. The cells constituting the masses described are the hæmatoblasts, while the depressions in the yolk or entoblast constitute the site of the subintestinal veins.

As the subintestinal veins are followed backwards they are seen to approach, with the mesoblast plates, more and more the middle of the line of the ventral side of the yolk and where the mesoblastic plates from

each side unite in the middle line, the veins form a single channel, till a point immediately in front of the anus is reached. In its course backwards the vessel is filled with cells closely packed and derived, in the same manner as those forward are, from the visceral layer of the mesoblast, although it is more difficult to exclude here the participation of the parietal layer in the formation of the hæmatoblasts. The mesoblastic plates again diverge at the anus and the venous trunk bifurcates, a branch running separately on each side of the cloacal cavity, the cells contained in them becoming less in number till, for lack of them, it is impossible to follow the veins any distance behind the anus.

When these veins and the cellular elements in them have attained the development described the heart is formed and beats. At first it contains no organized elements, the force of the beat being, apparently, exercised on what would appear to be serum. About the fifteenth or sixteenth day cellular elements in every respect like those found in the subintestinal veins are found in large numbers in the heart cavity and as the subintestinal veins are almost empty it is clear that the hæmatoblasts are derived from this source. It is, in fact, easy in series of sagittal sections of larvæ of the fourteenth and fifteenth days to see the detachment of the hæmatoblasts in the anterior portions of the subintestinal veins and their arrival in the heart cavity.

The hæmatoblasts are derived from this source alone. All the other vessels of the body have a different origin, that is, they are not formed by solid columns of cells exerting a pressure on the immediately adjacent mesoblastic elements, but rather by the extension of the subintestinal vessels and of the cavities of the heart. In *Amblystoma* larvæ therefore the hæmatoblasts are of mesoblastic origin alone and they are not increased in numbers by additions from the yolk elements or entoblast.

At first they are large, not differing from mesoblast cells in anything except their somewhat spherical shape. They contain in their cytoplasma a large number of yolk spherules which obscure more or less the nucleus. The latter is somewhat irregular, often amœboid in outline and richer, apparently, in chromatin than the ordinary mesoblastic cells of the same stage of development. To this greater richness in chromatin may be attributed the more abundant proliferation of these cells, for one can see that cell division is more frequent in them than in the neighboring cells. As the quantity of yolk spherules is limited, the repeated division, probably accompanied by a digestive action on the part of the cell on the spherules, produces a form of hæmatoblast (Fig. 16 and 17 *a* and *b*) in which the yolk spherules are few and in which nuclear chromatin is very abundant. It is in this stage that one finds the

hæmatoblast amœboid in outlines. Its cytoplasma is as yet undifferentiated and it does not possess a membrane although the peripheral portion gives evidence of its formation in the presence of a series of regularly arranged granule-like bodies affording a sharply outlined border.

In Figs. 19, 20 and 21 we see the hæmatoblasts of a later stage with much fewer yolk spherules and with specialization of form and structure allied to that in the mature red corpuscle. The outline is oval or elliptical and the peripheral portion is usually limited by a clear hyaline, somewhat thick membrane while the cytoplasma is differentiated into coarse or fine trabeculæ strewn along which are granules, some of them brownish in color like those found occurring in the mesoblastic and ectoblastic cells of this and later stages. Frequently the cytoplasma in the immediate vicinity of the nucleus is denser, stains somewhat more deeply than the remainder while it sends coarse prolongations in a radiating fashion outwards (Fig. 20). The corpuscles are not as yet flattened, but about the twentieth day the majority of them are elliptical in outline and flattened. When the larvæ of this date are fixed with Flemming's Fluid the discoplasma and nuclei of such blood cells are homogeneous, indicating that the latter are fully formed, or mature blood cells. These corpuscles are no longer capable of division and their nuclei give with alum-cochineal a reddish-brown stain and with hæmatoxylin a brown stain, in each case like that given in the red corpuscles of the adult animal. There still persist hæmatoblasts in which karyokinesis is very common and in which no specialization of form, such as that described for the remaining blood cells, is observable. These are the elements from which originate, not only the future blood corpuscles, but also the future hæmatoblasts. These elements form but a small proportion of the whole number of corpuscles and as they possess the power of division while the mature elements do not, the origin of these must now be considered.

In order to determine this, sections of larvæ of the eighteenth and nineteenth days hardened in chromic acid and stained with hæmatoxylin and eosin must be examined. If a section through the sinus venosus be under observation it will be found that that cavity contains a large number of blood corpuscles which, according to the staining effects of the two dyes, can be divided into two classes: one, the more numerous in which both nucleus and cytoplasma show a special affinity for the eosin, the former being often stained only with this dye; the other, comprising corpuscles in the nuclei of which the hæmatoxylin alone has reacted. Both classes of corpuscles are fairly represented in Fig. 15, *a* and *b*, the

greenish elements of the cytoplasma in both being yolk spherules colored by the reduction of the chromic acid. In the corpuscles at this stage karyokinesis is not more common than it is in ordinary tissue cells. It would appear that the more numerous class of corpuscles, *i. e.*, those reacting deeply with eosin, become converted into the mature blood cells existing in the larva up to the twenty-fifth day, for it is these cells only which illustrate the specialization of form and structure already described and partly represented by Figs. 19-21. The cells which react with hæmatoxylin alone constitute the persistent elements which ultimately become the frequently dividing hæmatoblasts of the later stages of development. The eosinophilous cells are apparently in a condition of degeneration, for the division of their nuclei is not always followed by a division of the cell (Fig. 18). Both classes of hæmatoblasts at this time do not specially illustrate division but those which stain with hæmatoxylin only seem to retain the capacity for proliferation while the eosinophilous elements gradually lose it within the next ten days.

At a period which seems to coincide with the formation of the liver as a vascular organ and with the development of tubules in it, the hæmatoblasts, which, from the sixteenth to the nineteenth day, when hardened in chromic acid, stain with hæmatoxylin only, now begin to acquire a capacity for proliferation far in excess of that which they previously had. It would appear that this change is associated with the appearance, in the blood vessels of the body generally and of the liver specially, of a serum which stains very deeply with eosin. This serum stains slightly with alum-cochineal but greenish-blue or green, like the yolk spherules, with the Indigo-carmine Fluid described in the foregoing pages. I regard this staining capacity of the serum as due to the solution of yolk or rather of that constituent of it which has been called hæmatogen by Bunge. This is but a reserve form of chromatin and as the undifferentiated hæmatoblasts float in the serum, it is reasonable to believe that they absorb the dissolved chromatin. It is from this time on that the hæmatoblasts begin to manifest the incessant divisions which characterize the stage represented by Figs. 9, 10 and 11. It is at this time also that the chromatic figures of the hæmatoblasts increase in size. Previously their figures were not larger than those of the other cells of the body. These facts can be explained in no other way than by assuming that the hæmatoblasts surviving as such, absorb the chromatin or "hæmatogen" which is dissolved in the serum and thereby entered on a phase of renewed vitality. The other cells in the body also exhibit divisions now more frequently than before this stage, though not by any means as frequently as the hæmatoblasts, and this increased capacity for proliferation may also be explained

by the more abundant supply of dissolved chromatin in the serum bathing them.

These hæmatoblasts are met with most frequently in those parts of the circulatory apparatus where the blood current is slow or where physical conditions retard their movement. Such conditions are found between the muscle trabeculæ stretching through the heart cavity after these are formed, in the concave portions of the aortic arches and especially in a minute branch of the arteria mesenterica distributed in a plate of tissue derived from the visceral layer of the mesoblast. This is the site for the future spleen. The origin of the spleen in the visceral layer of the mesoblast in the toad was pointed out by Goette* who described the cells of the organ as direct descendants of the yolk cells (entoblastic cells). My observations are not yet concluded in the development of the spleen, but they have progressed so far as to allow me to say definitely that the organ increases in bulk by multiplication of the capillaries arising from the branch of the mesenteric artery to accommodate the excessively large number of hæmatoblasts derived by division from the original hæmatoblasts which have been caught in the narrow spaces of the capillaries, early in development of the organ. At a date roughly corresponding to the interval between the fortieth and sixtieth days, sections of the organ fixed in Flemming's Fluid and stained with hæmatoxylin and eosin, contain a very great number of elements like those represented in Figs. 10 and 11. In fact sections of the organ thus prepared have a deep ochre-red or terra-cotta-red color, owing to the great number of mitotic hæmatoblasts present in it. At later stages of development hæmatoblasts are rarely found elsewhere than in the spleen, which is, from now on, the organ for their production out of the original elements whose history has been traced above and whose presence in the spleen is to be explained as I have pointed out. Whether there is a secondary formation of hæmatoblasts out of the cells of the original tissue of the visceral layer of the mesoblast, it is impossible to say, but as the hæmatoblasts and the spleen are both formed out of portions of visceral layer, such a secondary origin is not, theoretically, improbable. All that I can at present say is that early in the development of the spleen its vascular channels become distended with hæmatoblasts, which are also to be found in other vessels of the body where the blood current is slowed or retarded, that these hæmatoblasts undergo rapid divisions and increase thereby the size of the organ and that these divisions are quite sufficient to explain the occurrence there of all the hæmatoblasts observed. The first appearance of the organ in fact consists in the

* *Loc. cit.* p. 812.

presence of a few hæmatoblasts like those shown in Figs. 10 and 11 in the channel of the branch of the mesenteric artery.

As I have never found in adult caudate Amphibia hæmatoblasts in any other organ than the spleen and then only in its blood sinuses, these may be regarded as direct descendants of the hæmatoblasts which arise by proliferation of the cells of the ventral portion of the visceral plate of the mesoblast.

It is, I think, worthy of note that though there is but one source for all hæmatoblasts, yet there are two stages in their history, the second of which appears when the liver begins to take on its adult structure, the forms belonging to this stage being remarkable for their great capacity for division, while the first series of hæmatoblasts are, almost wholly, formed in the subintestinal veins and the great majority of them are directly converted into red cells, the remainder persisting to form the hæmatoblasts of the second stage.

IV. Conclusions.

1. The hæmoglobin of the blood corpuscles is derived from the abundant nuclear chromatin of the hæmatoblast.

2. The fusiform cells of Amphibian blood are derived from the red corpuscles, the latter in this conversion losing the cell membrane and the greater portion of the discoplasma.

3. The hæmatoblasts in *Amblystoma* are direct descendants of cells split off from the extreme ventral portions of the visceral mesoblast and they pass, at first, a portion of their existence in a specialized part of the original body cavity of the embryo.

V. Appendix.*

The foregoing paper was written, part in 1889, part in 1890. The publication of it now seems opportune since one of the conclusions contained in it has been fully confirmed by the results of my investigations during the last year. *The chromatin of every cell, animal and vegetable, is an iron compound* and this can be proved not only by the use of freshly prepared ammonium sulphide, as described in a communication sent to the Royal Society of London† last year, but also by other methods since discovered, the use of which excludes inorganic and albuminate iron and, at the same time, does not affect the iron in hæmoglobin or hæmatin. With the more recently discovered methods, so easy is their application

* Written Feb. 4, 1892.

† Proceedings, Roy. Soc., Vol. 50, p. 277.

and so definite their reaction, one may make permanently mounted preparations of sections of animal and vegetable tissues, in which the distribution of the chromatin is shown by the iron reaction. The latter may thus be quite readily employed instead of the staining methods with hæmatoxylin and other dyes which, when carefully used, are supposed to select only chromatin. The results which I have obtained with the new methods are so numerous and so important that I must reserve an extended description of them for another paper. *Suffice it at present to say that the fundamental life substance is an iron compound and that, inferentially, the chemical processes underlying life, in other words life itself, are to be referred to the constant oxidation and reduction of the iron of this compound.* This iron-holding compound being present in every living cell, the mystery of the appearance, here and there in animal and vegetable forms, of hæmatin* either free, or attached to a proteid as hæmoglobin, is explained.

It is to be noted further that the iron, though not held in chromatin as firmly as it is in hæmatin, is yet as tenaciously held therein as it is in the ferrocyanides, which also yield, under the same conditions, their iron to ammonium sulphide.

The methods referred to show further that the stainable substance which diffuses from the nuclei and mitotic figures in hæmatoblasts, is an iron compound in which the iron is less firmly held than in hæmoglobin, and that it persists for comparatively a long time as such, before becoming converted into the latter substance. There are also facts which seem to indicate that hæmoglobin is a degeneration product and not a substance formed in the synthetical processes of the hæmatoblasts.

The bearing of these conclusions on the currently accepted views as to the pathology of anæmia is obvious. Since hæmoglobin is a derivative product of chromatin, and since the latter is an iron compound all important in cellular life, anæmia cannot be, primarily, a deficiency in the formation of hæmoglobin, but, first of all, a deficiency in chromatin, not only of hæmatoblasts, but of every cell in the body. In other words the primary cause of all anæmias, other than hæmolytic, is *hypochromatosis* and the condition which Virchow called *hypoplasia* is as much a result of this hypochromatosis, as is the deficiency in formation of hæmoglobin.

Other points arising out of these investigations may be mentioned: the differences between animal and vegetable chromatin and between the chromatin of highly specialized animal cells on the one hand and that of lower forms of animal life, on the other, the occurrence of hæmoglobin

*Linossier and Phipson describe (Comptes Rendus Vol. CXII, pp. 49[illegible] 666) the occurrence of hæmatin-like compounds in *Aspergillus niger* and *Palmella cruenta*

chiefly in the higher types of animal life, the analogies between chlorophyll and hæmatin and the derivation of the digestive ferments from chromatin.

These and other related subjects I intend to discuss in a future publication.

EXPLANATION OF FIGURES.

Figs. 1–4 are drawn from preparations from the adult *Necturus*, and Figs. 5-7 are taken from larval *Amblystomata* (*A. punctatum*).

Fig. 1. Red disc from a cover-glass preparation of the blood. Corrosive sublimate, Indigo-carmine Fluid—×700.

Fig. 2. Red disc from splenic vein. Chromic acid, Indigo-carmine Fluid—×700.

Fig. 3. Red disc, cover-glass preparation. Chromic acid, Hæmatoxylin, Eosin—×700.

Fig. 4. Red disc cover preparation. Corrosive sublimate, Hæmatoxylin, Eosin—×700.

Fig. 5. Red disc from heart cavity. Flemming's Fluid, Hæmatoxylin, Eosin—×1,000.

Fig. 6. Red disc from gill vessel. Osmic acid, Hæmatoxylin, Eosin—×1,000.

Fig. 7. Cover-glass preparation of red blood cells. Fresh, acetic methyl-green—×1,000.

Fig. 8. Group of blood cells from a vascular sinus in a section of the spleen of *Necturus*. In the centre is represented a hæmatoblast in mitosis and with its chromatin so changed chemically that it takes the sulphindigotate portion of the reagent; *a*, a red disc, *b* a leucocyte. Chromic acid, Indigo-carmine Fluid—×700.

Fig. 9. From a free swimming *Amblystoma* larva.

a, Hæmatoblast from the concave side of one of the aortic arches, in division showing in the abundant chromatin as well as in the cytoplasma a slate or slate-brown reaction.

b, an endothelial cell from same aortic arch in same preparation undergoing mitosis and showing the normal reaction of the staining fluid.

Flemming's Fluid, Hæmatoxylin—×1000.

Fig. 10. Hæmatoblast from concave side of aortic arch in a free-swimming larval *Amblystoma*. Flemming's Fluid, Hæmatoxylin, Eosin—×1,000.

Fig. 11. Hæmatoblast from same preparation as last—×1,000.

Fig. 12. A dividing hæmatoblast in the last stage of its development, showing two kinds of chromatin in the nuclear figures. Cover-glass preparation, Corrosive sublimate, Hæmatoxylin, Eosin—×1,000

Figs. 13–14. Hæmatoblasts in the last stage of their development, showing a degenerated chromatin between the regular chromatin loops of the dividing nuclei. From the heart cavity of a free swimming *Amblystoma* larva. Flemming's Fluid, Hæmatoxylin, Eosin—×1.000.

Fig. 15, *a* and *b*. Two hæmatoblasts from the heart cavity of a very young *Amblystoma* larva (not free swimming). Chromic acid, Hæmatoxylin, Eosin. ×1250.

Figs. 16 and 17, *a* and *b*. Amœbiform hæmatoblasts from heart cavity of a very young larva (not free from envelope). The chromatin is very dense in the nuclei. The cavities in the cytoplasma were occupied by yolk spherules.

Flemming's Fluid, Alum-cochineal—×900.

Figs. 18 and 19. Two hæmatoblasts from the heart cavity of very young larva (not free swimming). Cavities in cytoplasma occupied by yolk spherules. Fig. 19 represents a more fully developed corpuscle with well defined contour and abundant chromatin. Chromic acid, Hæmatoxylin, Eosin—×1250.

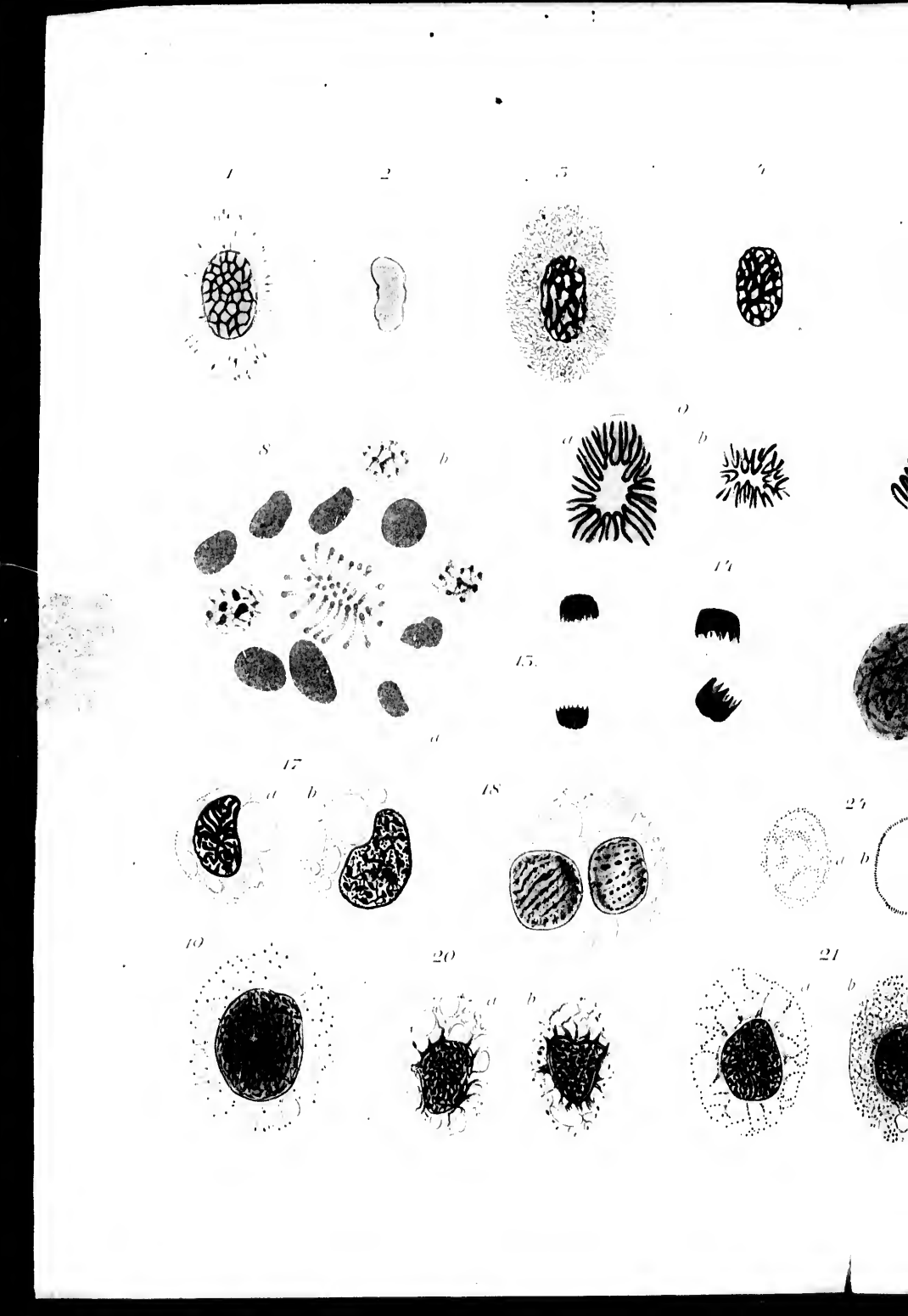
Fig. 20, *a* and *b*. Two hæmatoblasts, from a very young larval *Amblystoma*, with definite elliptical outlines, uncolored cytoplasma and the nuclei abundantly provided with chromatin. Chromic acid, Hæmatoxylin, Eosin—×900.

Fig. 21, *a* and *b*. Two hæmatoblasts from larva of same age as in last case. Flemming's Fluid, Alum-cochineal—×1200.

Fig. 22, *a–f*. Different forms of fusiform corpuscles met with in the same cover-glass preparation of *Necturus*' blood,—*b* was fixed while exhibiting, apparently, the slow vibratory motion of its thorn-like prolongations. Corrosive sublimate, Hæmatoxylin, Eosin—×1,000.

Fig. 23, *a—d*. Fusiform corpuscles of *Necturus*' blood exhibiting various intra-nuclear arrangements of its chromatin. Cover preparation, Picric acid, Safranin.

Fig. 24, *a* and *b*. A hæmatoblast (?) seen at two different optical planes exhibiting the peculiar yellowish granules (hæmoglobin?) apparently like those described by Cuenot as secreted from the nucleus—*a*, at the plane passing through the upper surface of the nucleus, *b*, at the plane passing the centre of the nucleus. There is very little cytoplasma in this cell. Fresh—×1000.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