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With the Author's
Compliments

AN INVESTIGATION

OF THE BLOOD OF

NECTURUS AND CRYPTOBRANCHUS

(*Necturus maculatus and Cryptobranchus alleghaniensis*)

BY

EDITH J. CLAYPOLE, PH. B., M. S.

This paper received the first prize in animal histology, awarded by
THE AMERICAN MICROSCOPICAL SOCIETY.

Mounted by the author

[EXTRACT FROM PROCEEDINGS. VOL. XV, 1893, pp. 39-71.]

WASHINGTON, D. C.:
JUDD & DETWEILER, PRINTERS.
1893.

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THE BLOOD OF NECTURUS AND CRYPTOBRANCHUS.

(*Necturus maculatus—Cryptobranchus alleghaniensis.*)

Awarded First Prize in Animal Histology by the Society.

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The investigation, the account of which is contained in these pages, is partly histological and partly physiological. Although there exists a large amount of literature on the subject of blood, it relates almost exclusively to that of mammals; the naturally predominant interest of man in man has caused the nature, peculiarities, and influencing conditions of this important tissue of the body to receive careful and exhaustive study in higher forms of life. In the forms below mammals, in some relations at least, the blood has not been so critically studied, and consequently a great many points are as yet obscure and undetermined. It has been the subject of this investigation to attempt to take a step into this relatively unexplored region and to make somewhat clearer the at present indefinite horizon. The conclusions have been drawn with as little bias as possible, yet the possibility exists that the facts as presented may bear other and better interpretations.*

The subject, as here considered, divides itself into three principal heads:

1. Coagulation.
2. Measurement and counting of corpuscles.
3. Ingestion of carbon by leucocytes and their subsequent distribution through the tissues.

1. Coagulation.

In respect to the first part, all discussions on the subject of blood in general give no mention as to whether the conditions of coagu-

*This paper contains the results of an investigation carried on in the Histological Laboratory of Cornell University, and was presented for the degree of M. S. in June, 1893. I wish to express my appreciation of the abundant material and facilities so generously put at my disposal.

lation are similar in these low forms to those known to exist in the case of mammals; simply statements as to the rapidity with which the process takes place are made. The conditions are definite and very well known in the higher forms, and the various authors agree as to the results obtained under various physical conditions and when acted upon by various chemical reagents.

Many difficulties exist in the investigation of amphibian blood, owing to its relatively small amount and its rapidity of coagulation. The restraining or preventive agent must be mixed with the blood immediately on the issue of the latter from the heart or vessels, and only comparatively small quantities can be used to demonstrate its action. When, however, some skill is attained as accurate results can be got as when larger quantities are used and as definite action takes place in regard to the chemical substances used.

Before entering on the discussion of coagulation *macroscopically* a few words as to the *microscopical* appearance of fibrin will here be appropriate. It is already known from the researches of Professor Gage, of Cornell University, that a great difference exists between the fibrin of mammalian and the fibrin of amphibian blood, in regard to the fineness of the constituent threads. The mammalian threads are much coarser than those of amphibian blood. As in the frog, the fibrin threads of Necturus and Cryptobranchus are so fine that they require careful observation with a $\frac{1}{12}$-inch oil-immersion objective to distinguish them. Even under the most favorable conditions a very fine network is apparent, rather than individual threads. Comparison with the fibrin of a mammal (see plate I) immediately shows the great difference between the two formations. Experiments were made with various per cent. solutions of neutral salts to determine, if possible, whether the delayed fibrin formed differently from that unmixed with chemicals. No difference in microscopical structure could be detected, either in fineness or any other respect. The fibrin thus formed had exactly the same appearance as that shown in pure blood preparations. The same methods of preparation were employed in preparing both mammalian and amphibian blood. Briefly, they were as follows: A small drop of blood, taken fresh from the animal, was put on a cover-slip; this was covered with another slip, leaving the two eccentric for convenience of handling; the unused blood was pressed out; the preparations were put in a moist chamber for from 10 to 30 minutes. When a neutral salt solution was used, a small drop was mixed with

the blood on the cover immediately, the subsequent treatment of the two being exactly alike. For mounting, the covers containing fibrin were washed with normal salt solution ($\frac{6}{10}$ per cent.) or water stained with eosin, dried thoroughly, and mounted dry in a cement cell. As both the mammalian and amphibian blood were subjected to precisely the same processes under similar conditions, the results obtained were quite comparable.

During some experiments made with various per cent. solutions of the neutral salt, magnesium sulphate (chemically pure), to find the one that best prevented coagulation and preserved the form of the corpuscles, some interesting peculiarities were observed. 1 per cent., 2 per cent., 3 per cent., 4 per cent., 5 per cent., and 6 per cent. mixtures were used, and the first three proved to preserve the form of the corpuscles well, the 2 per cent. being on the whole preferable, as the weaker one decolorized and the stronger distorted the cells more or less. With increased strength came increased distortion, although the color was much better preserved. After 12 to 14 hours the color was so far extracted from the corpuscles, even in the 2 per cent. solution, as to make the easy distinction of the cells impossible. With 5 per cent. the color was well preserved in the corpuscles even after 24 hours, and also in the 6 per cent. solution, but in the latter by that time a weak clot had formed, which increased slightly in 36 hours, remaining, however, distinctly marked even after the corpuscles were decolorized. On microscopical examination the characteristic appearance of fibrin was shown in the clot. Experiments were then made, keeping the same ratio of dilution, 1 part of blood to 50 parts of the salt solution, on the higher per cent. solutions of the salt, 7 per cent., 10 per cent., 15 per cent., and 25 per cent. The clotting took place in these also and with greater rapidity than in the weaker solutions. A firm clot appears in 6 per cent. in 6½ hours; in 7 per cent. traces of a clot appear in 2 to 3 hours; in 10 per cent., 15 per cent., and 25 per cent solutions in 30 to 40 minutes, though in 6 hours a distinct clot forms in all the solutions. After 24 hours in the strongest solutions the whole mass becomes jelly-like, most strongly so in the 25 per cent. solution, and decreasing to the 10 per cent., and only slightly appearing in the 7 per cent., the latter preserving most clearly the true appearance of the clot. Here it must be mentioned that in all the solutions the jelly-like formation increases slightly but definitely in time and the clot decreases in the same slow way.

In order to find out whether other neutral salts behave in like manner a similar series of experiments was carried out, using solutions of sodium sulphate (chemically pure) as the diluent. In this the only difficulty encountered lay in the much greater solubility of the magnesium salt; it takes 55 per cent. for saturation, while the sodium salt is saturate at 29 per cent. However, as the low per cents. were mainly used and the solutions made in an approximately uniform temperature, the difference between the two salts does not materially affect the results.

As far as preservation of form in the corpuscles is concerned, Na_2SO_4 was not nearly so successful as the magnesium salt. Even with the low per cents. distortion takes place. This may be accounted for in part by the difference in the molecular weights of the two compounds, that of $MgSO_4$ being 120, while Na_2SO_4 is 142. The density of equal per cent. solutions is consequently different, greater in the sodium salt, producing a distortion which does not take place in the lighter magnesium salt. In clotting also a slight difference is present. Indications appear in 5 per cent. solutions, while in 7 per cent. there is a firm, solid clot; the firmness increases in 10 per cent., while in 20 per cent it is a jelly-like liquid rather than a clot.

Experiments were made on the effect of these salts on frog's (*Rana catesbiana*) blood with approximately the same results. The clotting began in 6 per cent. and was very marked in 7 per cent.; it formed slowly and rather weakly in 10, 15, and 20 per cent. Na_2SO_4 and $MgSO_4$ in 2 to 3 hours. The *jelly-like* formation also took place less distinctly than in Necturus and Cryptobranchus, yet it was sufficiently clear to be unmistakable. A similar series of experiments was made on the blood of the ganoid fish, *Amia calva;* both salts were used with the characteristic results somewhat modified and not so distinct as in the frog. In $MgSO_4$ clotting began in 5 per cent. with a very slight trace, in 6 per cent. increased, in 7 and 10 per cent. still weak, while not till 25 per cent. was a good clot observed, when the jelly formation also appeared. In 5, 6, and 7 per cent. $MgSO_4$ and 7 per cent. Na_2SO_4 the clot appeared fine and granular, while the strongest clot was in a saturate solution of Na_2SO_4. In addition, the same series of experiments was made on cat's blood; the results were *uniformly negative*. No clot of any kind formed in any of these solutions, the mixture remained liquid, and even after the ninth day no tendency to clot was observed. That

TABLE I.

	Necturus and Cryptobranchus.	Frog.	Amia.	Cat.
$MgSO_4$.				
1 % ...	No clot; form of red corpuscles well preserved.	No clot.....	No clot.....	All experiments gave uniformly negative results.
2 " ...	" " " "	"	"	
3 " ...	" " " "	"	"	
4 " ...	No clot; form of red corpuscles *not* well preserved.	"	"	
5 " ...	" " " "	Slight trace of clot.....	Slight trace of granular clot.....	
6 " ...	Weak clot in 6 hours; jelly slight....	Weak clot.....	More traces of granular clot	
7 " ...	Firm clot in 2 to 3 hours; jelly slight; clot best preserved.	Weak clot and jelly.....	Granular and weak clot; weak jelly.	
10 " ...	Firm clot in 30 to 40 minutes	Weak clot and strong jelly in 2 to 3 hours.	Weak clot and weak jelly.....	
15 " ...	" " "	" " "	" " "	
20 " ...	" " "	" " "	" " "	
25 " ...	Clot present; jelly strongest.....	Strongest jelly and clot in 2 to 3 hours.	Strong jelly and clot	
Na_2SO_4.				
1 % ...	No clot; form of red corpuscles *not* well preserved.	No clot.....	No clot.....	
2 " ...	" " " "	"	"	
3 " ...	" " " "	"	"	
4 " ...	" " " "	"	"	
5 " ...	Slight traces of clot.....	"	"	
7 " ...	Firm clot and weak jelly.....	Firm clot; weak jelly.....	Granular clot; jelly.....	
10 " ...	Less clot and stronger jelly.....	Firm clot; stronger jelly...	Clot; stronger jelly.....	
15 " ...	Strong jelly.....	Weaker clot; strong jelly ..	Stronger clot; stronger jelly.....	
25 " ...	Strong jelly and weak clot.....	Weak clot; strongest jelly..	Strongest clot; strongest jelly.....	

the peculiarities were not shared by mammalian blood was clearly demonstrated.

The conditions under which this clotting takes place are exactly those which, according to all authorities on the coagulation of mammalian blood, favor the prevention of the process:

1. The addition of water to a greater amount than twice the bulk of blood.

2. The addition of neutral salts, 2 to 3 per cent. and upward; a large proportion entirely prevents the formation of a coagulum.

3. Agitation (shaking of vials to mix blood and liquid).

The proportion of the salts usually given as best are 25 to 28 per cent. $MgSO_4$, *concentrated* Na_2SO_4, *saturate* solutions of both. No statement as to the temperature under which the saturation takes place is given, however, and the amount of these salts soluble in water varies with the temperature. These solutions are commonly used in the proportion of ⅙ per cent. of blood, but owing to the small quantities dealt with here and the very rapid coagulation their exact use is impossible, as only by immediate mixing of the two liquids direct from the vessels of the animal can coagulation be prevented. But a moment's consideration shows that the methods used—dilution of 50 parts of water to 1 of blood, greater proportion than 2 to 3 per cent. of neutral salts, and thorough shaking of the vials containing the mixtures—combined the various retarding agencies and should have exerted a retarding action or have totally prevented the formation of the fibrin. The facts, however, show that even under these conditions the formation of fibrin takes place in from thirty minutes to four hours, according to the strength, in all solutions over 5 per cent., those mixtures under 5 per cent. remaining clear and liquid. Together with the appearance of the clot in higher solutions comes the jelly-like change previously noted. As to the exact stage when the strongest fibrin formation takes place, if the jelly is debarred from that name, a considerable variation exists, according to the salt and blood used. In Necturus and Cryptobranchus, with $MgSO_4$, 7 per cent. was approached as a maximum, on each side being a decrease in the clot; but in the strong solutions the jelly increased with the lessening of the coagulum, and also with the passage of time the true clot became to some extent slightly obscured, but never disappeared—a fact which tends to show that the jelly was not a secondary formation resulting from the destruction of the true fibrin, but rather an independent one, brought

about by conditions additional to those favoring the production of the ordinary fibrin. This may be called *additional fibrin*, and, according to Semmer's researches, which are now to be considered, does not differ in its essential nature from fibrin obtained by ordinary methods.

A paper by George Semmer, published as an octavo, in Dorpat, in 1874, entitled "*Ueber die Faserstoffbildung im Amphibien und Vogelblut*," gives the results of the author's investigations in a similar direction, including, however, as indicated by the title, bird as well as amphibian blood. Very careful investigations were made on the blood of the frog and domestic fowl under conditions, however, that vary somewhat from those given in the foregoing pages. In the first place, *defibrinated* blood alone was used for all the experiments; and, secondly, as a diluent a solution of soda. The jelly-like or *additional clot* formed under these circumstances also, and further investigations were made to determine, if possible, the exact origin of the substances from which it came. All the various known and possible sources of fibrin were eliminated—*i. e.*, by whipping the blood to defibrinate it in the ordinary way, then carefully decanting it to remove the serum and white corpuscles; and, further, by solution, the hæmoglobin of the red cells. With these parts eliminated the author induced the *additional fibrin* by means of the salt solution. In consequence of these results he concludes that the fibrin owes its formation to substances contained in the protoplasm of the red cells in the blood and set free by the solvent action of the salt which had been been added. In all essential respects he believes there is no difference between the fibrin thus artificially formed and that naturally produced, and that probably, if not exactly alike, they are very closely allied forms. These fibrin-forming materials belong to the red corpuscles of birds and amphibia, excluding those of mammals, which, under no similar conditions, show the least tendency to act as fibrin-bearing bodies.

If the experiments with normal are compared with those on defibrinated blood some very interesting facts become apparent. The small clot that so uniformly appears in the stronger solutions, beginning usually with 6 per cent., is the true fibrin; with the increased amount of the salt the solution of the red elements begins to take place, and consequently the formation of an *additional fibrin*,

which attains its maximum quantity in the highest per cent. solutions. Whether the true fibrin in these solutions is slowly dissolved by the neutral salts or the elements forming it are converted to the additional fibrin remains an open question. But probably both actions take place to some extent, since the clot in the higher solutions never appears as large as in the lower ones and also suffers a distinct though small decrease on the prolonged action of the salts. Another point which must also for the present remain unexplained is the much more rapid formation of the true fibrin in the stronger solutions. This is against all precedent in mammalian blood and is difficult to account for. That the neutral salts here play a significant part in hastening the fibrin-formation is beyond doubt, but their precise action on the different constituents remains for future investigation.

As a result of the above experiments the following can be stated :

1. In amphibian blood (*Necturus*, *Cryptobranchus*, *Rana*) and ganoid blood (*Amia calva*) true fibrin forms in the presence of strong solutions of neutral salts, (5 to 25 per cent. $MgSO_4$, 5 per cent. to a saturate solution of Na_2SO_4).

a. The formation takes place in the presence of a large quantity of water, (50 parts to 1 of blood).

b. It takes place with agitation in from 30 minutes to 6 hours, (shaking vials frequently).

2. In all solutions above 5 per cent. there is an additional formation of a fibrin-like body, the firmness of which increases in proportion to the strength of the salt solution.

From these facts it is seen that a wide gap separates the forms of life here represented from those which are usually examined for coagulation experiments. An interesting question arises as to the action of the same salts on reptilian and birds' blood. That the results would be comparable with those obtained from amphibian blood is highly probable, a probability that would make a generalization as to the greater distinction between nucleated and non-nucleated corpuscle-bearing blood, in regard to fibrin formation, perhaps possible. However, there exists an immense field in the various forms of fishes, Marsipobranchs, Elasmobranchs, Ganoids, Teleosts, and Dipnoans, where practically nothing is known concerning the action of the blood toward these salts. No more than a suggestion is as yet warranted.

2. Measurement and Counting of Corpuscles.

A.—Measurement.

A great difference exists between animals with non-nucleated and most of those with nucleated corpuscles as to the distribution of the blood corpuscles in larger or smaller elements. A difference also exists between reptiles and amphibia, and from this point of view, at least, the latter occupy a much lower position.

In general appearance the red corpuscles of Necturus and Cryptobranchus do not differ much, excepting in size, from those of other amphibia. They are oval, nucleated and plate-like, slightly biconvex and of a pale yellowish color. The stroma is finely granular and darker than the nucleus, which shows more or less distinctly a dark network (Pl. I). After a fresh preparation (prepared by simply covering a small drop of blood with a cover-slip and sealing with a neutral oil) has stood for half an hour or more in a moist chamber, an overlapping tile-like arrangement, which represents the rouleaux in mammalian blood, appears (Pl. I). In addition, a rosette form, commonly observed in the corpuscles of the frog, is also present, arranged about a larger or smaller mass of leucocytes.

Fresh preparations were used exclusively, and when simply sealed with castor oil the elements preserved their form and color excellently for several hours. All measurements were made by the Zeiss micrometer-ocular, with a movable scale and Reichert's no. 7 objective. The value of the divisions of the micrometer with this objective was determined by comparison with a Ewell stage micrometer ruled to .01 mm.

As in other questions of measurement, the results given in the following pages can be taken as of only relative correctness. The difference of instruments, circumstances, and of observers unite to form a considerable area for variations, even when the greatest care is taken to be accurate. The average size of fifty corpuscles in different individuals of Necturus and Cryptobranchus was obtained as shown in the following specimen table. L equals the long diameter, B equals the short diameter. In the ocular-micrometer scale one space equals three microns, with the combination of objective and eye-piece used.

TABLE 2.

Measurement of 25 Red Corpuscles.

Cryptobranchus.				Necturus.			
B.	L.	B.	L.	B.	L.	B.	L.
10.9	16.0	9.8	15.0	9.0	24.5	9.5	21.3
10.0	16.0	12.0	18.0	11.0	20.0	9.2	20.9
10.7	16.3	10.0	15.1	9.5	21.3	10.5	20.0
9.1	16.1	10.5	17.0	10.0	21.2	12.5	17.0
10.0	16.0	11.0	18.0	9.0	22.0	11.5	17.0
8.0	15.5	11.0	17.0	8.0	21.0	10.0	18.0
10.0	17.0	11.0	16.8	8.0	23.8	11.1	17.0
10.0	16.0	11.0	18.1	9.0	21.0	9.0	20.1
10.2	16.1	9.0	15.0	11.5	21.0	8.6	21.0
10.0	14.1	11.0	17.3	8.0	25.5	9.1	25.0
9.6	14.4	12.0	18.4	9.0	25.0	10.0	24.0
9.0	16.0	10.0	15.3	10.3	20.5	11.1	22.0
11.4	15.6	9.0	16.5	10.0	22.0	9.2	23.1
11.2	17.8	10.0	17.5	11.4	21.3	10.1	24.0
12.0	16.0	10.0	16.6	9.0	21.0	11.2	25.6
11.4	18.0	10 5	19.3	10.0	20.0	10.6	20.5
10.2	17.3	10.1	17.0	10.0	21.4	8.0	23.0
9.8	17.0	10.3	16.0	11.0	20.0	9.0	24.2
9.4	15.8	9.0	18.8	8.0	21.0	10.1	20.6
10.4	19.0	10.0	16.3	11.0	20.5	11.4	22.0
11.0	15.5	11.5	18.0	7.0	25.6	8.1	20.0
11.2	16.0	10.0	19.3	11.0	22.0	9.3	22.0
11.0	17.4	11.7	15.0	11.3	22.5	11.0	20.6
10.1	17.2	9.0	15.2	8.5	22.0	9.4	19.7
10.0	17.0	10.0	14.6	9.5	21.0	10.7	23.6
256.6	409.1	260.5	401.1	240.0	547.1	250.2	532.0

Av. of B = 10.35 × 3 = 31.05 μ. Av. of B = 9 80 × 3 = 29.40 μ.
" L = 16.20 × 3 = 48.60 μ. " L = 21.58 × 3 = 64.74 μ.

From fifty corpuscles in five different individuals of *Necturus maculatus* come the following results, (L = the long diameter and B = the short):

1. L = 50.9 μ B = 30.6 μ
2. L = 55.5 μ B = 31.8 μ
3. L = 58.8 μ B = 31.5 μ
4. L = 64.7 μ B = 29.4 μ
5. L = 62.1 μ B = 32.4 μ

An average of these different figures gives L = 58.4 μ and B = 31.1 μ. While a considerable amount of uncertainty exists as to the exact truth represented by these figures, they probably express approximately the true conditions. The cells exhibit so much variation that a large number of individuals is required to obtain more approximately true results. Only two individuals of Cryptobranchus were examined, 100 corpuscles in each being measured with the following result:

1.	L = 48.6 μ	B = 31.0 μ
2.	L = 48.7 μ	B = 27.5 μ

The chief variation here, it is seen, appears in the short diameter, the long one being nearly constant. According to Dr. Leon Vaillant, in *Siren lacertina* a range of variation occurs between L = 54–58 μ, B = 24–27 μ. This author quotes Gulliver as giving L = 62 μ, B = 33 μ, correcting the latter's figures by his own. Supposing the conditions of measurement to be approximately alike, the results obtained by both authors could be correct if the variations found to exist in the case of Necturus were assumed to be present in Siren also, an assumption by no means improbable. Brass gives the size of the corpuscle of *Proteus anguineus* as L = 58 μ, B = 33.7 μ, and those of *Megalobatrachus maximus*, the great Japanese salamander ,as L = 47 μ, B = 33 μ. Tabulating these results, the following is obtained by averaging the different results:

Proteus	L = 58.0 μ	B = 33.7 μ
Necturus	L = 58.4 μ	B = 31.1 μ
Siren	L = 58.0 μ	B = 28.0 μ
Cryptobranchus.....	L = 48.7 μ	B = 29.2 μ
Megalobatrachus	L = 47.0 μ	B = 33.0 μ

If from these figures a generalization be made, it is seen that in the external gill-breathers the red cells average 10 μ longer than in the forms in which external gills are absent. In width, however, results are contradictory, and, moreover, Riddel gives the corpuscles of *Amphiuma tridactylum* as the largest known—one-third larger than in Proteus. This statement has been corroborated by Lamb, and corpuscles preserved in glycerine jelly have been examined and measured by the writer. Comparing them with the corpuscles of Necturus similarly prepared, the great difference in size could be seen, the relative shrinkage being probably the same in each case.

If compared with the size of the red cells in the frog and largest mammals, the truly gigantic size of these corpuscles can more easily be realized. For the frog Ecker gives L = 24 μ, B = 17 μ; for the triton and salamander, L = 25 μ, B = 16 μ; Brass, for the sturgeon, L = 13 μ, B = 10 μ; Welcker, for the elephant, D = 9 μ (the largest mammalian red blood corpuscles). In camels, L = 8 μ, B = 4 μ.

TABLE 3.

Size in Microns of the Red Corpuscles in Different Animals.

Oval corpuscles.				Circular corpuscles.		
Animal.	L.	B.	Authority.	Animal.	D.	Authority.
Amphiuma	75			Elephant.	9	Brass.
Proteus	58	35	Brass.	Goat	4	Brass.
Siren	56	27	Vaillant.	Sheep	5	Brass.
	62	33	Gulliver.	Beasts of prey.	4.7 to 8.7	Brass.
Necturus	58.4	31.1	E. J. Claypole.			
Cryptobranchus	48.7	29.2	E. J. Claypole.			
Megalobatrachus	47	33	Brass.	Apes... Man...		
Rana	24	17	Ecker.			
R. esculenta	22	17	Stricker.			
R. temporaria	25	16	Brass.	Musk-deer.	2 5	Brass.
R. viridis	24.4	16.3	Hayem.			
Triton	25	16	Brass.			
Lepidosiren	41	29	Welcker.			
Carp	15	9	Brass.			
Sturgeon	13	10	Welcker.			
Lizard	15.9	9.9	Brass.			
Domestic fowl	12	7	Brass.			
Duck, Pigeon	15	6	Brass.			
Camel, Llama	8	4	Brass.			

To get comparative measurements of the leucocytes it is necessary for them to be spherical. By mixing the blood with a drop of 1 per cent. osmic acid or 4 per cent. $MgSO_4$ the cells assume the desired form, care being taken only to measure those presenting true circular outlines. The number of cells measured was not equal in the two animals; in Cryptobranchus the average obtained from 100 cells is D = 18.5; from only 20 of Necturus, D = 28.5. It can be seen that even by considering the latter but a rough approximation

to the truth an appreciable difference exists between the two, comparable with that already shown to exist between the red corpuscles in these animals. In the drawings also, made from dry preparations, it is apparent that such a difference exists, though no definite idea of the relative condition of expansion present can be obtained (Pl. II). In the study of living cells in amœboid phases the difference in size may also be noted.

B.—Counting of Corpuscles.

Before giving the results obtained from this part of the investigation a few words as to the methods here used will be appropriate.

For a diluent a 2 per cent. solution of $MgSO_4$ proved to be the most successful, if used within two to six hours; a stronger solution, although preserving the color better, produces such an amount of distortion and massing of the cells as to make accurate counting impossible. For preservation of form, 1 per cent., 2 per cent. and 3 per cent. solutions of this salt were good, 2 per cent. being the best, as usually no distortion appeared in it, which occurred to a slight extent in 3 per cent., and also there was no great loss of color. (For further details see the discussion on Coagulation, page 42.) By means of a carefully graduated tube a definite amount of the liquid, 1,225 c. mm. was put in a small homœpathic vial. From this another smaller pipette, graduated to show 25 and 50 c. mm., was filled up to the 50 mark from the previously measured liquid, and then carefully emptied to 25; (this is to insure the subsequent filling up to 50 and the immediate mixing of the blood and salt solution.) The pipette ready, the heart of the specimen is exposed, a large vessel cut, the pipette inserted, and 25 c. mm. drawn in. Immediately this amount is mixed thoroughly with the $MgSO_4$ in the small vial. By this process a mixture of 1 to 50 is obtained. After thorough mixing a drop is placed in the counting cell (25 mm. deep, ruled to 1 mm. squares), covered, and allowed to settle on a plane surface. Then by taking an average from the different numbers contained in 100 of the squares and calculating the cubic contents of the cell, the number of corpuscles in a cubic millimeter of the blood and salt solution is found. As a dilution of 50 times exists, multiplying the results obtained by the above process by 50, the actual number present in a cubic millimeter of pure blood can be found.

TABLE 4.

Counting of Corpuscles.

Necturus.				Cryptobranchus.			
1	2	2	3	4	9	6	7
4	1	4	3	10	9	10	6
1	3	1	2	5	6	6	6
1	0	2	4	7	7	10	12
3	3	5	3	6	8	10	9
2	2	1	3	8	4	8	5
0	4	1	2	5	9	5	7
3	3	3	4	6	8	5	8
3	0	2	3	11	10	7	7
2	3	2	3	7	10	7	9
2	4	3	3	8	6	10	8
3	2	3	4	7	5	8	4
3	2	1	5	7	7	8	9
4	1	3	2	8	8	6	6
3	3	1	2	6	6	4	6
3	5	6	5	6	6	7	7
2	2	2	5	3	6	8	6
4	5	3	3	6	4	8	7
4	2	2	3	8	4	7	9
6	4	3	4	6	6	7	7
2	6	3	5	9	9	7	7
4	2	3	5	8	7	9	10
1	4	0	3	7	6	7	4
6	5	4	4	6	7	4	6
3	4	1	5	7	6	7	9
70	72	61	88	171	173	181	181

291 in 100 squares.
291 ÷ 100 = 2.91 in 1 square.
Cubic contents of the cell = .1 × .1 × .25 = .0025 cubic mm.
2.91 = the average no. in 1 square.
2.91 ÷ .0025 = 1,164, no. of corpuscles in 1 cubic mm. of the diluted blood.
1,164 × 50 (amount of dilution) = 58,200, no. of corpuscles in 1 cubic mm. of undiluted blood.

706 in 100 squares.
706 ÷ 100 = 7.06 in 1 square.
Cubic contents of the cell = .1 × .1 × .25 = .0025 cubic mm.
7.06 = the average no. in 1 square.
7.06 ÷ .0025 = 2,824, no. of corpuscles in 1 cubic mm. of the diluted blood.
2,824 × 50 (amount of dilution) = 141,200, no. of corpuscles in 1 cubic mm. of undiluted blood.

The results obtained in Necturus from freshly caught, healthy animals are given below, the following precautions being observed: to make the dilution of the blood immediately on opening the

cardiac vessels, as the numbers resulting from dilutions made from the same individual before and after excessive bleeding are strikingly different, in all counts, to fill the cell twice to insure using a good sample of the mixture:

1.....	58,200
2.....	58,800
3.....	56,800
4.....	60,000
5	53,600
6.....	52,400

339,800 ÷ 6 = 56,633, average.

In these results a large variation occurs, which arises from two causes at least: first, the greater or less uncertainty in methods; second, the condition of the animal at the time of examination. The first cause is unavoidable. As can be seen at a glance, a very small error in the first figures of the calculation will bring a large difference in the final result, and when the chances of misjudging the exact amounts measured are considered, there is seen to be reason enough for a variation of this amount; but when the second point is examined yet greater chances for variation are apparent. Even assumuming the animals to be in the same condition, the following figures, obtained from the same individual in different stages of bleeding, are significant: The first count gave 53,600, the second 45,800, and the third 33,800 per c. mm.; a steady decrease is present, while in one specimen that had been bled for laboratory use and kept for a considerable time the small number of 21,000 was present. From these results it is apparent that a large amount of uncertainty exists in dealing with animals possessing so small an amount of blood and those in which coagulation occurs so rapidly. It is absolutely necessary to have some of the diluent in the pipette in which the blood is measured or else by the formation of a coagulum many corpuscles become entangled, and consequently the results are vitiated. Moreover, after bleeding has occurred to some extent enough corpuscles have been removed to leave the serum largely predominant, and small numbers again result from this cause. To obtain entirely satisfactory results a large number of experiments is needed on animals in the same physical condition, and that as nearly normal as possible.

When these figures are compared with those given of other forms a great difference is seen to exist. In mammals the number varies between 3,000,000 and 18,000,000 per cubic millimeter, depending on the kind of animal used. In birds the average lies between 1,000,000 and 4,000,000, while in fishes the number is still lower. From the researches of Professor Gage, of Cornell University, the numbers present in two forms of lampreys, *Petromyzon marinus* and *Petromyzon branchialis*, are, for the first, 362,889 as an average number, a variation existing between the two sexes, and 500,000 for the latter form. From personal observation in the frog, *Rana catesbiana*, there were found to be 229,000 per c. mm. Assuming that a maximum amount of hæmoglobin or oxidizable material is essential, then the inference that the larger the corpuscles the smaller the number present is made safely, since there is a physical impossibility forbidding the occurrence of both large cells and large numbers in the same animal, if the same amount of blood-forming material exists in both those possessing large and those having small corpuscles. On this principle, as would be inferred, a larger number (138,600 per cubic mm.) is present in Cryptobranchus than in Necturus (see table on page 10); but as this is obtained from only one count, and so small a range of observation forbids the acceptance of these figures otherwise than provisionally, they may, however, be received as indicating the tendency which the above generalization embodies.

3. Ingestion of Carbon by Leucocytes and their Subsequent Distribution in the Tissues.

The power of leucocytes by virtue of their amœboid movement to take up foreign substances, giving to them as it were the office of scavengers of the animal body among their other duties, possesses great significance in the eyes of the physiologist, especially from pathological outlooks. The phagocyte theory of Metschnikoff, which to-day has so many adherents, is based on this power and a large amount of pathological investigation has been undertaken to prove, if possible, the true part played by these cells in diseases which owe their existence to the presence of foreign organisms and foreign matter in the body.

In Necturus and Cryptobranchus the process of ingestion, both on account of the size of the cells and their activity in the ordinary

temperature of the room, can be most favorably observed. Mixing a little carbon with the blood, the leucocytes may be seen to take up the foreign matter while passing through their amœbôid phases. In Necturus especially these phases are most beautifully exhibited, and by quickly drying a preparation when the cells are well spread they can be preserved in their extended condition (Pl. II). The carbon is exclusively contained in the cell-body, although the nuclei also exhibit active amœboid phases. From stained preparations these can be distinctly seen (Pl. II). Often two or sometimes three nuclei are present in one cell (Pl. II). In the one figured, two of the three nuclei are in amœboid motion, while the one is in a resting state. From the active movement and continued extension of the protoplasm into delicate pseudopods the carbon becomes massed in the central part of the cell in many cases, giving the appearance of its being in the nucleus, but that such is not the case is shown by the gradual movement of the granules across the surface of the nucleus, and, if watched for a sufficient period, it will become evident that they are contained in the cell-body and not in the nucleus. Sometimes these cells containing two or even more nuclei divide while under observation; such a one is figured in plate III. The division of the single nucleus had, of course, taken place previously, but the gradual breaking away of the two parts of the protoplasmic body was observed and the times between the successive stages noted. Often in a double-nucleated cell division appears imminent, the two parts being united only by a thin thread of protoplasm; but this may gradually thicken and the cell remain undivided. Such observations give a vivid idea of the activity of life in these simple cells and make it better understood how they can play the part they are shown to take in the following experiments on ingestion of foreign substances.

To ascertain the ultimate fate of foreign substances when they are introduced into the animal body and the means by which the material is disposed of has been the aim of an immense amount of investigation. Experiments have been made and existing pathological conditions studied, resulting in a great mass of literature and, as usually occurs, many conflicting statements. From the different ways in which, pathologically, under natural conditions foreign material comes into the system—that is, by inhalation and a consequent abnormal state of the lungs, by external contact as through wounds, and by the setting free from the blood itself mate-

rial which circulates freely—a condition resulting in melanosis—this question has been attacked from the following standpoints:

1. By artificially introducing into the circulatory system, directly, different materials.

2. By introducing foreign materials through subcutaneous injection, indirectly, into the circulation.

3. By compelling the inhalation under artificial circumstances of various dust-like materials and by forcing into the lungs substances, organic as well as inorganic.

Especially in these researches is there a conflict of opinion as to the part played by the leucocytes or wandering cells, both as to their ultimate fate and their attitude toward the foreign bodies. Do they simply form a means of transport to the various tissues and there in some way discharge their load and pass again into the circulation, or do they suffer the same fate as the foreign substance, which is either deposition in the tissues or ultimate removal from the organism? Is their presence essential or non-essential? These are questions receiving various answers. Many questions also arise as to the relations of the cellular constituents of the various organs to the foreign matter, especially in those concerning whose individual structure controversy exists.

To give here an adequate idea of the already published results is impracticable; merely a few of those whose works have been consulted will receive especial mention. In investigations on the effect of introduction through the lungs, the nature of the foreign material differs according to the different investigators. Some introduce into living or dead animals soluble substances; others use finely granular material suspended in a liquid, (Slavjansky, Schestopal). Another series of investigators adopted organic forms, (Sommerbrodt, Nothnagel), and, finally, the last class adopted the inhalation of dust-like materials suspended in the air, (Ruppert, Schottelius, Arnold). The latest researches have been made by Dr. Julius Arnold, whose results were published in 1885. A long bibliography of 264 writings on this subject is included in his publication, which makes it particularly valuable.

In the method of vascular injection for the sake of easy recognition in the various organs, cinnabar suspended in normal salt solution received most general use. Ponfick, Hoffmann and Langerhans made investigations in this direction, the last two exclusively, the first chiefly, on mammals. The rabbit, guinea-pig, cat, dog, rat and

other animals have been used by these investigators, to which list must be added the indispensable frog.

The results of the different methods vary. In many respects they are contradictory, so that a general statement of the condition in which the work stands is impossible. Even the question as to the presence of a deposit of free coloring material remains in controversy. Ponfick emphatically states that no free deposit took place in his experiments, the foreign material being exclusively contained in cells of various kinds. On the other hand, Hoffmann and Langerhans, in examination of the respiratory organs, which were untouched by Ponfick, find the foreign coloring material in a free state. The distribution of the introduced substances also varies. Arnold does not think that transfer from the lungs to the general circulation takes place regularly, and from his extensive work the word of this author is of great weight. Slavjansky, on the other hand, considers this transfer a regular occurrence.

One practice, however, is common to all the investigators whose works have been consulted; that is the introduction into the system of a very large amount of foreign material—1.5 to 3 c. cm. into a frog and from 8 to 10 c. cm. into guinea-pigs and rabbits, in one case of a dog 210 c. cm. being introduced by successive injections. Under such circumstances, that free granules pass into the tissues is by no means surprising, nor, moreover, considering the weight of cinnabar, is it to be expected that the normal action of the various organs remains unimpaired. A pathological condition must result to a greater or less extent and the true action of the leucocytes toward the injected material be obscured. To ascertain with exactness the true normal relations existing between these elements and the foreign substances, no more than can be disposed of ought to be injected. If no free material enters the blood and afterwards free material or material in cells other than leucocytes is proved to exist, there is then a definite point on which to base a discussion. So far as possible these conditions have been preserved and the results obtained are given in the following pages.

The low forms represented by Necturus and Cryptobranchus have not (the frog excepted) been studied in regard to this point, in consequence, probably, of the strongly pathological aspect which the question presented to the investigators. From that standpoint the highest mammals available for experimentation showed conditions most similar to those encountered in human pathology; but on

account of the relatively simple structure and large size of the constituent parts in these amphibian forms, they offer very favorable conditions for an investigation of this nature. Especially is this true in regard to Necturus. The presence of ectobranchiæ affords an excellent opportunity for examination of the circulation without any injury to the animal, and by careful watching the times of the appearance of the coloring substances in and the disappearance of the same from the blood can be determined. The following modifications were made in the methods usually adopted:

1. The coloring material chosen was a mixture of gum arabic, lamp-black and normal salt solution (1 gr. carbon, 1 gr. gum arabic, 15 to 20 cc. H_2O + .6 gr. NaCl).

2. The injection was made in two places:

a. Hypodermically into the abdominal cavity.

b. Into the jugular vein.

3. At no time was more than 1 cc. of the mixture injected; usually ¼ to ½ cc.

In cases of abdominal injection the time was necessarily prolonged beyond that required by venous injection, since some days must elapse before the foreign substance enters the blood through means of the lymph circulation. One fact, however, is ascertained: that the carbon particles must enter the blood this way in cells, as even four days after injection, when the injected material was not in the blood, no free carbon appeared in the abdominal cavity, all having been ingested by leucocytes.

In a few words will be given the results of the experiments regarding the appearance of cells containing carbon in the circulation and the disappearance of free carbon from the blood. A variation to a considerable degree existed on the first point. The earliest time was six days after abdominal injection when ingested cells appeared in the circulation, and the longest nine days. After sixteen days a few cells still remained. In venous injection no free carbon was found after the second day, but ingested cells remained in a small number up to the eighteenth day, when the animal was killed. The tissues, however, in cases of venous injection were not so thoroughly investigated, so the results given apply chiefly to abdominal injection.

In the microscopic investigation of the different organs the first difficulty lay in the presence in nearly all the tissues of a large amount of natural pigment, which was confusing both on account

of the similarity in color and the necessary obscuring by such large masses of the structural parts. Caustic potash destroys melanin, but boiling is necessary, a process which also destroys the tissues. From consultation with various authorities, ether, alcohol, acids, and strong alkalies were found to decolorize melanin, but the last two have a destructive action on tissues and the first two produce decolorization so slowly that they are practically useless. By means of hydrogen dioxide (H_2O_2) most successful results were obtained. Placing the sections, when cut and fastened to the slides, in a small vial of the liquid (10 vol. or 2 per cent. sol.), the melanin could be reduced to a pale yellow color in from 6 to 48 hours, depending on the amount of pigment present and the light in which the glass stands; strong sunlight materially hastens the process. If the treatment is continued longer the coloring material can be entirely removed, and by watching the process a little carefully the decolorization can be stopped at any point before absolute removal. Practically it was found to be of great advantage to leave sufficient color to mark the position of the pigment cells. This liquid, strong as it is in action, does no appreciable injury to the structural elements; when the tissues have passed through the processes necessary to prepare them for examination the details are as distinct as under ordinary circumstances and no chance remains to mistake the ingested cells when they occur. That hydrogen dioxide likewise decolorizes the pigment of mammals, at least that in the choroid coat of the eye, was proved by experimentation on the eye of the sheep. A piece of the tissue was put in bulk into a vial of hydrogen dioxide and in a few days it presented a yellowish hue, the deep black color being entirely destroyed.

This difficulty removed, the investigation to determine the location of the carbon in the different organs could be begun. The following parts were investigated: the spleen, kidney, ureter, liver, lungs, stomach, muscular and epidermal tissues. It was found to be necessary, in order to determine exactly the location of the carbon, to make serial sections of the different organs; by this method the structure of the organs themselves also was much more easily studied and in many cases points otherwise obscure were made clear. In many cases what did not appear distinctly in one section became clear after a series had been examined, and facts were learned that without this method could not have been ascertained. In all cases nvestigated, if the carbon had made its way into the circulation,

the ingested cells were scattered through the various tissues. The largest amount appeared in the spleen, the next largest in the kidneys, next in the liver, and in the remaining organs in about equal amounts. Considering each organ in turn on this basis of distinction, the spleen receives first notice.

1. Spleen. (Pl. IV.)

In Necturus and Cryptobranchus, as in higher forms, this organ consists essentially of a strong, fibrous capsule, from which fibrous trabeculæ pass in all directions, forming a supporting framework for the cellular structures. In this perplexing organ, owing to the obscurity of structural details, great difficulty exists in determining the exact relation between the carbon and the constituent elements. The cells containing carbon could be separated into two kinds: (1) leucocytes; (2) spleen-pulp cells. Those occurring in blood vessels were undoubtedly leucocytes, and from their peculiarity the spleen-pulp cells could be definitely distinguished. They were close to the fibrous tissue composing the malpighian bodies and often upon it; they were large, with very long protoplasmic processes, in which the carbon granules were contained (Pl. IV). The method of distribution of the carbon granules in the leucocytes was very different from that present in the splenic cells; while in the latter the individual granules are scattered fairly evenly through the protoplasm, in the former they are massed into heaps and make the whole cell appear black. The peculiar, even distribution of the granules strongly suggested a free deposit in the fibrous tissue, the extreme transparency of the protoplasm in the splenic cells adding to the appearance, and only after long and careful observation was it ascertained that such was not the case. On the establishment of this fact the old vexed question arises as to how the carbon particles get into the true splenic cells. It has already been shown that all the carbon entering the circulation is inclosed in cells. By what means is a transfer made from these cells of the ingested material to the splenic-pulp cells? For the present this question must remain unanswered; later a suggestion will be offered.

2. Kidney. (Pl. V.)

As in all Urodeles the kidney in Necturus and Cryptobranchus is an elongated club-shaped organ, with one renal vein between the

two parts, receiving branches from both, and being continued into the post-cava. The malpighian bodies lie toward the ventral surface, and usually are not completely filled by the glomeruli, leaving a lymph space between the two parts. The neck of the urinary tubule leading from the malpighian body is lined with small epithelial cells, bearing cilia (Pl. V) that exceed in length the diameter of the tube. In addition to these parts, which exist in the kidneys in higher forms, there are the nephrostomes opening into the abdominal cavity and communicating by tubes with the first part of the urinary tubule after it leaves the glomerulus. One funnel may communicate with two tubes or *vice versa*. The funnel-like apertures are clothed with epithelium that has long cilia, whose vibrations produce currents that carry the abdominal liquids and any substances contained in them down the funnels to the tubes and thence to the urinary tubules with the organ's peculiar secretion, and thence to the ureters (Pl. V).* Ingested cells were present in the blood vessels of the glomeruli, in the lymph space between the glomeruli and the malpighian corpuscle, in the tubules, in the nephrostomes before their union with the tubules, and in the ureters just before their opening into the cloaca (Pl. V).

Three ways lie open for the passage of these cells, both ingested and free, into the urinary tubules: (1) through the nephrostomes direct from the abdominal cavity; (2) from the lymph space between the malpighian capsules and the glomeruli; (3) from the fine capillaries of the glomeruli with the glandular secretion. Doubtless the majority find their way in through the first two ways, but their passage from the glomeruli also increases the number that leave the system through the kidneys. It is owing to the peculiar structure of the kidneys in Urodels that these organs rank so high in the possession of ingested cells. With the exception of the spleen, no other organs contained so many. In the ureters the cells were massed together and were invariably present. Neither ingested cells nor free carbon existed anywhere in the kidneys outside the blood vessels and tubules.

3. The Liver. (Pl. IV.)

The liver is composed of remarkably large cells, attaining sometimes the length of 50 μ, even after the tissue is hardened. Often the tubular structure is not evident, but in some surface sections the

details appear fairly distinct. Ingested leucocytes in this organ are confined to the blood vessels; no indication of extra-vascular presence could be detected (Pl. IV).

Although in the remaining organs and tissues the ingested cells were not so numerous, their position affords valuable suggestions as to their ultimate fate.

4. The Stomach. (Pl. VI.)

In the stomach carbon-laden cells were present in the blood vessels, in the muscular tissue, in the submucosa, mucosa, and epithelium, and free on the mucous surface. No free carbon was present here (Pl. VI).

5. Lungs. (Pl. V.)

Practically the same results were obtained from examination of the lungs—ingested cells appear in the blood vessels, in the inter-vascular tissue, among the epithelial cells, and free on the mucous surface of the lung cavity. The simple structure of the lung (it is a somewhat folded bag) makes the direct course of the cells through the tissues to the mucous surface easy to trace (Pl. V).

6. Muscular Tissues and Skin. (Pl. VI.)

In sections made of the abdominal walls and other parts of the muscular system ingested cells were present in the blood vessels and capillaries, between the muscular fibers; in the latter case they may either be in the endomysium or lymphatic system. From the muscular tissues they pass to the epidermal cells through the malpighian layer, where there are large mucous glands, and finally are free on the surface of the skin (Pl. VI). That those present in the last-named place did not come accidentally after death is proved by the presence of isolated cells in small folds of the skin, where one cell occupies all the space between the apposed surfaces. With the rapid coagulation of the blood that exists it would be impossible for one leucocyte to be alone. Red corpuscles must be present in greater or less numbers, and the combined mass would be incapable of penetrating to such fine crevices.

In addition to the above locations with an abdominal injection, large masses of cells accumulated on all mesenteries, the outer sur-

face of the stomach, the intestines, large and small, the lungs, and to some extent on all peritoneal surfaces; also at the point where the needle of the hypodermic syringe entered there was an accumulation of carbon-laden cells, giving evidence of an inflammatory process to some degree.

The results of the investigation can be briefly summed up as follows:

1. No free carbon was present in any organ examined.

2. All carbon was contained in leucocytes except in the spleen, where true splenic pulp cells also contained it.

3. Ingested cells were both extravascular and intravascular except in the kidneys and liver.

4. Ingested cells were free on mucous and epidermic surfaces—stomach, lungs, skin.

5. Ingested cells were present in the excretory organs with waste products—kidneys.

The one point in which the above results differ from those obtained by all the authors consulted is the absolute limitation, with the one exception of the spleen cells, of the injected material to the leucocytes. This entire absence of free carbon in the tissues differs especially from the results of Hoffman and Langerhans, Arnold, and all those who, as the last-mentioned author, introduced coloring matter into the lungs. Ponfick, however, found no free cinnabar in his work. These authors show that the fixed connective tissue cells of most organs, the interstitial connective cells of all tissues, the epithelial cells of the lungs, some forms of pigment cells and bone-marrow cells, all can take up and retain the foreign material if the conditions of introduction remain practically the same.

When, however, conditions are so radically changed in regard to the amount of material injected into the organism it is apparent that the results may be greatly modified. In consequence it is believed, as the result of this investigation, that when no more material is present than can be disposed of by the leucocytes they exclusively ingest it and gradually dispose of it by wandering out on the mucous and cutaneous surfaces and by passing away with waste products. The presence of the carbon in the spleen is a secondary result, owing to the destruction probably of ingested cells in this organ, and either the subsequent taking up of the particles thus set free or else the ingestion of the corpuscles *in toto* by the splenic pulp-cells. It is believed, however, on the other hand, that when an overwhelming

amount of foreign material is introduced, these other forms of cells are pressed into service to help remove it from the circulation. Even free deposits of injected material may take place, owing to the inability of the cellular elements to cope with the large amount present. In this case the ultimate disposition of the injected material is not known, as usually conditions so profoundly pathological are produced that the experiments are prematurely ended by the death of the animals.

Considering the leucocytes as scavengers of the animal body in addition to their other functions, if their individual fate is inseparable from that of the waste material which they contain, they themselves become waste material when their active functions cease and must ultimately disappear from the organism. Already the two ways in which the leucocytes may ultimately pass away from active life have been suggested. In the first place, they wander from the circulation through the tissues to the epidermal and mucous surfaces and are carried away with the other waste products of the body. In the second place, they are destroyed in the spleen by the splenic cells. The large numbers found in various stages in the first condition and the relatively large numbers of ingested spleen-cells prove that the destruction of leucocytes is by no means insignificant.

It is at present impossible to tell what becomes of the carbon contained in the splenic cells. In all problems connected with the blood and circulation this organ seems to play an important part. Only by prolonged experimentation could it be ascertained whether the carbon gradually disappears or remains permanently in these cells; but that is beyond the scope of the present series of experiments. The problems as to the time during which the ingested cells remain in the circulation and in the tissues are also not solved. These require more time. They are for the present left out of consideration.

Summary of Whole Paper.

From the action of different per cent. solutions of the neutral salts $MgSO_4$ and Na_2SO_4 in relation to coagulation, an essentially different condition is found to exist in amphibian and fish blood from that known to be present in mammalian blood. True fibrin forms in the presence of neutral salts in solutions above 5 per cent. The rapidity of formation increases with the strength of the solutions, beginning in 6 per cent. in six hours and appearing in 15, 20, and

25 per cent. in thirty to forty minutes. The quantity also varies; in amphibia it increases toward 7 per cent. solution as a maximum; afterward a decrease in quantity is apparent. In the blood of *Amia calva* a slightly different condition is present (see pages 6 and 7). Simultaneously with the formation of this true fibrin an additional formation takes place. This is of a jelly-like nature and has been called the additional fibrin. This fibrin increases uniformly with the increased strength of the salt solutions. George Semmer has investigated a similar formation in the blood of the frog and domestic fowl under slightly different conditions. He considers that the additional fibrin is produced from the protoplasm of the red cells of the blood by the solvent action of the neutral salts. This additional fibrin is not formed in the blood of mammals, but occurs in that of birds, amphibia (Cryptobranchus, Necturus, Rana), and of a ganoid (Amia). This distinction perhaps makes it possible to draw a stronger line between the nucleated and non-nucleated corpuscle-bearing animals. The experiments should be extended to the large field of work offered among the Teleosts and other forms of fishes, and also to reptiles, in order to make a sure generalization on this point.

From the measurement and counting of the corpuscles in Necturus and Cryptobranchus the following facts were ascertained: The cells of Necturus are much larger than those of Cryptobranchus. Comparing this result with the measurements given by different authors of the corpuscles in other forms of amphibia, a distinction can be made on the basis of size of cells between those animals possessing external gills and those without; to which rule, however, a striking exception is found in Amphiuma. In enumeration, also, a difference is shown to exist between the large and small corpuscle-bearing animals, the higher numbers of corpuscles per cubic millimeter belonging to the latter class of animals.

From experiments made on Necturus and Cryptobranchus in the injection of small quantities of carbon into the abdominal cavity, hypodermically, the following results were obtained: In the first place, it was established that under the given conditions the leucocytes ingested all the foreign material and entered the blood circulation after varying periods. In the second place, it was found that from the blood the ingested cells are distributed to the tissues and ultimately pass away on mucous and epidermic surfaces with waste products of the body. Thirdly, that no deposit of free ma-

terial takes place, the foreign substance being contained exclusively in leucocytes, with the one exception of the splenic cells, where carbon is present. This latter position is a secondary result. From the above it is concluded that probably the fate of many leucocytes is to wander out on mucous and epidermic surfaces and to pass away with waste products. Many must also meet their end in splenic cells, as is shown by the considerable amount of carbon present there. The fate of this carbon is left undetermined from want of time to pursue the investigation further.

Methods.

Wherever it has been practicable, the methods of preparing the various objects to be studied have been inserted in the text. Those, however, have been omitted which concern the preparation of the tissue *in toto*, and also the details of the preparation of the sections when cut. The picric-alcohol method of hardening tissues was employed throughout as used by Professor Gage, of Cornell University:

95 per cent. alcohol.....	250 cc.
Water.....	250 cc.
Picric-acid crystals.....	1 gr.

The whole animal, with the abdominal cavity opened, was put in a jar of the above mixture from 2 to 3 days, the liquid being changed at least once. From this it was transferred to 67 per cent. alcohol from 24 to 36 hours, and then into 82 per cent. alcohol. Here it can remain indefinitely.

The following method of imbedding was used: Small pieces of the different tissues and organs were cut out; these were dehydrated in 95 per cent. alcohol 12 to 24 hours, soaked in chloroform 12 to 24 hours, infiltrated 4 to 5 days in an incubator, and imbedded in small paper boxes by pouring a small quantity of pure, hot paraffin over the pieces of tissue.

After the sections were cut they were fastened to the slides by a thin layer of albumen fixative.

The paraffin is removed by soaking the preparations in a jar of xylol 15 to 20 minutes. The xylol is then removed by 95 per cent. alcohol; that is followed by 82 per cent. alcohol, and finally by water.

For staining hydrochloric acid carmine was found to be most successful; hæmatoxylin proved to be too dark and was liable to be

confused with the carbon. After staining the preparations, they were thoroughly washed, dehydrated with 95 per cent. alcohol, the alcohol removed by clearer (2 vols. of carbolic-acid crystals, 3 vols. of turpentine), and mounted in xylol balsam.

The stained-blood preparations were made as follows :

Most of the preparations were made from Cryptobranchus, because it proved to show the points most distinctly. The specimen was left ten days after injection before it was killed—longer than any of Necturus were left, the latter being examined to find the times of the appearance of the ingested leucocytes in the circulation.

Bibliography.

1. Arnold, J. : "Ueber die Durchtrittstellen der Wanderzellen durch entzündete seröse Häute." Virchow's Archiv., Bd. 64, 1875.

2. Arnold, J. : "Untersuchungen über Staubinhalation und Staubmetastase."

3. Beale, —— : "Observations on the nature of red blood corpuscles." Trans. Mic. Soc., Quart. Journ. of Mic. Sc., 1864.

4. Brass, A. : "Kurtzes Lehrbuch der normalen Histologie des Menschen und typischer Thierformen." Bd. 3, 1888.

5. Bronn, H. G. : "Die Klassen u. Ordnungen des Thier-Reichs." Amphibien, C. K. Hoffmann. 1873–1878, Bd. 6, pp. 424, 430, 448.

6. Dekhysen, M. C. : "Uber das Blut der Amphibien." Verhandlung der anatomischer Gessellshaft, Juni, 1892.

7. Eberth, C. J. : "Ueber den feineren Bau der Leber." Centralbl. f. d. med. Wiss., 1866, p. 897.

8. Eberth, C. J. : "Ueber die Pigmentleber der Frösche." Virchow's Archiv., 1862, vol. 29.

9. Eberth, C. J. : "Die Pigmentleber der Frösche u. die Melanämie." Virchow's Archiv., 1867, vol. xl, p. 305.

10. Ecker, A. : "Anatomy of the Frog." Translated, with additions by Geo. Haslam, 1889.

11. Eimer, T. : "Ueber amöboide Bewegungen des Kœrnkörperchen." Arch. Micr. Anat., 1875, vol. xi, pp. 325–328.

12. Elam, —— : "On the admission and retention of foreign matters in the lungs." The Brit. Med. Journ., October, 1876.

13. Farrington, O. C. : "The nephrostomes of Rana." From the Trans. of the Conn. Acad., vol. viii, March, 1892.

14. Foster, M. : "Text-book of Physiology," part i, 5th edit., 1888.

15. Gage, S. H. : "Comparison of the fibrin filaments of blood and lymph in mammalia and amphibia, with methods of preparation." History, constitution, and membership and titles and abstracts of papers for the years 1888, 1889, 1890 of the Association of American Anatomists.

16. Gage, S. H. : "Notes on fibrin, oxyhemoglobin crystals, and collodion methods." Proceed. of Am. Soc. of Microscopists, 1890.

17. Gage, S. H. : "Picric and chromic acid for the rapid preparation of tissues for classes in histology." Proceed. Am. Soc. of Microscopists, 1890.

18. Gage, S. H. : "Blood corpuscles, amphibian, permanent preparations of." Journ. Roy. Mic. Soc., 1880.

19. Gamgee, A. : "Text-book of Physiological Chemistry of the Animal Body," vol. i, 1880.

20. Gegenbauer, C. : "Elements of Comparative Anatomy." 2d edit., 1877, trans. by F. Jeffery Bell. Translation revised and preface written by E. Ray Lankester, 1878.

21. Graham, ——: "On the existence of charcoal in lungs." Edinb. Med. Journ., vol. 42, 1834.

22. Halliburton, W. D. : "Text-book of Chemical Physiology and Pathology," 1891.

23. Hayem, G. : "Du Sang et ces Alterations Anatomiques," 1889, 8vo,

24. Hindenlang, ——: "Pigmentinfiltration in den Lymphdrüsen. Leber u. anderen Organen in einem Fall von Morbus maculosus," etc. Virchow's Archiv., Bd. 79, 1880.

25. Hoffmann, F. A., and Langerhans, P. : "Ueber den Verblieb des in die Circulation eingefuhrten Zinnobers." Arch. Path. Anat. and Phys., Bd. xl, viii, 1869, 23 pages.

26. Hoffmann, F. A., and v. Recklinghausen : "Ueber die Herkunft der Eiterkörperchen." Centralbl. fur die med. Wissenchaften, Bd. 31, 1867.

27. Kellogg, J. L. : "Wandering Cells in Animal Bodies." Am. Nat., xxv, 1891.

28. Kirke's "Hand-book of Physiology," by M. Baker and V. D. Harris, 13th edit., 1892.

29. Knüpffer, A. : "Ueber den unlöslichen Grundstoff der Lymphdrüsen und Leberzelle." Dorpat, 1891, Schnakenburg, 27 pages.

30. Küttner, ——: "Die Abscheidung des indigoschwefelsauren Natron in den Gewebe." Centralbl. f. d. med. Wissenschaften, 1875.

31. Landois and Stirling: "Text-book of Human Physiology." 2d Am. ed., trans. from the 5th German, by W. Stirling, with additions, 1886.

32. Loos, ——: "Leucocytes in the the tail of a tadpole." Abs. Journ. Proc. Mic. Soc., 1890, p. 158.

33. McKendrick, J. G.: "Text-book of Physiology," vol. i, 1888.

34. Mettenheimer, ——: "Ueber die Ablagerung des schwartzen Pigmentes in den Lungen u. dem Lungenfell." Archiv. fur Anat. u. Phys., 1866.

35. Moleschott, ——: "Erneuter Beweis für das Eindringen von festen Körpchen in die kegelförmigen Zellen." Moleschott's Untersuchungen Bd. ii, 1857.

36. Mosler, F.: "Melanæmia, Ziemssen, Cyclopedia of the Practice of Medicine," 1880.

37. Mügge, ——: "Ueber das Verhalten der Gefässe bei disseminirter Lungentuberkulose." Virchow's Archiv., Bd. 76, 1879.

38. Muir, R.: "Division of Leucocytes." Edinb. Journ. Anat., 1890–1891.

39. Neumann, ——: "Formation of Blood Corpuscles in Marrow." Journ. of Anat., iii, 1869.

40. Nothnagel, ——: "Zur Resorption des Blutes aus dem Bronchialbaum." Virchow's Archiv., Bd. 71, 1877.

41. Nussbaum, ——: "Nephrostomes of Amphibia." Zoöl. Anzeiger, 1880, No. 67.

42. Ponfick, E.: "Studien über die Schicksale körniger Farbstoffe im Organismus." Archiv. für Path. Anat. u. Phys. u. für klinische Medicin, Bd. xlviii, 1869.

43. Osterlen, ——: "Ueber den Eintritt von Kohle u. anderen unlöslichen Stoffen vom Darmkanal aus in die Blutmasse." Zeitschrift für rationelle Medicin, Bd. v, 1846.

44. Quincke, ——: "Zur Pathologie des Blutes." Deut. Archiv. für klinische Medicin, Bd. xxv u. xxvii, 1880.

45. Ranvier, L.: "Traite technique d'Histologie." Trans. into German, by Dr. W. Nicate u. Dr. H. von Wyss, 1885.

46. "Reference Hand-book of the Medical Sciences." Edited by A. H. Buck, 1885.

47. Reitz, ——: "Ueber die passiven Wanderungen der Zinnoberkörnchen durch den thierischen Organismus." Wiener akadem. Sitzungsberichte, Bd. 57, 1868.

48. Riddel, ——: "Journal de la Physiologie." Tome ii, Paris, 1859, p. 159. (Stricker's Manual of Histology, p. 266.)

49. Ruffer, Armand: "On the Phagocytes of the Alimentary Canal." Journ. of Micros. Science, new series, xxx, 1889–1890.

50. Ruppert, ——: "Experimentelle Untersuchungen über Kohlenstaubinhalation." Virchow's Archiv., Bd. 72, 1877.

51. Rutimeyer, ——: "Durchtritt suspendirter Partikel aus dem Blute ins Lymphgefässystem." Arch. für experimentelle Pathologie, Bd. xiv, 1881.

52. Schiefferdecker and Kossel: "Gewebelehre mit besonderer Berücksichtigung des menschliches Körpers," part i, 1891

53. Schestopal, ——: "Ueber die Durchlässigkeit der Froschlunge für gelöste u. körnige Farbstoffe." Virchow's Archiv., Bd. 75, 1879.

54. Semmer, G.: "Ueber die Faserstoffbildung im Amphibien u. Vogelblut u. die Entstehung der rothen Blutkörperchen der Säugethiere." 8vo, Dorpat, 1874.

55. Slavjansky, K.: "Experimentelle Beiträge zur Pneumonokoniosis. Lehre." Virchow's Archiv., Bd. xlviii, 1869.

56. Sommerbrodt, ——: "Hat das in die Luftwege ergossene Blut ätiologische Bedeutung für die Lungenschwindsucht?" Virchow's Archiv., Bd. 55, 1872.

57. Thoma, ——: "Ueberwanderung farbloser Blutkörper aus dem Blut in das Lymphgefässystem." Heidelberg, 1873.

58. Vaillant, ——: "Annales des Sciences Naturelles. Zoölogie." Quatrième series, 1863, pp. 328, 329.

59. Van der Hoeven, J.: "Les globules du sang du Menobranchus." Arch. neel. d. sc. exactes, etc. La Haye, 1867, ii, 288.

60. Van der Stricht, ——: "Nouvelles recherches sur la genèse des globules blancs du sang." Memoir couronné par l'academie royale de médecine, Belgique. Archives de biologie, Tome xii, 1892, Fasc. 2, S. 199–344.

61. Von Scheuppel, ——: "Melanæmic Liver"—Ziemssen. Cyclopedia of the Practice of Medicine, 1880.

62. Wallian, S. S.: "Studies in Æotherapeutics." New York Med. Journ., Saturday, November 26, 1892.

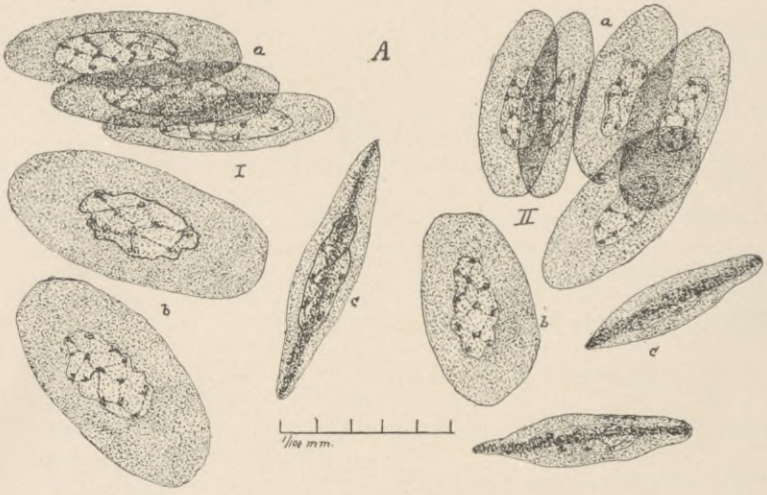
63. Werigo, ——: "Les globules blancs comme protecteurs du sang." Annales de l'institute Pasteur, année vi, Tome vi, 1892, No. 7, S. 478–511.

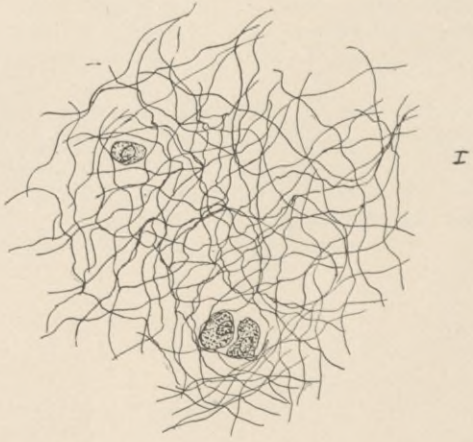
64. Wiedersheim, R.: "Elements of Comparative Anatomy of Verts." Adapted from the German by W. Newton Parker, additions by author and translator, 1886.

65. Wiesner, J.: "Ueber den microscop. Nachweis der Kohle in verschied. Formen u. über die Uebereinstimmung des Lungenpigments mit der Russkohle." (Sitzber. d. kais. Akademie in Wien, Math. Naturw., 1892.) Centralbl. for Physiologie, 11 February, 1893.

66. Zeiger, ——: "Leucocytes, multinucleated Forms and Repair of Wounds." Brit. Med. Journ., 1890, ii, p. 360.

PLATE I





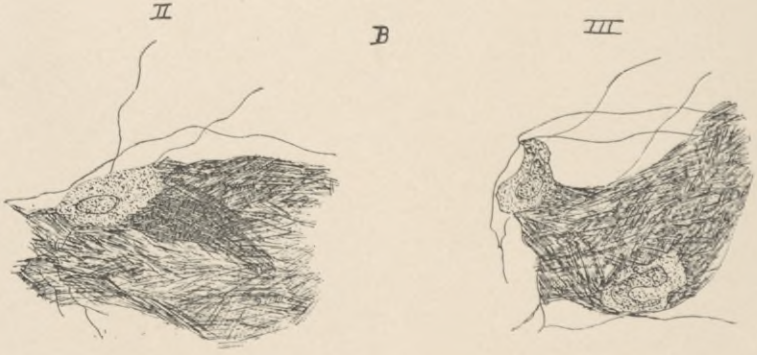


Plate I.

Red Blood Corpuscles and Fibrin Filaments.

A. Red blood corpuscles.
   I. Red corpuscles of Necturus.
      a. Overlapping tile-like formation.
      b. Cells seen flatwise.
      c. Cells seen edgewise.
   II. Red corpuscles of Cryptobranchus.
      a. Overlapping tile-like formation.
      b. Cells seen flatwise.
      c. Cells seen edgewise.
   Drawn from fresh preparations.

B. Fibrin filaments.
   I. Mammalian fibrin. Three leucocytes lie between the meshes of the network.
   II. Fibrin of Necturus, with leucocytes closely connected with the threads.
   III. Fibrin of Cryptobranchus, showing two leucocytes closely united with the threads.

"I" was drawn from a stained preparation. "II" and "III" were drawn free-hand.

Plate II.

Leucocytes.

A. Group of carbon-laden leucocytes, showing amœboid phases.
   a, *b*. Leucocytes of Necturus.
      n. Nucleus.
      p. Cell-body.
   c, *d*. Leucocytes of Cryptobranchus.
      n. Nucleus.
      p. Cell-body.

B. Group of Leucocytes, showing amœboid cell-bodies and amœboid nuclei.
   a, *b*, *c*. Leucocytes of Necturus.
      n. Nucleus.
      p. Cell-body.
   b. Shows three nuclei, two in amœboid movement and one resting.
   d, *e*. Leucocytes of Cryptobranchus.
      n. Nucleus.
      p. Cell-body.
   e. Has three nuclei, two amœboid and one resting.

Drawn from stained preparations.

PLATE II

PLATE III

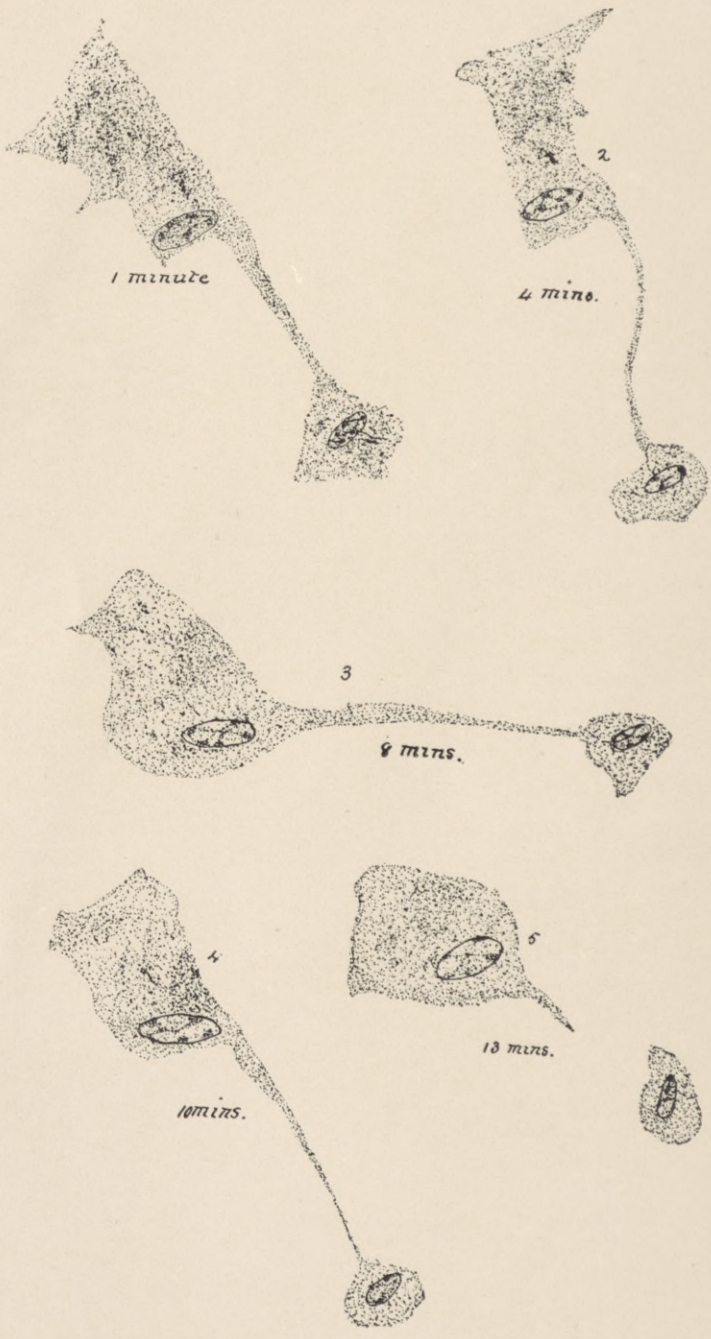


Plate III.

Direct Division of the Cell-body of a Leucocyte.

Drawn from freehand sketches made when the division was in progress.

The time taken to pass through the stages figured was thirteen minutes.

The unequal division of the parent cell is noticeable, the bud, as it were, being comparatively quite small.

Plate IV

A. Surface section of liver of Cryptobranchus.
- P. Capillaries of blood-vessels.
- T. Liver-cells.
- *b*. Red corpuscles in the capillaries.
- *c*. Ingested leucocytes in the capillaries.
- *d*. Small intercellular capillaries of the bile-duct.
- *e*. Epithelium of the larger bile-vessels.
- *h*. Hepatic cell-body.
- *n*. Nucleus of hepatic cells with nucleoli.

Note the absence of extravascular ingested leucocytes.

B. Vertical section of the spleen of Cryptobranchus.
- I. Part of the section near the surface.
  - 1. Peritoneum.
  - 2. Layer of fibrous tissue forming the capsule.
  - 3. Trabeculæ passing from capsule among the splenic cells.
  - *c*. Carbon-laden leucocytes.
  - *b*. Red corpuscles.
  - *l*. Leucocytes non-ingested.
  - *s*. Splenic pulp-cells.
- II. Ental part of the section.

  Lettering as above.

Note the ingested spleen pulp-cells and the different distribution of the carbon particles in them from that found in the leucocytes; also the absence of ingested splenic cells in the superficial part of the spleen.

PLATE IV


R
c
A
P
C
c
1/10 mm.
B
C
a
L
I
a
c
i
1/10 mm.
1/10 mm.
II
c
III
T
T
P
b
T
L
c
c
IV
S
c
g
P
P
P
c
T
T
P
P
O
P
T

Plate V.

A. Vertical section of the lung of Cryptobranchus.
   E. Ectal surface.
   R. Ental or respiratory surface.
   P. Blood capillary.
   c. Carbon-laden leucocytes.

Note the presence of ingested leucocytes in extravascular tissue as well as on the ental surface of the lung.

B. Section of kidney of Cryptobranchus.
   I. Transection of the ureters and cloaca, showing masses of ingested cells.
      a. Ureters.
      c. Carbon-laden leucocytes.
      l. Non-ingested leucocytes.
   II. Transection of urinary tubules.
      c. Carbon-laden leucocytes.
   III. Nephrostomic funnel, showing the ciliated mouth.
      c. Ingested leucocytes.
      l. Non-ingested leucocytes.
   IV. Vertical section of the kidney near the ventral surface.
      G. Glomerulus.
      P. Capillaries of blood-vessels.
      T. Urinary tubules.
      S. Lymph space around the glomerulus.
      O. Origin of a urinary tubule, with small, ciliated epithelium.
      b. Red corpuscles.
      c. Carbon-laden leucocytes.

Note the presence of extravascular ingested cells.

Plate VI.

A. Vertical section of stomach of Cryptobranchus, near the pyloric part.

I. Submucosa.

II. Muscularis mucosæ.

III. Mucosa.

c. Carbon-laden leucocytes.

Note the presence of the extravascular ingested cells.

The figure is diagrammatic in so far that the locations of the ingested cells are taken from different sections and put into one figure.

B. Vertical section of the skin of Cryptobranchus.

G. Large mucous glands.

c. Carbon-laden cells.

The ingested cells are wandering to the external surface from the blood-vessels.

PLATE VI