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CYTOLOGICAL STUDIES ON THE PLANARIAN NEOBLAST

By

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With 11 Figures in the Text

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Introduction

One of the basic problems in regeneration is the question of the sources of the cells used for building up the regeneration blastema and their activities during histogenesis. This problem, or at least the first part of it, is most easily attacked in the lower invertebrates. The material for regeneration is in many cases supplied by free, totipotent cells found in stock in the intact animals. This is for instance the case in coelenterates [interstitial cells (Tardent 1954)], in annelids [neoblasts (Stéphan-Dubois 1954)] and in planarians [neoblasts (Dubois 1949)]. In vertebrates the problem of the origin of the cells for regeneration is more obscure, but it is on the way to be clarified (Chalkley 1959) in the important case of limb regeneration in amphibians.

The great significance of a free, totipotent parenchyma cell for blastema formation in planarians is now generally accepted (Dubois 1949, Brøndsted 1955, Lindh 1957, Pedersen 1958). This cell, which has been variously named ("Bildungszellen", "Stoffträger", "Ersatzzellen", "formative cells", "Regenerationszellen", "cellules libres", "Stammzellen") is now most often called the "neoblast". The literature dealing with this cell is reviewed in Bronn (1917), Prenant (1922), Dubois (1949) and Brøndsted (1955). In most cases it is not difficult to identify the neoblast. The cell has a characteristic morphology (see below) and the scanty cytoplasm is strongly basophilic. However, cases are encountered where it is not possible to decide whether a given cell is a neoblast. Two cell types may under certain circumstances give rise to confusion, namely some nerve cells and some fixed parenchyma cells (see e.g. Murray 1931). So far, a way out of this dilemma has not been found.

To-day, the analysis of the fundamental biological phenomena has been extended to the molecular level through the rapid advances in analytical cytology, and also investigations on regeneration may greatly profit from the application of modern optical and histochemical methods. The present paper is concerned with a closer study of the planarian neoblast using such methods. Only the "resting" neoblast not engaged in regeneration has been dealt with, so only a static picture of this important cell has been obtained. The dynamics of the neoblast as revealed through the histogenetic processes in the blastema will be considered in subsequent studies.

Material and methods

Cloned individuals from the unpigmented triclad flatworm *Planaria vitta* were employed. The animals were unstarved or starved for a few days.

They were fixed as described in a previous paper (PEDERSEN 1959), and in addition the following two fixatives were used: Acetone and absolute ethanol 1:1 for 18 hours at 2° and neutral formalin-calcium chloride for 18 hours at 2° (HOLT 1958).

The planarians were appropriately washed, dehydrated, cleared in methyl benzoate and xylene or in benzene only and embedded in paraffin for 20 min to half an hour. The animals fixed for enzyme methods were quickly dehydrated and embedded for 15 min, the temperature never exceeding 58°.

The animals were most often cut sagittally at 7 μ, in a few cases frontally or transversely. When very thin sections were required (1 μ) planarians embedded in a hard paraffin were cut on a Sjöstrand ultramicrotome with the block previously cooled. In other cases methacrylate-embedded animals were cut on a serial microtome using an ordinary steel-knife. It was nearly impossible to make fresh-frozen sections on planarians because of the small size and fragility of these animals. In some cases frozen sections were made on neutral formalin-calcium chloride fixed animals embedded in acacia gum-sucrose (HOLT 1958).

It was often found convenient to use squash preparations for the enzyme methods. However the squashing had to be done very gently.

Histological staining methods. The methods used have previously been described (PEDERSEN 1959).

Cytological methods. *1. Method for demonstration of mitochondria.* CAIN's method was used (CAIN 1948). This is a modification of ALTMANN's acid fuchsin method.

2. Method for the Golgi apparatus. ELFTMAN's direct silver method was employed followed by goldtoning and light staining with hematoxylin (ELFTMAN 1952).

Histochemical methods. The methods for proteins and amino acids, nucleic acids and polysaccharides have previously been reported (PEDERSEN 1959). Moreover, some additional methods were employed in the present study. Naphthol yellow S (DEITCH 1955) and chromotrope 2R (WAGNER and SHAPIRO 1957) were used to demonstrate basic proteins. Protein-bound amino groups were demonstrated by the method of WEISS et al. (1954). In addition to alcian blue, MOWRY's improved method for demonstrating acid mucopolysaccharides was used. The method is a rather specific colloidal iron procedure (MOWRY 1958). The method for arginine adopted here was that of MCLEISH et al. (1957).

Methods for enzymes. *1. Alkaline phosphatase.* Both the cobalt-method (LILLIE 1954) and the azo dye method (PEARSE 1953) were used.

2. Acid phosphatase. The azo dye method described by PEARSE (1953) was used.

3. Unspecific esterase. The α-naphthyl acetate and naphthol AS acetate methods were employed (PEARSE 1953).

4. Succinic dehydrogenase. KAUFMAN and HILL's method (1959) was adhered to, using the agar substrate mixture method.

5. Cytochrome oxidase. The G-nadi reaction described by GOMORI (1952) was used.

6. Peroxidase. A benzidine method was employed (GOMORI 1952).

Methods for electron microscopy. The methods have previously been described (PEDERSEN 1959). Staining with uranyl acetate was tried in addition (WATSON 1958).

Results

I. Observations on living cells

The neoblasts were obtained by careful squashing of fragments of planarians or isolated blastemas between a coverslip and an object slide. The squashing was performed in a drop of MURRAY's salt solution for planarians (MURRAY 1931). After squashing, the coverslip was sealed by paraffin. In these preparations the living cells could be studied for several hours. No effort was made in this study to cultivate the neoblasts.

The cells were examined by phase-contrast microscopy (ZEISS). The neoblasts were best studied in blastemas about 6 days old. The most useful region to employ in the intact animal is a fragment between the head and the pharynx.

The neoblasts are very easily isolated from the tissues, being rolled out by the squashing process. They are always separated from each other and never connected by other cells. The neoblasts are rather small cells (about 11—12 μ in diameter) because of the scanty cytoplasm. However, the nucleus is rather large, about 9—10 μ in diameter and is most often round or slightly oval (Fig. 1). The chromatin is very conspicuous. It is evenly distributed as fine granules of approximately uniform size. The nucleoli are often difficult to identify, because

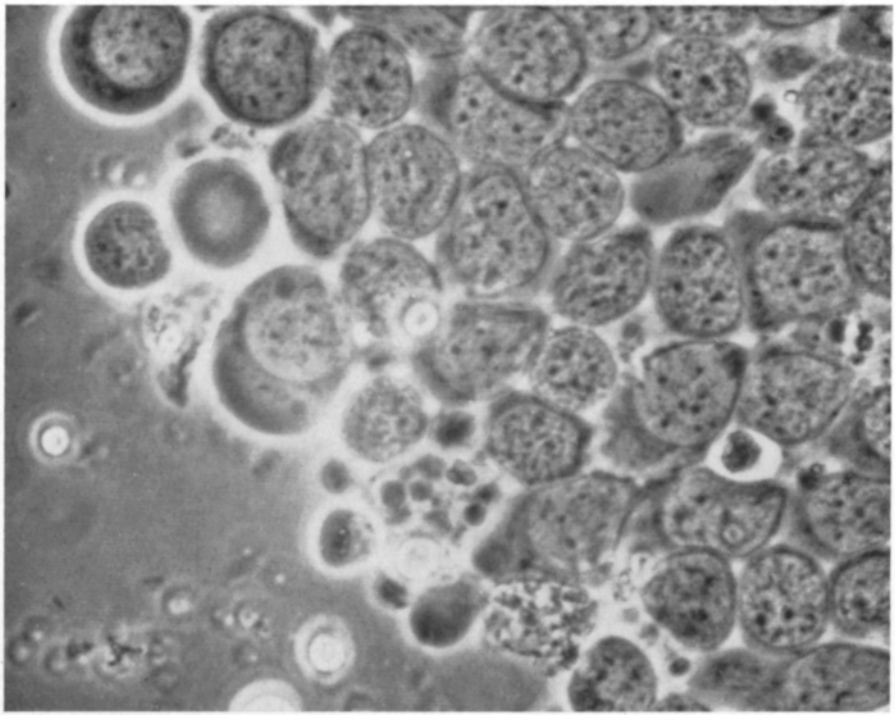

Fig. 1. Living neoblasts from an early blastema. Notice the granularity (mitochondria) in the neoblast at the upper left. 1575 ×

of the abundant chromatin. One big or two or more smaller nucleoli are usually present. As mentioned, the cytoplasm is very scanty in the neoblasts and it is sometimes only present as a narrow border around the nucleus. When the cell contains more cytoplasm it is usually excentrically distributed with a narrow border on one side of the nucleus and a more abundant portion just opposite. Neoblasts are also found containing the cytoplasm uniformly distributed round the nucleus. The cytoplasm is granular, but the granules are rather small. Most of these granules, which are rod-shaped or spherical, are undoubtedly mitochondria or represent clusters of small mitochondria. In a few cases one or two small fat-droplets are present. Cell processes were never observed. This is in accordance with MURRAY (1931), who was able to observe processes only after 24—26 hours in culture. MURRAY does not distinguish between the neoblasts and fixed parenchyma cells. She is of the opinion that there is no essential difference between these cells.

On squashing the parenchyma cells become spherical and isolated, thereby closely simulating the neoblasts, and it has not been possible with certainty to differentiate between neoblasts and parenchyma cells in the cultures observed in the present work. It should be stressed that this is the case only with living unstained cells. In fixed preparations a differentiation between neoblasts and parenchyma cells is generally easy enough after appropriate staining.

II. Morphology of fixed and stained neoblasts

It is notably difficult to fix the planarian parenchyma properly, and the same fact applies to the neoblasts. The best results have been obtained by using osmium tetroxide, Helly or Zenker fixation. The last two fixatives have been most extensively used routinely. The neoblasts are rather distorted by formalin or Carnoy fixation.

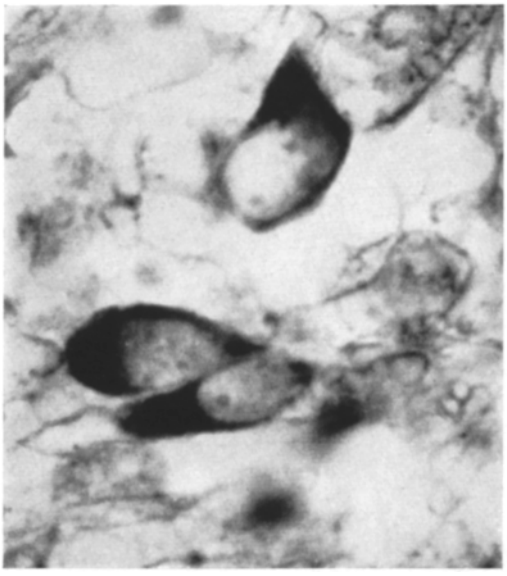

Fig. 2. A group of neoblasts. Azur A-eosin B staining. 1965 ×

The neoblasts occur free in the parenchyma, and as a rule their boundaries are easy to delimit, even in unstained sections using phase-contrast.

In routine stained sections the neoblasts show up well because of a strong staining of the cytoplasm by the basic (cationic) component in the stain. An excellent way to demonstrate the neoblasts is staining with toluidine blue after Zenker or Helly fixation. Gallocyanin is also well suited for the purpose, especially for the study of the nucleus. By far the best method, however, for a practically selective staining of the neoblasts is LILLIE's azur A-eosin B method (LILLIE 1954). This stain is buffered and by changing the p_H in the solution, the balance between the red and the blue component in the stained sections can be varied at will.

Zenker was preferred as fixative for this staining, and after some experimentation it was found that at p_H 5.0 in the staining solution (corresponding to p_H 4.5 of the buffer) the cytoplasm of the neoblasts was stained strongly blue as were also the nucleoli. The nuclei were, however, only slightly reddish-blue, and the blue colour was only exhibited by a few parenchymatous vacuoles and by the cytoplasm of some interesting gland cells containing round eosinophilic granules. These glands are the type 3 basophiles previously described (PEDERSEN 1959). All other histological structures were red except the nuclei, which were reddish blue to red.

It was concluded that at the p_H employed the method is rather selective for the neoblasts. The staining method is very useful for counting neoblasts.

The degree of cytoplasmic basophilia in the neoblasts is somewhat variable. Sometimes it may be nearly absent in morphologically well-defined neoblasts. The significance of this fact is still obscure, but may perhaps be better understood by following the neoblasts during different functional states and during differentiation.

As to the morphology of the neoblasts it has been previously mentioned that they are rather small cells, with a relatively large nucleus. However, this is variable in size. In fixed and stained neoblasts the shape of the nucleus is round or ovoid (Fig. 2). Otherwise, the picture conforms well with that of living neoblasts as seen in phase contrast.

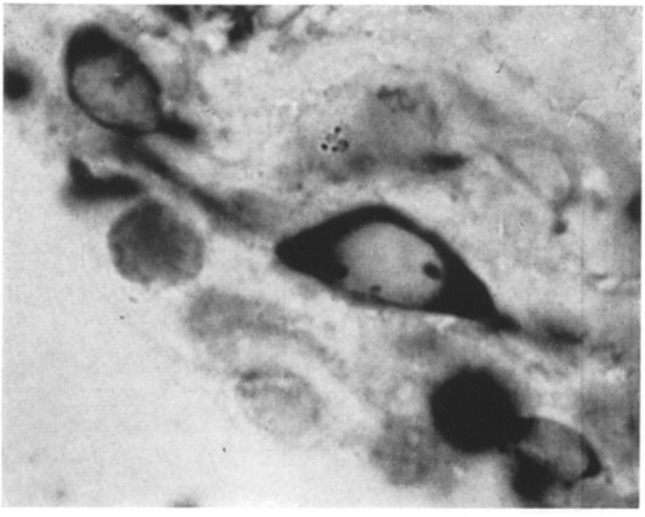

Fig. 3. A typical neoblast. Notice the three nucleoli. Azur A staining. 1965 ×

The scanty cytoplasm is strongly basophilic and distinctly granular. The shape of the cell is variable. Often it is round, the cytoplasm being equally distributed around the nucleus, forming a narrow border (Fig. 4). The most commonly encountered shape is that of a drop, a small dense border of cytoplasm around one end of the nucleus and a tapering process at the opposite pole (Fig. 2 and 3). Sometimes another picture is seen: a small cytoplasmic border at one pole and a broad flattened mass of cytoplasm at the other pole, exhibiting a distinct vacuolisation.

ELFTMAN's direct silver method for the Golgi apparatus was employed, both on intact planarians and, to get a high concentration of neoblasts, on early blastemas. Because of the scanty cytoplasm in these cells it is difficult to visualize the cytoplasmic organelles. It was not possible consistently to demonstrate any Golgi material.

CAIN's method for mitochondria was used both on ordinary 7 μ sections, on thin sections of about 1 μ and on squash preparations. In general, mitochondrial techniques are very unsatisfactory on planarian tissues. The acid fuchsin stains many inclusions in the parenchyma besides the parenchyma itself. Also the many eosinophilic gland cells are strongly stained. The differentiating agent cannot remove this staining without also abolishing the mitochondrial staining.

However, it was in fact possible to demonstrate the mitochondria of the neoblasts. They are uniformly distributed in the cytoplasm and consist of numerous very small round or rod-shaped elements. The rods are rather thick and the round mitochondria predominate. It is possible that at times they represent clusters of still smaller mitochondria. However, the mitochondria are best studied on the electron micrographs.

III. Distribution of the neoblasts

The distribution of the neoblasts was studied in serial sections of intact planarians. Both frontal, sagittal and transverse sections were examined. The neoblasts exhibited a characteristic distribution. They were most abundant ventrally, especially crowding about the nerve-cords. They are often found clumping together. They are sparse both cephalically and caudally. Apparently they are concentrated in front of and behind the pharynx on the ventral side. There are also many situated ventrally and lateral to the pharynx.

Apart from the sites mentioned the neoblasts are widely distributed throughout the parenchyma, but much more sparsely.

The exact distribution of the neoblasts in the species used in this paper and several other planarian species has been studied by BRØNDSTED (in preparation).

IV. Histochemical observations

Table 1. *Reactions for nucleic acids*

Method	Fixation	Nucleus	Nucleolus	Cytoplasm
Methylgreen pyronin	ac-alc	++	++ to +++	+++
	f	++	++	+++
	c	++	+++	+++
+ RNA-se	c	++	0	0
Feulgen	c	+++	0	0
	f	+++	0	0

Abbreviations. ac-alc: acetic-alcohol; f: buffered formalin; c: Carnoy. 0: negative; ±: doubtful; +: faint; ++: moderate; +++: intense.

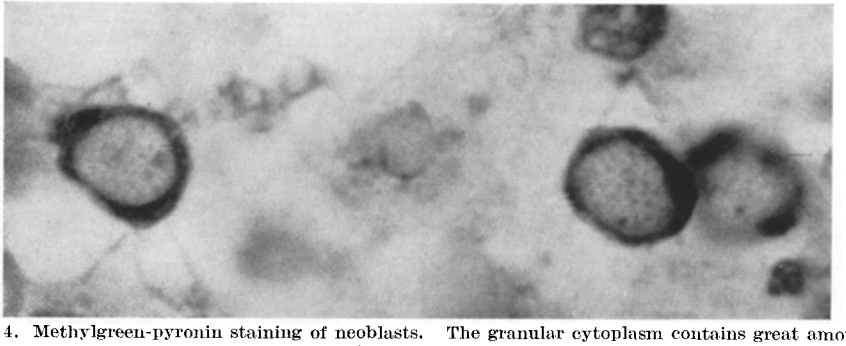

Fig. 4. Methylgreen-pyronin staining of neoblasts. The granular cytoplasm contains great amounts of RNA. 1965 ×

1. Methods for nucleic acids. The results have been tabulated in Table 1. DNA is present only in the nucleus, as indicated by the Feulgen reaction. RNA is present in great amounts in the cytoplasm and the nucleoli, as witnessed by the strong basophilia of these sites and by its subsequent removal after treatment with ribonuclease (Fig. 4).

2. Methods for proteins. The results have been arranged in Table 2. The tests were unanimous in demonstrating moderate amounts of proteins. MILLON'S reaction for tyrosine was only faint and the intensity equally distributed throughout nucleus, nucleolus and cytoplasm. No tryptophan could be demonstrated. The Sakaguchi reaction for the strongly basic arginine showed a moderate intensity. The 2-hydroxy-3-naphthaldehyde reaction specific for protein-bound amino groups exhibited only a rather faint reaction in the cytoplasm, while the nucleolus often exhibited a stronger reaction. The DDD-method revealed a moderate to

Table 2. *Reactions for proteins*

Method	Fixation	Nucleus	Nucleolus	Cytoplasm
Millon	f	+	+	+
GLENNER'S for tryptophan	f	0	0	0
Naphthol yellow S	z	++	++ to +++	++ to +++
	b	++	++	++
	h	++	++	++
	f	++		++
Dihydroxy-dinaphthyl-disulfide	f	+	+ to ++	+ to ++
	z	+	++	++ to +++
	tca	+	++	++
Chromotrope 2R	f	0	+++	+++
	z	0	+++	++ to +++
Sakaguchi	z	++		++
	f	++		++
2-hydroxy-3-naphtaldehyde	z	± to +	+ to ++	± to +
	acet-alc	± to +	+ to ++	± to +

Abbreviations. f: buffered formalin; z: Zenker; h: Helly; tca: trichloroacetic acid-alcohol acet-alc: acetone-alcohol.

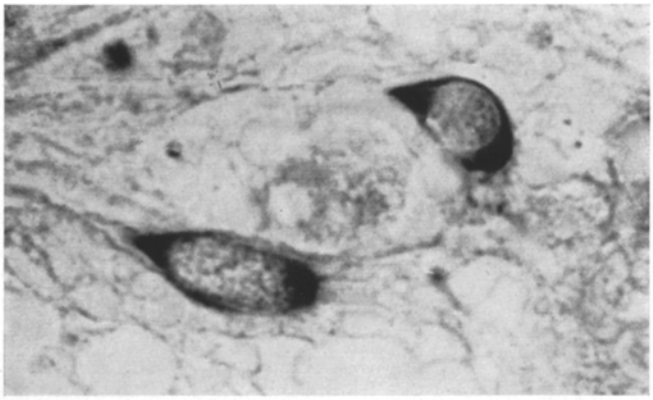

Fig. 5. Chromotrope 2R staining of the cytoplasm demonstrating basic proteins. 1965 ×

strong reaction, pointing to the presence of —SH-groups. The reaction was not much modified by reduction. Though not strictly histochemical, two staining methods for basic proteins were employed, the principle being staining at a low p_H with acid (anionic) dyes. The chromotrope 2R staining after Zenker fixation was excellent for demonstrating neoblasts (Fig. 5). Both this method and the

naphthol yellow S method pointed to a moderate or strong content of basic proteins in the cytoplasm and nucleolus. These methods do not distinguish between the basic amino acids contributing to the acidophilia at a low p_H, but by comparison with the amino acid end-group reactions mentioned, it can be concluded that both arginine, lysine and free amino end-groups contribute, and perhaps histidine too. Using these qualitative methods, it is not possible to decide what share of the eosinophilia should be ascribed to each of these amino acids. The different sensitivities of the methods used are an important factor in evaluating the predominance of one amino acid over another. Thus, a faint reaction with a rather insensitive method may in reality demonstrate a higher concentration of one amino acid than a strong reaction for another amino acid using a highly sensitive method. Only a quantitative approach can be trusted in this matter.

3. Methods for polysaccharides. Table 3 indicates the reactions used and the results obtained. The question as to the presence of PAS-positive material in the neoblasts was a very difficult one to answer because of the strong PAS-reactivity of the parenchyma, in which these cells are embedded. Osmium tetroxide fixed, methacrylate embedded sections demonstrated a PAS-negative cytoplasm in the neoblasts, in strong contrast to the intensely stained surrounding

Table 3. *Reactions for polysaccharides*

Method	Fixation	Nucleus	Nucleolus	Cytoplasm
Periodic acid-Schiff	h	0	0	0 to ±
	z	0	0	0 to ±
	b	0	0	0 to ±
	os + methacrylate	0	0	0
Alcian blue	f	+	0	0
	z	0	0	0
Alcian blue after perchloric acid extraction	f	0	0	0
Colloidal iron	f	0	0	0

Abbreviations. h: Helly; z: Zenker; b: Bouin; os + methacrylate: buffered osmiumtetroxide and methacrylate embedding; f: buffered formalin.

parenchyma. In Helly or Zenker fixed material embedded in paraffin the picture was more uncertain, but it was concluded that the presence of PAS-positive material in the cytoplasm is unlikely, though its complete absence cannot entirely be proven. The presence of acid mucopolysaccharides could not be demonstrated, neither with alcian blue nor with the colloidal iron technic. The slight alcian blue staining of the nuclei after formalin fixation was caused by nucleic acid, as witnessed by the disappearance of the reaction after extraction with perchloric acid, which completely removes both nucleic acids.

4. Methods for enzymes. The results have been arranged in Table 4. The histotechnical difficulties connected with the demonstration of enzymes in planarians were considerable, and especially in a single cell like the neoblast, which has very little cytoplasm. With an ordinary freezing-microtome, fresh frozen sections could not be made of the species employed. It was even difficult to make frozen sections after fixation. A still more serious problem was encountered in the widespread lipid droplets in the parenchyma. Most of the reaction products in

Table 4. *Reactions for enzymes*

Method	Fixation	Embedding	Reaction
Alkaline phosphatase (GOMORI)	acet-alc	P	0
	Unfixed	Squash	0
	fca	P	0
	fca	F	0
Alkaline phosphatase (Na-α-naphthyl phosphate)	acet-alc	P	0
	Unfixed	Squash	Diffuse and ±
	fca	P	0
	fca	F	Diffuse and ±
Acid phosphatase (Na-α-naphthyl phosphate)	As for alkaline	phosphatase	
Esterase (α-naphthyl acetate)	acet-alc	P	0
	Unfixed	Squash	Diffuse and ±
	fca	P	0
	fca	F	Diffuse and ±
Esterase (naphthol AS acetate)	As for esterase	(α-naphthyl acetate)	
Peroxidase	Unfixed	Squash	0
Succinic dehydrogenase	Unfixed	Squash	0
G-nadi reaction	Unfixed	Squash	± to +

Abbreviations. acet-alc: acetone-alcohol; fca: formalin-calcium chloride; P: paraffin embedding; F: freezing sections.

the enzymatic methods used are lipid-soluble, and thus considerable diffusion takes place and renders cytological localisation impossible. The succinic dehydrogenase reaction was performed on squash preparations. No reaction could be detected in the cytoplasm of the neoblast, but a diffuse red reaction was observed in a cluster of neoblasts as seen in an early blastema. Evidently the concentration of this enzyme is too low to be detected in a single neoblast with this method. The G-nadi reaction, which above all demonstrates cytochrome oxidase (GOMORI 1952), often exhibited a weak reaction with the neoblast cytoplasm. The peroxidase reaction was negative.

As to the hydrolytic enzymes, the reactions were negative for both alkaline and acid phosphatase even after prolonged incubation, and also unspecific esterase was undetectable with the methods used. It is planned to pursue the enzymatic cytochemistry of neoblasts during differentiation in the blastema, using cryostate technics.

The outcome of the enzymatic studies on the singly-occuring neoblasts in the intact non-regenerating animal is, therefore, that only traces of oxidative enzymes can be demonstrated with the present available methods.

V. Electron microscopical observations

The structure of the planarian parenchyma is extremely complex and accordingly the fine structure as revealed by the electron microscope is very difficult to understand. However, the neoblast occurring free in the parenchyma is easy to identify when present in its typical form. The cell is well demarcated from the surrounding tissue, and it is further characterised by an appreciable cytoplasmic electron density. Moreover the cytoplasm is scanty in most cases (Fig. 6, 7).

The nucleus appears round or oval on the electron micrographs. Indentations in the nuclear membrane are often found. The karyoplasm is finely granular with coarser blocks of chromatin scattered throughout. These blocks do not

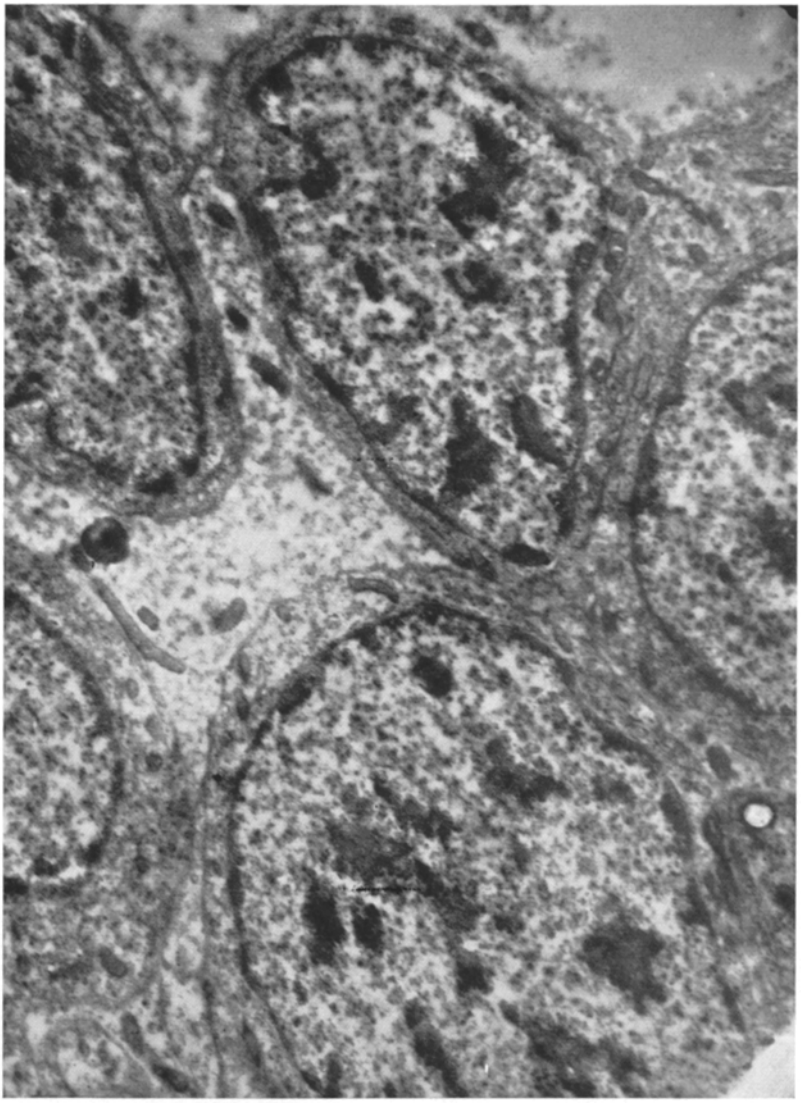

Fig. 6. Electron micrograph of a group of neoblasts. 12000 ×

vary much in size. Fine chromatin granules are often apposed to the nuclear membrane as a fine border. The nucleolus is composed of fine granules of uniform size. No reticular structure can be discerned. The nucleus is bounded by two opaque electron dense layers separated by an interspace of uniform width (Fig. 8). Small pores in the nuclear membrane can sometimes be discerned, but they are not very evident.

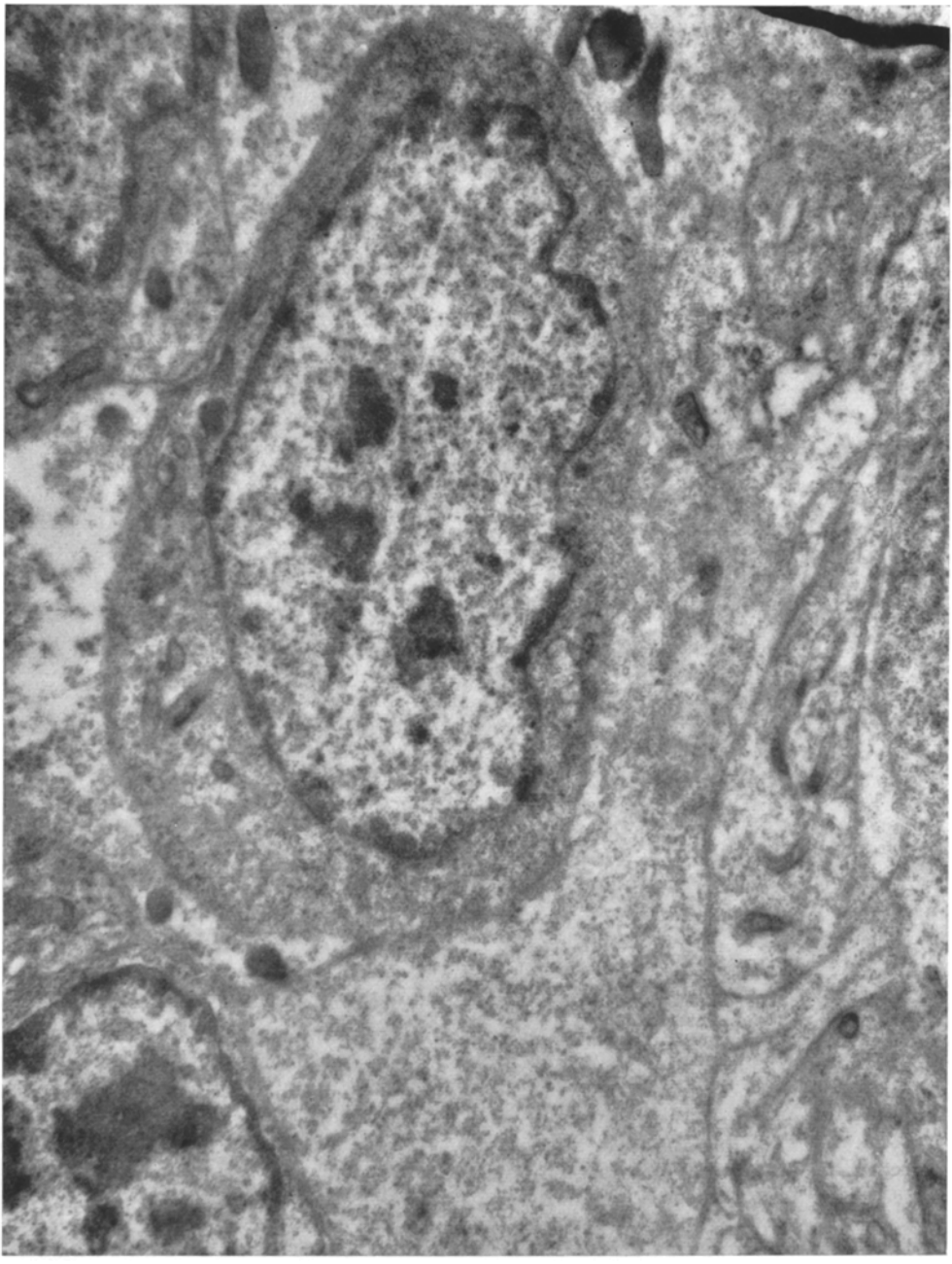

Fig. 7. Electron micrograph of a single neoblast. Notice its demarcation from the surrounding tissue and the strong electron density of the scanty cytoplasm. The mitochondria are few, small and irregular. 15 000 ×

The cell membrane of the neoblast presents something of a puzzle. As pointed out by ROBERTSON (1959), electron microscopists have defined the cell membrane in various ways, and considerable doubt is often present as to the meaning of

the term. When examined with the ordinary magnification (about 20000) the cell membrane of most cell types appears as a well defined 60—75 Å thick electron

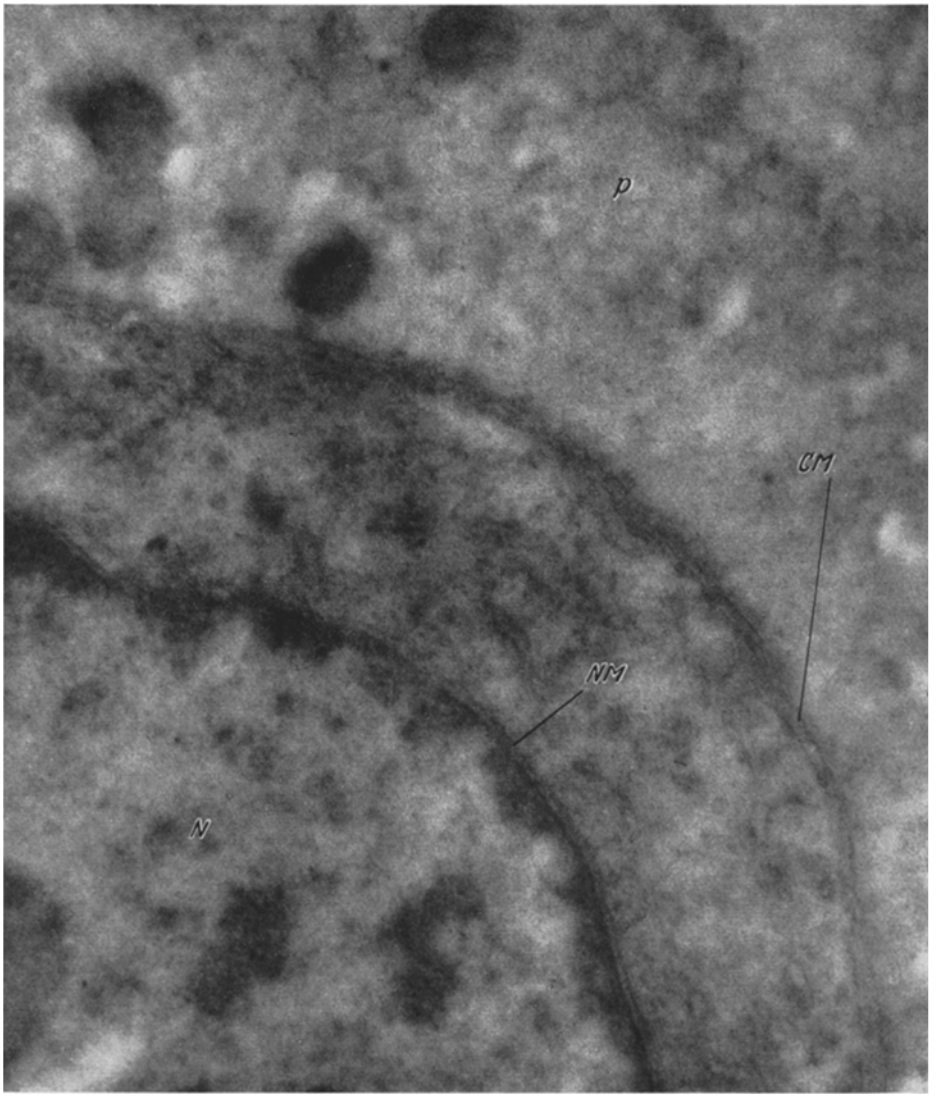

Fig. 8. Part of the nucleus and cytoplasm of a neoblast bounding the parenchyma. Notice the double nuclear membrane, the cell membrane (see text) and the electron dense cytoplasmic granules. 50000 ×. Abbreviations: *N* nucleus; *CM* cell membrane; *NM* nuclear membrane; *p* parenchyma

dense line. When high resolution is used and especially after potassium permanganate fixation, the cell membrane of different cell types exhibits a double structure consisting of two 20 Å thick lines separated by an interspace. However, in the neoblasts investigated in this work, the cell membrane exhibits a double structure already at the rather low resolution employed. The two lines are about 60—75 Å thick. They are very electron dense and are separated by

a less opaque interspace (Fig. 8). There is a very close similarity to the nuclear membrane. This very unusual feature of the cell membrane at the resolution

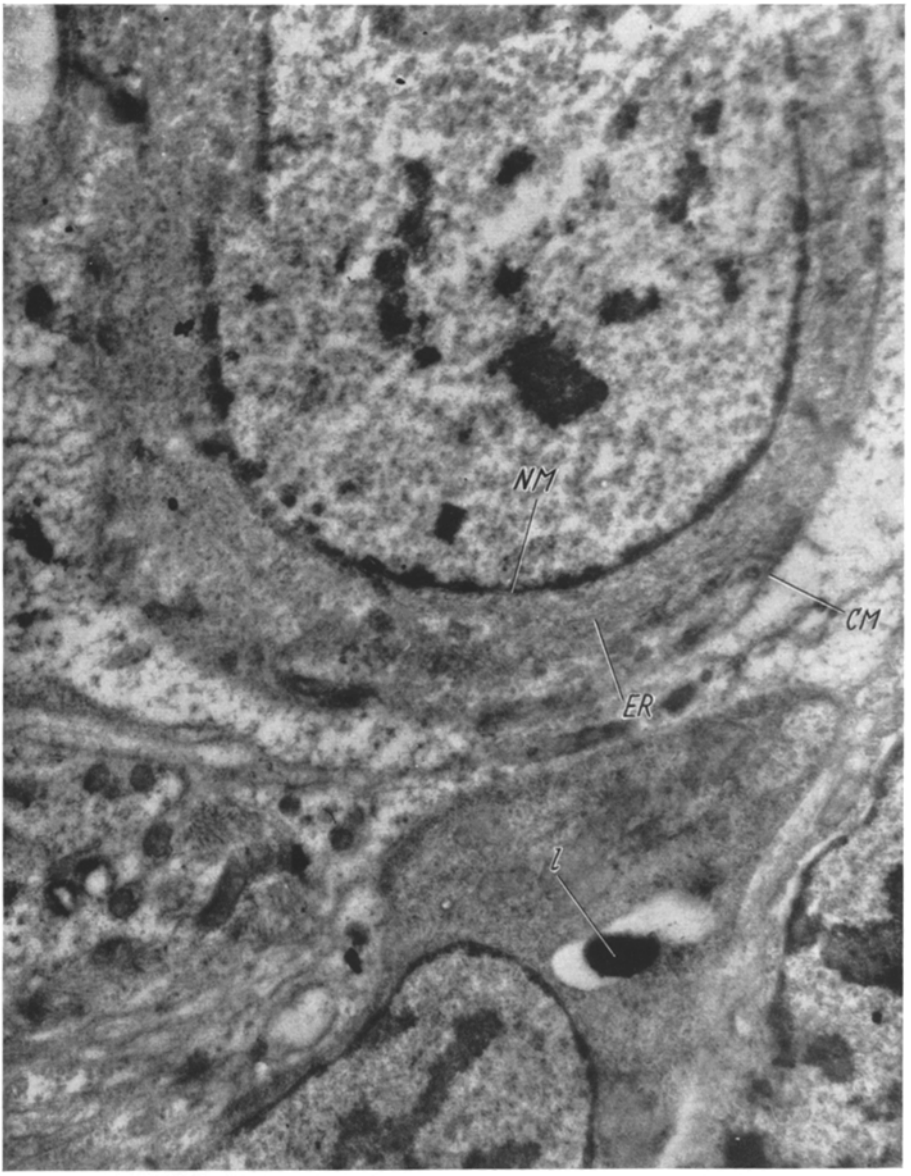

Fig. 9. Parts of two neoblasts are seen. The section has been stained with uranyl acetate thereby enhancing the electron density of the cytoplasmic granules. 15600 ×. Abbreviations: *CM* cell membrane; *NM* nuclear membrane; *ER* endoplasmatic reticulum; *l* lipid droplet

employed poses the question as to its significance. This question is indeed fundamental, as its answer is a key to some problems of the structure of the planarian parenchyma, namely: is the parenchyma a real syncytium; is it only composed of cells, or is an intercellular matrix present? These questions are not the subject

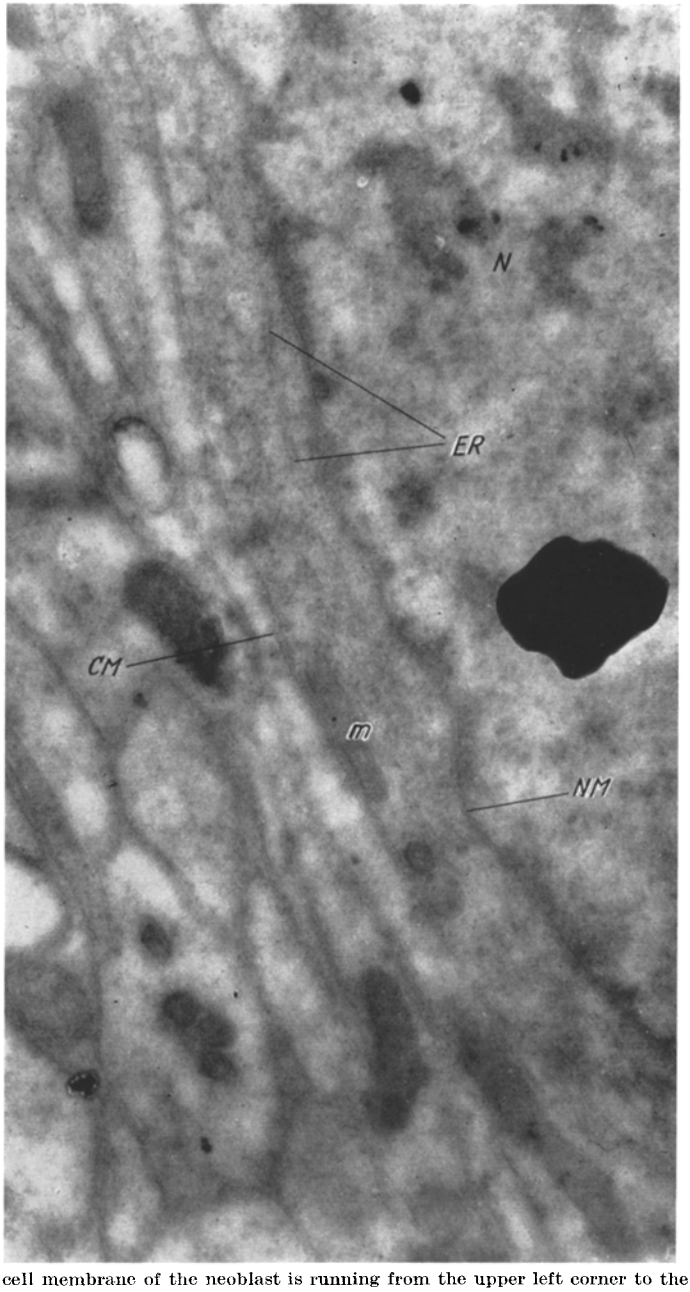

Fig. 10. The cell membrane of the neoblast is running from the upper left corner to the lower right. The endoplasmatic reticulum is well seen. 30200 ×. Abbreviations: *m* mitochondrion; *CM* cell membrane; *N* nucleus; *NM* nuclear membrane; *ER* endoplasmatic reticulum

of the present work and will be tentatively answered in a following study. Here it must suffice to say, that either the double structure delimiting the neoblast is the real cell membrane, and this would be a most unusual feature, or otherwise

only the inner membrane represents the cell membrane, and then the outer membrane belongs to an intercellular matrix. The latter hypothesis is at present

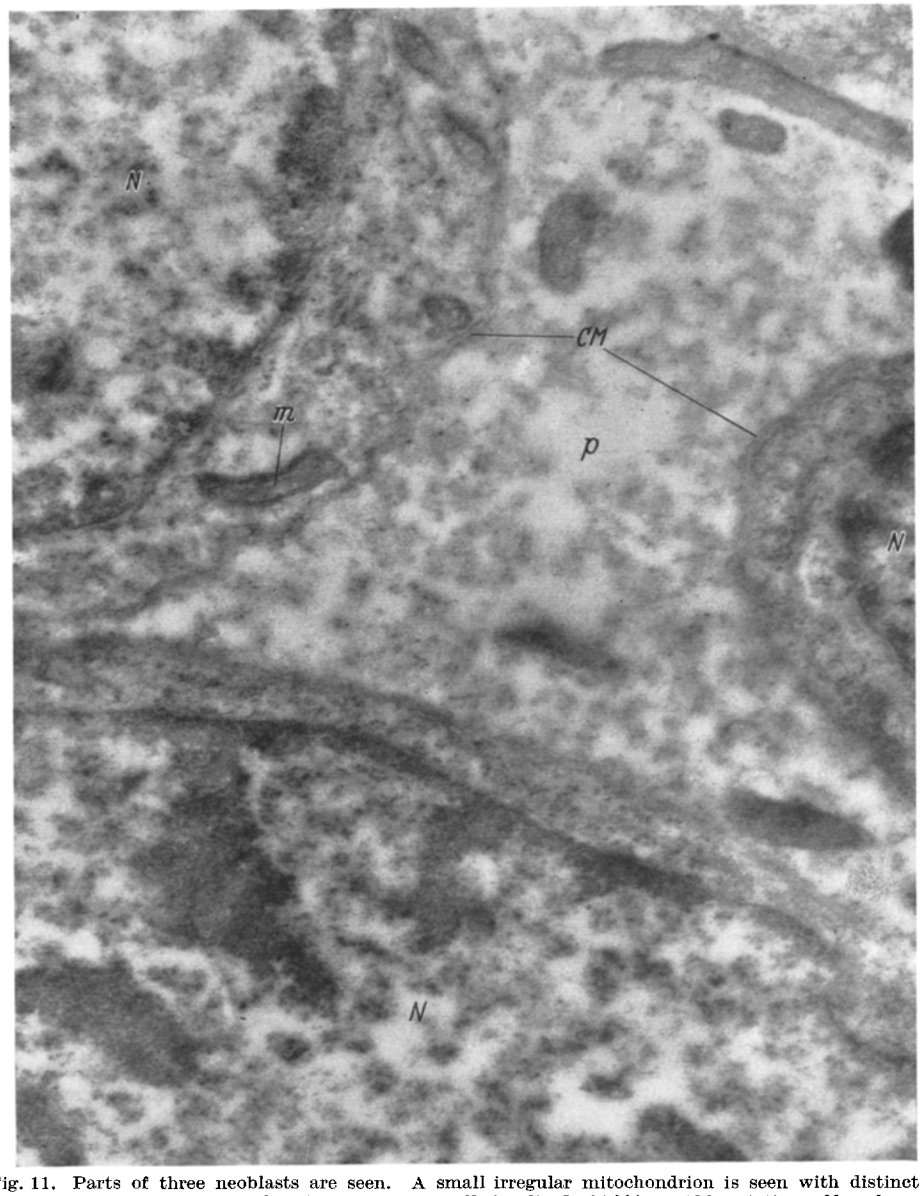

Fig. 11. Parts of three neoblasts are seen. A small irregular mitochondrion is seen with distinct double membranes. The cytoplasmic granules are well visualized. 34 000 ×. Abbreviations: *N* nucleus; *p* parenchyma; *m* mitochondrion; *CM* cell membrane

thought to be nearer the truth, and it is believed that the intercellular matrix is present in the form of vacuoles bordered by a cell membrane, as it perhaps represents isolated parts of cells. The reasons for this conclusion will be set forth in a following paper.

The cytoplasm is very electron dense, and the contrast can be even more augmented by staining with uranyl acetate (Fig. 9). The density is caused by fine granules about 150 Å in diameter. They are rather evenly distributed in the cytoplasm. Most of them occur free, but also many are membrane bound. These membranes of the endoplasmatic reticulum are present as double, closely apposed membranes 60—75 Å thick. The interspace is of constant width, about 180 Å. In this way very shallow compartments are formed. The endoplasmatic reticulum is characteristically distributed, mainly found concentrically around the nucleus parallel with the nuclear membrane and plasma membrane (Fig. 10). The membranes are continuous for long distances. The outer part of the membrane is covered with small granules. The corresponding free granules filling the cytoplasm occur so abundantly that the endoplasmatic reticulum is often somewhat obscured.

The mitochondria present are small and inconspicuous. On the sections they appear round, about 2000 Å in diameter or as short thick rods, about 5000 Å long and 2000 Å wide. The round structures may of course represent cross sections of the rod shaped mitochondria. In a few cases the mitochondria are long and slender. They are bounded by a complete membrane exhibiting double contours. The inner cristae are irregularly oriented, most often in the longitudinal direction (Fig. 11).

It was not possible with certainty to identify any Golgi material in the sections. Golgi material could not be detected until differentiating neoblasts were studied, and then was found only with difficulty.

Discussion

The picture of the neoblast obtained in the present study is that of a free cell with a characteristic morphology and characteristic cytochemical properties. The cytoplasm of the neoblast was strongly basophilic. Basophilia at a low p_H is generally due to RNA, DNA or acid mucopolysaccharides, but only RNA could be demonstrated in the cytoplasm of the neoblast, tests for the two last mentioned substances being negative. Protein was present in moderate or high concentration, and this protein displayed a highly basic nature. The amino acids demonstrable were cysteine/cystin, tyrosine, arginine, lysine and perhaps histidine. For theoretical reasons set forth previously in this paper, it was not possible to decide which of these protein-bound amino acids predominated.

The reactions of the protein point to a histone nature, and it is generally accepted that nucleic acids are linked to basic proteins, protamines or histones through salt-linkages, so probably part of the protein in the cytoplasm of the neoblast is bound to RNA. Fixation probably involves a dissociation of this nucleoprotein, so that the protein and nucleic acid moieties can be demonstrated separately.

The nucleoli exhibited a similar cytochemical nature as the cytoplasm and the reactions were frequently stronger still.

The high RNA content of the neoblast has previously been described (CLÉMENT-NOËL 1944, BRØNDSTED 1955, LINDH 1957, PEDERSEN 1958); LINDH has commented on the possible occurrence of cytoplasmic DNA in the neoblasts in another planarian species, Euplanaria polychroa. No DNA could be detected in the

species studied here, as evidenced by the negative Feulgen reaction. It may be mentioned that a high content of RNA has also been demonstrated in regeneration cells (interstitial cells) in Hydra and Tubularia (TARDENT 1952).

No hydrolytic enzymes could be demonstrated in the neoblasts by cytochemical means. Neither could oxidative enzymes be detected, with the exception perhaps of cytochrome oxidase. Of course oxidative enzymes must be present, but the concentration is probably too low for demonstration with the rather insensitive methods available for their study. The low concentration is consistent with the existence of the small and inconspicuous mitochondria to which the oxidative enzymes are bound.

The most distinguishing feature of the fine structure of the neoblast is the strong cytoplasmic electron density. This density is the result of the widespread occurrence of small granules, partly free and partly membrane-bound. It is assumed that the strong cytoplasmic basophilia is caused by these granules. The granules are probably composed of ribonucleoprotein. This is the case with other cells (PALADE 1956, HAGUENAU 1958, SIEKEVITZ 1959).

The fundamental problem as to the origin of the neoblast and its relation to the parenchyma cells is very difficult to solve. A discussion of this subject will be postponed to a following paper on the structure of the parenchyma.

The picture of the neoblast depicted in this study is that of a primitive, rather undifferentiated cell both on the level of light microscopy, electron microscopy and cytochemistry. The present approach to its study has not been able to reveal the basis for the generally accepted totipotency of the neoblast. It has only pointed to similarities with other morphogenetically active cells such as embryonic cells. Like these, the neoblasts contain great amounts of RNA and sulfhydryl-group containing proteins so vital for protein synthesis and perhaps also for inductive phenomena and differentiation during morphogenesis (BRACHET 1950, 1957).

Summary

The paper is a study of the cytology of the regeneration cells (neoblasts) in *Planaria vitta*.

The morphology of the living cells has first been examined to provide a reference for an investigation of the fixed neoblasts as studied by ordinary cytological, cytochemical and electron microscopical technics.

A rather selective staining method has been devised based on the strong basophilic properties of the scanty cytoplasm. The morphology of the fixed neoblasts and their distribution in the intact animal have been described, using this method.

The marked cytoplasmic basophilia was found to be exclusively due to ribonucleic acid, and not to desoxyribonucleic acid or acid mucopolysaccharides.

The cytoplasm contains moderate to considerable amounts of basic proteins. Tyrosine, cysteine/cystin, arginine, lysine and perhaps histidine were present, while tryptophan could not be demonstrated.

No enzymes could be demonstrated apart perhaps from cytochrome oxidase.

The mitochondria are small and inconspicuous and more or less evenly distributed throughout the cytoplasm. A Golgi apparatus could not be demonstrated.

The electron microscopic picture is very characteristic, because of the high electron density of the cytoplasm. This density is the result of the presence of a great number of ribonucleoprotein granules. Most of the granules are free and only a minor part bound to the membranes of the endoplasmatic reticulum. The interesting features of the cell membrane are discussed in relation to the structure of the parenchyma.

The cytochemical properties of the neoblast (RNA and sulfhydryl-group-containing protein) and the fine structure as revealed in the electron microscope characterize the neoblast as a morphogenetically active cell.

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