leaves. On the basis of his measurements on the light absorption in green algæ with only one single layer of plastids (Ulvaceae), SEYBOLD[1] estimated that the unproductive absorption in green leaves at the most amounts to 10 per cent of the illumination. This value appears to be rather reasonable if it is taken into account that the light, before entering the cells of the palisade tissue rich in chlorophyll, passes an epidermis which, apart from the guard cells around the stomata, does not contain any chlorophyll. Correcting the value of Q for an unproductive absorption amounting to 10 per cent of the illumination leads to $Q = 29{\cdot}5$, and the k values of the *Sinapis*, *Corylus* and *Fraxinus* leaves are then placed between 0·082 and 0·087.

In addition to the data presented above, some observations on photosynthesis in leaves made by BRIGGS in 1929[2] may serve as a basis for analogous calculations. BRIGGS' experiments were performed in light of wave-lengths 570 to 640 mμ. The assimilation was determined by air analysis, applying BLACKMAN'S palladium-black method in a hydrogen atmosphere with 5 vol. % carbon dioxide. Using the leaves of *Phaseolus vulgaris* (the only species on which several experiments were performed) and an average illumination of 33·5 cal/dm²/hr, he observed a mean value for the oxygen evolution of 1·03 ml/dm²/hr. Applying SEYBOLD and WEISSWEILER'S figure for the light absorption in *Phaseolus* leaves[3], we can calculate $k = 0{\cdot}077$ if no correction is made for unproductive absorption, and $k = 0{\cdot}088$ if a correction is performed in the manner described above. These values are in reasonable agreement with those calculated for the leaves of *Sinapis*, *Corylus* and *Fraxinus*.

The present calculations of the quantum efficiency in foliage leaves seem to indicate that the correct value is between 0·08 and 0·09. Thus, the results are in good agreement with numerous investigations on the quantum efficiency in monocellular algæ performed in recent years, especially those carried out with great thoroughness in methodical respect by EMERSON and LEWIS. There is much reason to assume that within the whole plant kingdom the conversion of one carbon dioxide molecule by photosynthesis requires about 12 quanta (12 quanta per molecule $\sim k = 0{\cdot}083$). Presumably, the true figure will never be determined quite accurately. With regard to the algæ, it will scarcely be feasible to measure the gas exchange during photosynthesis with sufficient accuracy; with respect to leaves, where this difficulty does not exist, the unproductive absorption, which eludes measurement, makes an accurate determination of the quantum yield impossible. Finally, the possibility cannot be excluded that an unproductive absorption exists also in algæ, and here it might also build a barrier, which would make impossible an exact determination of the quantum efficiency.

Zusammenfassung

Nach der thermodynamischen Gleichung für die Photosynthese kann die Quantenausbeute bei dem Prozeß (k = Anzahl von umgesetzten CO_2-Molekülen pro absorbiertes Lichtquant) höchstens einen Wert $k = 0{,}34$ haben. Für den Umsatz von 1 CO_2-Molekül müssen mindestens 3 Quanten verwendet werden. WARBURG und NEGELEIN fanden 1923 bei Versuchen mit einzelligen Grünalgen $k = 0{,}23$ und haben hieraus geschlossen, daß der Umsatz von einem CO_2-Molekül 4 Lichtquanten erfordere. Später wurde die Richtigkeit dieser Angabe bezweifelt, da zahlreiche Untersuchungen mit anderen Methoden einen k-Wert zwischen 0,04 und 0,14 ergaben. Die Diskrepanzen wurden durch EMERSON und LEWIS (1939–41) erklärt: sie fanden in den Versuchen von WARBURG und NEGELEIN einen methodischen Fehler. EMERSON und LEWIS beobachteten bei einzelligen Grünalgen k-Werte zwischen 0,07 und 0,11.

In der vorliegenden Arbeit wird darauf hingewiesen, daß man auch bei Laubblättern k-Werte von weniger als 0,23 erhält. Nach den Beobachtungen von BRIGGS und von GABRIELSEN sind bei *Sinapis*-, *Corylus*-, *Fraxinus*- und *Phaseolus*-Blättern k-Werte zwischen 0,08 und 0,09 zu berechnen. Diese Werte deuten ebenso wie die bei Algen erhaltenen darauf hin, daß der Umsatz von einem CO_2-Molekül bei allen grünen Pflanzen 12 Quanten erfordert; das entspricht einem k von 0,083.

[1] A. SEYBOLD, Planta (Berlin) *21*, 251 (1933).
[2] G. E. BRIGGS, Proc. Roy. Soc. London (B) *105*, 1 (1930).
[3] A. SEYBOLD and A. WEISSWEILER, Bot. Arch. (Leipzig) *43*, 252 (1942).

The Trephocytes and their Functions

By EMIL LIEBMAN[1], Princeton, N. J.

Though white blood cells had been seen before, T. WHARTON JONES[2] is usually credited with their discovery and their first brief description as cellular elements. WILLIAMS[3], in a careful study of numerous invertebrates, gives a detailed account of "nonlocomotive", more or less spherical leucocytes, filled "with oil molecules and granules" and stresses their large size, which in some species makes them discernible with the naked eye.

WILLIAMS, like his predecessor, also saw ameboid elements but considered them as "jagged and broken

[1] N. Y. Zool. Society.
[2] T. WHARTON JONES, Philos. Trans. *136*, 63 (1846).
[3] TH. WILLIAMS, Philos. Trans. *142*, 595 (1852).

cells" for they were then thought to represent artifacts or, more commonly, amebas. LIEBERKÜHN[1] is largely to be credited with disproving these misconceptions and demonstrating that the ameboid elements are actually leucocytes.

Ingestion of solid particles was considered an important characteristic of amebas and, to prove further the ameboid nature of leucocytes, it was repeatedly attempted to make them take up such substances. HAECKEL[2], by injecting indigo and carmine suspensions into several invertebrates, finally succeeded in demonstrating ingestion of particulate substances by the leucocytes, a phenomenon which later became known as phagocytosis.

Despite the occasional early distinction between nonameboid and ameboid leucocytes, both were generally held as identical or, at best, were confused with each other. Nonetheless, it was gradually realized that the leucocyte inclusions, held to be fat or albuminoid, are liberated into the body fluids—a view already conjectured by WILLIAMS. Thus a dualistic nature became vaguely associated with the leucocytes, viz.: ingestion of foreign matter, and liberation of nutritive substances.

The conceptions of this period are perhaps best reflected in the extensive study of CUÉNOT[3] covering practically the whole animal kingdom. Briefly, his conclusions were that the ameboid, hyaline elements are phagocytes and normally instrumental in excretion. Upon further development they evolve inclusions of nutrient matter, losing, *pari passu*, more or less of their ameboid capacity. The nutritive matter is released into the body fluids, the cell changing again into an amebocyte. He often considered the leucocytes as mediators between the digestive system and the rest of the body, especially where their origin or sojourn was linked with this system. Later, clearly under the influence of METCHNIKOFF'S theories, he reversed his stand on the nutritive function of the leucocytes and, often based on faulty technics, saw in all leucocytes phagocytes or excretory cells. KOLLMANN[4] has not gone so far as CUÉNOT in rejecting the nutrient function of leucocytes but his account is often confused owing to an attempt to use the size of the cellular inclusions or their tinctorial reactions as exclusive criteria.

METCHNIKOFF, starting with studies on ingestion and digestion of particulate food by the endorm-cells of lower invertebrates, later found that in the higher animals somewhat similar faculties are still retained by some mesodermal cells: the free and fixed macrophages and the microphages (special leucocytes[5], neutrophils in man). He also established the role of phagocytosis in metamorphosis. But his greatest contribution is the discovery that the leucocytes phagocytose and destroy bacteria and other pathogenic agents, thus acting in defense of the organism. Starting with the pioneer study on *Daphnia*[1] he rapidly extended his investigations to pathogenic organisms of higher animals and man and expounded what is known as the phagocytic theory or inflammation and immunity[2].

The great importance which METCHNIKOFF's work held for medicine caused all attention to be focussed on the phagocytic and defensive functions of the leucocytes and the notion became practically universal that they are all involved in these activities.

This trend lasted well over a quarter of a century. It was broken by the extensive studies on the leucocytic trephones of CARREL[3] and his school and was soon followed by investigations into the trophic function of leucocytes in invertebrates. A summary of these developments is presented here. Owing to their relative simplicity, conditions in invertebrates are dealt with first.

A. *The trephocytes of invertebrates*

Investigations of the present writer on representatives of annelides, insects, tunicates and the cœlenterate *Tubularia*[4] as well as other sources, reveal that invertebrates possess two main kinds of leucocytes: (a) hyaline and (b) granular (Fig. 1).

The hyaline elements are similar to the lymphoid series of vertebrates and can conveniently be termed lymphoidocytes. Functionally, these cells represent *phagocytes* as, with the possible exception of their youngest forms, the lymphocytes, they are able to ingest foreign and noxious matter, an activity known as *phagocytosis*. Phagocytosis is particularly evident in the largest of these cells, the macrophages.

The granular elements are neither ameboid nor phagocytic. In cases where they develop from a common stem cell with the phagocytes, the intermediate forms may be both ameboid and phagocytic, but the fully developed ones of all cases are neither ameboid nor phagocytic. Their morphological and more detailed physiological properties, as well as similarity throughout the animal kingdom, have been outlined by the writer and the term *trephocytes* suggested for them as they produce and ultimately liberate nutritive and growth substances. Their function is referred to as *trephocytosis*.

[1] N. LIEBERKÜHN, Müllers Arch. Anat. Physiol. *1854*, 1, 349.
[2] E. HAECKEL, Die Radiolarien. Berlin 1862.
[3] L. CUÉNOT, Arch. Zool., expér. 2me sér. *9*, 13, 593 (1891).
[4] M. KOLLMANN, Ann. Sci. nat. (Zool.) Année 84 (9 sér.) *8*, 1 (1908).
[5] In order to avoid contradictions and confusion stemming from terminology based on staining reactions, which vary greatly in these elements, depending on the species, this term will be used henceforth. It is particularly suitable as *these* leucocytes are special feature of the vertebrates only.

[1] E. METCHNIKOFF, Virchow's Arch. *96*, 177 (1884).
[2] E. METCHNIKOFF, Leçons sur la pathologie comparée de l'inflammation. Paris 1892. – L'immunité dans les maladies infectieuses, Paris 1901.
[3] A. CARREL, J. Am. med. Ass. *82*, 255 (1924), et ante.
[4] E. LIEBMANN, Zool. Anz. *69*, 65 (1926); Zool. Jb. (Physiol.) *44*, 269 (1927); Zool. Jb. (Anat.) *54*, 417 (1931); J. Morph. *70*, 151, *71*, 221 (1942); J. exp. Zool. *91*, 373 (1942); J. Morph. *73*, 583 (1943); Growth *9*, 43 (1945); Ib. *10*, 291 (1946).

The trephocytes as a rule are larger than the corresponding phagocytes in the same species and apparently represent the largest blood elements in the animal kingdom. Their shape is spherical, sometimes ovoid or flattened. They are non-ameboid when fully developed though a slow and slight change in form may be noticed during prolonged observation, even in the older forms.

The nucleus is round or oval. It is small in relation to the cytoplasm and often smaller than the corresponding nucleus of the phagocytes, features particularly evident in the fully grown elements. It stains rather weakly, showing only few chromatin granules. In the mature cell, the nucleus is often elongated, fragmented into two or more portions, or pycnotic; sometimes it dissolves in the cytoplasm, the cell remaining anuclear. In fixed material, a central body and a mitom appear as regular features of these elements.

The cytoplasm is filled with endogenous inclusions ranging in size from coarse granules to a single large globule, filling the bigger portion of the cell. In the latter case, the globule may develop by accretion of smaller droplets or by growth of a single minute inclusion, the various stages being found in the same individual. In some species the individual cells may contain inclusions ranging from granules to big globules; exceptionally, the inclusions are fine and appear more or less uniform in size. The tinctorial reaction of the inclusions varies from weak acidophilia to basophilia, the latter apparently being more common. Metachromasy has been observed in an oligochæte and a tunicate. In some species the individual trephocytes contain granules staining in a diverse manner; even single inclusions may exhibit areas or layers of diametrically different staining affinities. The inclusions of young trephocytes are often chromophobe. All this suggests the relative unimportance of staining reactions as general criteria.

The chemical nature of the inclusions is of far greater significance. Protein and lipids, often combined, appear to be most common; phospholipins, glycogen and mucin are also frequent. Guanine, claimed to be a growth substance, is reported from trephocytes of numerous annelides. Basophilia of the inclusions and dissolution of the nucleus in the cytoplasm suggest the presence in these cases of nucleoproteins. Old cells often contain pigment granules and urates which apparently represent end products of the cells' own metabolism. The inclusions are frequently colored, which gave rise to assumptions that they contain a respiratory pigment. This seems to be established for echinochrome found in echinoderms (MACMUNN, CANNON, and others). In highly specialized groups like the insects, derivatives of trephocytes are suspected of producing hormones and enzymes.

In older trephocytes the inclusions, together with the nucleus and cytoplasm, often fuse into spheroid, compact concretions. In *Tubularia*, these are almost homogenous and appear to represent one of the two modes of trephocyte development (Fig. 2); here they are known as "pseudo-cells". All these formations represent globular masses of nutrient matter to be utilized in growth.

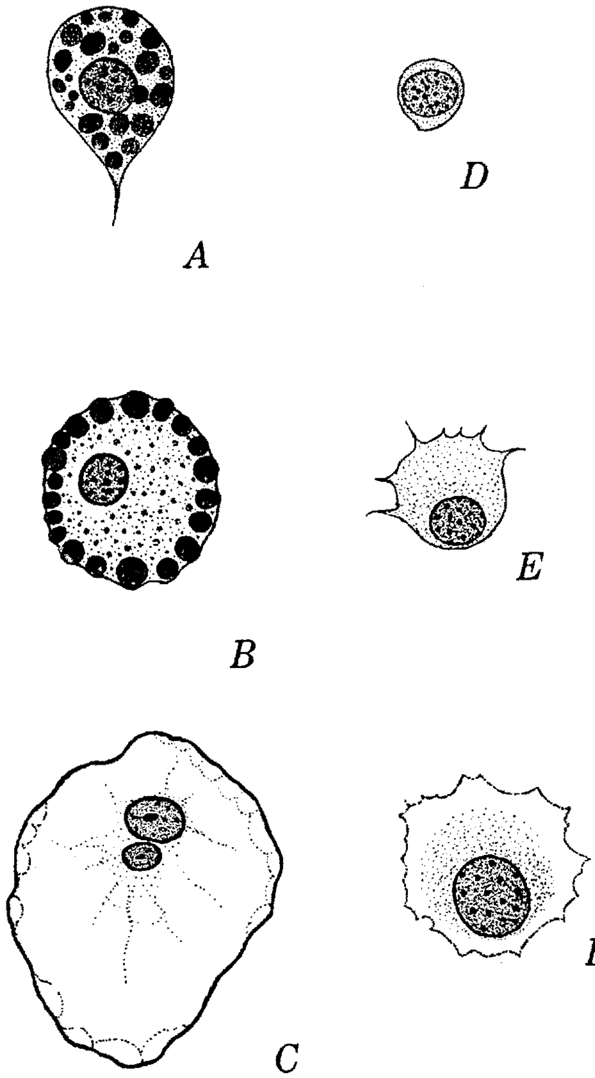

Fig. 1. Left series: trephocytes, right series: phagocytes, of the earthworm *Eisenia foetida*, drawn to the same scale. *A* a young, *B* a mature trephocyte; *C* a mucocyte; *D* a lymphocyte; *E* an intermediate form (young macrophage, monocyte); *F* a macrophage. Original.

The trephocytes often form aggregates: these have been investigated in the earthworm *Eisenia foetida*. Here they float in the cœlomic fluid and though not bound by a limiting membrane their form and position in the body are practically stable. In the adult they are active during regeneration only and may thus be regarded as regeneration organs. There are two such organs in this species each located at opposite ends of the body and each exhibiting characteristic cytological and cytochemical traits of its own: a permanent "head" organ and a transitory "tail" organ. The latter is formed after amputation and utilized in regeneration of the posterior part of the body. Very young individuals invariably possess a tail organ which is used up during the growth of the posterior segments.

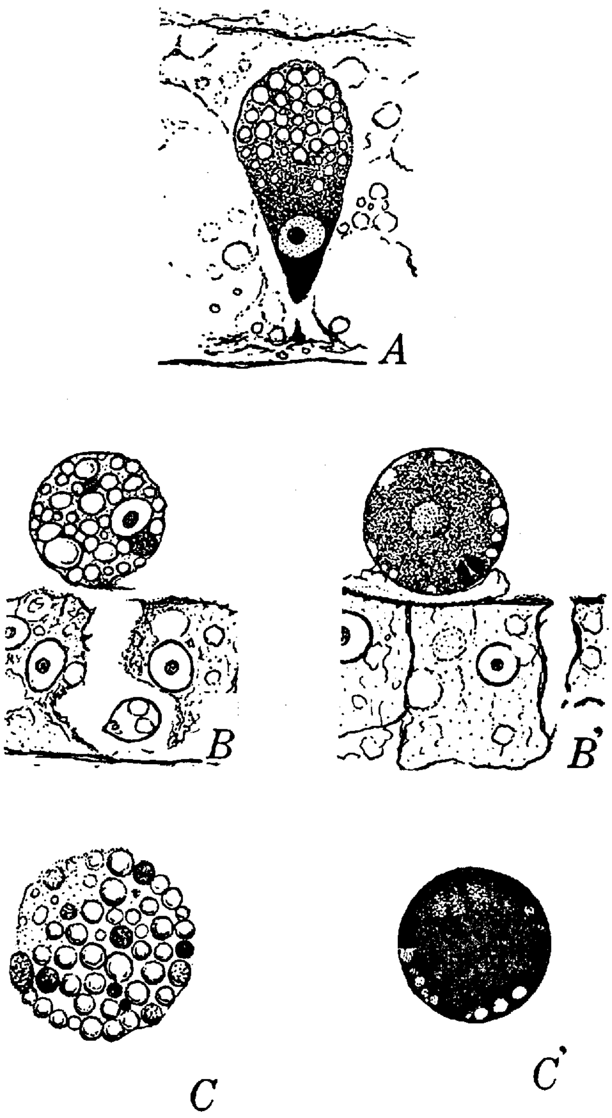

Fig. 2. Trephocytes of *Tubularia* sp.; *B*, *C* granular variety; *B′ C′* homogenous, compact variety ("pseudo-cells"); *A* endodermal stem cell of both. Original.

Similar aggregates have long been known in insects and termed fat-bodies, a misnomer as, besides fat, their cells contain protein, glycogen and other substances (WIGGLESWORTH). Their organization varies, according to the species, from loose aggregations like those in *Eisenia*, to compact cell masses covered with a membrane (SNODGRASS). Exceptionally, as in *Aleurodes*, no fat body is present and the trephocytes (fat-cells) float freely in the body fluids.

In the tunicate *Styela partita*, mature trephocytes were found by the author to settle in the connective tissue where they attain large size; this phenomenon resembles conditions in the vertebrates, where the tissue mast-cells also reach extreme proportions. The staining reactions in this particular case are also similar to those of the mast-cells. This turning of free trephocytes into fixed cells does not apparently represent an isolated case amongst invertebrates and also seems to occur in molluscs.

The substances formed by the trephocytes are released into the body fluids, organs and tissues or directly into other cells. As a rule, it is mostly the fully grown elements which liberate their inclusions. During this process the cell often disintegrates, thus its whole content, including the nucleus, serves as nutrient. In cases where the cell survives the process, its cytoplasm appears hyaline, liquid and poorly or non-stainable. These emptied cells are usually called mucocytes (Fig. 1 *C*).

The body fluids of numerous invertebrates contain very little nutritive matter in dissolved form. In these species, which probably comprise the majority, the trephocytes represent the main or sole carriers of nutrient substances within the organism. In those where the dissolved nutrients attain a higher percentage, the action of the trephocytes, though not entirely lacking in normal conditions, becomes very patent in sites and periods of increased growth, as in wound healing, regeneration, metamorphosis, sexual and asexual reproduction.

The ovarial eggs during their growth period offer very suitable objects for the study of both increased activity of the trephocytes and their penetration into, and assimilation by, another cell. In *Tubularia*, the so-called pseudo-cells are found in great numbers in the growing eggs, where they gradually become assimilated (Fig. 3*A*). Trephocytes were also detected in the ovaries and eggs of several oligochætes investigated; the deutoplasm of these eggs shows cytochemical characteristics similar to those of the trephocyte inclusions, from which it largely derives. In the cases mentioned, the trephocytes apparently penetrate passively into the egg. In *Styela*, however, and probably in other tunicates, the process is apparently an active one, the trephocytes penetrating when they are still in the young, ameboid stage (Fig. 3*C*). Here, as in the two other tunicates studied, the trephocytes actually seem to grow for some time within the egg before they disintegrate and become incorporated into the cytoplasm. Trephocytes appear to furnish substances to the growing eggs of other groups also (sponges, myriapodes, spiders, etc.).

The ingestion and assimilation of trephocytes by other cells is apparently not confined to the ovum.

Young phagocytes of annelides were observed *in vivo* to nip off portions of live trephocytes and to assimilate them. This apparently represents a form of feeding on the part of the phagocytes and can hardly be considered as phagocytosis in the accepted sense, which usually implies removal of dead or noxious matter. Trephocytes migrate into the epithelium of the digestive tract of oligochætes, where they are often found within the cells in a dissolved condition and are assimilated by them. A more frequent occurrence is that of the trephocyte applying itself to another cell or tissue and giving off its content directly to them or to the pericellular fluid.

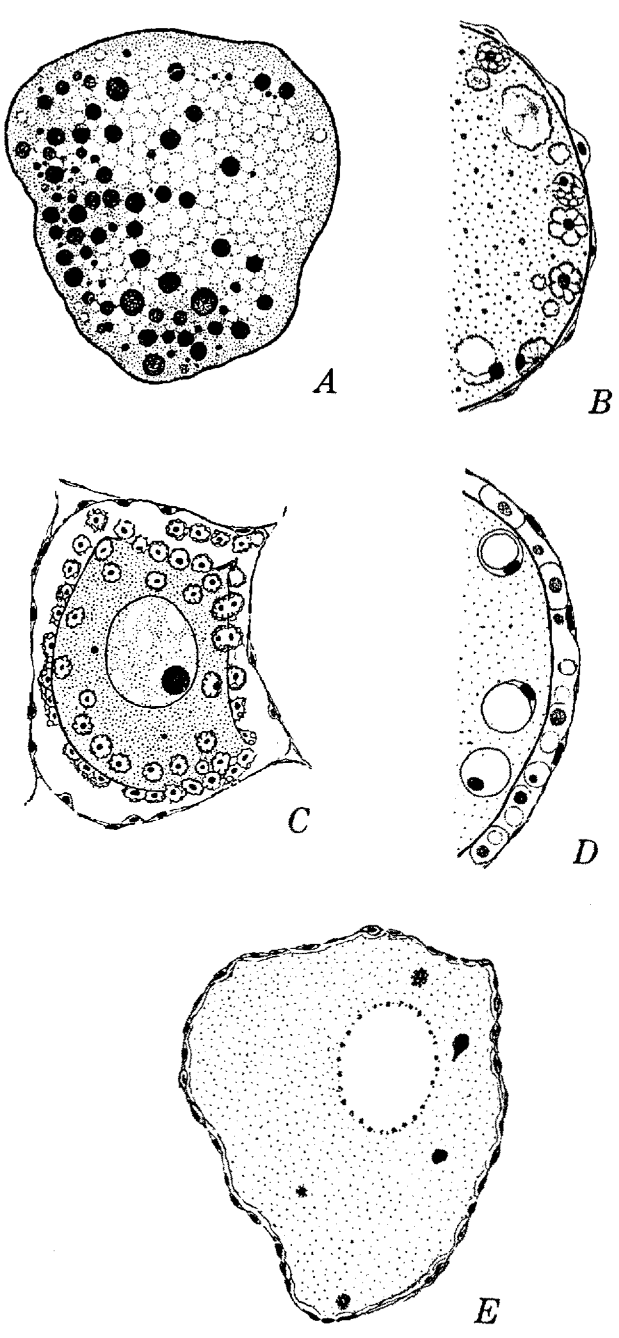

Fig. 3. Trephocytes in ovarial eggs. *A* of *Tubularia* sp.; *B*, *C*, *D* of the tunicates *Perophora viridis*, *Styela partita*, *Molgula manhattensis*; *E* lymphocytes (dark spots) in the egg cytoplasm of *Triturus viridescens*. Original.

Trephocytes are also instrumental in asexual reproduction. In tunicates they are reported to move into the winter-buds and to form aggregates filling their cavity (SPECK[1]). SPECK's assumption, however, that they are "totipotent" cells from which all the tissues of the emerging organism develop is untenable in our opinion, since the trephocytes represent specialized end cells.

There are some isolated observations that elements of the trephocyte type play some role in wound healing in a variety of animals (echinoderms, crustacea, insects). In *Eisenia*, where conditions are best known, they accumulate near the wound in great numbers, after the macrophages have eliminated the cellular debris caused by the injury. Except for greater intensity, their action is essentially the same as in normal conditions and consists in supplying nutrient to the cicatrix during the healing process.

During regeneration of the same species the trephocytes act as elements of special aggregates or organs described earlier. In addition to their characteristic morphology, these organs are quite specific in their function. They are not affected by, nor do they become active in, wound-healing or the developing egg, and it is evident that the individual trephocyte, when organized into an aggregate, loses its pluripotent trophic capacity and is capable of supplying nutriment to the regenerant only. The aggregates are functionally polarized and act exclusively in one direction, which coincides with and possibly determines the direction of regeneration; their extent and quantitative gradient appear to be the immediate determinants of the extent and gradient of regeneration. When no aggregate is present, no regeneration is observed to take place.

The study of trephocytosis throws some light on the still obscure problem of growth-correlations. In *Tubifex* and *Eisenia* continued egg production depletes the reserve of trephocytes and the mother tissue producing them: the chloragogue. This in turn causes a decrease of regeneration capacity in the depleted region. Vice versa, amputation of the posterior end attracts great numbers of trephocytes for the ensuing regeneration process, thus withdrawing them from the egg, and oviposition ceases during the main period of regeneration. In other words, the presence of one type of intensive growth consumes the available trephocytes (and ultimately nutritive matter) thus inhibiting the simultaneous occurrence of other types of growth. As similar growth correlations are of com-

[1] J. SPECK, Arch. Entwmech. *111*, 119 (1927).

mon occurrence, it is not improbable that a like mechanism underlies these cases.

The trephocytic aggregates of the insects, the fat-bodies, apparently represent the highest degree of development in the animal kingdom. This seems to be linked with the presence in this group of periods of highly increased growth during moulting and metamorphosis and it is in these processes that the activity of the fat body is most conspicuous. It often breaks up into individual cells and the reserve material stored up in the latter becomes liberated so that the blood assumes a thick, creamy appearance. In some cases the individual fat-cells disintegrate, as is the case with trephocytes in general. The substances released into the blood are apparently utilized in the extensive upbuilding processes which take place during this period. In the adult, the fat body is often larger in the female than in the male; this may be connected with egg-production as suggested by the writer for mosquitoes. Starvation and hibernation cause a considerable depletion of the reserve substances from the fat body.

*

It should be stressed here that the trephocytes are not merely nursing cells in the usual sense but that they appear to enter some close physio-chemical relation with their surroundings or the object they nurture. So far, the indications for such a nexus are few but nevertheless highly significant. The phenomenon that in *Eisenia* both trephocytic aggregates, though lacking a membrane or other bonds, are kept in shape and position notwithstanding the movements of the body and the lymph, points to some as yet unknown forces in the internal milieu, effecting this stability. In this species the trephocytes of the head aggregates differ considerably from those of the tail, though both originate from identical free elements: those of the tail have characteristic lipid inclusions, whilst those of the head are devoid of them. Similarly in tunicates, the trephocytes within the eggs develop in a different way, and their inclusions frequently show tinctorial and cytochemical reactions varying from those in the elements outside the ovum.

The significance of trephocytosis in the invertebrate organism may be judged by the numerical prevalence of the trephocytes over the phagocytes. In oligochæte annelides, crustaceans, insects, tunicates and some smaller groups, they are the predominant type of leucocytes. In other forms, where they are not as numerous, they and their activity apparently become prevalent during periods of increased growth activity.

The morphologic and physiologic pecularities outlined above clearly demonstrate that the trephocytes are specific cells and not merely transitory forms of phagocytes, as surmised by early investigators. Actually they represent highly specialized end cells, as witnessed by the loss of the nucleus, a phenomenon again encountered in the mammalian erythrocyte. The fact that in several groups they arise independently from the phagocytes (in *Tubularia*), often in special regions or even in peculiar organs (in oligochætes and polychætes), only stresses their specific character. Finally, their occurrence in the lowest invertebrates suggests that they are phylogenetically the first specialized hemocytes in the animal kingdom.

B. *The trephocytes of vertebrates*

Notwithstanding the great number of investigations into the vertebrate leucocytes, our knowledge of their trephocytes and trephocytosis is in many respects far more limited than that of invertebrates. In consequence, much of the evidence offered here is fragmentary and some conceptions expressed debatable or of a hypothetic nature.

As will be shown, the mast-cells, amongst the vertebrate leucocytes, reveal the closest possible resemblance to the invertebrate trephocytes. They are not, however, the only vertebrate blood elements exhibiting a trophic activity and the lymphocytes appear to elicit similar functions. This duplication does not stand alone in the vertebrates; a similar case is the emergence in this group of the special leucocytes and their supplementing—and in some respect superseding—the function of the macrophages. Thus there are apparently in the vertebrate organism two kinds of trephocytes: mast cells and lymphocytes, and two kinds of phagocytes: macrophages and special leucocytes.

(a) *The mast-cells*

It is generally agreed that in the lower vertebrates the tissue mast-cells originate from those of the blood: the mast leucocytes or basophils. As for the mammals, the majority of hematologists maintain—largely on the basis of solubility of the cellular inclusions—that each represents a distinct cell type.

In rodents and some other groups, however, a separation of mast leucocytes from mast-cells is difficult and a migration of the former into the tissues has been repeatedly observed (TÜRK; HELLY, JOLLY, LEHNERT[1]); in inflammation this seems to be of general occurrence. There are also claims that forms intermediate between the two kinds are encountered (MARCHAND, ZIMMERMAN, and others). The numerical correlation between the blood elements and those of the tissues, observed in mammals, adds further support to the idea that here too both kinds are genetically related. And, finally, our own observations on the variations of invertebrate trephocytes and their inclusions, de-

[1] For a detailed account on earlier investigations, see: A. MICHELS, The Mast-Cell, in Downey's Handbook of Hematology *1*, 232 (1938).

pending on the surroundings, make it very doubtful whether the argument of variability can be applied against a unitarian cenception of the mammalian mast-cell. Consequently, both the tissue mast-cells and those of the blood will be considered as representing variants of a single type throughout the entire vertebrate section.

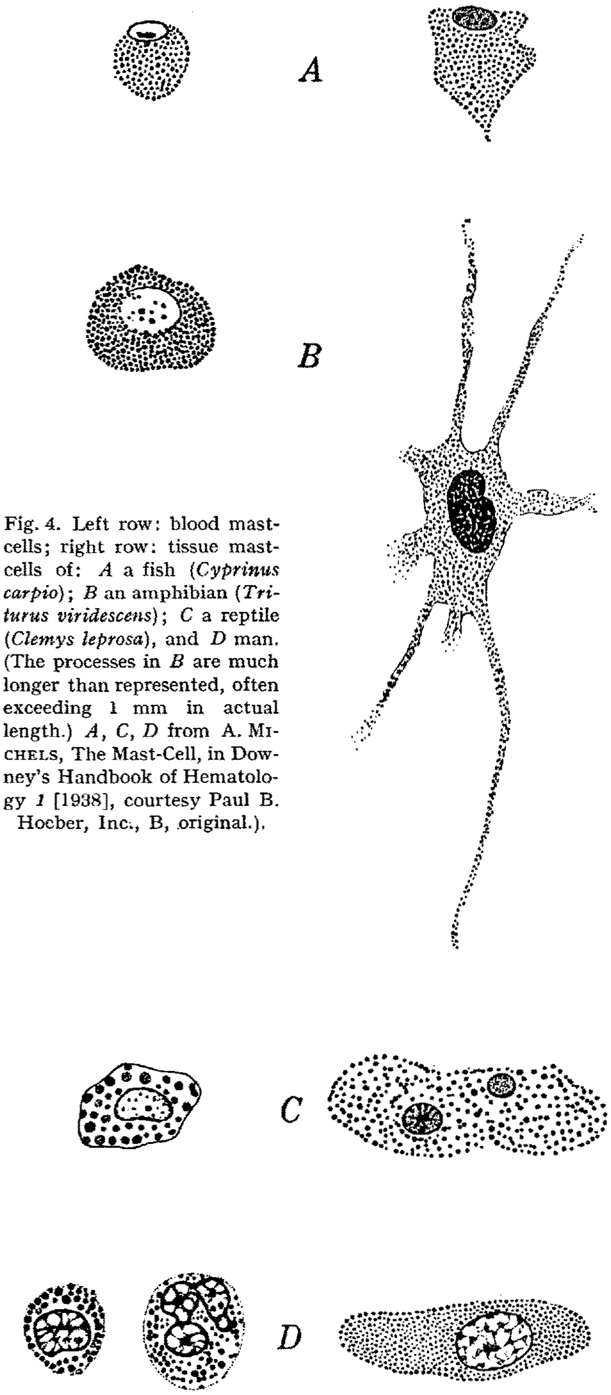

Fig. 4. Left row: blood mast-cells; right row: tissue mast-cells of: *A* a fish (*Cyprinus carpio*); *B* an amphibian (*Triturus viridescens*); *C* a reptile (*Clemys leprosa*), and *D* man. (The processes in *B* are much longer than represented, often exceeding 1 mm in actual length.) *A*, *C*, *D* from A. MICHELS, The Mast-Cell, in Downey's Handbook of Hematology *1* [1938], courtesy Paul B. Hoeber, Inc., B, original.).

The blood mast-cells vary considerably in relative size, sometimes greatly exceeding all the other leucocytes, a trait observed occasionally even in the mammals (pig, camel). The tissue mast-cells, however, appear to be invariably larger than the corresponding mast leucocytes, often attaining gigantic size (Fig. 4). In the blood, their shape is spherical or oblong; in the tissues of similar form, polymorphous or branched. There is general agreement that both the blood and the tissue elements are poorly or non-ameboid and non-phagocytic. The tissue mast-cells very often form aggregates.

The nucleus is round, oval or indented; in the blood mast-cells of the higher vertebrates it is often lobated or moderately polymorphous. In the tissue variant, which probably represents the fully grown element, the nucleus is small in relation to cell size, often fragmented or pycnotic; a centrosome is also found in this form.

The cytoplasm is packed with granular, occasionally spherular, inclusions which usually vary in size within the species or the individual cell. Tinctorially they are all basophilic and usually metachromatic. The inclusions are variously described as containing albumin (EHRLICH, BALLOWITZ, V. MÖLLENDORFF, NAKAJIMA, and others), glycoprotein, specifically mucin or a mucoid substance (WERMEL and SASSUCHIN; PAPPENHEIM, HARRIS, STAEMLER, and others) and fat (LOMBARDO, CIACCIO, TUMA). Basophilia of the granules suggests, according to recent views, the presence of ribonucleic acid; several early investigators (MEYER, VAN HERWERDEN, ROMIEU) assumed that the inclusions contain nucleoprotein. Some of the above claims, especially regarding fat, are admittedly not uncontested. Recent studies by JORPES, HOLMGREN, and WILANDER[1] strongly suggest that the metachromasy of the granules is indicative of heparin. By this we have not nearly exhausted all the claims, which include: glycogen, pigment, elastin, oxydase, peroxydase, etc.

Amongst the numerous and controversial claims about the nature and function of the mast-cells, two views are almost unanimously upheld: (a) that they are not phagocytic and (b) that they release portions of their cytoplasm and inclusions to the surrounding, the elements acting as unicellular glands. This and the great similarity of the mast-cells to the invertebrate trephocytes, both as regards morphology and the chemical nature of their inclusionn, leaves little doubt that the mast-cells are trephocytes.

The notion that the mast-cells are linked with the nutritional condition was already voiced by EHRLICH[2] who was the first to demonstrate the specificity of these elements and who was the originator of their name. This view, albeit not uncontested, is held by many investigators and is supported by ample though

[1] E. JORPES, H. HOLMGREN, and O. WILANDER, Z. mikr. anat. Forsch. *42*, 242, 279 (1937).

[2] P. EHRLICH, Arch. mikr. Anat. *13*, 263 (1877); Arch. Anat. Physiol. (Phys. Abt.) *1879*, 166.

admittedly general evidence. To quote only some: in frogs, feeding causes increase of mast-cells as compared with the fasting period (STASSANO and HAAS). Similarly, mast-cells in the gut of lower vertebrates were considerably scarcer after hibernation (MICHELS). They were also found to be extremely active in assimilation and transportation of food (WEILL). A decrease in the number of mast-cells and disapearance of fat from their inclusions was observed in avitaminosed rats (TUMA). Rats fed on cows' milk showed a doubling in the number of peritoneal mast-cells (NAKAJIMA).

In so far as growth is concerned, investigations have been limited almost exclusively to neoplasms. It has repeatedly been observed that the mast-cells accumullate around cancerous growth, whether spontaneous (FROMME, WEILL, HIGUCHI) or experimental (BIERICH, FABRIS, and others). It is assumed that they have a defensive role here. Recently CRAMER and SIMPSON[1] based such statement on the presence of heparin in the mast-cells. *In vitro*, this substance was found to retard the growth of sarkoma cells and histiocytes (ZAKRZEWSKI; FISCHER).

During regeneration of the salamander *Triturus viridescens*, tissue mast-cells in the vicinity of the cut were found to shed a great part of their granules; at the same time, similar inclusions appeared in the fibroblasts of the regenerant, which multiply intensively during this period (LIEBMAN, unpublished observations). Whether these inclusions are given off directly to the fibroblasts has not been definitely established, but there are some indications that this may be the case. BRODERSEN[2] claims that in the mouse a transition of granules from the mast-cells to the fibroblasts takes place under normal conditions.

We have limited our account to the functions of nutrition and growth, the main activities with which the trephocytes in general are concerned; these too take up a considerable part in the study of the mast-cells. Other phenomena have occasionally been linked with these cells, such as: pigmentation, respiration, lactation, etc. Considerations of space and the secondary place these functions occupy in the investigations of the mast-cells, exclude dealing with them at present.

The similarity, both morphological and functional, and the identity of substances found in both, strongly suggest that the mast-cells are homologous to the invertebrate trephocytes. Though many particulars especially those regarding the function of the mast-cells are little known and probably for this reason highly controversial, the picture as a whole is too convincing to permit any other conclusion.

It is at the same time evident that, numerically at least, the mast-cells represent a vestigial cell-system, especially in the higher vertebrates. Whilst in the blood of some lower vertebrates they still make up one-third or more of all leucocytes, they seldom exceed 3% in mammals; in man they vary between ½%–1%. This decrease is also claimed to be patent in the tissue mast-cells. The limited information available seems, however, to suggest that in the vertebrates the lymphocytes supplement, substitute or even supersede the mast-cells and their function.

[1] W. CRAMER and W. L. SIMPSON, Cancer Res. *4*, 601 (1944).
[2] J. BRODERSEN, Z. mikr. anat. Forsch. *14*, 60 (1928).

(b) *The lymphocytes*

CARREL and his school, based primarily on tissue culture studies, demonstrated the growth-promoting action of vertebrate leucocytes. He also made it probable that the leucocytes produce trephones, substances assumed to be both trophic and activating in character and similar to, or identical with, protein hydrolyzates. He did not however make it clear which type of white-blood cell is instrumental in producing growth increase and suggested alternately leucocytes, lymphocytes and macrophages as involved in this process.

Recent investigations indicate that of the types mentioned above, the lymphocytes function as trephocytes within the organism, a view expressed earlier by several hematologists (RENAUT, WEIDENREICH, JOLLY).

The vertebrate lymphocytes show essentially the same traits as those of the invertebrates (Fig. 1*D*): small size, relatively large, round nucleus and little cytoplasm. The ameboid and phagocytic properties are lacking or poor. As in the invertebrates, the lymphocytes of vertebrates appear to possess an embryonic, pluripotent character. There is an increasing body of evidence, admittedly not uncontested, that besides other functions they give rise to all blood cells, including the macrophages (see: DRINKER and YOFFEY[1]). Opinions are not divided on this question, at least with regard to the lower vertebrates.

That lymphocytes shed portions of their cytoplasm has long been known and it was even conjectured that this may represent a trophic activity (WEIDENREICH, DOWNEY). During this process they may disintegrate completely, including the nucleus, or they may change into plasma cells, elements very similar to lymphocytes, often enlarged and showing an achromatic region (HOF) around the nucleus, the latter phenomena reminiscent of those in the mucocytes. There are numerous indications supporting the view that the plasma cells actually represent spent and senile lymphocytes.

The phenomena of cytoplasm-shedding and dissolution of lymphocytes were long overlooked or regarded as abnormal. Recent investigations of WHITE and DOUGHERTY[2] have demonstrated that this is a

[1] Z. K. DRINKER and J. M. YOFFEY, Lymphatics, lymph and lymphoid tissue. Cambridge, Mass., 1941.
[2] A. WHITE and T. F. DOUGHERTY, Ann. N. Y. Acad. Sci. *46*, 859 (1946) et ante.

normal activity controlled, in laboratory mammals, by pituitary-adrenocortical secretion. The lymphocytes were found by these authors normally to contain highly nutritive proteins (γ and probably β- globulin) and an intensified disintegration of the lymphocytes resulted in an increase of serum γ- and β- globulins.

In immunized animals the lymphocytes release antibodies (EHRLICH; DOUGHERTY and associates). Thus it appears that this aspect of the defense mechanism against pathogenic agents is linked with trephocytes and not, as assumed earlier, with the phagocytes[1] and represents merely a special form of trephocytosis. When disintegrating, lymphocytes undoubtedly liberate nucleic acids which are present both in the nucleus and cytoplasm (BRACHET) and are highly important in protein synthesis and in growth.

The vertebrate lymphocytes form numerous aggregates and organs. In the simplest cases, as subepithelial follicles (lymphoid tissue), they are found primarily along the alimentary tract, have no lymph vessels and usually no proper limiting membrane. In some respects they resemble the trephocytic aggregates of invertebrates. Their function may represent nothing but the sum total of the activity of the individual lymphocytes making up these accumulations. The more composite organs of the higher vertebrates: the lymphnodes, thymus, spleen, are also too heterologous in function to be dealt with here, even briefly.

Individual lymphocytes are frequent in most tissues, including the epithelioid. It is generally maintained that they wander here between the cells. LORETI[2], however, claims that within the renal and intestinal epithelium of fishes and Amphibia, some penetrate into the cells and disintegrate, a phenomenon which he considers to represent a trophic process. Recently ANDREW and associates[3] stressed again that the lymphocytes in the intestinal epithelium of mammals are for the most part intracellular in position; they degenerate and are claimed to pass into the lumen and to act as defensive cells. I found them to be quite frequent in the epidermis of salamanders, where they are occasionally located within the cells. No discharge to the exterior was observed here. Macrophages are generally known to ingest fragments of, or whole disintegrating lymphocytes, a process which may represent a normal way of food intake. The inclusion-bodies of malignant cells are considered, by some, to be ingested lymphocytoid cells (HUZELLA, VADASH).

[1] E. METCHNIKOFF found himself compelled, in his later work, to modify his phagocytic theory of immunity and assumed that the macro- and microphages, in addition to their phagocytic activity, liberate, upon disintegration, hemolytic and bactericidal enzymes (his macro- and microcytase). The notion that phagocytes release antibody is steadily losing ground in favor of the lymphocytes.

[2] P. LORETI, Z. Anat. Entwgesch. *103*, 679 (1934), Arch. Ist. Bioch. Ital. *7*, 407 (1935).

[3] W. ANDREW and associates, Anat. Rec. *93*, 251 (1945); *96*, 445 (1946).

A more decisive conclusion as to the trophic nature of lymphocytes found in other cells is gained from their relation to the ovarial eggs recently studied by the writer in *Triturus viridescens*[1]. They are found in the ova during the latter's growth period, which occurs in the autumn, and are particularly numerous at the height of this period, in September (Fig. 3*E*). Within the egg they undergo a progressive fragmentation and are incorporated into the cytoplasm. During regeneration in the same species, they begin to appear prior to, and their disintegration rate runs parallel with, the mitotic activity of the fibroblasts (LIEBMAN, unpublished observations). It is apparently common that mitotically active tissues contain lymphocytes. The involution of the thymus and of the lymphoid tissues with age likewise suggests a using up of their contents in growth processes.

A connection between cancer and lymphocytes has repeatedly been observed and also experimentally approached (MURPHY and associates, and others). Generally, an increase or decrease of the number of lymphocytes corresponds with an increased or decreased incidence of tumors. It remains to be elucidated whether this suggests that the lymphocytes exert a defensive function here or, what seems more probable, stimulate the growth of neoplasms by furnishing them with protein, nucleotides and other substances.

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The lymphocytes are easily the most numerous leucocytes of the higher vertebrates. In the mammals, where conditions are well known, they admittedly occupy the second place in the blood; on the other hand, however, they comprise almost the exclusive cell content of the lymph and the lymphoid tissue, the latter alone amounting to 1% or more of the total body weight.

A considerable number of the lymphocytes normally disintegrate in the body fluids, in the tissues, or within other cells, thus acting as trephocytes. These, together with the mast-cells undoubtedly make up a large portion of the total number of the leucocytes. Thus we have in the vertebrates conditions resembling those in the invertebrates, where a considerable part, often the majority, of the white blood cells are trephocytes. It should be added here that the two remaining types of vertebrate leucocytes, hitherto unmentioned: the eosinophils and the thrombocytes (platelets in mammals) also liberate substances into the organism. Thus, out of a totality of six main types of vertebrate leucocytes, only two are phagocytes, the others acting as free, unicellular glands.

Conclusions

A study of conditions in the animals reveals the presence in their organism of poorly or non-ameboid

[1] E. LIEBMAN, Am. J. Anat. *77*, 273 (1945).

and non-phagocytic leucocytes: the trephocytes. An outline of their morphology is presented. Their function consists in liberating endogenous, nutritive and growth substances into the body fluids, the tissues and into individual cells. This activity is referred to as trephocytosis. There is evidence that the trephocytes are also involved in respiration and other functions.

The trephocytes and the phagocytes represent the two main and often sole groups of white-blood cells of the invertebrates. In several major groups, as in the oligochætes, in Crustacea, insects and tunicates, the trephocytes make up the majority of the leucocytes. In all cases, however, they and their action are predominant when growth, and anabolic processes in general, take place.

Morphologically, the mast-cells of the vertebrates are identical with the invertebrate trephocytes. Their function, though little known and controversial, also seems to be trophic. They appear, however, to represent a vestigial and waning cell system within the vertebrates. Our limited information suggests that here the lymphocytes supplement and even supersede the mast-cells and their functions.

In animals having body fluids with a low content of dissolved nutrients, as seems to be the case in most invertebrates, the trephocytes are the sole or main carriers of nutritive matter. Their function is nonetheless still considerable in forms possessing blood rich in nutrients, including the lower vertebrates and mammals.

The study of trephocytosis reveals some fundamental points regarding the nutrition of the animal cell. It appears that, in addition to utilizing substances in solution, the cells within the organism also take up and assimilate whole trephocytes or corpuscular matter liberated by them (granules and cell fragments). This has so far been observed to take place in ovarial eggs, and in mesodermal and epithelial cells. The nutrient of some animal cells thus appears to be made up of both chemical as well as biological components. It may thus become rather difficult to explain nutrition of the animal cell in purely chemical terms.

Résumé

Certaines recherches ont démontré qu'il se trouve dans l'organisme des animaux des leucocytes peu ou non amiboïdes et non pnagocytaires: les tréphocytes. L'article présent donne un aperçu de leur morphologie. Leur fonction est de transmettre des substances endogènes, nutritives et de croissance aux fluides du corps, aux tissus et aux cellules individuelles. Cette activité est connue sous le nom de tréphocytose.

Les tréphocytes et les phagocytes représentent les deux groupes principaux et souvent uniques des leucocytes des invertébrés. Dans certains groupes importants (Oligochètes, Crustacés, Insectes, Tunicates) la plupart des leucocytes sont des tréphocytes. De toute façon, les tréphocytes et leur fonction sont prédominants durant la croissance et les procès anaboliques en général.

Du point de vue morphologique, les mastocytes des vertébrés sont identiques aux tréphocytes des invertébrés. Leur fonction, quoique peu connue et de nature controverse, paraît également être trophique. Néanmoins, ils semblent représenter chez les vertébrés un système cellulaire décroissant. Il résulte de nos recherches limités, qu'ici les lymphocytes supplémentent et remplacent même les mastocytes et leur fonction.

Chez les animaux dont le sang a un bas contenu de nutriments dissouts, comme cela paraît être le cas chez la plupart des invertébrés, les tréphocytes sont les porteurs uniques ou principaux de matières nutritives. Leur fonction est non moins considérable chez les formes à sang riche en nutriments, y compris les vertébrés inférieurs et les mammifères.

L'étude de la tréphocytose révèle quelques points essentiels au sujet de la nutrition de la cellule animale. Il apparaît que les cellules des organismes n'utilisent non seulement les substances dissolues, mais ingèrent et assimilent en outre des tréphocytes entiers et des matières formées, libérées par les tréphocytes (granules ou fragments cellulaires). Jusqu'à présent, ce fait a été observé dans les œufs ovaires et dans les cellules mésodermiques et épithéliales. Les substances nutritives de certaines cellules animales seraient donc en partie chimiques et en partie biologiques.