ON THE PHYSIOLOGY OF DIGESTION, RESPIRATION AND EXCRETION IN ECHINODERMS

H. C. VAN DER HEYDE

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ACADEMISCH PROEFSCHRIFT

TOT HET VERKRIJGEN VAN DEN GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE UNIVERSITEIT VAN AMSTERDAM OP GEZAG VAN DEN RECTOR-MAGNIFICUS, Dr. J. K. A. WERTHEIM-SALOMONSON, HOOGLEERAAR IN DE FACULTEIT DER GENEESKUNDE, IN HET OPENBAAR TE VERDEDIGEN OP VRIJDAG 5 MEI 1922, DES NAMIDDAGS TE DRIE URE PRECIES, DOOR

HENRI CHRISTIAAN VAN DER HEYDE,
GEBOREN TE HEM.

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AAN MIJNE MOEDER EN
AAN DE NAGEDACHTENIS
VAN MIJN VADER.

De gelegenheid, in dit proefschrift openlijk mijn dank te brengen aan allen, die in mijn academischen studietijd hebben bijgedragen tot mijn wetenschappelijke vorming, grijp ik volgaarne aan.

Hooggeleerde Weber, Sluiter, De Meyere, Dubois, Verschaffelt en Stomps, gij allen hebt mij de wetenschap van het levende doen zien vanuit uw standpunt en veel heb ik uit uw lessen geleerd, wat mij in de toekomst van onschatbare waarde zal zijn. Dat ik tenslotte het voetspoor van geen uwer ben gevolgd, was niet uw schuld, maar de mijne.

Hooggeleerde Sissingh, Holleman, Smits en Korteweg, uw lessen hebben het tekort mijner gymnasiale opleiding aangevuld. De moderne biologie, ontgroeid aan het beschrijvend en ontledend stadium, voelt meer en meer een dringende behoefte aan contact met uwe wetenschappen. Te betreuren is het daarom, dat de nieuwe Hooger-Onderwijs-wet het mogelijk maakt, dat een geslacht van biologen gaat opgroeien, dat niet voldoende vertrouwd is met de beginselen van de „exacte" wetenschappen. De woorden van den grooten plantenphysioloog Osterhout, tot een mijner vrienden gericht: „First study mathematics, then physics and chemistry, finally some botany and I'll make you a plant-physiologist", hebben mij intuitief voor den geest gestaan toen ik onder uw leiding mij voor het candidaatsexamen in de scheikunde bekwaamde.

Hooggeleerde Buytendijk, gij zijt het geweest, die een grooten invloed op mijn wetenschappelijke ontwikkeling hebt gehad. Was mijn neiging naar dier-physiologie en -psychologie al in den aanleg aanwezig, toen ik u vroeg, onder uw leiding te mogen werken, gij hebt aan dien onbewusten drang vorm en bewustheid gegeven. Onder uw leiding heb ik mijn eerste wetenschappelijk werk mogen verrichten en uw felle kritiek — vaak misschien te fel — op de fundamenten van het gebouw, waarin de biologen zich veilig wanen, heeft mij de betrekkelijkheid van vele hunner waarheden leeren beseffen.

Gij, hooggeleerde Jordan, hebt in uw voordrachten mij doen verstaan, wat vergelijkende physiologie is en hebt mij vervuld met bewondering voor deze groeiende, jonge wetenschap.

My dear Dr. Morse, the time I spent in your department as an instructor in physiology and physiological chemistry and our subsequent journey through the "East", will remain one of my most pleasant memories, even if I do not return to your country. Your valuable help and advise, the time you spent in trying to enable me to carry out the work I had hoped to do, have always been very much appreciated by me and I will never forget them.

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Hooggeleerde Sluiter, hooggeachte promotor, u ben ik dankbaar, dat gij dit werk, al is het niet onder uw leiding tot stand gekomen en al ligt het niet in uw lijn van onderzoek, toch hebt willen aanvaarden als proefschrift. Uw colleges hebben mij in het begin van mijn studententijd, toen ik nog geheel onder den invloed verkeerde van Häckel's Anthropogenie en Welträtsel, vervuld met de grootste bewondering. Ook later heb ik altijd uw morphologische lessen ten zeerste gewaardeerd; geen zoologische physiologie is mogelijk zonder een goeden morphologischen grondslag, zooals gij dien weet te leggen.

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La morphologie constitue la base des sciences biologiques, mais on ne peut la considérer comme leur aboutissement; la „science de la vie" a pour objet l'étude des fonctions, l'exposé de leurs relations réciproques dans un même être et l'histoire de leur évolution passée.

Victor Willem in Arch. Néerl. de Physiol. T. II.

INTRODUCTION.

> Trotz der bedauerlichen Unvollständigkeit unserer Kenntnisse über die Ernährung der Echinodermen bietet doch schon das wenige was bisjetzt darüber festgestellt ist, ungewöhnliches Interesse dar und läszt weitere Untersuchungen höchst wünschenswert erscheinen.
>
> Biedermann in Winterstein's Handbuch.

More than anything else perhaps the young science of comparative physiology needs systematic work, in which the old literature is taken into consideration, but where the results are controled and verified by newer and often times better methods. This literature is on account of the fact that in the last 35 years biologists have almost exclusively been specialising on comparative anatomy and related sciences, in most cases either very old or very recent. The old literature, for a large part French, contains unknown treasures, but is frequently difficult to find. Many of the older papers will prove to be completely untrustworthy, be it because the methods are no more up to date, be it because the writer, not thoroughly acquainted either with chemistry or physics or with the anatomy of the group studied — the latter is often the case with work of medical physiologists — has made more or less fundamental mistakes.

This work must not only consist of a „Nachprüfung" of the work of previous authors and its appreciation, but also in a supplementing of those parts in which our knowledge is still incomplete. If all comparative physiologists work together in this way, we will in a short time have a basis from where to start, we will have formulated our problems and will be able to explore new fields more fruitfully than this has been done till now.

Ideas like these have induced me to study a problem like: Digestion in Echinoderms, a little more closely. I do not claim that my work contains an answer to burning questions or is concerned with such problems as occupy the modern biological mind, but as „clearing work" it may have some value.

The study of one definite group more in detail is especially desirable because other relations and other functions can be studied at the same time, so as to give a clear picture of the whole process of food intake, digestion, metabolism, excretion and respiration, a view of a definite complex of functions of the animal machinery. The whole literature contains an abundance of single facts, but real problems as there are hundreds, have seldom been attacked.

For a phylogenetic physiology the Echinoderms are one of the most interesting groups. The great importance they have for general physiology has already been realised clearly by von Uexküll, Jordan and other authors. Their study throws a light on many burning questions of comparative physiology-not to speak of the rôle their eggs play in genetics-because one organ has many functions and one function many organs — see f. i. the chapter on respiration —; specialisation and integration take place before our eyes. Their absolutely independent anatomical structure complicates this study still more.

Many of the older investigators have made more or less serious mistakes. One of those workers who by their work and by the authority they had, have done more evil than good, is Krukenberg. This author who by his astonishing working capacities, has been quoted by a great many authors, worked without a sufficient knowledge of the histology and anatomy of the animals he studied. As an example I may mention one of his experiments in which he calls the well-known yellow genital organs of Cucumaria: *liver* — no gut appendices are found in Holothurians —. Starting from this preconcieved idea he finds enzymes in them. He also finds these in the waterlungs, which as we can find in every text-book, are organs of respiratory and perhaps excretory function. In a glycerin extract of these organs he finds a *peptic* enzyme, but no trypsin and no amylase. One page before this statement the same author says emphatically that „alle Versuche, aus den Polischen Blasen, den Cuvierschen Organen, den *Wasserlungen* und dem Blute der Holothurien, Fermente zu extrahieren, nur zu negativen Resultaten führten".

He finds proteolytic enzymes in the digestive juice, but is unable to detect them in a glycerin extract of the intestine. He concludes that the enzyme present must be derived from the food, from which it gets free by autodigestion, which is the more improbable because the larger part of the food of the Holothurians are plant-tissues. Remarkable is his result that glandular cells are present in the blood vessels of the Holothurians, which secrete the enzymes. He does not try to explain how these enzymes get through the wall of the gut, but the work of Enriques 37) has elucidated this question sufficiently.

Krukenberg's result that a peptic and a tryptic enzyme are present simultaneously in Echinoderms as in many other groups he studied, will be discussed in chapter 6. a.

The unreliability of Krukenberg's work is a pity, because of the great influence he had in his time and because his work contains so many precious data, which are of not much value in that way.

Another author whose conceptions the present author is not prepared to follow, is Otto Cohnheim. His plan, the study

of invertebrate groups just for the sake of clearing questions of mammalian physiology, is wrong to my opinion. The many errors in his work will be discussed in various chapters, it is of no use to discuss them here.

These examples may serve to show the desirability of a careful re-testing of all the work of older investigators. Many mistakes have passed unnoticed and came into the text-books.

A summary of the literature which generally appears in the beginning of a somewhat larger paper like the present one, will not be given in this introduction, because there is practically no important literature and because the data which can be found scattered in the different journals, have been summarised in an excellent way by Biedermann in Winterstein's Handbuch 135) and by Jordan in the first part of his: Vergleichende Physiologie 142).

The writer does not flatter himself to have collected the whole literature and to some important papers little attention has been paid. This was necessitated by his desire not to drown the personal observations in the immense literature.

The groups of the Crinoids and the Ophiurids have been left out for practical reasons, because no appropriate material could be obtained.

2. SPECIES USED.

As a representative of the group of the Asteroidea I have used *Asterias Forbesii (Desor) Verrill,* a starfish, very common around Woods Hole. It is as far as appearance is concerned, practically identical with our A. vulgaris, but for the madreporic plate which is more clearly visible in the American species by its brilliant orange color.

The sea-urchin which I used, *Arbacia punctulata (Lm.) Gray,* is also very common in the neighbourhood of Woods Hole. It is a rather small form of a dark violet color with long spines. Its shell is semi-spherical in form, rather thick and provided with a so-called epistroma. The periproct is oval, the place of opening of the anus, covered by numerous sceletal elements in other urchins, is in the family of the Arbaciidae covered with four triangular plates. The interradia are open in the neighbourhood of the apex.

The spines are flattened — in the way of a spatula — on one side at their top. The tuberculae are not perforated, secondary tuberculae are not present.

Thyone briareus. Lesueur (= Anaperus br. Pourtalès = Sclerodactyla br. Ayres, Verrill) is the dendrochirote Holothurian, which I studied in Woods Hole. It is dark brown-greenish in color, almost black, ascidian-like; its size depends largely on the state of contraction of the musculature of the

body wall, animals of 15 c.M. eventually are as short as 6—7 c.M. Numerous small tube feet are found scattered all over the surface of the body. Except for the enddiscs of the tube-feet no calcareous bodies are found, according to Selenka. Ayres finds numerous long plates with a kind of crown in the middle. The members of the calcareous ring are fused together completely. Five calcareous teeth are found in the anus. The behavior of this species has been described in detail by Pearse 98).

In Bermuda I had occasion to study *Stichopus moebii. Semper,* a flat and very large aspidochirote Holothurian. It has 18 gray tentacles; on the back only very few tube-feet are found. One Polian vesicle and one stone-canal are present. Its color is reddish-grey with black spots in the West-Indian specimens — one on a hundred specimens in the Bermuda-islands also has this coloration —, absolutely black in most of the Bermuda specimens. Very strong calcareous elements are found in the tube-feet.

3. ANATOMICAL DETAILS ON THE DIGESTIVE TRACT [1]).

Related as the Echinoderms may be in anatomical respect („This is one of the best characterised and most distinct Phyla of the Animal Kingdom." Ray Lankester) by their pentagonal symmetry, by the structure of their body cavities and by their larval forms, physiologically there is a big difference. The feeding habits are widely divergent in the different groups, as will appear from the short descriptions, given in the next chapter. Stichopus eats calcareous sand; Thyone, other cucumbers and the crinoids catch small planctonts; the starfishes are the voracious carnivores of the group; the urchins the rodents. Such differences must give rise to big differences in morphological respect and in fact the digestive tract is, at least as far as the macroscopical anatomy is concerned, almost entirely different in the various groups.

In the starfishes the organs of digestion have a very peculiar structure. This is on one hand due to their „external digestion", which will be described in the next chapter, on the other hand to their peculiar star-like shape. It consists chiefly of a large, strongly muscular „stomach" in the disc and of the „radial sacs" or „pyloric coeca", which usually lie in pairs [2]) in the

[1]) This chapter does not pretend to be complete. It is beyond the scope of the present paper to give such account; the interested reader can find enough details in the numerous papers of Hamann 50), 51), and 51a), of Tiedemann 127), Frenzel 41), Jourdan 71) and 72), Koehler 74), Prouho 103) and others. A good key to this literature is found in 140).

[2]) In some cases — probably on account of mutilations — I found three of these organs present in one arm.

five arms [1]). The mouth situated at the ventral side of the animal, leads by a narrow neck, called the „oesophagus" [2]), into a voluminous sac, sometimes divided in two parts by a circular sphincter, called the „stomach". In front of the mouth we find the proximal members of the adambulacral series of sceletal parts, the so-called mouth-spines. The stomach is produced into five „stomach pouches", sometimes forked, projecting into the arms. These pouches and therewith the whole stomach can be retracted by means of two very thin and long muscles, lying ventrally and lengthwise on the ambulacral groove and fixed to the median ridge of the ray. The stomach pouches are continued by one or two ducts — in the place of bifurcation there is considerable variation — running out into the arms. Here they form the so-called pyloric coeca, accumulations of small pouches the little ducts of which all come out on the chief duct. These radial sacs are dark greenish yellow in color and attached to the dorsal wall of the coelomic cavity by suspensory bands of membrane, called mesenteries, simple continuations of the peritoneal lining of the coelom. Beyond the pyloric coeca the alimentary tract is continued as a slender „rectum", ending in the anus. Both are thin, relatively small and not of great importance; they are absent in the Astropectinidae and the Porcellanasteridae. The rectum gives off 2—5 pouches, sometimes forked, which are called rectal or interradial coeca. They mostly contain a dark, brown fluid; their function will be discussed in the chapter on excretion, since they probably have excretory function. The radial coeca of the stomach are very constant in structure and number; the rectal coeca vary largely. In Asterias we find two simple fans, in the Pentacerotidae there are five forked coeca, in Asterina five simple sacs and in the Echinasteridae and Astropectinidae one five-lobed coecum.

The histology of the digestive tract of the starfishes is in its general features identical with that of all Echinoderms. We can distinguish (from outside to inside): 1. an endothelial layer, a simple continuation of the peritoneal lining of the coelomic cavity, showing a. mucus cells and b. simple ciliated endothelial cells, 2. a thin layer of connective tissue, 3. a layer of muscles and 4. the epithelium which consists of long cylindrical cells, ciliated in most parts, with nerve fibres in their basal part. The mouth is opened and shut by means of an internal circular sphincter and of external radial dilatators; in other parts the longitudinal muscles lie at the inside, the circular ones are external. In the stomach we find a very strong musculature;

[1]) If more arms are present (their number can be as high as 25, e. g. in the Heliasteridae and Brisingidae), their number of course follows that of the arms.

[2]) Sometimes (in Echinaster and Cribrella f. i.) the oesophagus shows ten pouches, five radial and five interradial ones.

in the radial sacs there is no mucle layer (Cuénot) or a very thin one (Hamann). The epithelium of the oesophagus shows three types of cells, all of which are shorter here than in other parts: a. simple epidermal cells, ressembling those of the skin, b. mucus cells with one to three cilia, but without cuticula and c. granular cells, with brownish or yellow grains, K. C. Schneider 141) in his comparative histology of animals distinguishes these three groups as „Nährzellen", „Scheimzellen" and „Eiweiszzellen". The cells of group a., the „Nährzellen" of Schneider, are ciliated (in Echinaster spinosus f.i.), they are very slender and cylindrical, have a thin membrane and a „Stützfibrille", connecting flagellum and nucleus. The membrane forms a kind of collar, a „Kragen", sticking out from the epithelium, reminding weakly of the choanocytes of the sponges. They seem to play a role in resorption (Cuénot). Those of group c. have the same structure and cilia as those of group a., but their cytoplasm is reticular and their secretory activity is betrayed by the numerous granulae which they contain. The granular cells are much longer and much more typical in stomach and radial sacs where the epithelium almost exclusively consists of such „glandular cells". Their granulae can be seen lying in two or three regular lines and are very different in the different stages of secretion (Schneider).

In the sea-urchins the digestive system is altogether different. The mouth is situated at the centre of a peristomial membrane which forms the so-called lips. Generally we distinguish five of these lying between the five teeth. These are simple folds of the skin; if the lantern is retracted they can close together so as to make the whole lantern invisible. The gut commences with a short vertical tube, a stomodaeum or pharynx, which is surrounded by the upper ends of the teeth and their supporting ossicles, the whole of which is called Aristotle's lantern, a masticating apparatus of extreme complexity. Its mechanism has been studied in detail by von Uexküll 128). The gut has pentagonal form as long as it runs through the lantern and on the corners of this pentagon we find columns of connective tissue. A small piece between this stomodaeum and the stomach is frequently called oesophagus. It is separated from the stomodaeum by a small muscular ring which narrows the lumen at that place. It is cylindrical in shape.

The gut itself first turns around clockwise in one direction, then bends sharply and comes back anti-clockwise. The first part is a baggy, flat tube, sometimes called the stomach, running horizontally round the animal supported by strings of tissue from the coelomic wall. These mesenteries connect it at the same time with the gonads, so that the whole hangs down in a series of festoons, five in number. The second part, frequently

called the intestine, runs around in opposite direction and parallel to the first. Its festoons alternate with those of the stomach; in Arbacia they are usually full of faeces. The gut ends by a short rectum, opening into the dorsal anus on a space, called the periproct. Parallel to the stomach and opening into it at both ends runs the so-called „siphon" or „accessory intestine" (Nebendarm), discovered in 1825 by Delle Chiaje. This structure is in some species (e.g. in Dorocidaris papillata, see Prouho 103)) merely a gutter, comparable to the endostyle of the Tunicates, in others a narrow, cylindrical tube lined by cilia. A blood-lacune runs lengthside of this tube (Hamann). In the Regulares it follows the stomach, in the Clypeastroidea it makes a short-cut. Its function is completely unknown, several possibilities will be discussed in chapter 21.

The histological picture of the oesophagus ressembles that of the starfishes very much. Outside of the layer of circular muscles which is also present in tbe pharynx, we find here longitudinal fibres and the ciliated peritoneum, both derived from the lining of the coelomic cavity. The inside layer of connective tissue and circular fibres is often called the visceral or splanchnic part; the longitudinal fibres plus endothelium the parietal peritoneum. The layer of connective tissue is separated from the epithelium by a very thin, hyaline basal membrane (Hamann). The epithelium of pharynx and oesophagus contains two types of cells: 1. glandular cells, full of granulae and 2. „Stützzellen", simple supporting epithelial cells. It is about ten to fifteen times as high as all the other layers together.

Both types of cells are ciliated. The glandular cells are especially frequent in the five corners of the pharynx and in the neighbourhood of the mouth. At their base we find a layer of nerve fibres. The granulae have been studied with much detail by Hamann 51a), the same author gives an accurate picture of the act of secretion. In Arbacia we find a pigment present at the outer rim of the cells.

The first or direct curvature, as the stomach is frequently and more rightly called, shows the same picture except for two peculiarities: 1. between the layer of connective tissue and the peritoneal lining we find a whole system of lacunar spaces, full of „lymph cells"; 2. the cells of the epithelium have almost exclusively the glandular type, all cells are high, ciliated and loaded with granulae of all colors. The first curvature is usually much darker than the intestine; this is on one hand due to the presence of pigment and secretory granulae in its epithelium; on the other hand to the blood-vessels which are especially frequent here. This indicates that this part of the gut plays an important role in enzyme secretion and absorption. It also shows many folds, in these folds the glandular cells are especially accumulated (Hamann).

In the second or recurrent curvature the mucosa is much thinner again and the epithelial cells do not have the highly specialised type of those of the „stomach". [1])

In the siphon the ciliated cells do not show any special granulation.

The rectum is characterised by a thick layer of circular muscles, its histology is the same as that of the rest of the gut.

The alimentary tube of the Holothurians shows four distinct parts: 1. a short oesophagus, passing through the calcareous ring (= Aristotle's lantern), frequently folded longitudinally; 2. a so-called „muscular stomach" (J. Müller), very short (less than 2 c.M. in Thyone), initiated by a constriction and characterised by its musculature; 3. the so-called intestine (Frenzel also calls it „Chylusdarm"), very long — the whole gut is frequently longer than 75 cM. in our small Thyone —; 4. the „cloaca" or „rectum", a widened part, connected to the body-wall by muscular bands transversing the coelom. The whole intestine is suspended by bands of membrane, called „mesenteries" and shows the same general curvatures and clockwise turning as in all Echinoderms, as a study of cross-sections shows. Only in the Synaptids it runs nearly straight from mouth to anus.

In the stomach the food is thoroughly mixed with the visceral fluid, kept there for some time and passed through in small quantities. This is the case in Thyone where the food is swimming about here and there in little clumps in the large quantities of digestive juice. In Stichopus however, we find the whole gut always full of sand, the process of digestion seems to be continuous here. The extreme thinness of the intestinal wall is very striking and is common to many animals which eat mud and sand for the sake of organic matter which they contain (e. g. Sipunculus, Spatangus). Peristaltic movements, stronger perhaps than those in Vertebrates, are characteristic for the Holothurian intestine.

The histology of the gut of the Holothurians has been studied by Jourdan 71). Externally we find the peritoneal lining of the body cavity, a pavement endothelium. It has the same two types of cells which are present in the other groups. Most of them are simple endothelial cells which lie in a single layer: they have cylindrical shape and carry vibrating cilia. In the second place we find mucus cells, which have first been described by Semper.

The second layer is the fibro-muscular one; it has the same structure as in the other groups. It contains internal longitudinal

[1]) A special gland or coecum occurs in the digestive tract of the Spatangoidea; it is situated on the boarder of oesophagus and first curvature. This coecum contains a clear brownish yellow fluid, slightly acid, which contains proteins since a precipitate is formed by heat and alcohol (3—4 c.c.; V. Henry. 53)).

and external circular fibres with on both sides a layer of connective tissue. In the outside layer we find the so-called marginal vessels, at the inside the general lacunar system and many nerve fibres.

In some places of the intestinal wall the peritoneum leaves an open space of ellipsoid form, bringing the external connective tissue (and with it the marginal vessels ?) in contact with the coelom, a so-called „button" (Hérouard). By them the marginal vessels communicate with the general lacunar system and here the circular musculature is perforated.

The cells of the epithelium are very long and have cylindrical shape. They are covered with a fairly thick cuticula and show two types. Many cells show a fine granulation and appear to be secreting cells ; others have the character of mucus cells. In pharynx and cloaca we find normal epidermis cells.

Note. Of special interest is the presence of amiboid migrating cells probably of mesodermal origin-though Frenzel denies this- in the visceral epithelia of all Echinoderms. Biedermann in Winterstein's Handbuch 136) emphasises this point particularly. Frenzel 41) already found numerous red migrating cells in the normal epithelia of Toxopneustes and Spatangus, which he calls „rothe Wanderzellen". The color of their guts is very different in different circumstances and physiological conditions. In the same species of Toxopneustes f. i.. Frenzel found the gut in one case black, at another time pink with black spots, sometimes again the whole gut reddish-brown. This partly accounts for the difference in color of the gut as a whole. St. Hilaire 112) [1]) also observed them and found the same, cells present in the perivisceral fluid. Cohnheim and Cuénot found the same thing and found the protein crystalloids, studied bij List 81) and a fatty substance present in them. Are they carriers of food? Most probably not, because they only move towards the lumen, as Frenzel himself states. Possibly they carry excretion-products to the lumen of the gut ; this is rather probable from their movements which can even be observed in vivo according to Frenzel. Enriques 37) brings them in connection with the peculiar enzyme secretion described by him in Holothuria tubulosa. According to him

[1]) Saint Hilaire makes a sharp distinction between the „granular cells" and the „phagocytes". The granular cells according to him do not have any phagocytical activities; they are so to say „unicellular glands", formed in the peritoneal epithelium and dying and dissolving in the intestinal lumen. They do not participate in the clotting process and do not play a rôle in digestion: they are neither „Fermentzellen", nor phagocytes, neither do they contain any reserve substances. They should take care of the excretion by taking up foreign substances from the surrounding fluids, storing them as granulae and carrying them through the gut-wall.

we have to do with something also found in other forms; in the duodenum of the frog he described analogous cells 36). Frenzel also calls them „Sekretzellen".

The argument of Hérouard that these groups of corpuscles do not have any duct and consequently can not have excretory function, is of course valueless. His opinion that they would serve for the transport of food, is not proved by any experiments.

4. BIOLOGY. PREY.

The old story of the starfish devouring a bivalve, is too old to be repeated again in full detail. As early as 1826 Eudes-Deslongchamps 38) already described the process in the charming, primitive way of the observing biologist. He found starfishes on groups of Mactra stultorum. L. in the act of devouring this prey. „Je remarquai qu'elles avaient introduit entre ces valves de grosses vésicules arrondies, à parois très minces et remplis d'un liquide transparent." Here he means the stomach-edge of course, which is inserted between the valves and applied directly to the soft parts of the prey. He takes them for sacs in which there is a little opening through which the liquid content, a "humeur engourdissante", drips out gradually.

The opening of such bivalves is still always a problem when we take in consideration the enormous force of the adductor muscles of these animals. Many authors believe that a toxic substance of some kind helps here — this problem will be discussed in a separate chapter —, Schiemenz (Mitt. d. deutschen Seefischereivereins. XII. 1896. p. 102. Quoted from Mc. Bride in: Cambridge Natural Hist.) has figured out the dynamics of the process. He showed, 1. that while a bivalve can resist a sudden pull of 4000 grams, it yields to a long continued pull of 900 grams; 2. that a starfisch can exert a pull of 1350 grams; 3. that a starfish is unable to open a bivalve unless it can raise itself into a hump so that the pull of the central tube feet is at right angles to the prey. To prove this he mentions the interesting case of a starfish, kept between two glass-plates, walking around all day dragging about a bivalve, but unable to open it.

Mc. Andrew and Barret 2) soon found that the „vésicules" observed by Deslongchamps, are nothing else but the stomach, protruded as it is filled with the perivisceral fluid. It has considerable motility and can even penetrate to way up in the shell of a Littorina, as I could observe myself in one case.

About the completeness of this „extra-intestinal digestion (Jordan)", practically nothing is known. Whether it is complete as in Carabus 67) and Dytiscus-larvae or incomplete as in the case of the mesenteric filaments, „Saeptalränder" of the Actinians,

where only a desintegration takes place, is not sufficiently known. I did not get a chance to study this part of the problem, because only a few times I succeeded in securing animals in the act of eating.

An important rôle in the capturing of the prey is played by the pedicellariae the mechanism of which has been studied in detail by Perrier, von Uexküll 129) and Jennings 62). The latter author gives a photograph of a starfisch which has captured five little crabs (Hippa analoga) in this way and keeps them fixed on its back, Many smaller animals, as annelids, copepods and others are captured in that same way, also by the sea-urchins.

Even the seemingly completely inaccessible urchins, can be captured by a starfiish. A very interesting picture of a fight between these two has been given by Prouho 104). The starfish allowed the gemmiform pedicellariae of its prey to get hold of its body, then wrenched them off, and so on, till the urchin was a helpless prey.

Even fishes can occasionally be captured by starfishes, as Jennings decribes in his famous monograph on the behavior of Asterias Forreri. Dead prey is also occasionally taken according to Delage and Hérouard 136) p. 65. Cases of cannibalism have been described by von Uexküll 130) in brittle-stars, by Cuénot in Strongylocentrotus and by Prouho 103) in Dorocidaris.

Small animals (snails), like Littorina, Terebra, Strombus, Murex etc. are frequently digested inside of the stomach. The shell is then removed by the mouth, not by the anus. Shells up to 3 c.M. in width can pass through the mouth of an Astropecten.

Tremendous destroyers as the starfishes are, it sounds strange, that they have almost no enemies, at least not in their adult form. This may be due to the toxicity of their skin, most starfishes have moreover the premonitory color which is typical for animals protected by chemical means (Heliconia, Eolis, catterpillars and insects, the Gasteracanthidae among the spiders). The only enemies capable of attacking them with success, that I know of, are the acid-secreting sea-snails, as Dolium and Tritonium. Semon 119) reports that these attack starfishes, urchins and cucumbers with much success.

Starfishes have considerable negative economical value. Collins f.i. (Bull. U. S. Fish. Commission. Vol. 9, 1889. Quoted after Ludwig-Hamann) estimates the damage done by starfishes in one year (1888) on $ 631.500.—. Cuénot 21) saw that a natural oyster-bed of 10—12 K.M. length was completely destroyed by starfishes, incidentally introduced by fishers in their nets. Hamann found 10 specimens of Pecten, 6 Tellina, some Conus and 5 Dentalium in the stomach of one Astropecten.

The food of sea urchins is very different in different species. The only food that I actually observed our Arbacia eating, was Clione sulfurea, a yellow calcisponge. The study of the faeces however, will reveal us many other preys. The European species, Echinus esculentus, chiefly feeds on the brown fronds of Laminaria and other sea weeds (Mc. Bride in: Cambridge Natural Hist.) and its small inhabitants, chewing them all up with its teeth. According to Scott 117) it eats sea-weeds and sand. Chadwick 13) considers this species as carnivorous and found Balanus-shells in their guts. If some specimens are put into an aquarium, in which some Balani are present, they will immediately attack them and chew them up.

Not only such peaceful prey is taken however. The Neapolitan species, Spaerechinus and Toxopneustes, even capture such alert crustaceans as Squilla mantis, as Dohrn 32) reports. They cover themselves up with mussels and other harmless objects and move around camouflaged in that way. Suddenly they attack their prey which can not escape any more. If a dozen Toxopneustes were put into one bassin with a dozen Squilla, it did not take more than eight or ten days for all the crustaceans to disappear. The remarkable coordination apparently present in these sluggish animals is very interesting from the point of view of animal behavior and has been described in detail by Dohrn.

These crustaceans are caught by means of the ambulacral feet: as soon as one of them gets hold of a prey, all others are loosened. Some smaller animals are caught by the pedicellariae, some by the spines. H. Eisig (Kosmos. 8. 1883. p. 28) describes the capturing of a little annelid by Echinus lividus by means of its spines.

Sponges, Gorgonidae, fishes, Crustaceans etc. are also prey of some urchins (Prouho 103) for Dorocidaris papillata). Even calcareous algae are sometimes digested. Echinus miliaris bores holes in rocks, covered with crusts of Lithothamnium polymorphum, for the sake of food, according to Hesse 58), maybe for the sake of Ca and of protection against the waves, according to Jordan 142).

Quite a different food is taken by the Spatangoidea and the Clypeastroidea, They live in the sand and eat themselves through it. Since no representative of these groups is studied in the present paper — the common sand-dollar, Echinarachneus parma, is too small for most purposes — I do not consider it desirable to describe their very interesting habits here; the reader can find them described in a paper by Hornyold 61) and in von Uexküll's Umwelt und Innenwelt.

Holothurians usually eat mud. It may be of some interest to describe the feeding habits of our Thyone a little more in detail.

Whereas our Bermudian species, Stichopus moebii, eats mud, or rather the calcareous sand of the coral rifs and monotonously continues eating it day after day, this species shows a little more complicated behavior.

In its natural habitat this species lives in mud in shallow places. This mud is of the darkest and richest kind, almost blue in color and full of detritus. If one puts some specimens in an aquarium the bottom of which is covered with this mud, one can observe that after a little while part of the animals have buried themselves in the mud and on dissection the gut will be found full of this material. After some time however the majority of the animals is found partly buried in the sand, but with their ring of tree-like tentacles sticking out of the mud. These tentaculá arborescentia are in constant motion and a closer study of their behavior reveals a most interesting game.

Like nets these little arms move through the water in a fan-like and slow movement, apparently for the purpose of fishing small planktonts and débris out of the water. As soon as one of these bumps against one of the tentacles, the organ slowly bends downward and disappears into the mouth.

Of the ten tentacles the two ventral ones appear to be very much shorter. Shortly after one of the large tentacles has disappeared into the mouth, it comes back, and the small tentacles now functionate as „scratchers" and whipe the returning tentacle off. Another tentacle again disappears and the same rhythmic game goes on for hours and hours at a stretch.

Has a Thyone once found a suitable place, it can stay there for weeks and weeks, fishing in uninterrupted rhythm, once about every $7^1/_2$ minutes (Pearse 98)), a most interesting and fascinating spectacle. Cucumaria planci according to Dohrn 32), has preference for thick bushes of algae in which they hang for months.

From these observations it will be evident that Thyone is by no means an exclusive mud-eater, and it can not astonish us any more that, as we will see in chapter 19, the dried contents of the stomach contain much more nitrogen pro gram of substance than the mud in which the animals live.

Many other Holothurians live only on mud or sand, i.e. on the organic material contained in it. The importance of this process, the amount of bottom material „worked through" by Stichopus moebii, has been estimated by Crozier 18).

Stichopus moebii. Semper, a very large species, such as are eaten by the Chinese as „trepang", occurs in great abundance in the Bermuda islands on the shallow littoral bottom. Its sits on the sand and eats it by means of its 18—20 shovel-like tentacula peltata. Crozier determines the amount of sand present in the gut when it is filled completely. Then he observes how many times a day the gut is filled. This is possible because

there appeared to be a peculiar synchronism in the feeding habits of different specimens. On certain moments of the day almost all the animals had their gut either completely full or halfway full or empty. In this way he could observe that as a rule the contents of the gut are changed about three times a day. By determining the number of animals present in different localities around the Bermuda's — this is very casy since the big animals are clearly visible through the transparent water of the tropical sea —, he could figure out the amount of sand displaced in a certain amount of time. Restricting himself as far as necessary in his assumptions, he came to the conclusion, that in areas frequented by this species, roughly 6 to 7 kilo's (dry weight) of sand passes through Holothurian guts per year and per square meter. In the enclosed sink, Harrington Sound, he finds that the quantity eaten annually, is something like 500 to 1000 tons.

These figures illustrate more clearly than speculation could the tremendous importance of these animals and show „that the feeding activities of these animals may have an effect on the sea-bottom not unlike that, so carefully described by Darwin, which earthworms produce in the soil." This is the more probable, because as we will see later on, the gut contents of this species are fairly acid, so that calcium carbonate is brought into solution.

5. TOXICITY OF THE STARFISH STOMACH.

In almost every popular description of the attack of a starfish on bivalve molluscs, we are told that the starfish after having opened the shell, drops a few drops of „poison" on the prey and facilitates his work in that way. It seemed to me to be worthwhile to investigate this question a little more closely from the experimental side. The question, formulated more strictly, is: Does the stomach of the starfish secrete a substance which is poisonous to the muscles or in general, to the contractile tissues of the prey? In that way the secretion of the stomach would have a double function: 1. to kill the prey or at least to abolish the tonus of the adductor muscle, 2. to dissolve the tissues more or less completely in order to make them fit to enter into the stomach or the radial sacs.

The fact that as Eudes-Deslongchamps reports if an oyster is taken away soon enough, it is dead even if it has not been dissolved, pleads in favor of this hypothesis. If it had been dead previously, it would smell as strongly as all decaying sea material does; this is not the case. W. Hesz 59) also believes in the presence of a toxic substance. Cuénot 22) speaks of a toxic mucus („glaire"). The same mucus is according to him

secreted by the skin (p. 381 ; uric acid?). Schiemenz 115) does not believe in any toxic action.

A sea-watery extract was made of a large number of starfish-stomachs. Since the substance to be studied might be destroyed by heat, the extract was made in the cold by vigorous shaking. In order to avoid decay the extraction was not continued longer than about four hours. The mass was now filtered and the filtrate used for the following experiments.

In the first place the action of this extract on the heart of the bivalve Pecten was studied. This proved to be a very typical one.

The heart of Pecten can be isolated very easily. If one pulls the two shells which the animal always keeps open in its natural environment, apart, one easily succeeds in tearing the adductor muscle into two. If this is done carefully, the heart is found in absolutely uninjured condition right under the muscle. It is found beating in its pericard which contains a fairly large quantity of „coelomic" liquid. If left alone the heart will continue to beat for a considerable length of time, as control experiments showed.

By means of a small injection-syringe the stomach extract was now dripped on the beating heart in a fine stream. The first beats are perfectly normal and do not show any effect of the fluid on the beating. But by and by the beats come at shorter intervals and finally a highly accelerated and irregular beating results. Though no counts were made, I dare say that the heart beats more than three times as fast as usually. If the irrigation is continued, the reverse happens after some time. Gradually the rate of heart-beats slows down, till it falls below the normal rate and finally the heart stops in a contracted condition. The whole process takes place in only a few minutes and strongly impresses one that it is due to a toxic action of some kind of the extract. The poison would in that way have a stimulating effect at first and finally cause the stopping of the heart in contracted condition.

Experiments of the same kind were made on the adductor muscle of Pecten. Here however I did not succeed in obtaining satisfactory results, though I was impressed of the fact that the muscle weakened down after an initially vigorous reaction to the fluid. Several trials for a graphical registration of this fact gave disappointing results. By means of a light pulley the movements of one free shell — the other shell was clamped in a stand, while the open side of the shell was pointed upwards — were transmitted to a counterbalanced lever, writing on a drum, but the quite irregular behavior of the muscle made these experiments a failure.

Better results were however obtained with a frog's gastrocnemius. At regular intervals of 30 seconds the muscle was stimulated

and the twitches registered on a drum. From above the muscle was slowly irrigated with an extract of the starfish stomach in frog's saline. In a control experiment the same thing was done with saline only.

The results of these experiments are clearly visible in fig. 1 and fig. 2. In the case of the extract we see how the muscle after a short time falls into a series of almost tetanic contractions, allthough the signal indicates that I only stimulated once. The muscle remains in this semi-contracted condition for a long time, regularly responding to electrical stimulation. The response however, becomes weaker and is frequently irregular, after

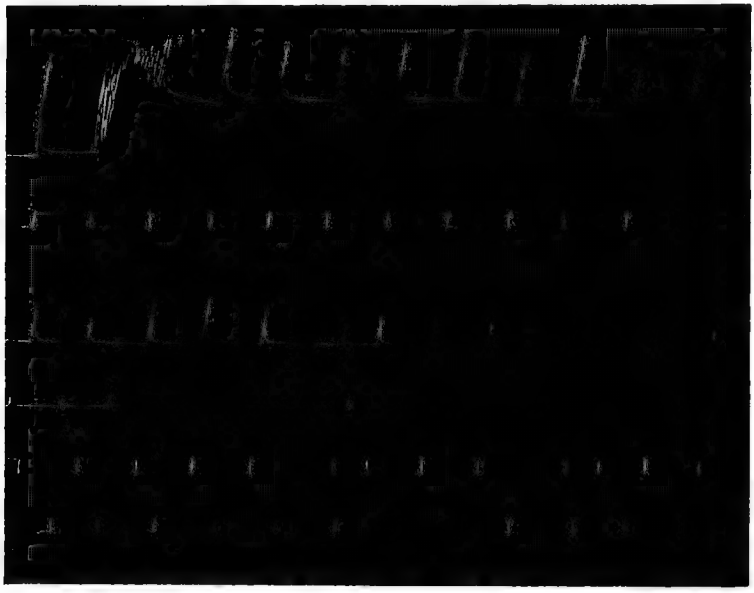

Fig. 1.

Poisonous action of extract of starfish stomach on frog's gastrocnemius.

about half an hour no response whatsoever is obtained for several minutes. After the experiment is stopped, the muscle does not even respond to direct stimulation any more and is apparently exhausted or „killed".

A control muscle did not show as irregular a behavior, gives the same response after half an hour as in the beginning, could be stimulated both directly and indirectly after this time and did not show the peculiar contracting phenomenon.

From this evidence the writer is inclined to believe that a toxic action of some kind can be attributed to the stomach of the starfish. Time for a closer study of this problem could not

be found: by the nature of the work in a summer laboratory one can scarcely expect to exhaust a subject like the one taken up here.

I must mention however that the phenomena observed resemble closely those caused by the toxic action of the „poison-glands" of the Cephalopodes.

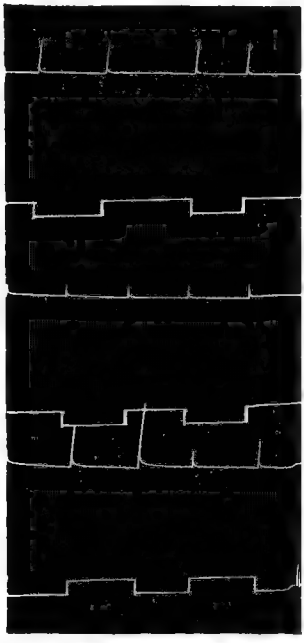

Fig. 2.
Control-muscle.

R. Krause (Zentralbl. f. Physiol. 9. 1895. S. 276), who was the first to study these, observed „zuckende Bewegungen der Extremitäten" in poisoned animals, followed by a gradual weakening till death. The same initial stimulation is indicated here.

Lo Bianco (Mitt. Zool. Stat. Neapel. Bd. 13. 1899. S. 530) describes a „incertezza della locomozione, subentrando poco dopo movimenti convulsivi, con un tremito rapidissimo in tutti i piedi toracici," which is also followed by death.

A. Briot (C. R. Soc. Biol. 1905 (T. I), p. 315) also mentions the „trémulations des membres".

These few indications will serve to show that there is a certain analogy between these poisons, at least as far as their action is concerned. Frogs seem to be sensitive to the cephalopode-poison, according to Krause. Briot however denies this, Baglioni 3) solves this controversy by showing that the poison can only act here after 10—20 minutes, when true typical attacks of clonic contractions occur. In our experiments we also find contractions occurring after a considerable length of time.

The poison of the cephalopodes, is according to Baglioni, a poison of the C. N. S., which first produces clonic contractions, followed by a „Lähmung" of the central functions afterwards. It seems to be of phenolic nature and this is the more probable since crabs are very sensitive to phenoles.

The analogy between these two poisons has also been seen by Piéron 100), who gives as a possible explanation „une égale action chimique provenant de sécrétions, liées à l'appareil digestif, sécrétion de l'estomac chez l'Astérie, sécrétion de la glande salivaire chez le Poulpe."

A closer investigation of the relation of this poison and the „omnipresent" substance in these animals, which is colored blue by Folin-Wu's uric-acid reagent, seems most promising.

6. THE ENZYMES IN THE DIFFERENT GROUPS.

a. Proteolytic enzymes.

In my introduction I said that the work presented in this paper would not only consist of the study of some new aspects, but also of a checking up of the old data in the literature. This is especially true of the present chapter; much was known about enzymes in our group already. Nevertheless I have tried to study some as yet obscure points and have come to results different from those of previous authors in one case.

That there is a proteolytic enzyme present in the starfish, is evident already from its biology. It has more over been found repeatedly already, f. i. by Frédéricq 40), Cohnheim 17), Chapeaux 15) and Griffiths 48).

Not clear however is the exact relation of radial sac and stomach as far as the secretion is concerned: this question is to be discussed in the chapter on the resorption in the starfishes.

About the chemical nature of the proteolytic enzyme we are not very well informed either. Here as in almost all groups of invertebrates, we find Krukenberg, who assumes that two enzymes are present simultaneously, i. e. pepsin and trypsin. Roaf and Bourquelot came to the same conclusion. How this is possible, why he assumes the presence of pepsin when the reaction of the digestive juice is on the alcaline side of the neutral point, is not clear. Neither how these two enzymes can act simultaneously while they require an absolutely different hydrogen-ion concentration. All such assumptions of Krukenberg have been critisised severely by several authors, among which Jordan 65), his „Helicopepsin", „Isotrypsin" and „Homaropepsin" have been shown to be fictions.

His ideas on the simultaneous presence of pepsin and trypsin at the same spot and in the same organ have also been critisised from several sides. In Protozoa, where an acid reaction in the vacuoles preceeds a later stage of alcalinity — as first discovered by Metalnikof 83) —, he assumed the same thing. Later investigations, like those of Greenwood 47) and Nirenstein 94), have shown clearly, that the acid period only serves for the „killing" and preparing of the prey, but that no digestion takes place during this time. The digestion and resorption take place in the alcaline period.

In order to study the nature of the proteolytic enzyme present here a little more in detail, several digests were made. Some of them were acidified with 1.3 pro mille HCl, another part was kept alcaline by means of sodium carbonate. As a substrate either gelatin or egg-white — raw — were added. Of lateral sacs and stomach equal quantities were used; 100 c.c. of water were added in each case and some toluene.

After four days the digests were taken out of the 37° in-

cubator and in the protein-free filtrates tests for peptones — biuret — and for amino-acids — ninhydrin — were made. The proteins were precipitated by means of an excess of trichloracetic acid and filtered off.

The results of these tests are given in the following table:

Table 1.

Peptone and amino-acid tests in several digests of starfish material.

Reaction.	Material.	Biuret.		Ninhydrin.	
		Egg-white.	Gelatin.	Egg-white.	Gelatin.
Alcaline digest.	Stomach.	—	+	—	+
	Radial sac.	—	+	—	—
Acid digest.	Stomach.	+ ?	+	—	—
	Radial sac.	+ ?	+	—	—

From this table three things are evident. In the first place we see that qualitatively at least there is no difference between the activity of the stomach digest and that of the radial sacs. We will come back on this point in the chapter on the resorption in the starfishes.

Secondly we see that gelatin is much better as a substrate than egg-white, which does not astonish us when we remember from human physiology, how relatively indigestible raw egg-white is.

In the third place we find that either the enzyme present acts just as well in an acid medium as in an alcaline one, or that Krukenberg is right in assuming the simultaneous presence of the two proteolytic enzymes.

Therefore we must try to put the question on a more quantitative basis, which I did in the following way:

$11^1/_2$ gr. of radial sac and of stomach material were ground up with sand. Equal quantities of gelatin, toluene and 100 c.c. of water were added to each digest, one of which was acidified as described above, the other one kept alcaline. After 24 days they were removed from the incubator, the proteins pecipitated with an excess of 5 % trichloracetic acid and filtered off.

In the protein-free filtrate total-nitrogen determinations were made on an aliquot, according to Folin's method. The Nesslerisation was direct.

In the acid digest 25.4 mgr. of non-protein nitrogen were present, in the alcaline one 144 mgr.

This proves clearly that only one enzyme is present, i. e. trypsin, which as we know, works over a relatively wide range. If pepsin were present, we would expect much more non-protein

N and not about one sixth of what we find in the alcaline digest. Not improbable however is that the trypsin present here should work over a wider range than the trypsin of mammals.

Another fact which proves that trypsin is at work here, is the regular occurrence of amino-acids in the acid digests: pepsin, as we know, does not carry the hydrolysis any further than up to the peptones.

In order to make sure that trypsin can work in this degree of acidity, I ran a control in which pure trypsin (Merck) was used. After three days already I could demonstrate the presence of peptones and amino-acids, after two weeks I found 16 mgr. of non-protein N.

It does not seem improbable though, that in these lower forms we may have to do with enzymes which do not fit into the classical division of trypsin and pepsin. I have the impression that probably there is a whole series of enzymes, working at very different optima of P_H, of temperature etc.

An interesting paper by Rakoczky 106) has given some experimental evidence for this view. He compares the pepsin of the dog and of the pike. Both had been extracted in exactly the same way, yet there were very decided differences in their activities. Some proteins are attacked more easily by one, some by the other enzyme. On the whole the dog's pepsin has a greater latitude of possibilities. The optimal acidity is higher for the dog's pepsin. Pike pepsin is destroyed much more easily by higher temperatures, on the other hand it stands freezing much better.

All these properties are extremely purposeful („zweckmässig"), from the biological standpoint. It seems as though everywhere such enzymes are present as are necessary or desirable in the conditions. A detailed investigation of the lower forms from this point of view seems very worthwhile.

In Echinoidea the same proteolytic enzyme is also present, but the enzyme appears very weak here when we compare its action to that of the strong enzyme of the starfish. After eight days no amino-acids could yet be demonstrated, though a peptone-biuret was positive. Later on, especially when dialysing tubes were used, they could be demonstrated very easily. Roaf 108), Henry 53) and Krukenberg (Toxopneustes) also found a protease in the species they studied.

In Holothurians the presence of proteolytic enzymes has repeatedly been denied. One of the authors who were not able to find it, in Holothuria tubulosa, is Cohnheim 17). He tried to find it in several different ways, but came to the conclusion that no such enzyme is present. In other chapters — that on

autolysis and that on the nitrogen metabolism of Holothurians — we will discuss his arguments more in detail; here I only mention my own experiments.

Several digests were made in which either egg-white or gelatin, Na_2CO_3 or HCl were used as described before.

After one week and a half the following tests were made:

Table 2.

Reaction.	Substrate.	Biuret.	Ninhydrin.
Na_2CO_3	gelatin.	+	—
	egg-white.	+	—
HCl	gelatin.	+	—
	egg-white.	+	—

The amino-acids which could not be demonstrated in the protein-free filtrates soon appeared to be present in dialysates.

Summarising our results on the proteolytic enzymes, we may say, that only one enzyme is present, i. e. trypsin, which possibly works over a wider range of P_H than the same enzyme in mammals, but that no pepsin is found. The enzyme is present in all groups studied.

b. Sucrolytic enzymes.

Invertases are also present in the three groups. Experiments of exactly the same nature as those described above, were made, in which cane-sugar (glucose free, the so-called rock-candy) was used as a substrate. The enzyme is much stronger in the starfish and Thyone, than in Arbacia in which form it is *very weak*. The same observation was made by Cohnheim in Sphaerechinus granularis. This author considers the invertase in the starfishes as an adaptation to a sweet, but not reducing sugar of unknown nature present in bivalves around Naples.

As far as the *amylolytic enzymes* are concerned our experiments were complete failures. I do not know whether I must blame this on my method, because I used the organs themselves, be it after having crushed them with sand, instead of extracts, as they have mostly been used by previous authors [1]). At any rate, I did not get any positive Fehling's tests in the protein-

[1]) Frédéricq 40), Griffiths 48), Chapeaux 15) and Clerc 16[a]) found an amylase present in starfishes; Krukenberg, Cohnheim 17) and Henry 53) in sea-urchins; Clerc 16[a]) and Cohnheim 17) in cucumbers.

free filtrates of any of the digests. In many cases I could convince myself, that starch which was added as such or after it had been boiled for a few minutes, was still present. The blue coloration which the digest assumed with J in KJ proved this clearly.

Though Fehling's test was entirely negative in all cases, Benedict's reagent gave results which were neither positive nor negative. In some of the digests I found that the blue coloration had disappeared and in its stead a red color was seen. Maybe this is to be explained in the following way: Biedermann 8) found that „durch das Mitteldarmsecret einer Raupe aus Stärke fast nur Erythrodextrin und nur sehr wenig Zucker gebildet wird." He concludes that we have to do here with an amylolytic enzyme entirely different from that in snails and other animals. This might account for my observations which greatly puzzled me during my stay in Woods Hole. Since however Biedermann's paper came too late to my attention, unfortunately I could not study this problem more in detail.

c. Lipolytic enzymes.

For these I refer to chapter 16.

7. ARE THE ENZYMES PRESENT IN THE FREE FORM? PHAGOCYTOSIS IN ECHINODERMS.

A question of great importance is whether the enzymes on which we have reported in the preceding chapter, are present in the free form.

We know that in unicellular organisms the whole process of digestion is an intracellular one. In a single food-vacuole everything is digested, all enzymes are secreted and even different degrees of acidity are realised here successively.

In pluricellular organisms, in their most primitive forms, the same state of affairs persists. This is especially true of the lower worms, the coelenterates and the sponges. The entoderm-cells of these animals can easily be compared with protozoa of different groups, which take care of one of the functions in the service of the organism as a whole. The chemical destruction of the food which prepares the same for the assimilation by the living substance, is in that way originally a purely cellular one.

The excellent study of Jordan 66) on digestion in Actinians, gives a clear idea of phagocytical processes of this kind. And yet the process is in some way complicated here; the large prey of these animals necessitates a device of some kind for breaking them down. Mesnil's hypothesis (Ann. Inst. Pasteur. T. 15. 1901 p. 352—397), that a kind of dissection and fragmentation by the rims of the saepta would take place, aided by a small quantity of proteolytic enzyme, secreted on contact,

seems to be wrong. Jordan could show this by folding a prey in blotting paper; this prey was broken down just as well though less vigorously.

One of the first authors who clearly realised the importance of this way of digestion and understood it as the phylogenetic precursor of the process in higher organisms, was Élie Metschnikoff 84) in his paper on intracellular digestion in coelenterates. The epithelium of the coelenteric cavity of these animals may be called an amiboid epithelium, in some species (e. g. Praya diphyes, a Siphonophore) a true plasmodium is even formed. In Ctenophores the food is carried further into the body by mesodermal cells, as in Sponges.

An excellent monograph on the intracellular digestion in the flat-worms has been given by Saint-Hilaire 113). The food taken by these animals is found as such in the cells of the lining of the gut. Blood-corpuscles f. i. keep their characteristic shape and only later they are gradually broken down. The products of digestion are collected in special vacuoles and deposited there in the form of crystalline structures. Numerous cell-inclusions appear to grow at the expense of the food as it is broken down. [1])

In the „higher" animals conditions are totally different. Apart from the Gastropods (Enriques, Biedermann and Moritz) digestive phagocytosis has been observed in no group of the higher animals. Digestion has become an extracellular act: it takes place in the „interior exterior" and it is doubtful whether in the resorption the separate protoplast plays a rôle as such. Here the free enzymes are not, as in the Actinians, a preliminary adaptation, a „Hilfsmechanismus", but the very essential thing in digestion.

In snails we find a kind of transitory stage. The so-called „liver" of these animals („Hepatopankreas"), has become an organ of special interest since the investigations of Biedermann and Moritz. In the digestive juice of the representatives of this group all enzymes — up to a cytase studied specially by Alexandrovicz 1) — are found free except for the proteolytic one. The proteolysis is localised in the liver. Protein is actually digested, as a careful analysis of the faeces and the food-N shows; fibrin is not digested however, if treated with the stomach fluid or with a watery extract of the liver. A free acid is formed, as we will mention in chapter 17, the whole mass becomes gelatinous, but no „digestion" takes place. Completely different is the result however if the shreds are placed between two cut surfaces of the liver. In less than no time it

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[1]) 1. Even a vacuole is not always necessary, as le Dantec showed for Gromia and Pfeffer for Myxomycetes in the case of crystals of asparagin.
2. Excretion also takes place by the same epithelium in many of these forms.

is digested and melts away to a greyish mass which can scarcely be discerned from the surrounding liver material. This problem has also been studied by Stübel 125). These experiments seem to show that the protein can only be dissolved by living liver-cells and shows something of the nature of the activity of the mesenterial filaments of the Actinians, as it was pictured by Mesnil. Jordan 69) made moreover phagocytosis in these animals probable.

From this point of view an investigation of the Echinoderms in this respect seemed most promising. It appeared however that all the enzymes are found free here; this group ranks high as far as the physiology of its digestion is concerned.

In order to get some definite information on this question, I made a series of experiments on the digestive juice, as secured from the intestine of fresh specimens. In Thyone and Arbacia this is very easy, one simply punctures an isolated loop of intestine, washed in sea water, which as a rule are full of liquid. In Asterias the liquid was collected by pressing on the outside of the stomach, after the arms had been cut off. The few drops of liquids which appeared on the oral disc were then collected. In this way I did not succeed of course in separating the digestive juice completely from the perivisceral fluid. The method however seems to be the only practicable one.

To these liquids a certain quantity of substrate was added in a deep-depression slide and a droplet of toluene. The whole was covered with a cover-glass and as a rule evaporation was prevented by the use of vaseline. The slides were then put into the incubator at 37° and inspected after different intervals of time.

It soon became evident that all the enzymes are present in the free form. Our trypsin-like enzyme is present in a free form in the digestive juices of every one of our three species. Small shreds of fibrin were dissolved within 24 hours, the same happened to pieces of gelatin. A positive ninhydrin — which test was negative in controls — demonstrated the presence of the products of enzymatic action. In some cases a positive biuret for peptones could be obtained.

No evidence whatsoever could be obtained of an amylolytic action of the visceral fluid. A microscopical examination of starch grains which had been digested with the digestive juices for one, two or three days, did not reveal any changes. A slight swelling takes place in the grains, probably due to the alcalinity of the medium, but no trace of corrosion can be observed. In one case I found a reduction of Fehling, but a control showed that the starch used here was not entirely trustworthy.

These results are the same in starfish, urchin and cucumber

and even continued digestion does not give any evidence of the presence of amylolytic enzymes in free form.

Strange enough the same thing is true in many cases if cane sugar is used as a substrate. In the previous chapter we have seen that an invertase is certainly present in the representatives of this group. It could however not be proved so readily that this enzyme is present in free form, in Thyone I only got one positive result on six tests, in Arbacia one on five [1]). In Asterias no positive evidence was obtained.

I am at a loss for an explanation of this fact. Maybe it means that there is a certain periodicity in the enzyme secretion.

For experiments of the same nature on fat I refer to the chapter on the digestion of fat.

When we look over the general result of these experiments, we see that those enzymes which are present in the watery digests, are (occasionally) found in free form.

On the other hand: the enzymes present are rather weak; we will be more impressed of this fact when we come to consider the gut-contents of Thyone and the faeces of Arbacia.

The mucous membrane in which the food is included in the digestive juice of the sea-urchins according to Scott 117) and Roaf 108), is other evidence in favor of the occurrence of free enzymes. These membranes are sometimes formed already in the first part of the gut, mostly however later on. In such conditions there is no possibility of phagocytosis or anything of the kind; digestion and resorption must take place in and from this food-vacuole.

Whether or not phagocytosis plays a rôle in the digestive processes of our group, is hard to decide. I started the present investigation with the idea of finding very primitive conditions. For this reason I made many experiments in which I fed urchins or starfishes on suspensions of carmin or bone-black. In the large amount of material collected from such animals I found but one section in which the grains of carmin could be seen lying inside of the cells (Fig. 3 of our colored plate). One positive experiment of this kind of course does not prove much; a closer investigation of the importance of phagocytosis in the digestion of the Echinoderms seems very well worthwhile however.

The fact that carmin is found in granular form inside of the cells, does not always prove phagocytosis. H. Eisig 35b) in the case of Capitella assumes, that carmin is absorbed in dissolved form and reprecipitated in the cell-body. In fact no other evidence whatsoever of phagocytosis was obtained.

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[1]) Roaf also failed to find any evidence of the presence of a saccharase in the Echinoidea.

8. AUTOLYSIS IN ECHINODERM GUTS.

Discussing the presence or absence of proteolytic enzymes in Holothuria tubulosa Cohnheim 17) states that he could not observe a trace of autolysis in their guts, if they were kept under aseptic conditions. This observation is doubtlessly right; I could observe something similar in our Thyone, its gut can be kept for an almost indefinite length of time in sea water under toluene without showing signs of profound changes. Is this however an argument in favor of his contention that Holothurians should have no proteolytic enzyme in their gut and that the N-metabolism of this group does not amount to anything?

I do not believe it. In the first place we must remember that the gut of these animals is strongly muscular and that muscle-tissue autolyses much less completely and rapidly than any other kind of tissue. It is very well possible that the epithelium of these guts autolyses away while the muscular wall keeps its coherency and gives the impression of being the whole gut. As a matter of fact we can observe a change in color and other changes in these guts allthough they do not fall to pieces.

To settle this argument and to find out whether Cohnheim is right in his conclusions, I decided to study autolysis of the guts of Asterias and Thyone a little more in detail. In Asterias nobody will doubt the presence of a proteolytic enzyme and yet autolysis proves to be exceedingly slow even in this case.

For this research I ground up a large quantity of radial sacs and of stomachs of Asterias with sand. Some water was added to these masses, a large quantity of toluene and a trace of Na_2CO_3 in order to keep the reaction of the digests in the neighbourhood of neutrality.

The digests were then put into the 37° incubator and left there to autolyse. At different intervals I made tests for peptones and amino-acids by means of the biuret reaction and ninhydrin on the protein-free filtrate. The proteins were as a rule precipitated with 5 % trichloracetic acid. The liquid as it came through the filter was clear, but not completely colorless, perhaps on account of the fact that a small quantity of a water soluble pigment which proved to be very troublesome in all these experiments, was not precipitated out.

After six days all these tests gave completely negative results. After nine days I got a positive peptone-biuret in the radial sac sample, but no evidence whatsoever was seen of autolysis in the stomach sample.

The radial sac sample became one homogeneous mass, but the stomachs did not seem to be liquified, they could still be seen separately.

The same result was invariably obtained for a period of about two months; in the protein-free filtrates I never got positive results with ninhydrin tests.

The digests had a slightly acid reaction which is optimal for autolysis. Yet autolysis seemed to proceed exceedingly slowly. This may of course have different reasons: it is possible that the proteins of these tissues have a great resistance against proteolytic enzymes. We saw in the chapter on the enzymes that other proteins are digested much more rapidly, this might be an indication in this direction. It is also possible that the conditions of reaction were very unfavorable; from what we know about autolysis in other forms, this does not seem very probable however. In the third place it is very well possible that the quantity of enzyme at hand is very small.

Autolysis occurs however, and this was more evident yet when I dialysed a small quantity of the digest through a thin collodion sac. A strongly positive ninhydrin test in the outside liquid showed that amino-acids though they did not show up in the protein-free filtrate are surely present.

The same thing proved to be the case in autodigests of Thyone guts. No trace whatsoever of autolysis could be obtained in the protein-free filtrates. The guts remained in unliquified condition for a long time and nothing indicated that autolysis were it even very slight, was going on. Yet when I dialysed part of the liquid, I obtained a positive ninhydrin reaction in the outside liquid.

From this evidence I have concluded that autolysis goes on very slowly in the guts of Asterias as well in those of Thyone. There is no doubt however as to whether it actually takes place. And there can in that way be no doubt that a proteolytic enzyme must be present in their guts.

But one thing is very evident on the other hand, i. e. that in these form this process does not take place with the same intensity and rapidity as in the higher forms on which biochemists have been working up to the present day. The solution of the problem why this is the case, must be left open for a more detailed investigation.

9. CONTENTS OF THE GUT OF THYONE.

In studying the contents of the gut of a particular species, we can on one hand get some information about the natural food of the species, on the other hand we may get an idea about the enzyme activity in their gut.

For the present study I used animals which had been brought into the laboratory on the same day on which the observation was made. Their gut-contents were put on a slide and examined through the microscope.

Apart from numerous sand-grains many pieces of decaying plant-tissue were found, recognisable by the cell-walls and by their brownish appearance. On the other hand however I could frequently see different species of unicellular green algae, which were perfectly intact and still in the possession of green chloroplasts, a nucleus and all other structural details. Many empty diatom shells were present, also a flagellate species, empty, with cirri sticking out. Many diatoms however were perfectly intact just as the green algae. Encysted Protozoa (?), perfectly round bodies, also frequently occurred. A complete copepod was seen, not moving, but very complete up to the smallest brittles, the eye full of pigment. Nothing gave the impression of being dissolved or dissolving, the heart was not beating. Pieces of other Crustaceans were also found, sometimes nothing but the chitinous sceleton.

Large quantities of detritus were present in another animal of which I studied the contents of the rectum. Here also complete green algae, uni- as well as pluricellular forms were found. Diatoms, either empty shells or in full possession of all the cell constituents, green algae, thread-like and with contracted cytoplasm — as if in a hypertonic solution —, were also very abundant. Pluricellular red algae with contracted cytoplasm, but uninjured and in full possession of the natural colors were also found occasionally.

To this list Pearse 98) still adds nematodes and an ostracode. In every animal I dissected many alive Protozoans were found in the rectum, moving around with great speed in the visceral fluid. According to Pearse they belong to the genera Gymnodinium and Lichnophora.

Ulva-leaves digested with the digestive juices of Arbacia and Thyone did not seem to be very much attacked. Sometimes the cells at the outer margin had a slight brown color, the whole leaf, even after digestion at 37° for one or two days, remained green however and kept all its structures.

10. COMPOSITION OF THE FAECES OF ARBACIA.

A study of the faeces of Arbacia was made with a double purpose. In the first place it may give us an idea as to the intensity of action of the enzymes in the intestine, secondly it may give us some information about the feeding habits of this species.

The faeces of this species are most easily secured. Shortly after the animals have been brought into the laboratory, their back is as a rule covered all over with small, oval bodies of a white color which prove to be their faeces. Even after 5—6 days the gut is still always not empty and faeces occasionally produced. According to von Uexküll 128) defecation takes

place by the pressure caused by retraction of the mouth-membrane.

Several samples were studied a little more closely. Some contained practically nothing but sand and some detritus, others however were full of interesting substances, most of which could easily be identified. Some were full of calcareous material, as a foaming with hydrochloric acid proved; others did not contain anything of the kind.

As examples of materials which I could easily identify, I may mention the following constituents.

Several species of diatoms were seen. Some of these moved around very actively. Though I tried to avoid all contamination with the sea water of the aquarium, some planctonts may have come into the material; for this reason I am not inclined to consider this observation as very important.

Otherwise it would be a very convincing demonstration of the weakness of the action of the digestive juice. Some unattacked unicellular algae were also found. Sand-grains are present in nearly every sample. Brittles, maybe of Bryozoa, are abundant. A hydroid was found (Sertularia?). In some of the compartments a seemingly intact, but contracted individual was seen. Spiculae were seen, probably derived from sponges. Red algae, also seemingly intact, frequently occurred. The granulation of the cell-contents and the chromatophores were clearly visible. It was a pluricellular branch: it did not seem to have been attacked severely.

Peculiar honey-comb-like structures, like minute plant-tissue (Cyanophyceae?), could not be identified. A leg of a copepod seemed to be attacked and the flesh dissolved away.

From this evidence we are once more impressed of the relative weakness of the enzymes present in the free form in this group. Of the large amounts of material which pass through the gut only a very small part can be attacked succesfully, some substances pass without serious changes. If really alive organisms are present, this is the most convincing proof; this does not seem to be very probable to me, though. [1])

As far as the feeding habits of the species are concerned, we see that it is practically omnivorous. Most probably it eats nearly everything, chews algae, also calcareous species, sponges, Bryozoa etc., by means of its strong masticating apparatus, and in this way also gets hold of its microscopical inhabitants and some other materials. The only thing which I actually observed an Arbacia eating, was Clione sulfurea, a calcisponge.

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[1]) The same incompleteness of the digestion has been found by Plateau (Recherches sur les phénomènes de la digestion chez les insectes. Bruxelles. 1874) in catterpillars and by Knauthe (Untersuchungen über den Stoffwechsel der Fische. Zeitschr. f. Fischerei.. 5. 1897. 189) in fishes.

11. HYDROGEN-ION CONCENTRATION OF THE GUT CONTENTS.

The enormous importance of the concentration of the hydrogen-ions in almost every biological process, so clearly realised by the numerous investigators who followed the example of Sörensen, justifies a closer investigation of the digestive liquid of the Echinoderms in this respect and a comparison with the very scarce studies on other invertebrates.

One reason why these studies have always been so very scarce, is the fact that methodologically biologists were so very poorly equipped. It took a long time before the P_H work penetrated through all the branches of biology, so that allthough Sörensen's work appeared already in 1902, even biologists with the physiological attitude, have been going on using the old fashioned methods, chiefly the mere coloration of indicators, for the determination of acidities of media to be investigated.

Plateau (Mém. de l'Acad. Roy. de Belgique t. 41. 1874) studied a series of myriapods, arachnids and crustaceans as to the reaction of their digestive juices in this way. He found that in all articulates the gut contents are alcaline, sometimes near neutrality, but never acid. In a later paper he states that frequently a weakly acid reaction is found in carnivores and omnivores, but that the digestive juice of herbivorous species is always alcaline.

Other authors came to similar results, mostly the contents of the fore-gut are more acid than those of the other parts. On this point I will come back presently.

The only investigator who ever studied the reaction in Echinoderm guts, was Roaf 108). Though his method was relatively up to date in his days, it does not give us as complete information as one might desire. The argument of his investigation was the question which we have discussed in the chapter on the enzymes, whether it is possible to have a tryptic and a peptic enzyme present, working at the same time. He succeeded in accomplishing what I never could get done, his starfishes and urchins took fibrin colored with different indicators. He now determined at the changing point of which indicator the P_H of the intestinal juice lies. The following indicators were chiefly used: dimethyl-amido-azo-benzene, congo-red, litmus, neutral-red, phenolphthalein.

In the case of the sea-urchin whose feeding reactions this author describes in detail, he found by comparing the results obtained with different indicators, that the hydrogen-ion concentration of their intestinal juice is about that of the changing point of neutral red, i.e. $P_H = 8$. This indicator which is colored yellow by sea water, turns red in the gut of the urchins. The intestinal juice is alcaline to litmus however.

The indicator diffused out of the food masses, so that no definite information could be obtained as to the reaction of the middle- and end-gut.

Asterias was fed on fibrin which had been colored with congo-red. The initial reaction proved to be on the alcaline side of this indicator, that is $P_H > 4$. The rectal coeca are blue from the beginning and remain blue. This is interesting in connection with their function as uric acid excreting organs, which will be discussed in chapter 23. On treatment with alcali however they contract and squeeze out a liquid which instantaneously changes the indicator into red. After their initial alcalinity the food masses are transported into the interior of the radial organs, which show a distinctly blue color, „as if containing a blue solution". This would mean a very high acidity, which Roaf does not quite seem to realise, since the changing point of congo-red is at $P_H = 4$. This acidity has however also been found by other authors. „Das Sekret reagiert schwach sauer". Schneider 141). p. 653. „Les coecums pyloriques sécrètent un liquide à réaction acide." Delage et Hérouard 136). p. 66. Also Stone 124) [1]). Chapeaux finds the secretion alcaline.

The same strongly acid reaction was observed in the small species, Porania pulvillus, which was fed on vesuvian-brown (changing point also at $P_H = 4$). Roaf concludes that the reaction of the pyloric coeca is about that of a decimolar solution of sodium di-hydrogen phosphate. Here also the rectal coeca appeared to have a brown color, which shows an acid reaction.

Of very recent date is a paper of Jameson and Atkins 61[a]) on the physiology of the silk-worm. These authors used the more recent method of Clark and Lubs [2]), — see for a description of these methods the excellent book of Clark on the determination of hydrogen-ion concentration 134) —, comparing the color of a drop of digestive juice added with some indicator to the colored plates of Clark 134). A decided acidity could be observed in the gut-contents of the imagines, the $P_H = 5.2$ — 5.8. In the larvae however the digestive secretion proved to be alcaline ($P_H = 9.0$ — 9.8); in the hind-gut somewhat less strongly alcaline ($P_H = 8.4$). The acid mulberry leaf is apparently „over-neutralised" in the intestine.

My own experiments were made with the same indicator method. I had the privilege of using a set of Sörensen-standards, made up by Dr. J. B. Collip of the U.o. Toronto

[1]) Stone found the reaction of the secretion of the pyloric coeca slightly acid: they turned litmus blue. This acidity must be due to some organic acid since tropaeolin OOO which is extremely sensitive to mineral acids showed no change of color. Lactic acid? v. d. H.

[2]) J. Bact. 2. 1917. 1,109,191.

and used by him in his work in the M. B. L. The standards were kept for about a month in Jena Erlenmeyers closed tightly with rubber stoppers.

Juice of the different parts of the gut of Thyone and of Arbacia was secured. One drop was put on a white porcelain plate on which a series of standard drops had been laid out previously. Equal quantities of indicator were added to the different samples—I used a narrow capillary tube for this purpose—and the color comparison made. As an indicator I used phenol-red.

On Thyone four sets of determinations were made. A series of figures was secured for the different parts of the gut. This is especially easy in these animals on account of the length of their gut and because their intestine is nearly always full of liquid. The results obtained in this way are represented in table 3.

Table 3.

Hydrogen-ion concentration in Thyone gut.

	1.	2.	3.	Average.
Stomach.	7.4	7.8	7.5	7.6
Intestine	7.0	7.6	7.1	7.2
↓	7.8	7.8	7.4	7.7
	8.0	8.0	7.7	7.9
Rectum	8.2	—	—	8.2

In the urchins it proved to be very difficult to secure liquid from definite parts of the gut, partly on account of the more complicated anatomical relations, partly because these guts usually do not contain much liquid. I obtained two series of figures, but I do not give them any more value than just figures of the P_H of an arbitrary drop of digestive juice. Their values are represented in table 4.

Table 4.

Hydrogen-ion concentration in Arbacia gut.

1.	2.
7.2	7.7
7.5	7.8
7.4	7.6

In the starfishes it is most difficult to secure any digestive juice. Since, as mentioned before, I did not succeed in feeding these animals artificially, I had to be content with a drop of liquid eventually falling out of the organ to be tested. Considering the roughness of such procedure, it is rather remarkable that even these drops had a P_H very much different from that of the perivisceral fluid by which, though the utmost care was taken to avoid this, it might have been contaminated. The P_H of the radial sac fluid appeared to be 7.3; that of the contents of the stomach 7.1, 7.6 and 7.7 in three samples.

One fact was very striking in all tests. After the color comparison had been made, the apparatus was frequently left at the same place for some time, before it was cleaned. Then a comparison of the colors would have given entirely different results: the digestive juices which at first were rather far on the acid side of the sea water, more and more turned over to alcalinity. I do not know definitively how to explain this remarkable phenomenon, since the standards were exposed to the same amount of evaporation etc. Maybe it is due to a loss of CO_2 which might be the cause of acidity, since phosphates are not present in quantities large enough to account for the acidity, as we will see in the next chapter. Gas-bubbles present in the intestine of Tenebrio, have been supposed by Biedermann 8) to consist of CO_2.

It will be noted that all these P_H's are on the acid side of that of sea-water, which in Woods Hole appeared to have a P_H of 8.2—8.3. In the experiments of Roaf mentioned above we have seen the same thing in his experiments with neutral-red. For such acidity there must be an explanation and in the next chapter we will endeavor to find such reason.

First I want to mention another interesting phenomenon, i.e. the relatively high acidity of the true intestine, just behind the stomach in Thyone. In Stichopus we even find real acidity according to Crozier (on p. 388 of 18)), where he says: „The yellow fluid, contained in the stomach of an „empty" Stichopus gives with indicators an apparent acidity of 5.0—6.5. Fluid obtained by centrifuging the stomach contents of animals engaged in feeding showed acidities varying from 4.8—5.5, the latter being the most common." Here of course, we have to do with a specific adaptation to the calcareous sand, taken as food by these animals, which as we pointed out in the chapter on the biology of our group, may have considerable economical importance, because the calcium present in nature in undissolved form, may be brought into solution in this way. Rain-water present everywhere in nature as dissolving medium, only has a P_H of 6.0; the water dripping from the tip of stalactites one of 7.9—8.0 (Crozier).

But something of the same nature is also the case in our

Thyone which also eventually feeds on calcareous matter. The aquarium in which the fresh Thyone's were put, frequently contained an immense amount of small and very characteristic calcareous bodies on the next day. I have not been able to identify these, but according to some morphologists who were especially acquainted with the local fauna, they ressemble very much the calcareous sceletal elements of Alcyonarians.

The same phenomenon, an acidity in the beginning of the middle gut has also been observed by other authors in other forms. Basch [1]) found it in the digestive tract of Blatta orientalis. The secretion of the salivary glands, the contents of oesophagus and fore-gut are acid. In the middle-gut („Chylusmagen") the reaction is neutral in the first part, alcaline further on. Basch explains it by an alcaline secretion of the crypts of the middle-gut, Jousset de Bellesme assumes that an acid liquid, containing pepsin, is poured into the beginning of the middle-gut by the small coeca, found right behind the „Kaumagen".

Biedermann 8) fed Tenebrio larvae on flour, added with litmus. About two thirds (the first part) were colored red, the rest blue. Similar results were obtained by Kovalevsky in Muscidae, Blattidae and Tenebrionidae.

These few references will serve to show, that an original acidity, gradually passing over into alcalinity is by no means rare in lower animals. The alcalinity in the very first part of the gut of our Thyone is of course a complication due to the fact that we have to do with a marine form here. The sea water taken in with the food accounts for this alcalinity.

In the next chapter we will report on our trials to find the cause of the relative acidity in the present case. It does not seem to be due to free acid in most cases; congo-red which indicates free mineral acid, was never colored blue in Biedermann's experiments — this might be due to the low P_H (4) of the changing point (v. d. H.) —; Günzburg's reagent (2 gr. phloroglucinol, 1 gr. vanillin and 30 gr. absolute alcohol), also indicating free mineral acid, especially hydrochloric acid, gave the same result. It is not due to butyric or lactic acid fermentation either, since lack of carbohydrates in the food does not change the hydrogen-ion concentration.

12. THE DIGESTIVE FLUID.

Studies on the composition of the digestive juice in invertebrates are not so very abundant; the only ones that have come to my knowledge, are those of Biedermann (and Moritz) on the intestinal contents of snails and of the larva of Tenebrio.

The first question of importance is: „What causes the relative acidity of these juices?" Though not absolutely acid, they are

[1]) Sitzungsberichte Akad. Wien. 33. No. 25. 1859. p. 234.

more so than the sea-water and more so than the perivisceral fluid, which both have a P_H of 8.2-8.3. This acidity is strange is so far as the secretion of free acid has never been shown to occur in lower organisms. This is true, of course, as far as the digestive juice is concerned; the secretion of acid by sea-snails etc. (Semon 119)) and analogous phenomena are not to be discussed here. Even in animals as high in the scale of phylogenesis as the dogfishes, it has not yet definitely been settled whether free acid actually is present or not. In a controversy between Weinland and Miss van Herwerden — see van Herwerden 56), Weinland 132), and van Herwerden and Ringer 57) — the former author has been critisising the Sjöqvist method for determination of free acid, used by Miss van Herwerden, as not being reliable if earth-alcalies are present. Van Herwerden and Ringer have answerred that the $P_H = 1.69$ which is the same as that of 0.02 N acid and given many other arguments in favor of the presence of free acid.

Yet the acidity of the digestive juices in our group must have a cause of some sort and maybe a chemical investigation of the digestive fluid will furnish us some information. Since the material was not available in large quantities, I had to run my tests microchemically. A certain quantity of the digestive juice was secured; separate drops were used for the tests to which small quantities of the reagents were added from capillary tubes.

All the tests gave the same result in the digestive juices of Arbacia and Thyone. For this reason I do not treat these two species separately.

The first possibility to be thought of, is the presence of phosphates. They are present in large quantities in the gut of Tenebrio, where a weakly acid reaction has also been found [1]). These acid (dihydrogen-) phosphates can, if magnesia is present at the same time, be demonstrated very easily by a white crystalline precipitate of „tripel-phosphate" formed after the addition of ammonia. They are absent however in the digestive fluid of the snails and do not seem to be present either in our Echinoderms.

Ammonia did in fact give a precipitate. But this precipitate was probably nothing but simply calcium hydroxyde since it did not have the typical appearance of the „Tripelphosphat". Other tests were equally negative, Mg-mixture did not give any precipitate, with Ur-nitrate a very slight precipitate was obtained. This might indicate that a trace of phosphates is present, but at the same time, that this quantity must be very small.

An ammoniacal solution of ammonium oxalate gave a heavy

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[1]) A fluid behaving chemically just like the digestive juice of these animals, could be obtained by adding a trace of ammonia to a solution of mono-sodium phosphate.

precipitate. This proves that Ca must certainly be present. This is not strange since sea water also contains a fairly large quantity of calcium.

Carbonates are not present in large quantities, since if a small quantity of the liquid is evaporated, the residue when treated with hydrochloric acid, does not foam,

Chlorides of course are present in large quantities, silver nitrate gives a heavy precipitate.

In the digestive fluids of Gastropods a large quantity of proteins seems to be present. The same thing is true of the larva of Tenebrio, even when the animals have been starving for some time. It does not seem to be the case with the Echinoderms. On heating no precipitate is formed before evaporation takes place. A xanthoproteic- and biuret-test gave negative results. Since the biuret-test is negative, no peptones are present either. A ninhydrin is negative, no amino-acids are present in that way, though the animals from which the juice had been obtained, apparently were in full digestion.

Di-sodium phosphate gives a precipitate in an ammoniacal solution; this may indicate the presence of Mg, but since Ca is present, it may be due to this metal.

The precipitate with sodium carbonate, also indicates the presence of Ca. Bichromate does not give a precipitate, Ba is not present.

The results of my own experiments and those of Biedermann have been represented in table 5.

Table 5.

Composition of digestive fluids of different species from personal observations and data of Biedermann.

Species.	Proteins.	Ca.	Mg.	CO_3.	PO_4.	Cl.
Helix pomatia L.	Large quantities.	+	Trace?	+	—	—
Tenebrio molitor. L. Larva.	Rich. Globulins?	?	+	?	+	—
Arbacia punctulata. Gray.	None.	+	Trace?	—	—	+
Thyone briareus. Les.	None.	+	Trace?	—	—	+

All constituents mentioned above do not explain the observed acidity satisfactorily. The very small quantity of phosphates, if they are present at all, does not account for an acidity which as Roaf 108) states may be as high of that of a decimolar solution of di-hydrogen phosphate.

In the chapter on excretion we will discuss the importance of the excretion of uric acid into the lumen of the intestine. In connection with the results reported on in that chapter it is

interesting to mention the work of Sitovski 121). This author found an acid reaction in the rectum of the catterpillars of the moth, Tinea biselliella, which he fed on wool which had been permeated with a solution of litmus. In the last two segments a distinctly acid reaction was found. Sitovski supposed that this acidity is due to uric acid.

The question is whether we may make the same hypothesis for the Echinoderms. This does not seem very probable. In its favor pleads for instance the acid reaction which Roaf found in the rectal coeca of the Asterids. In chapter 23 we will see that these organs probably are the place of uric acid excretion in the starfishes. My own findings on the P_H of these organs do not quite agree with his, I never found it lower than 7.3, but my method is doubtlessly much more crude. The same thing appears to be true for the urchin and the cucumbers however; we saw that the acidity is highest in the middle-gut and decreases towards the end-gut. This does not plead in favor of his hypothesis, since we will see that the acid is chiefly excreted into the rectum. Moreover we must remember that uric acid is an very weak acid so that it is scarcely possible that in a „buffer-mixture" like this fluid — carbon dioxide and a trace of phosphates are present! — it should have any noticeable influence on the P_H.

The only possible explanation of the low P_H which seems left in this way, is the excretion of CO_2 into the gut. As a matter of fact the samples described in the previous chapter changed in reaction if left alone. They appeared to become more and more alcaline which would prove the presence of a volatile acid.

13. TRANSPORTATION OF THE FOOD. THE PERIVISCERAL FLUID.

A definite blood system is not present in Echinoderms and a special propulsatory organ is certainly lacking. Two systems represent this one in this group: 1. a so-called blood-lacunar system and 2. the perivisceral fluid. Opinions are different as to whether these two systems communicate. Cuvier believed in their communication, more recently Vogt and Yung in their Lehrbuch der vergl. Anatomie (1880. Braunschweig. p. 523) have defended the same opinion. Hamann denies it, he does not consider injection experiments as convincing since the holes may be caused by the force of the injection. Many other investigators found elements present in the blood system which are absent in the perivisceral fluid, Enriques 37) f. i. finds green-red dichroitic granulae of enzymatic nature in the blood system of Holothuria tubulosa, partly carried by amibocytes and transported to the gut-wall by the oscillations of the fluid;

H. Ludwig reports on the presence of „clotting" substances in the „blood" of cucumbers.

One of the most important characteristics of a blood system is completely absent in Echinoderms. A propulsatory apparatus has never been demonstrated, neither valves, though the axial organ has for a long time been called „heart". Tiedemann 127) was the first to see rhythmical contractions in the blood-vessels of Holothuria; Cohnheim also saw them in those of Cucumaria, but as he says, no definite movement in either direction was seen if one corpuscle was spotted. Enriques 37) discusses his observations in detail and comes to the conclusion that a definite movement actually exists (see the chapter on resorption in Echinoidea and Holothureoidea).

At any rate, these movements are exceedingly slow and not to be compared to those in vertebrates and higher invertebrates.

The importance of this blood system in resorption is to be discussed in chapter 20. As far as its respiratory function is concerned, Ludwig in Bronn's Klassen und Ordnungen etc. in the case of the Holothurians is very sceptical on this point. The wonder-nets (retia mirabilia, Wundernetze, réseaux admirables) found around that left respiratory tree, are so loosely attached to the lung, the one can scarcely expect them to have any such importance. In the chapter on respiration I hope to demonstrate that the perivisceral fluid actually plays the rôle of internal respiratory medium, maybe also the water-vascular system. Perhaps it plays a rôle in excretion; the excretion by means of phagocytes -after physiological injections and perhaps also in normal conditions- takes place through the water-lungs [1]).

The perivisceral fluid is of much more importance; 175 c.c. of the contents of a Sphaerechinus, measuring in toto 225 c.c., is according to Cohnheim perivisceral fluid. Physiologically the contents of the coelomic cavity must play the rôle of blood, in as far as it is a fluid medium, interposed between intestine and consuming tissue and tissue and organs of excretion. A little closer investigation of the chemistry of this medium shows that apart from the corpuscles which are present in abundance in all groups and which show a phenomenon of clotting, entirely different from that in mammals-yet leading to the same end result (see f. i. the paper of Geddes 42) on this point)-, it has practically the same composition as sea water. Cohnheim already found that this is the case. It contains the same percentage of chlorides and the same inorganic constituents. In as far as it is physiologically „blood" we must investigate if it carries any food- or waste-constituents and whether it plays a rôle in respiration. The latter topic will be discussed in chapter 24, for the present will test for food and waste constituents.

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[1]) See the references in the chapter on the respiration.

Though no special organs are present for this purpose, the perivisceral fluid is by no means stagnant and without currents. The whole peritoneum is ciliated and these cilia of course cause currents. That such ciliar currents can take care very well of the distribution of dissolved substances, was shown by Widmark 132) for jelly-fishes in a detailed investigation.

A ninhydrin test for amino-acids in the perivisceral fluid invariably has a negative result in animals which have been in the aquarium for more than a day. In some very fresh specimens one occasionally gets a slight 'pinky color; in one starfish that was caught while it was eating a Littorina, a strongly positive result was obtained. Fehlingh's test is, as Cohnheim found already, always negative, no monoses appear to be present. A biuret test invariably gives negative evidence; neither the violet color which indicates the presence of proteins, nor the pink of peptones was ever seen. This proves that no proteins are present in the filtered perivisceral fluid, as moreover a negative xanthoproteic reaction showed. This result is in contradiction with the assumption of many of the older investigators: Cuénot 23) [1]), Semper 120) Geddes 42), Mourson and Schlagdenhauffen 90) and Williams (Philos. Transact. 1852). No free fat is present; the P_H is in most cases the same as that of sea-water (see chapter 24). Its specific gravity is also the same as that of sea-water (Geddes). An analysis of the ash compounds has been given bij Griffiths 49) and by Mourson and Schlagdenhauffen 90). Griffiths however finds 0.042—0.049 % fibrin (!) and Mourson and Schlagdenhauffen 0.010—0.013 % urea, both of which are now known to be absent. More CO_2 and less O_2 is of course found in the perivisceral fluid as compared to the sea water (M.&S.)

As far as the waste constituents are concerned, no more ammonia is found in the perivisceral fluid than in sea water. Nessler's reagent gives a white precipitate of Ca-hydroxyde with sea water. If however a trace of ammonium sulfate has been added, the precipitate has a deep orange color.

The precipitate is just as pure white in the perivisceral fluid as in sea water. No creatinine is present as Jaffé's test, which demonstrates the presence of this substance even in a dilution of 1 : 5000, is negative.

Thus far it seems that none of the constituents which are present in most bloods, occur here. One of the waste constituents however is present, i. e. uric acid. Folin-Wu's phosphotungstic-phosphomolybdic acid gives a blue coloration in the alcalinised liquid. The intensity of this color is very different in different cases, but it always occurs. It is not due to polyphenoles, since ether extraction does not take away the color.

[1]) This author changed his opinion later on.

Cohnheim discovered that the only substance present in the perivisceral fluid and more than in the sea water, is a substance which gives a precipitate with phosphotungstic acid. It is not arginin or any other amino-acid (no precipitate with $AgNO_3$), since as we saw above none of these are present. Is this perhaps the same substance? Phosphotungstic acid in fact precipitates uric acid (Carl Oppenheimer. Handbuch der Biochemie. Jena. Gustav Fischer. 1909. Bd. I. p. 625).

He also found slightly more N in the perivisceral fluid of our animals than in the sea water, as the following table shows:

Table 6. After Cohnheim.

100 c. c. sea water (Kjeldahl). . . .	0.4 mgr. N.
100 c. c. Holothuria „blood"	2.5 mgr. N.
100 c. c. urchin „blood"	5.7 mgr. N.

He was unable to explain such excess of N; possibly it is, at least partly, due to uric acid.

Mourson and Schlagdenhauffen found urea in the perivisceral fluid of Strongylocentrotus lividus. Brandt and Toxopneustes lividus. Lacken in a concentration of 0.010 %—0.013 %. I have not been able to detect any by means of Nessler's reagent after hydrolysis (see also the results of Griffiths in chapter 23 and remember the scarcety of urea in lower animals). They also find a „ptomaine [1])" present which has a toxic action on frogs. The same ptomaine is also found between the shells of bivalves. Whether or not our substance is meant here, the present writer does not dare to decide.

The occurrence of uric acid in the perivisceral fluid of the Echinoderms is very interesting, because it may throw a light on the dark problem of their excretion. I refer to chapter 23 for this topic.

All the tests mentioned above have been made on Asterias, Arbacia and Thyone with the same result. We see that in these representatives of the chief groups of the Echinoderms, the coelomic fluid is really nothing much more than sea water in which almost constantly a small quantity of uric acid is present and occasionally some of the products of hydrolysis of the food, depending on the feeding conditions.

A fairly constant blood picture is only characteristic for the

[1]) I do not understand what these authors mean here. Ptomaines are as far as my knowledge goes, basic substances, products of bacterial decomposition of protein, such as cadaverin and putrescein. I can not concieve how such substances present only in decaying material and highly toxic for every living organism, should be found in the „blood" of the representatives of this group. Since however uric acid has some reactions in common with these substances — the authors do not indicate which test they have used — it is possible that this might be the explanation.

Vertebrates; in these lower animals there is a very strong variation. As yet unpublished experiments of Dr. Morgulis who was working in the same laboratory last summer, showed that the blood picture of many invertebrates of various groups is not constant at all, but shows a wide variation, due most probably to the moment's feeding condition.

Note. The perivisceral fluid of Strongylocentrotus and of Toxopneustes is said by Mourson and Schlagdenhauffen to be used in the Midi of France as a tonic. What might account for such activity, I do not know.

14. ARE THERE ENZYMES PRESENT IN THE PERIVISCERAL FLUID?

„Bei frischgefangenen, also in Verdauung begriffenen Seeigeln fand ich mehrmals in der Leibeshöhle ein diastatisches, bei Holothurien ein diastatisches und ein invertierendes Ferment". (Cohnheim 17) p. 42).

Bacterial action was excluded, because toluene was present; no hydrolysis took place if the liquid was boiled. These experiments according to Cohnheim, show the presence of enzymes in a solution which except for some inorganic salts, does not contain any other substances. „Dieser Befund ist chemisch recht interessant, weil er das Vorhandensein von Fermenten in einer Lösung zeigt, die weder Eiweisz noch Kohlehydraten und überhaupt organische Substanz nur in Spuren enthält und so einen Beweis mehr für die Nichteiweisznatur dieser Körper liefert." He compares them to the „Fermentschlacken", found more or less regularly in the blood and the urine of mammals.

As a matter of fact, such result would be exceedingly interesting, but I believe that Cohnheim in wrong in the interpretation of his observations. I am more inclined to believe that a small amount of corpuscles or remainders of corpuscles present in the liquid may account for his result.

In order to ascertain whether this could be the case I made some experiments of my own on this question.

Two large samples of perivisceral fluid of Thyone and Arbacia were secured. One of each was centrifuged repeatedly with a strong centrifuge in order to get rid of the corpuscles. This is as a matter of fact, not as easy as one would suppose it to be. One is almost never sure that no more corpuscles are present. A part of the corpuscles seems to be crushed or „laked" which is especially clear in Arbacia where the liquid is never colorless. This is what made me think that an error of this kind might have been made by Cohnheim.

Cohnheim reports that he could observe this enzymatic activity only in freshly caught specimens. For this reason I used

Arbacia's which had been brought into the laboratory on the same morning. The Thyone's I used, however, were in the aquarium already for a week.

The corpuscle samples and those which had been freed from corpuscles, were added with liberal quantities of cane-sugar and starch. Some toluene was added to each of them; after that they were placed into the incubator at 40° and kept there for some days. In case either an amylolytic enzyme or an invertase were present in the liquid, one might in that way expect a reduction of Fehling's solution.

After two days a Fehling's test was made on an aliquot of all the samples. All were negative at first sight, on standing however a precipitate was seen which was much heavier in the corpuscle samples in both cases.

Two days later a complete reduction was seen in the corpuscle sample of Thyone. In the others the reduction was incomplete and the precipitate only became visible on standing. In the urchin this precipitate was at least five times as heavy — estimating this roughly from the quantity of precipitate — in the corpuscle sample as in the otherone. On the next day the difference was still more striking, now at least ten times as much was present in the corpuscle sample.

This experiment shows clearly that the corpuscles must explain the remarkable observation of Cohnheim. Since this author does not mention whether or not he filtered his samples, this seems to be very well possible.

15. RÔLE OF THE CORPUSCLES.

Several assumptions have been made with regard to the functions of the amibocytes of the Echinoderms. Some of these do not have any direct bearing on the problems studied in the present paper. Some authors have described special calcigenous cells, carrying reserve-calcium for the formation of sceletal elements. Others have emphasised their function as phagocytes. The original experiments of Metschnikoff 84[a]) on phagocytosis which gave rise to his very illuminating conceptions of this process were conducted on this very group. As a matter of fact they seem to play a very important rôle in the metamorphosis of this group. Secondly he could show that foreign bodies, bacteria, dying mesodermal cells, etc. can be swallowed by these corpuscles. Mammalian corpuscles injected into these animals were surrounded by a kind of plasmodium of amibocytes and digested; I myself could observe something of the same nature in Stichopus after bone-black injection (see chapter 23). The rôle which the corpuscles play in excretion will be discussed in in chapter 23; that they may carry waste has been proved by Durham 34) and 35) and Saint Hilaire 112).

Their rôle in the metabolic processes has doubtlessly been exagerated, especially by Cuénot 23) who assumed that they take care of the transportation of the food to the tissues. Chapeaux 15) also has exagerated their rôle in the assimilation of fat; his hypothesis that fat would be infiltrated by the gut-epithelium, passed unchanged, given off to the coelomic liquid and be digested there by the corpuscles, will be discussed in the next chapters. They doubtlessly contain enzymes; see chapter 14. But this is the case just as well with mammalian leucocytes. Müller and Jachmann (Münch. med. Wochenschr. 1906. 1393, 1507 and 2002) demonstrated the presence of a proteolytic enzyme in leucocytes; Haberlandt (Pflüger's Arch. 132. 1910. 175) and Mancini (Biochemische Zschr. 26. 1910. 140) found a diastase.

All data concerning movements of the corpuscles unanimously prove that they only move from inside to outside (see chapter 3). I myself never succeeded in finding any amibocytes in the gut-contents samples studied for the experiments in chapter 9 etc. From such considerations it is not probable that the amibocytes would participate to any extent in the transportation of food; on the contrary, their life-circle goes the inverse way; born in the lymph-glands they pass into the coelomic fluid and leave the body by different ways (see the chapter on excretion) loaded with waste.

Possibly they also play a rôle as store of reserve substances (Cuénot). Echinochrome and other fats are found in them, the protein-crystalloids will be discussed in chapter 25. I can not concieve however how the two functions of excretion and storing of reserves can be located in the same organ however.

16. THE DIGESTION AND UTILISATION OF FATTY SUBSTANCES. EMULSIFYING ENZYMES?

The digestion of fats is one of the most difficult and obscure chapters of biochemistry and up to the present time the question has not yet been settled whether or not the fat is hydrolysed before it passes into the cells of the intestinal epithelium. There is about no subject in which so many controversies exist or have existed.

It is beyond the scope of the present paper to discuss the problems which have stirred the minds of the medical physiologists. But it may be justified to discuss briefly some of the discrepancies and differences of opinion in invertebrate physiology.

The comparatively simple problem whether or not Protozoa digest fat, has been studied by many authors and almost every one of them came to different conclusions. Nirenstein 95) in his paper on fat-digestion and -storing in infusoria, is of opinion that fat is digested easily and eagerly by these animals. In the

first place he finds that fat is normally present in them, as a simple test with Sudan III in 80 % alcohol shows. This fact in itself does not prove anything however; we know that fat may be derived just as well from carbohydrates or proteins as from fatty food: see f.i. Chanievski 14). Moreover, Nirenstein himself shows that the quantity of fat in Paramaecium increases just as well after a prolonged feeding on rice-starch and on albumin from eggs (Albumin aus Eiern. Merck), as by a fine oil-suspension.

Nirenstein however states most emphatically that he has observed the *digestion* of fat in his animals. He studied this process with egg-yolk. As long as the reaction of the vacuole remains acid, the fat droplets remain unchanged; as soon as alcalinity is established, the fat droplets gradually form a homogeneous solution with the „Vacuolenschleim". No emulsification is observed, but Nirenstein believes in a hydrolysis and recombination within the cell-body. Animals fed on sodium oleate and glycerin deposit fat in their cells, the same thing happens if Na-oleate alone is used. Saint-Hilaire 113) came to conclusions of the same kind for the planarians, Mesnil is also one of the advocates of the hydrolysis-hypothesis in invertebrate physiology.

Bütschli however already saw, that fat-droplets are thrown off just as they come in. The same observation has been made also by Stanievicz 122); A. Drz. (Drzewina? v. d. H.) 33) in the Revue Scientifique, abstracting this paper, seems to be of the same opinion. Stanievicz has extended his experiments over a large quantity of animal and vegetable oils and seems to be quite careful in drawing his conclusions. He completely agrees with Nirenstein as far as the ingestion of fats is concerned. While however bacteria and algae, taken up at the same time, are digested pretty soon, the fat-droplets remain unchanged, no emulsification occurs and no hydrolysis.

„Après un certain temps", he says, „la membrane se dissout, les bactéries et les algues sont partiellement digérées, par contre, les globules de graisse ne sont point altérés et après plusieurs heures sont éliminées avec des parcelles non digérées." After feeding olive oil to Paramaecia, he does not find any increase in fats!

The fact that Sudan III is decolorised in the vacuoles, does not prove hydrolysis according to this author. It is probably due to a reduction of the dye, some of these dyes are even dissolved out of the fat and eliminated by way of the pulsating vacuole. Moreover Mesnil and Mouton have shown experimentally that a lipase is not present in Protozoa.

After feeding of egg-yolk and of carbohydrates, he finds fat. His negative results with regard to the digestion of fats and these findings, force him to conclude, that the fat which is natu-

rally present in these infusoria, is built up from carbohydrates or proteins.

This example may illustrate the extreme difficulty of drawing conclusions in problems of this kind. Biedermann in his studies on the digestion of Helix and of the lar'va of Tenebrio, is more definite in his statements. Yung already found that fat can be emulsified by the contents of the gut of snails, Biedermann and Moritz show that a steapsin is present. This enzyme is more essential to their opinion.

Milk to which the digestive fluid of these animals is added, turns acid and coagulates. The residue, treated with osmic acid, does not turn black. Fatty acids can actually be demonstrated by ether extraction, addition of KOH or NaOH gives a turbid solution or a jelly of the soaps. The free acids can be precipitated from the solution by means of HCl (What about the lactic acid which is known to occur in these digestive juices? v. d. H. — see the chapter on this topic —). In the cells fat-droplets can be seen „in zierlichen Reihen".

„Man kommt so zu der Anschauung dass es sich bei der Fettresorption nicht sowohl um eine Einlagerung von reinem Fett in die betreffenden Zellen, sondern vielmehr um eine Art von Infiltration von neugebildetem Fett in eine andersartige Grundsubstanz handelt, eine Vorstellung, die freilich mit der herkömmlichen einer direkten Aufnahme von genuinem, fein verteiltem (emulgierten) Fett nicht wohl in Uebereinstimmung zu bringen ist".

These arguments sound rather convincing, the last word however has not yet been spoken. I'll pass the numerous data in the literature on the subject — in nearly every order some authors have reported the presence of emulsifying enzymes, others that of a steapsin, while a third group denies its capacity of digesting fats —, but first report on some experiments of my own on Echinoderms.

As a substrate I have used a neutral olive oil. I believe that this substrate is very much better for the study of steatolytic enzymes than commercial esters, like ethyl butyrate, which have frequently been used for this purpose, because the latter substrates only give us the right to draw conclusions about esterases, not about a lipase.

Fat is either taken up in hydrolysed form or as such. In the latter case emulsifying enzymes must be present enabling the cells of the wall of the gut to take hold of the small fat-globules.

For this reason it is in the first place necessary to make sure whether such emulsification can be demonstrated. For this purpose I secured samples of the intestinal juice of Asterias, Thyone

and Arbacia in the way described in chapter 7. A deep-depression slide was now filled with this fluid and a drop of olive oil added. The sample was covered with a cover-glass and put into the incubator. At different intervals these tests were inspected.

In none of these tests I could see a dispersion or emulsification of the oil amounting to anything. Occassionally the drop was found to be split into two, sometimes even more (5 or 6) parts. A true emulsification which would have a biological importance could never be seen however. Shaking of the samples was of course avoided as much as possible.

Another possibility is the assumption of the hydrolytic dissociation of the fats. Griffiths 48), Stone 124), and Clerc 16[a]) in fact report that they found steatolytic enzymes. I studied this problem on digests of the same kind as those described in the chapter on the enzymes, using olive oil as a substrate.

A large quantity of starfish radial sac material was ground up with sand and put into an incubator after olive oil and an ample quantity of toluene had been added. At regular intervals the mass was shaken vigorously and 20 c. c. samples of the homogeneous mass taken. These were titrated with 0.05 N KOH. The results obtained are represented in the following little table:

Table 7.

Titrable acidity of digests of radial sacs and fat.

Moment.	c. c. 0.05 N KOH.
Control on initial sample. Monday evening.	1.20
Tuesday, 6 p.m.	6.35
Wednesday, 6 p.m.	8.05
Thursday, 7 p.m.	8.40
Friday, 7 p.m.	8.36

These figures show a regular increase in acidity and superficially one might consider them as a proof for the presence of a lipolytic enzyme. The fact that the process goes on rapidly at first, then slows down, would be explained by the assumption that the enzyme had been destroyed by the accumulation of the products of its action. It is strange however, that the process takes place with such surprising rapidity, practically the whole acidification has taken place on the first day.

For such reasons it seemed advisable to me to run a control. A similar digest was made and no olive oil added this time. Toluene was added as always and some water. The titrations were not made every day, since I was only interested in the end-result. The results of this experiment are given in table 8.

Table 8.

Control on the experiments of table 8.

Moment.	c.c. 0.05 N KOH.
Saturday evening, control.	2.10
Next Tuesday morning.	11.77

In all these titrations I used phenolphthalein as an indicator.

This control experiment shows clearly that the acidity developped, *may* be due to other than fatty acids, resulting may be from autolytical phenomena. Remarkably enough Chapeaux found such digests *alcaline*.

The next thing to do was to try separating out the olive oil plus formed fatty acids from the material. This was done by extraction with liberal quantities of ether; the ether was evaporated away from the extract and a titration was made. A control-titration was also made on the same amount of olive oil.

The result of these titrations gave me more courage again and made it probable that at least part of the acidity might be caused by fatty acids.

Table 9.

Titrations of ether extracts.

Material.	c.c. 0.05 N KOH.
Ether extract of the material studied.	6.12
Control: same amount of olive oil.	3.60

One more control was necessary however. It should be proved that the „other acid" was insoluble in ether, in other words, the same proceedure should be repeated while no olive oil had been added. For this purpose I used the material which had been used for the autolysis experiments of p. 26. A large amount of this material, containing many more radial sacs than the other digests, was extracted with ether in exactly the same way. The final titration gave 12.80 c.c. of twentieth normal alcali.

From these critical experiments one can see how careful we must be in judging about the presence of lipolytic enzymes in our group. These difficulties have not been seen by other authors who have been working on our group; for this reason I am inclined not to take their results too seriously. The formation of an acid of some kind in these digests of the radial sacs,

makes the analysis of this problem especially hard. In a special chapter I will discuss briefly the importance of this development of acidity in dying tissues; for the present I will confine myself to a report on analogous experiments on Arbacia and Thyone.

Experiments of the same kind as the ones described above, have been made on the guts of these two species. The guts were crushed with sand, taken up in some water and added with toluene, olive oil and some water.

In the case of Arbacia 8.5 gr. gut was used — wet weight —. After two weeks the etheric extract of the material appeared to need 11.34 c.c. of twentieth normal KOH for its neutralisation whereas a control sample of the same amount of olive oil only required 1.80 c.c.

The same thing proved to be true for the cucumber. The amount of gut used here was 19 gr. — wet weight and including the „calcareous ring" —. After $1^1/_2$ week this digest required 16.3 c.c. of 0.05 N KOH for neutralisation, while for a control 8.0 c.c. were needed.

Whereas this series of experiments gave rather undefinite evidence, there are others which have given me the absolute proof that a digestion of fats takes place in Echinoderms. Starfishes and sea-urchins were fed on olive oil. The methodology of such experiments will be described in chapter 20 and 21; it is rather difficult to inject a syrupy fluid like olive oil, but after some practice one easily succeeds in forcing the animals to take it.

At different intervals the animals were dissected and their guts taken out. They were fixated in Flemming's fluid. The usual imbedding proceedure in paraffin could not be used because I wished to avoid the passing through the alcohols, which might dissolve out the fats. For this reason I used a very old method, recently very much improved by Mc. Junkin 72[a]).

This method is based on the principle of imbedding the material in soap. This can take place by passing the material right away from water or even from a fixating fluid which is volatile (f.i. formaldehyde) into the soap. The method has been described with much detail by Mc. Junkin; the great difficulty is the choice of the right kind of soap. The author claims that if one has once succeeded in finding a suitable kind, sections may be cut thinner even than in paraffin. The soap is hardened in a saturated salt-solution, before the sections can be cut; the ribbon is dissolved away in water and the sections floated on slides. This procedure takes a lot of practice, which is a decided disadvantage of the whole method.

In sections of the starfish radial sacs enormous quantities of fat could be seen. The sections showed a typical greyish, almost black color, which on closer examination appeared to be due to numerous little fatdroplets in the epithelium. A similar obser-

vation has been made by Biedermann and Moritz on the liver of Helix 9): „So erschienen etwas dickere Schnitte einer mit Osmium behandelten Schneckenleber fast gleichmässig schwarz und undurchsichtig"; only in very thin sections could they see the separate droplets. After about 10—20 hours this loading of the epithelium with fat has reached its maximum, I obtained the best results in specimens which had been injected in the late afternoon of one day and dissected early in the morning of the next. The staining of the fat by Flemming's fluid — one day — was very incomplete in my sections, under high power the droplets were perfectly clear and not black. The soap sections freed from the soap, could easily be used for a test with Sudan III and I have used this reagent in order to make sure that the droplets which I had observed, actually were of fatty nature. Sections thus treated gave a most splendid and enchanting sight, the hundreds of droplets all colored reddish could be seen everywhere and in every cell of the epithelium. In some of the sections of later stages, the cell-border turned towards the intestinal lumen, was fairly clear and free of globules. The fat then began to accumulate in the sub-epithelial connective tissue in the same way as many other substances — see the chapter on resorption in starfishes —.

Some of the guts of Arbacia which had been treated in exactly the same way, but which were imbedded in paraffin — so that the alcohols through which they passed, might have dissolved away the fats —, gave very nice sections. The fat was stained very completely here and the sections immediately showed the presence of fat by the many pitch-dark globules they contained. A piece of a section of this kind has been drawn by Mr. H. van Laar, who also made the other pictures for me [1]). (Fig. 4 of our plate).

These results seem to indicate with a fair degree of certainty, that fat is actually used and digested, but they do not give us any evidence as to whether it is ingested as such after having been emulsified (phagocytosis?) or whether it is hydrolysed first. Our titration figures would prove that the latter took place were it not for the formation of acid even in digests without olive oil. Since however no emulsification takes place, I am inclined to believe that hydrolysis actually occurs.

There are some facts, however, which deserve a more general attention in connection with the whole problem of the digestion of fat. I mean the experiments of Churchill 16) on the absorption of fat by fresh-water mussels.

Mussels were kept in soap solutions, prepared from olive oil, both unstained and stained with Sudan III. Histological examination of such mussels and of controls revealed the fact that fat is absorbed abundantly by all epithelia and carried all over

[1]) I am very much obliged to him for this service.

the body by the blood-corpuscles. Sections of mussels kept in such solutions for short periods, e.g. 18 hours, showed such heavy loading of fat in the epithelium of gills, mantle and foot, that it seemed very probable, that the cells of these epithelia absorbed the fat directly from the solution. This could in fact be demonstrated experimentally. Mussels with the valves wedged open and suspended so that the mouth did not come down to the surface of the liquid, showed the same absorption.

Such results are surely remarkable, for no steatolytic enzyme can be present here making the fats „fit" for resorption. They indicate that at least *some* epithelia can absorb fat without previous hydrolysis. Perhaps this is a general phenomenon, perhaps there is some complicated colloid-chemical procedure which forces fatty substances to be absorbed by every epithelium. In that case our experiments would show nothing with regard to the actual *utilisation* of fats by Echinoderms.

The presence of fats in itself of course never proves the presence of lipolytic enzymes. They may have been built up just as well from carbohydrates, proteins, fatty acids and many other substances as from fats. Biedermann found this true for the liver of snails e.g.; after a heavy meal of flour he always found the liver cells full of fat droplets.

There is still another thing which might favor this view, namely the abnormally high respiratory quotient which Pütter observed in his Cucumaria grubei. He explains it by the assumption of a butyric acid and methene fermentation [1]), in chapter 26 we shall will hear of another possible, indeed, a more probable explanation, in the third place it may also be caused by transformation of carbohydrate into fat. Cane-sugar f.i. can yield one third of its weight in fat and at the same time $^2/_5$ of the total quantity of its energy (Moore 87), etc.). If such processes take place, much more CO_2 is produced than corresponds with the oxygen-intake. The R.Q. consequently is > 1.

17. LACTIC ACID DEVELOPMENT IN AUTOLYSATES OF THE LIVER.

In the preceding chapter we saw that an acid of some kind was formed in our digests which made it hard to judge whether fatty acids were actually produced or not. This spontaneous development of acid seems to me to be of particular interest, since I believe that we have to do here with a phenomenon of a more general importance than one might expect.

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[1]) Such fermentations are not very frequent and I am not impressed by the validity of his arguments. To quote Fränkel 138): „Sumpfgas (Methan, CH4) entsteht nur bei Pflanzenfressern und Omnivoren im Darm, nicht aber in dem der Fleischfresser ...." .„Es entsteht wohl nur aus Cellulose..." (p. 207).

Dr. Withrow Morse and the writer 89) studied the changes in reaction in dying tissue, in connection with the senior author's work on autolysis. The liver of guinea pigs was taken out immediately after they had been killed, and ground up in a mortar. Sometimes they were first frozen by means of an ethyl-chloride spray, sometimes used without freezing. They were taken up in a small quantity of destilled water and the whole mass was then put in the cup of a Leeds and Northrup potentiometer, type K. P_H readings were made at different intervals. It appeared that very soon after death occurred, a relatively tremendous acidity was established, even up to $P_H = 4$, but that afterwards this acidity gradually diminished to near the neutral point. No trial could be made to study more in detail the nature of this acid on account of our poor equipment; we supposed that we might have to do with lactic acid.

Lactic acid is also formed in autolysates of the snail's liver. Biedermann and Moritz 9) found that if the digestive fluid of Helix was left alone, even with shreds of fibrin in it, no decay could be observed. If decaying pieces of fibrin were put into that liquid, the decay stopped in a very short time. A strongly acid reaction which he found in these samples solved the problem. Whereas e. g. in crabs the digestive fluid decays at once here the acidity prevented all bacterial growth. He show showed that lactic acid was the cause of this acidity.

A digest of crushed liver substance also appeared to contain a large quantity of this acid. It did not digest proteins, but a coagulation of the material was observed caused by the acidity. That lactic acid was also present here, could be shown by means of Uffelmann's reagent, a 2 % phenol treated with dilute ferric chloride till of an amethyst violet color. This reagent is decolorised by mineral acids, but colored yellowish by lactic acid and some other organic acids. The development of this acid is due to bacteria according to Biedermann: chloroform and thymol prevent its formation.

Other experiments showing this same phenomenon came to my attention in a paper of Lindemann 80). This author finds in 100 gr. autolysed liver of a rabbit an acidity titrable with 7.6 c.c. decinormal NaOH. Estimated as butyric acid this would mean 66.9 mgr.

As we see, this phenomenon occurs rather regularly in the most divergent groups. In our digests of starfish „liver" we found, as mentioned above, an acid of some kind to be present. This acid is soluble in ether — this is in fact the case with lactic acid. From a small quantity of material I extracted the acid by means of ether; Uffelmann's reagent lost its violet color and changed into yellow. These facts make it rather probable that here we have to do with lactic acid, though they do not prove it definitively, since many other organic acids may give the same reaction.

There is still another remarkable thing to be mentioned in this connection; it has been observed by Enriques 37) (p. 11), where he says: „Del resto, il succo gastrico non putrefà o putrefà soltanto dopo molto tempo, anche senza nessuna precauzione, certamente a causa della sua acidita". The same thing proved to be true for extracts of the retia mirabilia.

This fact shows a remarkable analogy with the results of Biedermann on the gastric juice of the snails. I have made the same observation repeatedly on samples of digestive juice, as used for the experiments described on p. 30 and 34. They never had that hideous smell of decaying marine material, the remainder of these samples were left standing in the room for more than a week, they were yet fresh at the end. As the paper of Enriques, who states that it is due to acidity, came too late to my attention I could not test for lactic acid in my material.

In one point I came to conclusions different from those of Biedermann, The acid mentioned in the preceding chapter was formed in an autolysate *in the presence of toluene*. The same was the case in Lindemann's experiments. Bacterial action seems to be excluded in that way; most probably we have to do here with a spontaneous development of acid of as yet obscure nature.

These few remarks do not pretend to be built on a very strong basis. They only indicate a problem on the solution of which I hope to be able to work more carefully in the future.

18. IS THERE A LIPOLYTIC ENZYME IN THE CORPUSCLES OF THE STARFISHES?

In the chapter on the resorption in the urchins and the cucumbers I shall mention the conceptions of Chapeaux 15) as to the rôle of the corpuscles in the digestion and transportation of fats. In this chapter I only want to mention one of his findings because I came to different results.

Chapeaux studied the rôle which the corpuscles play in the digestion of fatty substances. He found that fat-droplets are taken up eagerly by the corpuscles and digested in the cell-body. He finds a lipolytic enzyme present in them and observes the development of acidity in plasmodia to which olive oil has been added. This acid reaction was not found in a control.

In order to amplify my own experiments on the digestion and utilisation of fatty substances in our group, I made some experiments of my own on this question.

A large quantity of starfish amibocytes was separated out by means of a strong centrifuge; they were taken up in 100 c.c. of destilled water and a large quantity of neutral olive oil and toluene were added. The whole was put into an incubator

at 37° and at certain intervals titrations were made on 20 c.c. samples. On 20 c.c. of the original liquid a control titration was made.

The results of this series of titrations are given in table 10.

Table 10.

Titrable acidity of a digest of olive oil and corpuscles.

Day.	Hour.	c. c. 0.05 N KOH.
Control. Td.	6 p.m.	0.80
Wd.	6 p.m.	0.80
Thd.	7 p.m.	0.97
Fd.	7 p.m.	1.01
Td.	3 p.m.	0.98

These figures indicate very clearly that if there is any increase in acidity, which does not fall within the limits of error, that this increase is too slight to have any biological meaning. A priori it is not improbable that in these amibocytes which are primitive and independent cells without any special function, a lipase should be found, just as well as in an Amoeba. More important, however, is the question, whether this enzyme plays a rôle in the digestive processes of the animal as a whole. This seems to be very improbable, considering our figures.

I have also tried to study the same problem in two other ways. Two fairly large quantities of coelomic fluid of Arbacia were secured. One was centrifuged in order to remove the corpuscles, the other one was left as it was. To both an equal quantity of olive oil was added and some toluene. Then they were placed for two days in an incubator at 37°. After that time both gave exactly the same color with a piece of litmus paper, only to neutral litmus paper they showed a very, very slight acidity. If there was any difference at all, it was too slight to be worthwhile mentioning.

After this I repeated the experiment in a way similar to that which Chapeaux employed. A large quantity of amibocytes, collected by means of a centrifuge, was put into a deep-depression slide, a drop of olive oil was added, the whole covered with a cover-glass and put into the incubator. A very distinctly acid reaction was found after 24 hours. After my previous results I was rather surprised to find this and therefore I decided to run a control without olive oil. The same acid reaction appeared after a short time. Most probably the decaying material had produced an acid of some kind, which had escaped my attention in the titration experiment on account of its great dilution or which did not appear there on account of the presence of toluene. If toluene was added to the samples,

the acidity did not appear; for this reason I am inclined to believe that this is the source of error in Chapeaux's experiments.

19. THE NITROGEN METABOLISM OF THE HOLOTHURIANS.

In his paper on resorption, digestion and metabolism in Echinoderms Otto Cohnheim 17) discusses the nitrogen metabolism of the holothurians. He found no proteolytic enzyme present in holothurian guts. „Holothuriendärme geben auch nach wochenlangem Stehen an Meereswasser kein Ferment ab, das rohes Fibrin zu lösen imstande ist". Fibrin was not dissolved by such digests in one day at 37°, if toluene was added. Neither were pieces of mussel changed. Since no glands are present which could secrete a proteolytic enzyme, Cohnheim concludes that there is no such enzyme. Another proof in favor of his view is the negative result of autolysis experiments with Holothuria-guts. This result, namely that autolysis proceeds almost imperceptibly and that such guts can remain unchanged for weeks at a stretch, the present author is prepared to affirm, as mentioned before.

Other negative results were obtained in experiments on resorption of nitrogenous materials. Ammonia was not resorbed; in the case of proteins, no protein could be demonstrated at the outside and the total Kjeldahl-N did not increase.

From these experiments Cohnheim draws the conclusion that „die Resorbtion N-haltiger Körper sehr gering ist, im Vergleich mit der reichlichen Resorbtion und Verbrennung von Kohlehydraten, zu gering jedenfalls als dass ich sie erfolgreich untersuchen konnte. Wie und in welcher Form die Holothurien Stickstoffhaltige Nahrung aufnehmen, vermag ich danach nicht anzugeben".

In our chapter on the enzymes we have seen that a proteolytic enzyme is surely present in Thyone. And in the following chapter we have demonstrated that it even occurs in a free form.

It is clear that these results are in the most evident contradiction with those of Cohnheim. Theoretically the possibility of course exists, that there may be a specific difference between the two species. Considering the fact, however, that in many other instances we will see that this author has made more or less serious mistakes, I am inclined to have my doubts about his results, the more because Enriques 37) found a proteolytic enzyme present in the same species on which Cohnheim worked.

Assuming at first that Cohnheim was right in his experiments, but not satisfied with his attitude of resignation, I studied the

whole question over again. Cohnheim himself states that all nitrogenous excretion takes place through the gut. How is it possible that nitrogen is excreted, if it is not assimilated? This would mean a constant loss of nitrogen, which we can not understand. That an organism as highly organised as a Holothurian should have no nitrogen metabolism seems to be excluded a priori. Growth and formation of proteins are not possible then and yet these substances are present.

There is still one possibility of error left in Cohnheim's experimental procedure. The work of Enriques has shown that the vasular reticula play a very important rôle in the enzyme secretion (see chapter 20). One of the enzymes secreted by them is a proteolytic one. If Cohnheim has removed these plexusses, he may have missed the opportunity of finding the enzyme.

In our last chapter we shall discuss the application of Pütter's doctrine to Holothurians. Thinking of Pütter's work and assuming that actually no proteolytic enzyme is present, I made some experiments on the resorption of ammonia and nitrates by Thyone guts. The possibility of the binding of such substances to carbohydrates f. i. as a source of nitrogenous body constituents, should be studied.

For this purpose I secured Thyone guts. These show the same active movements which mammalian intestine shows in the living organism and move over the table like worms. Pieces were cut out, tied off at one end with a soft, but strong thread, then filled by means of a hypodermic syringe with the solution to be tested. It was then tied off at the other end with a knot made previously. The loop was now washed sea water and then put into a watch glass in some sea water.

In this way it became evident that at various concentrations ammonium sulfate diffuses through easily. At the end of the experiment I tested for ammonia in the liquid at the outside with Nessler's reagent and invariably got positive results. The gut was not dead, for very frequently it could still be stimulated by pinching it with a forceps. Neither were there leaks since, the liquid did not disappear from the gut though the muscles were in constant tension.

The same thing holds true for nitrates, for which I tested with the ferrosulfate-sulfuric acid ring reaction. The rate of this resorption is somewhat slower than in the case of ammonia, but most assuredly resorption took place.

However, we do not have to take refuge in such more or less probable speculations, since there is a proteolytic enzyme and since it is even present in the free form. Amino-acids do diffuse through the intestinal wall, as experiments of the nature of those mentioned above showed. Such experiments were made with a digest of gelatin by means of trypsin (without toluene). A strongly positive ninhydrin-test in the outside liquid took away

all doubt. This shows that the products of hydrolysis are also actually resorbed, so that the evidence in favor of my opinion was complete.

There is still another way of demonstrating the actual resorption and this way is the more biological one. I collected (1) a certain amount of the mud in which the animals are found in nature, (2) a sample of the contents of the fore-gut, -chiefly from the stomach- and (3) one from the end-gut and rectum. These materials were dried for some weeks in an exiccator, ground up, dried again and finally weighed. After that a total-nitrogen determination was made, based on the principle of the method of Folin. The digestion which could not be carried to completion with Folin-Wu's digestion mixture, became complete after the addition of a little crystal of copper sulfate. In a preliminary experiment I found that direct Nesslerisation was impossible because a heavy precipitate was formed. For this reason the distillation method was used.

Knowing the amount of material used and the resulting color, it was very easy to figure out the amount of total N in one gram of the three samples. In one gram of the mud as it is found in nature, 3.1 mgr. of total-nitrogen were present, in one gram of a composite sample of each of fore-gut contents as much as 21.1 mgr. were present, and in the end gut 1.0 mgr. These figures of course represent samples and do not pretend to have an absolute value, though the samples were of course obtained from many animals. Nevertheless they show very clearly that, at least in certain periods, withdrawal of nitrogenous substances from the mud takes place. The samples 2 and 3 were secured from the same animals, whose fore-gut and rectum were emptied at the same time.

This withdrawal can be observed even with the naked eye. As long as the material was wet, all samples had the same color. The dried substances showed great differences in color: it was clear, that almost everything present in the stomach sample had been dissolved away except for the sand.

It will be noted that the quantity of N in the contents of the fore-gut is much higher than that of the mud found in the animal's natural environment. This fact has been explained in chapter 4.

20. THE RESORPTION IN ECHINOIDEA AND HOLOTHUROIDEA.

In the chapter on the perivisceral fluid we have compared this liquid to the „blood" in higher animals. The question is whether it actually has the function of transmitting the resorpta from the intestine to the consuming tissue or whether the corpuscles have this function. Cuénot 23) considers the cor-

puscles as the chief transporters of food, other authors also attribute considerable importance to their activity in the service of digestion. Among these authors is Chapeaux 15), who considers them as especially important in the digestion of fats. In chapter 16 we saw that in some respects he exagerated their importance and that I have never been able to observe the emulsification of fats by the intestinal juices which he found. He states that the emulsified fat passes through the gut-wall without any chemical change but gives no experimental proof for his contention.

„La dissolution s'opère dans la cavité générale"; hydrolysis takes place here by the corpuscles, as the change in reaction — which I have critisised in chapter 18 — indicates. If olive oil is injected into the perivisceral fluid, the amibocytes „eat" it. That such experiments do not prove much, will be discussed in the chapter on excretion.

There is one small problem, however, which he does not attack. Is there any increase in fat in the corpuscles after fat-feeding? in other words, can we actually prove his hypothesis that the fat would not be changed chemically during its passage through the intestinal wall, that it would then enter into the perivisceral fluid and finally be „eaten up" and hydrolysed by the amibocytes? I have made experiments of this kind repeatedly (on Arbacia), but always with negative results. Except in two cases in which the coelomic cavity contained very large drops of oil, probably on account of the breaking of the intestinal wall, I *never found a trace of free oil.* Neither could I observe an increase of fat in the corpuscles. Centrifuged corpuscles of injected animals were put on a slide and treated with Flemming's fluid, as was a control sample. I never could see any difference between two such samples though they were withdrawn at various periods of time after the feeding. For this reason I am rather inclined to believe that Chapeaux is wrong in his hypothesis.

In the chapter on the perivisceral fluid we saw that this liquid is by no means absolutely stagnant and motionless. This makes it possible that such functions as the distribution of food could be taken care of by this medium.

To study this problem I made some very simple experiments on Arbacia. Food solutions (e. g. solutions of cane-sugar or of casein as the most simple carbohydrate and protein) were injected into the digestive tract of the animal.

Feeding experiments never had any success, because the animals, urchins, starfishes and cucumbers refused to take any food in captivity, even if their natural food was presented to them. Starfishes, put into one basin with either mussels or small sea snails (e. g. Littorina), never ate any of them.

In this respect I was very unfortunate. All plans to feed animals stained with some vital stain to our Echinoderms for

the study of resorption or for the hydrogen-ion concentration work, could not be carried out.

Twice only I did obtain an animal feeding in its natural way. They were both brought in with their prey. Once I got a starfish, in the act of devouring a little snail, in its perivisceral fluid aminoacids appeared by a positive ninhydrin-test to be present. The other case was an Arbacia in the act of chewing up a Clione sulfurea, a calcisponge. A ninhydrin-test again gave positive results, no monoses could be demonstrated by means of Fehling.

Apart from these two cases I had to feed the animals artificially. Several substances were injected in that way for the study of the resorption and in order to find out whether phagocytosis takes place in these forms.

The injection of such liquids is very easy in the case of the sea-urchins. If one lays an Arbacia on a table with the oral side up, the lantern will start to perform rhythmical movements up and down, accompanied by a circular movement of the distal part of the lantern in a vertical plane. When the lantern is in its most distal position, the teeth are closed tightly. When it moves down again, i. e. towards the body, the jaws open up widely. This is the moment to slip in the needle of an injection syringe. With some practice one easily succeeds in bringing it down just as far as the masticating apparatus reaches, without injuring the tender gut behind it. The animal now tries to bite this intruder, sometimes also brings it out of the centre of the five teeth, pushing it down between two of them, so that it leans against the circular „lip" and easily falls down.

These movements resemble closely those described by Gemmill 43) and 44) in his papers on the locomotor function of the lantern. He saw animals moving around by lurches on the table — the same thing can also frequently be observed in our Arbacia — and studied this method of locomotion a little more in detail. The five little teeth, brought down in closed position, form a powerful central stilt, which lifts the body. This body of course capsises in a certain direction, which direction is determined by the circular movements in a vertical plane, described above. This method of locomotion seems to be rather common in urchins; not only Echinus esculentus. L. and Echinus miliaris. Gmel., studied by Gemmill, show it, but also our Arbacia. They move in that way in a rather surprisingly quick way; in my work I was sometimes suddenly scared by one of the animals dropping down from the table, from the centre of which it had reached the rim. Even loaded — up to half a pound — and on an inclined plane, they seem to be able to execute these movements (Gemmill). In protruding the lantern, the animal relaxes its posterior retractors and contracts the anterior protractors. These same protractors serve to close the teeth.

The contrary of course happens when the lantern is pulled inward.

It looks to me as if there is a great analogy between the movements which I described above and those described by Gemmill. It seems to be a general reflex of the urchins if taken out of the water; perfectly purposeful in case the ventral side is down, but senseless if the animal lies on its back.

Whether or not these same movements play a rôle in respiration, when the animal is under water, has not been proved experimentally, but it seems probable from the description of von Uexküll. In the chapter on the respiration I shall describe the so-called internal gills of the urchins. These baggy prolongations of the peristomium are filled with water when the lantern is raised and the water is expelled, when the lantern is lowered. One indication in favor of their respiratory importance is that in water with low oxygen-concentration the animals move their lantern up and down without changing their position. Interesting from the general physiological standpoint is the fact that these movements seem to follow van 't Hoff's rule with regard to temperature, as Roaf has shown 108).

Returning to our injection experiments, we may say that in the case of proteins a strongly positive ninhydrin-test after a little more than an hour showed that they have actually been broken down and resorbed and that the perivisceral fluid has the function of conveying them to the tissues.

In the case of the sugar injections I have never yet succeeded in demonstrating glucose in the perivisceral fluid by means of Fehling's test. This may be due to several circumstances. Possibly the larger part is „burned up" in its passage through the intestinal wall, as Cohnheim assumes for the case of Holothuria; it may also be due to the quick resorption of this substance from the perivisceral fluid, which we will discuss in chapter 22.

About the resorption in Holothurians we have two studies at hand, and strange enough the two authors contradict each other in nearly every detail. Cohnheim 17) was the first to attack this problem, Enriques 37) in the Archivio zoologico discusses his results and comes to absolutely different conclusions, notwithstanding the fact that both authors worked on exactly the same species.

Enriques' paper came too late to my attention: in that way I was not able to perform any but very superficial experiments on this question; since however it seems to me that this problem has enough importance, I may be justified in giving here a short summary of their controversy.

One of the chief points of their discrepancy is the question of the function and importance of the „blood (i. e. the lacunar)" system. Cohnheim does not pay any attention to it and simply states, „dass bei den Seeigeln und Holothurien jede Circulation überhaupt und imbesondere jede circulatorische Verbindung zwischen dem Verdauungskanal und dem übrigen Körper

fehlt. Wasser und in ihm gelöste feste Stoffe, die bei der Verdauung entstehen, haben keine andere Möglichkeit zu den übrigen Organen, die sie ernähren sollen, zu gelangen als dass sie aus dem Darm in die Leibeshöhle eintreten und durch diese hindurch zu den andern Organen gelangen (p. 18)".

Enriques has made a very extensive study of the anatomy and function of this system, which consists of a dorsal mesenterial and a ventral anti-mesenterial complex of vessels. On the dorsal side we find a dorsal marginal vessel over the whole length of the gut, adhering so firmly to its wall that it can not be torn off. Between the „loop" of the intestine, it forms a so-called transversal vessel. In the „oesophageal ring" there is a blood-ring by which it communicates with the ventral vessel. From this ring five vessels radiate out, following the five ambulacrae. There is also present a ventral marginal vessel which splits further on into a left and right ventral marginal vessel. These communicate by means of the anterior and posterior transverse vessels. From the right ventral marginal vessel the rete mirabile runs over the whole gut and mesenteries.

Other vessels go the water-lungs and to some other organs; the genital organs are also richly vasculated.

Something of the same nature is also found in the sea-urchins and starfishes. Here the whole system is much less complicated; in the starfishes it took a long time before it was discovered by Cuénot 25).

Enriques first mentions that in studying the movements of a body suspended in a liquid, one does not get a clear idea of the real movements of such liquid (inertia). He succeeded by means of a small syringe in bringing into one of the vessels an oil drop which filled the whole vessel so that no water could flow between the wall and the drop. He then registered the movements of these drops photographically. He finds that the oscillating movements of the blood, the pulsating of the vessels, finally causes a gradual movement in one direction, moreover, that the drop is broken down into two parts as soon as the vessel branches into two. This process goes on and in this way the fat is finally distributed all trough the blood system. This is another proof for the effectiveness of the mixing in the blood system.

The vessels pulsate especially when they are full of liquid as he could demonstrate by experiments in which he injected a small quantity of sea water into a vessel. This is the case during the period of resorption.

A very interesting process of secretion (of enzymes?) is described by Enriques. Greenish dichroitic granulae showing much similarity to the liver secretion of Molluscs (Enriques) and whose enzymatic nature is made probable by the fact that they stain brownish with osmic acid, are formed in the cells of the wall of the vessels; they are carried to the gut-wall, sometimes by amibocytes, and penetrate into the lumen. Their number increases during inanition.

Let us now proceed to discuss Cohnheim's experiments on absorption. A remarkable thing in all his experiments is that he nearly always fills the intestine with 20—30 c.c. of liquid. Now,

according to Enriques, the gut of very large animals does not contain much more than 10 c.c. In that way Cohnheim must have made his experiments on distended guts. This is according to Enriques one of the chief sources of error in his experiments: the epithelium after death has allowed the contents to be pressed out through small holes by the surviving musculature. He himself seems to be conscious of this pressure, for he says (p. 24): „Ich füllte ihn (i. e. den Darm) unter geringem Druck aus einer Bürette".

Cohnheim finds that if sea water is put both inside and outside the gut, it is resorbed almost completely within 24 hours. Enriques does not fill the guts with as much liquid, and *does not find any such resorption.*

This discrepancy is rather striking and surely proves that one of the authors must have made more or less serious mistakes. Enriques warns against using the guts for 24 hours or longer, they frequently fall to pieces after that length of time.

Other experiments have been made both by Cohnheim and by Enriques on the resorption of various substances dissolved in the sea water. Some of Cohnheim's experiments are very unnatural: e. g. he injects a 1 % solution of sodium iodide in sea water and a $^2/_5$ % solution of sodium phosphate. Experiments of this kind can not possibly elucidate as important a question as the present one. His results are nevertheless remarkable, he finds that from such hypertonic solutions the dissolved substance as well as the solving medium passes to the outside.

This is almost pure resorption as we find it in vertebrates and of substances which, if not toxic, are certainly indifferent. On the other hand these salts are used in concentrations which are much too high and their toxic action may easily have killed or harmed the gut wall.

The experiments of both authors on the resorption of sugar etc. also contradict each other in many points and it is hardly possible to get a clear and unprejudiced picture of the process.

Enriques severely critisises Cohnheim's work and shows many contradictions in his experiments. In some of these experiments Cohnheim uses solutions the osmotic pressure of which is much too high. Sugar solutions in sea water are used e. g. of 3—18 %. Such solutions are beyond the range of physiology and results obtained therewith only give an insight in a „pathological physiology", as Enriques calls it. Against such osmotic potentials no semipermeability of the gut-wall can resist.

In the experiments on sea urchins too serious operations are made; since they gave the same result of those on the cucumbers, I will not discuss them here.

The outcome of the differences in their experimental results is

an entirely different conception of the process of resorption. Cohnheim considers the gut wall merely as a diffusion-membrane through which substances pass equally well in both directions. There is no „polarity"; there are no substances which are resorbed against an osmotic difference. On the other hand there is an active *water*-resorption, „die sich aus den osmotischen Kräften allein nicht erklären lässt". The diffusion of dissolved substances takes place just as in every diffusion membrane, without any „orientation", but water is resorbed quite independently and simultaneously.

Enriques pictures the process of resorption in an entirely different way, very much as it takes place in mammals where this remarkable, and as yet physically unintelligible process was discovered by Cohnheim (Zschr. f. Biol. 36. 1898. 129 and 37. 1893. 443) and by Waymouth Reid (Philos. Transact. Ser. B. 192. 1900. 211).

He makes the very interesting observation that during the period of digestion the Cl-percentage in the visceral fluid is lower than in sea water. This phenomenon is most pronounced during the actual digestion, after resorption has taken place the difference is only very slight. I could myself verify the latter stratement, but at the same time I observed that it still always exists, in some hasty experiments which I made as soon as Enriques' paper came into my hands. On the same day on which the animals were brought into the laboratory, he eventually found as little as I. 84 gr. $^0/_0$ of Cl, in older specimens kept in captivity for some time, the concentration of the chlorides sometimes was the same as that in sea water, i.e. 2.11 $^0/_0$. The same thing holds true for the sulfates which he titrates with barium chloride. He explains this peculiar phenomenon thus: during the period of digestion when the larger molecules are broken up into smaller ones by hydrolysis, the osmotic pressure inside the gut-wall increases. Consequently water diffuses out into the gut, diluting the sea water which was present at first. Consequently the percentage of chlorides in this liquid, decreases. It does not increase in the coelomic liquid, however, as experiments show. The assumption is therefore made, that regulatory processes take place either through the water-lungs or through the many other places where the perivisceral fluid comes in close contact with the surrounding medium, the sea.

Gradually the products of hydrolysis are resorbed now and the contents of the gut become hypotonic. Practically no chlorides can enter into the gut, but *the water is resorbed back with the food substances in it.*

The blood system becomes full of liquid, consequently the vessels begin to pulsate — see above — and distribute the food all over the larger part of the body. Part of it is given

of to the perivisceral fluid, which also takes care of the distribution of the food.

Real *resorption* though in its kind primitive takes place here in that way. „L'assorbimento e dunque necessariamente nelle Oloturie un processo fisiologico." This is still more evident from the following set of experiments. Enriques demonstrated that sugar can be resorbed from solutions of equal osmotic pressure or even *lower*. For this purpose he prepared artificial salt-sugar solutions, the osmotic pressure of which he controlled by freezing point determinations. The osmotic exchange takes place chiefly by means of water passage, the chlorides do not pass as quickly. In one of Enriques' experiments as much as 2.3 c.c. of water had passed over to the outside and only 0.004 gr. of NaCl into the gut. As a rule such osmotic differences as are used in the experiments will not occur in nature. Thus we can conclude that the Holothurian intestine possesses as Enriques calls it, an „absolute semipermeability", and is not capable of allowing either salts or food substances to pass through by „diosmosis". To quote his own words: „La membrana intestinale presenta dunque in modo palese due proprieta apparentemente contradittorie: impermeabilita per diffusione, permeabilita per assorbimento." A true „physiological" resorption occurs the mechanism of which, as in Vertebrates, is as yet completely unknown.

This resorption question is one of the big problems of general physiology and it is to be hoped that in the next decennia comparative physiologists will study the genesis of this remarkable property of the living gut wall more in detail. It is in the various groups of invertebrates that we must observe it at its origin. It is by no means primitive: in the lower forms (Sponges, Coelenterates, Plathelminthes, etc.) the „assimilation" of the food occurs by means for phagocytosis, later on we find special organs developped for resorption (as the „Mitteldarmdrüse" of Crustaceans and Molluscs, the „liver" of the starfishes — see the next chapter —, the coeca of Aphrodite, etc. etc.) and only in the higher invertebrates do the first traces of resorption over the entire gut wall occur. In the lower forms the gut is just as long as is necessary for a safe conduction of the food to the organ of enzyme secretion and resorption and for a rapid elimination of the waste. Frequently it is even covered by completely impermeable substances, such as chitin.

Only in animals in which the gut begins to be much longer, may we expect to find the solution of this big problem. And even in many of these three characteristics of this function are absent: 1. the polarity of the intestinal membrane, 2. the resorption of fluid together with the food substances and 3. the rapidity of the process, as Jordan states for the case of Helix. The study of the gradual development of these three properties in the different groups — in Helix e. g. sugar can be taken up

from an isotonic solution by the intestinal wall (Jordan 69)) — may give us the key to the explanation of this phenomenon as yet unexplained on a physico-chemical basis. Such instances as Helix may be especially instructive, their gut epithelium does not take either iron, carmine or fat. These properties have also been considered as characteristic for resorbing epithelia (Cuénot. Arch. de Zool. exp. et gén. (3). 25. 1899. 7; Biedermann and Moritz 9)).

Among the many, perhaps unreliable, results of Cohnheim, one is very interesting. If sugar is injected into the perivisceral fluid, he finds it back inside of the gut. I have made similar experiments, using cane-sugar for the injection. Two interesting facts could be observed in such animals: 1. If the gut contents were secured after about eight hours and hydrolysed, a strongly positive Fehling's test indicated the presence of sugar; this proves that Cohnheim did not make a mistake in his observation, 2. Some of these animals showed a peculiar swelling, as though they had taken up water to compensate for the excessively high osmotic pressure of their coelomic fluid. This phenomenon was not observed regularly, but was very striking. It even attracted the attention of some visitors to the laboratory.

The first result is interpreted by Cohnheim as proof in favor of his conception of the gut wall as diffusion-membrane. I am inclined however to believe that another explanation is more probable and I was glad to find that Enriques had made a remark of the same kind. In our chapter on excretion we shall see that uric acid, a normal excretion product of the Echinoderms, is excreted into the lumen of the gut. Probably, as we shall there see, this is the normal way of excretion in this group, which is certainly true in many other invertebrate groups. The same thing may hold true for sugar, the excess of non-utilisable sugar — see the chapter on enzymes in the perivisceral fluid — being possibly excreted here, just as in mammals in cases of alimentary glycosuria.

The swelling phenomenon then might be explained by the assumption that the animal is not capable of „working through" so much sugar at once but is capable of taking up water by means of its many water-permeable membranes. In that way a preliminary dilution would make the high osmotic pressure less harmful. Since I could not study this problem more in detail, I shall leave this explanation in a very hypothetical form; the recent work of Dekhuyzen 30), however, on the Sipunculidae makes such hypothesis rather probable.

Note. Enriques has kept some of his Holothurians in a vessel filled with sea water containing milk. After some time he finds fat droplets in the animals blood-vessels and later on in its perivisceral fluid. Still later the quantity of fat in the

coelomic cavity has increased, but not so that in the blood system. To him this is a proof in favor of his conception of the importance of the blood system in resorption, as one can easily understand. I mention it in connection with my own experiments on sea urchins.

Enriques finds the fat in free form in the coelomic cavity. *It is not taken up by the corpuscles,* according to him. This agrees very well with my own experiments on sea urchins; there also I did not find any increase in fat in the corpuscles after fat feeding, it disagrees however with the experiments of Chapeaux, mentioned previously. I did not find any free fat in the coelomic cavity of my urchins however.

21. THE RESORPTION IN ASTEROIDEA. FUNCTION OF THE THEIR „LIVER".

There is an old question in comparative physiology which Jordan 68) calls the „Leberfrage". The numerous appendages of the middle-gut of invertebrates have usually been called „liver" by the older, especially by the morphological writers. This can not astonish us, since in most groups they are located exactly where the liver is found in higher animals. Furthermore, their secretion frequently contains a yellow or brown pigment and tastes bitter. One of the things which chiefly accounts for this assumption is the fact that they frequently contain glycogen. Their secretion also contains sugar by which even Claude Bernard 6) and 7) was misled.

Later on it appeared that the secretion of these organs resembled more that of the pancreas of the Vertebrates; in order to recognize this fact the name „Hepatopankreas (Max Weber)" was accepted and is used up to the present day in the leading text-books of zoology.

One of the chief reasons why this belief was not sooner abandoned is the fact that with the word *gut* one generally associates the notion of a tube, such as is found in vertebrates. A ramified gut was known to occur in many of the lower groups, e. g. the flatworms and the coelenterates, but the idea that these „livers" are nothing much else but ramified middle-guts, did not at once succeed in getting a foot-hold. Yet, just as in many of these lower groups the respiratory organs branch out all over the body, so to a certain extent, does the digestive tract. In many instances we have to do here with an adaptation to the lack of circulation, the intestine follows the form of the body in order the assure an equal distribution of the food.

Now in this connection it is very remarkable that organs of the same nature are found in the starfishes, just where the problem of food distribution is most difficult owing to the strange shape of the body.

In the Holothurians we find that the body wall is simply a sac in which the gut is folded many times. The urchins are also simple, round containers and even here the gut shows two curvatures and winds around in every direction. But in the starfishes it would not be possible for sufficiently large quantities of food to reach the tips of the arms on account of the lack of circulation, if the gut did not extend into these arms. The „Mitteldarmdivertikel" are a logical postulate here.

These middle-gut sacs are on the one hand a place of enzyme secretion, but on the other hand, chiefly organs of resorption. The first authors who clearly realised this important fact, were Biedermann and Moritz 9) in their almost classical monograph on the function of the so-called „liver" in molluscs. They showed that the anatomical relations are such that the food is forced to enter into the „liver" up into the smallest ramifications. This can easily be shown, if e. g. the animals are fed on flour and the iodine test is applied to microscopical sections.

Numerous structures which have always been a puzzle to anatomists and on which a very particular light is thrown by this discovery, have since been explained by it. Two very interesting cases have been described by Jordan 64).

In case this „liver" is not present, i. e. in those groups to which the collective name of „injecurata" has been given, other devices take their place in such groups as have an insufficient circulation. In this way the ramifications of the gut of the flatworms, the coeca of Aphrodite, the curvatures in the digestive tract of sea urchins and sea cucumbers etc. may be explained.

It has frequently been stated that bile pigments are found in these livers. This is not true and later authors (Hoppe-Seyler, Frenzel, Plateau, Voit, Frédéricq et al.) always failed to find them.

Summarising this brief introduction we may say that these middle-gut sacs and the liver of the vertebrates have no *specific* function in common. These so-called livers are nothing but extensions of the middle-guts for the function of resorption, a system of blind-guts.

From the very beginning of this work I suspected that the radial sacs of the starfishes would appear to have that same function. To get some more definite information on this question I used different procedures partly of a positive, partly of a negative nature.

Two different views have been held in the past; they were either considered to be organs of secretion or organs of resorption. The first view is the one held by nearly all authors f.i. Griffiths, Frenzel, Frédéricq, Krukenberg, Cuénot (in 1887) and others. Biedermann in Winterstein's Handbuch, summarises the earlier views thus: „dasz die Radialanhänge des

Magensackes bei den Seesternen in erster Linie der Absonderung des wirksamen Verdauungssaftes dienen, während der riesige Magen selbst lediglich als Behälter der aufgenommenen Nahrungskörper dient und nur insoweit eine active Rolle spielt, als er sich vorstülpend, die oft sehr groszen Beutetiere noch auszerhalb des Körpers umschlieszt . . . . ."

Krukenberg, about whom we have given our opinion already in the introduction, denies emphatically that food ever penetrates into the „Lebergänge" of the starfishes. He fed fibrin stained with different dyes to these animals and the result of his observations was, „dasz die sogenannten Radialanhänge des Asteridenmagens reine Ausführgänge der Leberdrüse sind". In some cases he found parts of the water vascular system colored even out to the tips of the arms, but no dye present in the „liver".

Similar observations were made by Frenzel and Frédéricq who also believed that the radial sacs only have secretory and no resorbing activity or that they are the only secreting organ.

Chapeaux, Hamann and Cohnheim have come to a different conclusion. Chapeaux found that after the ingestion of fat the cells of the radial sacs were all full of it. Cohnheim proved by autodigestion-experiments that enzymes are present just as well in the stomach as in the radial sacs.

If the radial sacs are the exclusive or even the chief organ of enzyme secretion, it must be possible to demonstrate a difference in enzymatic activity between equal quantities of stomach and radial sac material. On the contrary, if such difference is found not to exist, if both prove to be about equally active, it is not very probable, though yet concievable — on account of the relatively enormous quantity of radial sac material present in one animal —, that a difference can be shown.

An equal quantity of stomach and of radial sac material was secured. Equal quantities were taken rather than proportional parts, because there is in reality about 7 or more times as much radial sac as stomach material which would not give an unfair comparison. Both were ground up with sand, to both an equal quantity of gelatin, water, sodium carbonate — to keep the reaction slightly alcaline — and toluene were added. The digests were now placed in the incubator at 37° and kept there for 24 days. After they had been taken out of the incubator the amount of total non-protein nitrogen present in them was determined. The proteins were precipitated with an excess of 5 % trichloracetic acid. In an aliquot the amount of total nitrogen was determined by means of the method of Folin. The diluted digestion mixture was used and direct Nesslerisation.

In both digests in which 11.5 gr. of tissue had been used, almost exactly the same amount of nitrogen proved to be present. In the digest of the lateral sacs. I found 144 mgr. of nitrogen, in the

other 138 mgr. The difference between these two figures is most probably within the limits of error. If not, it is sufficiently explained by the fact that proportionally more epithelial tissue is present in the same quantity of radial sacs, since the muscular wall of the stomach of course has also been included.

These figures certainly do not favor of the assumption that the radial sacs are the organs of enzyme secretion; on the contrary they prove that relatively just as much enzyme is secreted by the stomach. In the chapter on the histology of the two organs we have seen that the „granular" cells are present in the stomach just as well as in the radial sacs; this affords other evidence in the same direction [1]).

There are, however, in the literature certain statements which seem to make the other view more probable. One of these we find in Cohnheim's paper. Cohnheim observed that if one puts small pieces of mussel flesh or fibrin into the stomach of an Asteropecten aurantiacus, „so wird es nicht verdaut da die Fermente nicht in den Magen gelangen. Für die Thätigkeit des Seesternmagens ist ein complicirter Bewegungsmechanismus nötig die das von dem übrigen Tiere losgelöste Organ nicht mehr leisten kann". He states that with some practice it is easy to prepare the stomach free. I never succeeded in accomplishing it without tearing the wall. I do not understand either how one can bring a solid particle into these stomachs which contract their sphincter as soon as the animal is treated roughly.

For these reasons I have made similar experiments in a somewhat different way. The arms of a starfish were cut off at a little distance from the disc. Ventrally each arm was now opened carefully near the centre of the disc. The ducts of the radial organs were located and prepared free from the dorsal mesenterium. This operation is exceedingly delicate and I had to eliminate several animals before I succeeded in accomplishing it. The ducts are very fragile and break at the least touch. A ligature was now passed around the ducts after which they were tied off firmly. The ducts were now cut distally and the remainder of the arm was eliminated which is very easy since they naturally break off at the articulation of the arm with the disc (see p. 70).

After all rays had been treated in this way, the stomach was filled with a food substance in liquid form, be it a sugar solution or a solution of some protein for which I usually took casein — dialysed carefully — or K. L. I. M., a commercial milk powder. The injection was made by means of a small hypodermic.

These experiments had a double purpose. In the first place I wanted to find out whether, as Cohnheim found, the isolated

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[1]) Chapeaux could extract enzymes even from oesophagus and rectum.

stomach is incapable of digesting food, secondly whether it is capable of resorbing the products of hydrolysis.

The preparation after having been filled with the solution to be studied, was put into a shallow watch-glass in some sea water with the oral side up — in order by gravitation to keep the liquid in the stomach —. After various periods of time tests were made for glucose or amino-acids in the surrounding liquid.

As far as the digestion is concerned, completely positive evidence was obtained. A Fehling's test and a ninhydrin both gave positive results in so many cases that there does not seem to be any reason for doubt as to the capacity of the isolated stomach for digesting food substances.

As far as the resorption is concerned, I believe that we are not entitled to draw sufficiently justified conclusions from a few experiments like these. There are three important possible sources of error. In the first place we do not know how long the stomach actually remains alive; its wall might finally become a dead membrane through which everything passes; in the second place the products of hydrolysis may have escaped through the mouth and thirdly the stomach pouches and the remnants of the ducts may have taken care of the resorption. One fact especially makes me doubtful, i. e. that it always took a long time for the substances to make their appearance.'

The anatomy and histology of the radial ducts does not give us much information either. In the snails the anatomical relations are such that the food masses are forced to enter into the „liver" (see Biedermann and Moritz" 9)). The same thing is true for the crawfish (see Jordan 64)). Here we do not find anything of the kind. I have fixed some of these ducts including a little piece of the stomach and of the radial sacs in acetic-alcohol and cut them up in a serial section. Though these sections were very poor, partly maybe on account of the fixing fluid, they showed at least this, that there is no particular device for forcing the food into the stomach neither is there a filtering apparatus („Reusenapparat") The ducts are simply hollow tubes, leading streight from the stomach into the radial sacs. The lining epithelium is ciliated everywhere: possibly. these cilia by their movements account for the transport of the food from stomach to radial sac. In the sections moreover small pieces of organic material, stained with the eosin employed to color the sections, were seen lying within the tubes. To me they looked like food particles on their way from the stomach to the radial sacs.

If all these data do not give us any positive evidence whether or not the radial sacs are organs of resorption, there are others however which to me are absolute *proof for my thesis that they have this function.*

I have fed starfishes on various substances and I could observe

that all these substances actually penetrated into the radial sacs. Since the animals did not take any food voluntarily, I had to feed them artificially. The animal was laid down on the table with its mouth turned upwards. The needle of an injection syringe was now introduced into its stomach. As the animals on such rough mechanical treatment frequently withdraw their oral sphincter, I was often obliged to remove some of the spines surrounding the oral cavity. The fluid was then injected, in some experiments the animal was put into the water again soon after the injection, sometimes the mouth was closed with recently melted paraffin of a low melting point. The animal frequently succeeded in soon getting rid of the piece of paraffin, but yet, the paraffin did prevent the immediate squeezing out of the injected substances.

In some of these experiments the animals autotomised their arms, probably on account of the unusual stimulation by injection and unnatural food. This peculiar autotomy takes place in starfishes along the interradial saepta which divide the coelom of the disc in five parts. It takes place very easily in some species; Luidia ciliaris, according to Cuénot 22), is almost never caught in intact condition. They are like folds projecting inwards from the interradial spaces; they are stiffened by a calcareous deposit and are the areas of lateral adhesion of the arms. In them the stone-canal and the axial sinus are found. If all the arms are removed in this way, only an exceedingly small part of the disc is left as central part.

It needs not to be stated that „abnormal" animals of this kind were always excluded.

One of the injected substances was the saccharate of iron. This substance has frequently been used in experiments on resorption, on account of the fact that it is so very easy to make iron visible in microscopical sections. As examples from invertebrate physiology, I might quote the papers of Jordan 69) on the resorptive function of the middle-gut gland of Helix pomatia and that of Steudel 123) on absorption and secretion in the gut of insects.

The substance was prepared according to the U. S. Pharmacopea (carbonas ferrosum saccharatum). In injecting such preparations one must be very careful to use a very small dosis, since even traces of iron are toxic (see also Jordan 63)). A very dilute solution of the preparation was used for this reason, which has a light brown color.

At different intervals after the injection, the animals were opened, inspected and, if they looked promising, the radial sacs were fixed. It was most interesting to observe how the material entered the radial sacs through the ducts. The small iron particles which had not gone into solution could clearly be seen through the wall of the ducts and eventually one could even see them move.

The material penetrated into the very tip of the „livers"; a small piece of the tip of one of these organs was dissolved in nitric acid and gave a strongly positive prussian-blue reaction. When one has actually seen an experiment of this kind, one immediately realises the importance of the radial sacs as organs of resorption.

The fixed material was cut up in Amsterdam where Mr. K. Boedyn obliged me very much by his valuable and experienced help. It appeared that up into the smallest ramifications of the radial sacs little iron particles could be seen, which can easily be made visible as blue grains -prussian-blue- by a treatment of the sections with ferro-cyanide and hydrochloric acid, described by Tartakowsky 126) [1]). But this is not the only thing visible in these sections. In fig. 5 of our plate, we see one of these sections in its natural colors, resulting from a combined treatment with eosin and the prussian-blue procedure. In this figure we also see that the material is actually resorbed. The cuticula of many cells shows a dark blue coloration, the iron seeming to diffuse out into the gut epithelium. Then we can not follow it any more till we see it again in the pavement endothelium of the coelomic cavity. This layer has in some sections a very dark blue color. No considerable quantities of iron are found in the layer of connective tissue, but the coelomic endothelium, the „stain-layer", seems to store the materials which have been resorbed, before giving them off to the perivisceral fluid.

Something of the same nature could be observed in some experiments in which a solution of ammonium carminate was injected. On dissection the radial sacs of such animals appeared to be absolutely red. They were fixed in alcohol, in which, as we know, ammonium carminate is insoluble. They could in that way be passed through xylol and paraffin without fear that the stain would be dissolved. In these sections one of which has been pictured in fig. 6 of our plate, we again see something of the same nature. The carminate has been resorbed by the epithelium and passed over to the „membrane nutritive", as Cuénot has called an analogous layer in other forms. It is seen here not only in the coelomic endothelium, but also in the layer of connective tissue. Unfortunately these radial sacs were taken out at a late stage of the resorption. The actual resorption process can not be observed as in the iron sections, but the result is as clear as it could possibly be. The presence of the carminate everywhere in the coelomic lining investing the radial sacs, is the most conclusive proof that these organs have resorbed it.

[1]) The sections, after having been freed from paraffin and xylol, were put into a $1^1/_2$ % solution of potassium ferro-cyanide for 15 minutes, then for 10 min. into a 0.45 % HCl solution.

Another series of injections was made with olive oil. This substance also was seen to enter the radial sacs, which looked like a kind of oily gland shortly after the injection had taken place. For fear that I might dissolve away the fats by passing the fixed radial sacs through the alcohols and xylol, they were fixed in Flemming, washed and imbedded in soap. The recent method of Mc. Junkin described in chapter 16 was used for this purpose.

Also in these fat preparations the same thing could be observed again. The whole epithelium appeared to be full of fat in drops of all sizes. It was so full that the sections, notwithstanding the fact that the Flemming had stained the fat only incompletely, appeared to be dark greyish even on superficial examination. The addition of Sudan III gave a most brilliant picture as described in chapter 16 which one could not possibly reproduce in print. The whole epithelium appeared like one mass of reddish droplets.

From all this evidence we see that *without any doubt this „liver" like so many other invertebrate livers is primarily and chiefly an organ of resorption.* It is rather remarkable that Krukenberg 78) came to results which are absolutely contradicting mine in experiments of the same kind. He fed Astropecten pentacanthus and aurantiacus and Asteracanthion glacialis on fibrin stained with different dyes and never saw them enter into the radial sacs. He concludes: „dasz die sogenannten Radialanhänge des Asteridenmagens reine Ausführungsgänge des Leberdrüsen sind und dasz in sie am normalen Tier kein Speisebrei gelangt." In connection with my remarks in the introduction, I do not consider this as a very serious objection to my results. Just as in the snails the food substances here enter into the liver to be resorbed there.

One peculiarity, however, of the snail's liver is absent here, Biedermann and Moritz describe were definite and active movements of the separate pouches of the Helix liver, causing currents which move the food through the whole organ. They compare the liver of an animal in full digestion to a kind of boiling jelly, bubbling here and there. This does not occur in starfishes as far as I could observe. Their radial sacs are completely motionless during the period of food ingestion, probably the cilia do most of the work here (compare also the chapter on the histology where we mentioned that muscle fibres are either absent or only occasionally present in these organs).

Nearly all natural food in starfishes seems to be digested very completely. The anus is almost never used and is very narrow. Shells if taken up are removed by the mouth. This also might furnish evidence in favor of a very complete resorption into and digestion in the radial sacs. In the other groups of Echino-

derms we always find very large faeces and a very incomplete digestion — see chapter 9 and 10.

Note: 1. Griffiths 48) has considered the function of the radial sacs to be partly of pancreatic, partly of excretory nature. The first author who supposed that they have the function of kidneys, was Johannes Müller 91). Griffiths demonstrated — see the chapter on excretion —, that uric acid is present in them. After injection of acid fuchsin, indigo-carmine and methyl-green Cuénot found the epithelial cells of the pyloric coeca of A. rubens full of these substances; this surely proves that they must play some rôle in excretion. The same thing was observed in urchins; the cells of the last part of the gut (second curvature and rectum) also appeared to contain these substances. Jordan 63) in his paper on the excretory function of the „liver" of Astacus fluviatilis, points out that one must distinguish between the true function of an organ, an accessory function and a merely incidental activity- between: „Hauptfunktion, Nebenfunktion and Nebenerscheinung (i.e. zufällige Reaktion auf zufällige Bedingungen)". As a matter of fact, there is reason enough to think of an excretory function on the part of these invertebrate „livers". Kovalevski 75) finds indigo-carmine, injected into Squilla, in their liver, de Saint-Hilaire 111) finds peptones there in crawfishes, so does Bruntz 12) with other substances. Darboux 29) finds uric acid and urates in the coeca of Aphrodite and describes „cellules excrétrices" in these organs. For Arachnoidea it has been established with an almost absolute certainty by Berlese 5), that their „liver" has excretory function. He demonstrated uric acid in these livers. Plateau, Bertkau and others also found guanin in them.

Mc. Munn 93) comes to similar conclusions for Molluscs and decapod Crustacea. Jordan himself finds injected methylen-blue back in the liver of Astacus. When we remember that all these „livers" are nothing much more than simple appendages of the mid-gut, serving for the extension of its surface and that the Malphigian tubes, the organs of excretion in arthropods, are also appendages of the mid-gut, except as far as their ontogenis is concerned, we must believe that the mid-gut plays a rôle of some kind in excretion. Jordan believes that this excretory function, at least in Astacus, is a „Nebenerscheinung", not a true function — just as in the case of the excretion of small quantities of urea, anisoil, alizarin etc. by the mammary glands —.

Delage 31) is opposed to their excretory function as far as the physiological injections are concerned, he calls it pharmacology, Cuénot 24) explains the excretion of dyes as due to the desire of the organism to get rid of the excess of water, in which the colored substance has been dissolved. As far as

the excretion of iron is concerned, Jordan is certainly right; the presence of uric acid however, a very important excretion product in lower animals, in the radial sacs of Asterias, in the coeca of Aphrodite etc. makes me believe, that this question is not yet completely settled, the more because Roaf 109) found creatinine to be present in many of these organs. This fact seems to me to need further confirmation, considering the fact that this substance only rarely occurs in lower animals; if it appears to be true, however, it is another indication in the same direction.

2. The function of the so-called siphon in the urchins has never been investigated and is still completely unknown. The assumption of Perrier 99), that it might have respiratory function, does not seem very probable to me, because at least in the species I have studied, one never observes any active movements in them [1]) and because other organs of respiration are known in Echinoidea.

A priori it might be thought of as an organ of resorption. This is not probable however for two reasons: 1. its histology is more primitive than that of the main-gut — see the chapter on the histology —, 2. in sections of guts of animals which had been fed on ammonium carminate and other substances I always found these substances present in large quantities in the main-gut.

Thirdly, one might assume that it is a kind of hydrostatic apparatus for the transport of the food in the gut. The food is in our sea urchins transported in round masses, included in a mucous membrane — food-vacuoles as observed already by Roaf and Scott; see p. 33 —. They fill the whole gut and owing to the incompressibility of fluids, it becomes necessary to have a device of some kind to permit their forward movement, the siphon might serve for this purpose.

22. THE RESORPTION FROM THE PERIVISCERAL FLUID.

When we take for granted that the perivisceral fluid has the function of „blood", of distributor of food substances, it seems very strange that we never find these substance in it. In the chapter dealing with this liquid we have seen that it contains almost no organic substances, except for a small quantity of uric acid and some corpuscles. Ordinarily no monoses, no amino-acids nor peptones nor proteins are present.

In the chapter on resorption in Echinoidea and Holothurians we have learned that after the artificial ingestion of pure food

[1]) V. Henry 53) however saw rhythmical contractions.

substances into the digestive tract these products of hydrolytic dissociation can as a rule be demonstrated very easily. This suggests a rapid withdrawal from the blood by the tissues and it seemed to me worthwhile to investigate this problem a little more closely.

We know that in nature the Echinoderms obtain their food at very irregular intervals and I have often been impressed by the fact that almost all marine animals are, so to say, in a state of constant starvation.

If this is true it must be of the utmost importance for the animal to secure whatever it gets and whenever it get sit, in the most rapid and effective way possible. Seen from this standpoint, this rapid withdrawal of substances is without any doubt a property which is highly purposeful in the struggle for life.

There is one very essential feature by which these bloods are entirely different from mammalian blood. They are in no respect a store of anything, they are only a transmitting medium. Nothing is stored in dissolved form; the protein crystalloids, described by List 81) and the fats contained in the interior of the corpuscles, are not yet present in dissolved form, but in formed elements.

These bloods are not the carefully balanced system which the blood of vertebrates represents; they are just dissolving and transporting media. The question whether they are absoluty stagnant as has frequently been supposed, has been discussed on p. 39.

To study this question of the withdrawal of food constituents from the perivisceral fluid and to get some information about the food-consumption by the tissues, I made the following set of experiments on starfishes. Various quantities of either glucose or glycine, being the most easily available monose and amino-acid, were injected by means of a syringe into the coelomic cavity. They had been dissolved in sea water and were distributed as equally as possible over the whole system. To avoid the objection that part of the injected fluid might have escaped through the wound made by the injection, I first made the following control experiments. Several starfishes into which a large quantity of glucose had been injected, were put into a finger-bowl in a minimal quantity of seawater. After an hour or so this liquid was tested with Fehling and always gave a completely negative result, even if Benedict's qualitative reagent was used. The same tests in the case of glycocoll gave a negative ninhydrin.

Possibly this retention of injected fluids is due to a contraction of the muscle tissue which is found everywhere under the peritoneal epithelium, very much like when in intraveneous injection in mammals no blood escapes. It may still have another explanation. Cuénot 23) and 27), in his paper on the biological importance

of the clotting of the urchins blood, describes the formation of a clot in animals from which he removed a very small part of their shell. After two hours he found the hole covered with a crust just as a wound in mammals is covered up. All kinds of corpuscles were seen in these clots, the cells do not any longer form amiboid pseudopodia, but are gradually transformed into connective tissue („semblent déjà en voie de transformation conjonctive"). The same thing takes place in many other species — the original experiments were conducted on Echinus acutus. Lmk. —. It does not take place if the wound is too large. Then the rims of the wound are closed, but the animal dies, before it has been able to repair the damage.

The biological importance of such protective clotting is very evident, especially in the case of the Spatangidae, whose very thin-walled alimentary tube seems to be especially liable to be hurt by the sharp particles which are frequently taken in with the natural food. Something of this nature may take place in the case of injections of fluid into the coelomic cavity.

In order to avoid all objections however, I injected the liquid from very near the tip of the arm with a fairly long needle. Close to the proximal side of the opening, I tied off the arm with a cord. In that way it was not possible for any fluid to escape. About one fifth of the total quantity was injected into each arm and with some force so that one might suppose that it was fairly well equally distributed through the whole perivisceral liquid.

At definite intervals a sample of one c.c. was drawn from the coelomic fluid, with a syringe. In these samples the quantity of sugar was determined by means of Folin and Wu's micro-sugar-method for blood filtrates. In this method the liquid to be tested is boiled with an alkaline copper solution for 6 minutes. Phosphomolybdic-phosphotungstic acid is added to dissolve the cuprous oxide. This is oxidised and the molybdic reagent reduced with the production of a blue color, which is then compared with a standard. This standard was in the present case made up in sea water.

The corpuscles were first removed by means of a hand centrifuge; after that they were washed once more with about one c.c. of seawater. When sodium carbonate was added in order to produce the blue color, a slight precipitate came down. This precipitate was removed by means of the centrifuge, before the colometric reading was made.

In that way the results represented in table 11 were obtained in a series of glucose experiments. The quantities injected were 75, 150, 350 and 700 mgr., which covers a wide range. On the average — 6 animals were used — a starfish appeared to contain 22 c.c. of perivisceral fluid. On this assumption and given the quantity of liquid in which the sugar had been dis-

solved — 5 c.c. —, it was possible to figure out the quantities of sugar present in one c.c. of coelomic fluid at any desired moment. Such figures are represented in table 11; in the first column the time which has elapsed since the injection, is indicated, in the second the quantities of glucose remaining at that time in one c.c. Diagrams have been plotted which illustrate these relations still more clearly (Fig. 7).

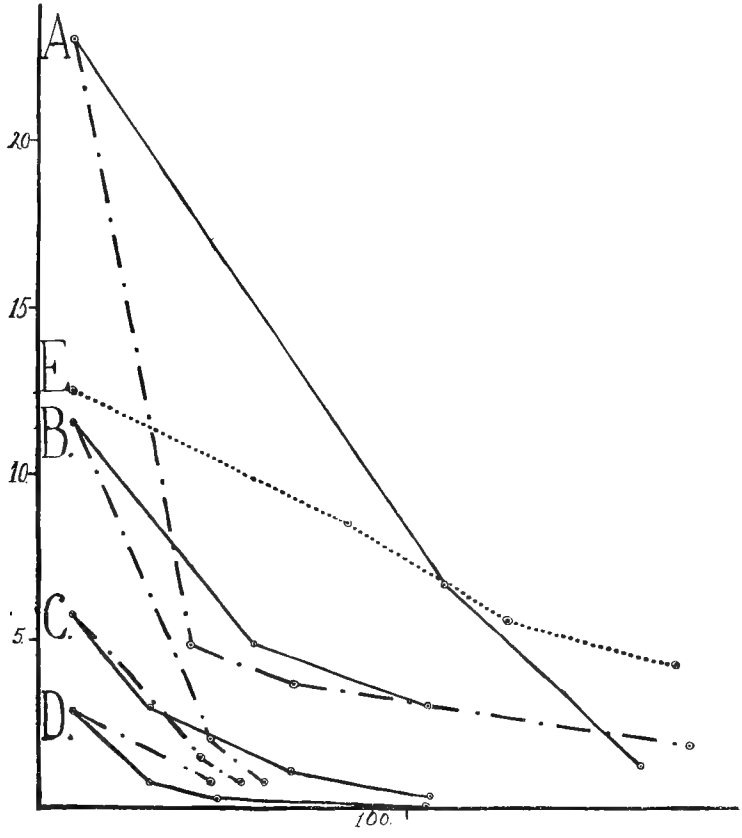

Fig. 7.

Resorption of food materials from the perivisceral fluid.
For explanation see text.
——— glucose.
—·—·— glycine.
····· corpuscle experiment.
A. 700 mgr.; B. 350 mgr.; C. 150 mgr.; D. 75 mgr.

Similar experiments have been made with glycocoll. The same quantities were added and the whole experimental procedure was the same. The quantity of amino-acids present was estimated by means of Sörensen's formol-titration method.

Table 11.

Resorption of glucose from starfish coelom[1]).

75 mgr. injected.		150 mgr. injected.		350 mgr.		700 mgr.	
T(ime).	R(emaining). Q(uantity).	T.	R. Q.	T.	R. Q.	T.	R. Q.
0	2.88	0	5.77	0	13.46	0	26.92
16	0.71	23	3.00	54	4.88	67	**5.83**
43	0.29	65	1.08	106	3.01	111	6.67
106	0.048	107	0.31	—	—	170	1.21

This method depends on the amino-acids having their basic character destroyed by formaldehyde and thus bringing about greater ionisation. A neutralised formalin solution is added in order to bind the NH_2 groups, the COOH group is then titrated in the usual way. A correction was made for the titrable alcalinity of the sea water. The results of these experiments are represented in table 12 and fig. 7.

Table 12.

Resorption of glycine from starfish perivisceral fluid[2]).

75 mgr. injected.		150 mgr. injected,		350 mgr.		700 mgr.	
T(ime).	R(emaining). Q(uantity).	T.	R. Q.	T.	R. Q.	T.	R. Q.
0	2.88	0	5.77	0	13.46	0	26.92
41	0.74	38	1.48	41	2.09	35	4.88
—	—	60	0.19	57	0.74	66	3.67
—	—	—	—	—	—	184	1.86

This rapid withdrawal of materials from the „blood" is really very striking. The most essential feature of vertebrate blood is lacking, for we do not have a balanced and storing system here, but everything that comes in, leaves the transmitting medium just as rapidly as possible. Everything coming in, is consumed with surprising rapidity, the waste products also disappear rapidly and there are no „threshold substances". This consumption is not only due to the tissues, but also, and in large part, the corpuscles seem to take away the food substances. I could make sure of this by the following experiment. The

[1]) The time-figures indicate minutes, the quantities are given in milligrams.
[2]) Figures as in table 11.

total quantity of perivisceral fluid of a starfish was secured. This can easily be done by cutting off the tips of the arms and allowing the „blood" to drip out. A cut in another arm facilitates this dripping out. 23 c.c. were secured. To this 5 c.c. of sea water were added in which 350 mgr. of glucose had been dissolved. The liquid was now put into a small Erlenmeyer flask and shaken constantly by means of a hot-air automatic shaker, in order to prevent the clotting of the corpuscles and to mix the liquid thoroughly. From time to time a sample of one c.c. was removed, as in the experiments mentioned above. The corpuscles were centrifuged of and the sugar determination made, as described above.

The results of this experiment are given in table 13 and plotted in fig. 7 (E).

Table 13.

Consumption of glucose (350 mgr.) by the corpuscles of a starfish.

T(ime).	R(emaining) Q(uantity).
0	12.5
82	8.56
130	5.6
180	4.3

These experiments have given us an opportunity, exceptional from the general physiological standpoint, to study the disappearance of food substances from the „blood" into the tissues. One fact is very striking: that the consumption is not, or at least not primarily, dependent on the quantity which is offered. In the 700 mgr. experiments we have after the lapse of a certain time just as little left as when 75 mgr., less than one ninth of the amount, is injected. *There is no proportionality between quantity offered and consumption,* the law of mass action can therefore not be applied to the present case. The needs of the tissue seem to be the factor of primary importance.

They explain at the same time why the blood picture of the invertebrates is so very inconstant, as I have already pointed out in the chapter on the perivisceral fluid, when mentioning the experiments of Dr. Morgulis. The concentration of the products of hydrolytic cleavage of food substances and of waste constituents depends largely or wholly on the feeding condition of the moment. Constantly starving and having almost no reserves — as we will see in another chapter —, these animals live from day to day and have to fight for existence.

23. EXCRETION IN ECHINODERMS. FUNCTION OF THE RECTAL COECA OF THE ASTEROIDEA.

About excretion in Echinoderms we are, as far as my knowledge of the literature goes, only very poorly informed. One thing however is absolutely clear: the representatives of this group do not posses any definite kidney for the elimination of nitrogenous waste. These kidneys, present in most other divisions of the animal kingdom, are, as comparative morphology teaches us, specialised parts of the coelomic epithelium; in many cases this appears from embryology, in other cases part of the coelomic wall still participates in the excretory activities. Since in the Echinoderms specialised organs have never been demonstrated, the whole coelomic lining apparently must have excretory function. Cells from this peritoneal wall are constantly thrown into the surrounding fluid, where they move about. They are called from the way in which they move, amibocytes and they seem to have a great importance in excretion. Durham calls them sphaeruliferous corpuscles. Substances like indigo-carmine which in the higher groups, are eliminated by the kidneys are here found to be absorbed eagerly and to be stored by these „nephrocytes [1])". Later these cells in starfishes accumulate in the dermal gills, the papulae, in Holothurians in the wall of the waterlungs, where they move to the outside — diapedesis — and degenerate. Sometimes they form large clumps containing the material to be eliminated, as I myself could observe in Stichopus. Part of them also disappear through the ambulacral feet. Sometimes the papulae when loaded with these „nephrocytes", are autotomised.

The picture given above was, in its general lines, first concieved by Durham 34) and 35), also by Chapeaux. Though nobody has ever tried to demonstrate that this method of elimination which has beer observed in the case of physiological injections — of suspended substances —, is the normal one, it sounds rather probable that some natural waste-products might be eliminated in this way.

The rôle which in the starfishes is played by the papulae, is played by the water-lungs in the holothurians. These have been considered to be organs of excretion by a great many authors. Pourtalès 102), Oken, Huxley, Bartels 4), Hérouard 55), Schultz 116), Bordas 11) and Danielssen and Koren 28) all found that migrating cells loaded, either with artificially injected substances (ink etc.) or with normal granules left the coelomic cavity through the waterlungs [2]). A beautiful description of this process of elimination has been given by

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[1]) This name has been given by de Ribaucourt. C. R. Biol. 19 Janv. 1901.

[2]) Jourdain 70) also assumes an excretory function for the migrating cells.

Schultz 116). Jourdan 72) also saw these cells loaded with yellowish, refringent granules in the wall of the water lungs.

It must therefore be considered as definitely established that such elimination takes place, the more because Hérouard found definite stomata for the outlet of these phagocytes. Bordas demonstrated uric acid and urates in them by means of Garrod's test, Schultz however finds that they do not give a murexide test. Neither are they guanin, as Carus supposed [1]), nor calcium salts, as Jaeger assumed (De Holothuriis, Inaug. Diss. 1833), or carbonates — they do not dissolve in HCl.

It does not astonish me either, that such elimination takes place, since these nephrocytes leave the body at almost any place where there is a thin wall — as we shall see presently —. That it is not the only way of elimination, however, is proved by the fact, that in many Holothurians — the Spatangidae und Clypeastroidea — the respiratory trees are completely absent. Our own conception of the process of excretion will be discussed later on in this chapter.

Perhaps all these phenomena can most easily be explained in the following way. All „Fremdkörper", all foreign bodies are grasped by the amibocytes. All amibocytes are, as general physiology teaches us, positively chemotropic to oxygen. Consequently they will all tend to move towards places where the oxygen tension is highest. Possibly it is even justified to assume a certain „Umstimmung" of these cells after they have taken up the excretion products.

Now, it is very remarkable indeed that almost all the organs mentioned above as supposed organs of excretion are at the same time organs of respiration. This is true for the papulae and for the water lungs, as also for the „ciliated funnels" (Wimpertrichter) of the Synaptidae where respiratory trees and podia are lacking. These little funnel-shaped organs, attached to the mesenteries in the region near the body-wall, are supposed to maintain a water-current which aids in respiration through the skin. This same fact, the high oxygen tension in the neighborhood of these organs, might account for the accumulation of the amibocytes at that particular place, which otherwise would seem strange. This hypothesis would also account for the statement of some authors, among them Chapeaux, that the phagocytes, not only those loaded with injected substances, but also young ones — eventually leave the body through the stone-canal, and madreporite, notwithstanding the fact that the current in this tube is directed inward — this current caused by the cilia of the endothelial lining, is supposed to take care of the internal pressure of the water vascular system —. This

[1]) He found this substance in the organs of Cuvier which probably is a mistake (Bordas).

also explains why sometimes the water vascular system has been considered to play the rôle of an organ of excretion — because it also contained these amibocytes. — The importance of this system for respiration can not be doubted, as we shall see in the chapter on respiration, especially not because hemoglobin, whenever it occurs in this group, is found in this very system. I myself could see this once very beautifully in a starfish which had been fed on ammonium carminate; the whole dissepiment of the water vascular system appeared on dissection to be colered brilliantly red even into the very tips of the arms.

The well known paper of Kovalevski 75) on organs of excretion in lower organisms, has given rise to a whole series of analogous investigations. This author injected small quantities of a certain dye into the animals to be studied, and observed where it went to. That such organs would have the function of excretion is, however, not necessarily true. On the contrary, the diversity of the results obtained by such methods with different dyes, makes this rather improbable. Cuénot 23) distinguished between „néphrocytes à carminate" and „néphrocytes à indigo". These nephrocytes, according to him, desintegrate in the coelomic liquid, are taken up by the phagocytes and eliminated through the skin-gills, as described above.

One fact must however be mentioned as the result of many such experiments, i.e. the frequent accumulation of the injected dyes in the so-called axial organ [1]) and in the bodies of Tiedemann. Injecting a suspension of carmine or Bismarck-brown into the water vascular system by means of one of the tube-feet, he found the bodies of Tiedemann stained in the starfishes; injecting such substances into the coelomic liquid of urchins, he found them in the axial organ. Leipoldt 79) denies their excretory function, pointing out that these organs do not have a glandular epithelium and do not stand in connection with the perivisceral fluid; furthermore that the current of the stone-canal moves inward. It is rather remarkable to observe here that again we have to do with an organ in the neighborhood of the stone-canal, which should thus be furnished with much oxygen.

The great objection to all such experiments is that they are „unnatural" and that, though they have given valuable information in mammalian physiology, they may not have any value in these groups, because we only have to do with a kind of vital staining. The same thing also holds true for many experiments on the amibocytes as excretors, especially for the experiments of Metschnikoff who made his original studies on phagocytosis on this very group. The fact that the phago-

[1]) The axial organ or ovoid gland has been supposed to have excretory function by a great many authors, among which Jourdan, Perrier, Koehler, Hamann, the Sarasin's etc.

cytes eliminate dying mesodermal cells, and that they take up all kinds of foreign bodies, dyes and bacteria, does not prove that they are the only, or even the chief, organ of excretion. Something of this nature is also done by the mammalian leucocytes, and yet they are not considered to take care of excretion. This is especially true in cases where foreign bloods or pathogenic bacteria are injected.

> The same thing has already been pointed out for the cases in which these phagocytes take up food substances. Even if these are digested as in the cases of injection of starch or olive oil emulsions, this does not prove that they play a rôle in the natural process of digestion.

The possibility that the „liver" plays a rôle in excretion, has already been discussed in chapter 21.

Not satisfied with the little evidence known about excretion in our group, I made some experiments of my own on this problem which have led me to absolutely different conclusions.

In our study of the perivisceral fluid we have seen that it is nearly the same as sea water, except for a small quantity of uric acid which discloses itself when the fluid tested by means of Folin's uric acid reagent. This substance is always present though in varying concentrations and in all three of our species. This proves that is a regular waste product in their metabolism.

The next step was to find out in what way it is eliminated. Faeces of Arbacia were collected. shaked with destilled water in a test-tube, then the liquid was filtered. In the filtrate Folin's uric acid test always gave positive evidence, in a few cases the color was rather faint, but generally it was strongly positive.

The same material proved to be present in the rectal coeca of Asterias. It is difficult to isolate these organs as they are very small and lie in the neighborhood of the anus. The whole stomach of the starfish is rather fragile, but the rectal part always sticks to the dorsal tegument. This consists not only of the rectal coeca, but also of a part of the rectum. The whole mass was simply taken as such and extracted with cold destilled water. This extract after having been filtered, showed the same typical blue coloration.

In looking up the literature on the subject I found a paper of Griffiths 48) in which the same uric acid is reported to be present in the five pyloric coeca of Uraster rubens, a fact which I mentioned in the chapter on the function of these organs. He examined chemically and microchemically the secretion of the five stomach pouches and the radial sacs and came to this conclusion from the following evidence:

1. Treatment with NaOH gives a flaky precipitate. Crystals of various forms appear to be present. If treated with alcohol, only rhombic crystals are found. With HNO_3 they give the murexide test.

2. The secretion is evaporated down, treated with absolute alcohol, then filtered. The residue is taken up in boiling water, then filtered. Typical uric acid crystals result.

These two concordant and independent observations make the presence of uric acid probable, if not certain. They show that it may be excreted into different parts of the digestive tract.

> Whether or not the uric acid is excreted into the radial sacs and passed over to the stomach pouches or excreted into the pouches themselves is not stated by Griffiths. Neither whether these„ livers" are capable of destroying uric acid, like many true livers, especially those of the dogfish (Scyllium catulus); see Scaffidi 114).

The same substance was also found in our Thyone. If the liquid contents of the rectum, which forms a distinct swelling in this species, or of the end parts of the gut are collected and a test is made for uric acid in them, strongly positive evidence is as a rule obtained. The same thing is frequently true of the contents of the middle gut. Here also uric acid seems to be excreted into the intestine. Remarkably enough Cohnheim did not obtain a positive murexide-test in the faeces of Holothuria tubulosa.

From all this evidence I have concluded that uric acid plays a very important rôle in the meta- or catabolism of the Echinoderms. It seems to be the solution of the dark problem of excretion in this group. None of the other waste products of the animal machinery could be detected. In our chapter on the perivisceral fluid we mentioned already the absence of ammonia in detectable quantities. Neither does urea seem to be present. Griffiths searched for it in vain in the radial sacs of the starfish which he considered to be organs of excretion. Four tests: 1. the mercuric nitrate test — no white precipitate, 2. the Na-hypochlorite test — no N-bubbles, 3. the absence of urea nitrate after treatment with nitric acid, 4. a negative Nessler, all gave negative results which makes the presence of this substance very improbable. Neither are guanin or Ca-phosphate, the regular products of excretion in Cephalopodes and Lamellibranchiates, present here (compare p. 81 on the Holothurians).

The gut, especially the end parts, seems therefore to be the organ of excretion in Echinoderms and the most important substance excreted uric acid.

This method of excretion is by no means rare or strange. In almost all arthropods we find just the same thing and the Malphigian tubes in insects and other groups, seem to be merely parts of the mid-gut, specialised for the purpose of excretion. They are however by no means the only organs with excretory function, for the whole mid-gut may be involved in the like duty, as stated by von Gorka 46) on p. 329 of his paper on the physiology of the Malphigian tubes, where he says: „Hieraus folgere ich. dasz auch der Mitteldarm an der Ausscheidung der Harnsäure teilnehmen kann".

Sitovski 121) in a paper on the biology of the catterpillars of the moth Tineola biselliella, has explained the acidity of the contents of their end-gut as due to the presence of uric acid. This does not seem very probable considering the very low degree of dissociation of this acid, but it seemed to me to be desirable to ascertain whether those parts of the gut which excrete uric acid actually have a lower P_H. Therefore I made some determinations especially of the rectal coeca of the starfishes. When we remember that in the cucumbers there is a very decided alcalinity which increases towards the rectum, we must agree that here at least the evidence is entirely negative. But in these rectal coeca some authors have occasionally found a rather strongly acid reaction. I have made some determinations for the solution of this question in exactly the same way as described in the chapter on the hydrogen-ion concentration. To one single drop of the rectal-coeca-preparation described above was added a certain amount of indicator and its color compared with that of a set of standard drops. The following values were found in different experiments: 7.2; 7.4 and 7.4. These figures may demonstrate a slightly stronger acidity than that of the stomach contents, but I do not believe that this difference has any significance at all. It would be very dangerous to draw from this conclusions like Sitovski's especially since Roaf occasionally found a strongly alkaline reaction in the liquid squeezed out of the rectal coeca on treatment with NaOH.

The importance of uric acid as a product of excretion in Echinoderms may be greater than one would at first suppose I refer here especially to some very interesting observations of Mangold 82) on auto-intoxication in sea urchins. The form on which he worked was Arbacia pustulosa a species rather closely related to our A. punctulata. His observations hold equally true for our form, and I would have described these phenomena if he had not done it.

In Arbacia the faeces, greenish or in our case white, round bodies, are expelled through the cloaca which is situated in the middle of the periproct. The spines show an avoiding reflex — first described by von Uexküll — as soon as one of these little bodies comes in contact with them. In this way and by the aid of the trifoliate pedicellariae the bodies are carried over and dropped down gradually. In nature, of course, the waves promptly remove them, so that here this complicated system of remo al is of not much use.

Arbacia is a rather weak form and soon dies in quiet water. This is doubtless due to a toxic action of the faeces on the skin. Even in a well aerated aquarium the animals die because the removing force of the waves is lacking. Irritability

and capacity for reaction disappear, the skin shows traces of decay and the spines fall off one after another.

These phenomena can be prevented by regularly cleaning the animal with sea-water. After the faeces had worked for *one* hour, this recovery, according to Mangold, could no longer be obtained.

This doubtless proves that a toxic action of some kind is exerted on the animal by its own faeces. Since faeces of other aminals do not cause the same phenomena, we can not have to do with a mechanical lesion, but a substance must act here, „deren physiologische Wirkung auf die lebende Arbacia der Wirkung von Säuren (! v. d. H.) gleicht" (Mangold).

„Ueber die Natur dieses Stoffes etwas bestimmtes aus zu sagen, ist vorerst nicht möglich, die Frage wird sich aber jedenfalls erledigen müssen, wenn einmal die Verdauung der Echinodermen überhaupt die langerwünschte Bearbeitung gefunden hat."

It seems probable that these phenomena may be due to the presence of uric acid. Isolated pieces of shell, with the spines on them, live much longer than the intact animal, even when no care is taken to change the water. Spines and pedicellariae keep alive easily for four days on such pieces. The following observation is interesting in this connection: A specimen of Arbacia was brought into the laboratory in a badly injured condition. Almost half of the shell, including the periproct, was lacking. This piece of animal remained alive for 5—6 days, while normal animals frequently already begin to show loss of spines and other symptoms of bad health in less time. It is a beautiful illustration of the autonomy of the different parts of the urchins body, of a coordinated functioning of this „republic of reflexes" without central impulse, on the other hand it shows that the symptoms observed in the intact animals are due, if not exclusively, yet chiefly to the accumulation of faeces on their body.

> Something of the same nature has been observed by von Uexküll, who saw brittle stars moving around for days after the major part of the disc, including the stomach, had been removed.

Arbacia seems to be much more liable than any other genus to injure itself by its own faeces. This might be due to two of its peculiarities: 1. the spines of this species converge toward any stimulated point (von Uexküll) and in that way will prevent the faeces from falling down, 2. the ectoderm is devoid of cilia so that the faeces are not swept off by ciliary action. These forms are largely dependent on the washing produced by the ripples on the shore.

The substance which von Uexküll postulates as se-(ex-)-cretion of the skin of the Echinoderms, may be identical with this uric acid. The pedicellariae of the starfishes are known to take hold of every strange object whatever it may be and to hold

to it: they are the fighting members of this „republic of reflexes". Yet they will never attack any part — tissue, pedicellariae or spines — of the animal which bears them nor of animals of the same species. This phenomenon has been called by von Uexküll „autodermophily" and he explains it as the result ot a negative chemotropism towards some substance, which he calls „autodermine". Uric acid, the toxic substance present here, might be the autodermine, in fact the substance can be dissolved away by means of hot water, after which a spine of the animal itself is attacked just as vigorously as anything else. It may be however that in this case we have to do with some other substance as yet unknown; the gemmiform pedicellariae also contain toxic substances in their „poison-glands" (von Uexküll 129)), the bite of one of them may stop the frog's heart, kill the ischiadicus etc. Little animals (marine snails etc.) are killed at once. Cuénot has come to the conclusion that the toxix substance was secreted by certain glands of the skin, called „glandes mûriformes". Parker 96) could kill young animals, e.g. cats, by means of extracts of the skin of starfishes [1]).

Other experiments of Dr. Hiroshi Ohshima, unpublished as yet, in which touching the skin of Arbacia made their eggs unfit for fertilisation and, indeed, proved to be lethal for them, may furnish additional evidence in favor of my view. If one removes the skin of Arbacia from the shell and tests a watery extract of it for uric acid, one invariably gets a positive result.

Further experiments will be necessary for a definite decision.

Note: 1. There is one other phenomenon which I want to mention in connection with this chapter. I have observed it frequently but have not found it mentioned anywhere in the literature.

If one takes a starfish from an aquarium and cleans it thoroughly with a clean towel, it is as dry as dry can be. Keeping it for some time in his hand, one can observe that water begins to exsude through the skin. After a little while one observes, especially on the aboral side of the arms, long lines of water-drops. This process goes on until finally drop after drop falls from the tips of the arms. Meanwhile the arms loose their rigidity.

Whether or not this process is a normal one, I do not dare to decide. It might be due to the breaking of the papulae or other thin membranes under the internal pressure of the perivisceral fluid. It may, however, also be a process which takes place under normal conditions and then it must be a great help in excretion, unless the papulae etc. are completely semi-

[1]) Notwithstanding its toxicity the skin of the starfishes carries parasitical Protozoa and a Caprella species, which must in that way have a kind of natural immunity for their poison.

permeable. Further work will be necessary to clear this question.

2. The arguments collected by the Italian investigator Russo 110) to prove that the genital organs in Holothurians would have an excretory function, have not convinced me. It may be that one of the accessory functions (see p. 73) of these organs is to store or possibly even to excrete certain substances, but my solution seems to me to be the more probable one.

In sea-urchins Giard 45) also observed crystals of calcium phosphate in deutoplasmatic bodies in the genital organs.

24. RESPIRATION.

a. General observations.

It has repeatedly been suggested that the perivisceral fluid might play the rôle of a carrier of oxygen, of an internal respiratory medium. But this is only one of the numerous guesses which have been made with regard to respiration in Echinoderms, for no experimental work has ever been done as far as the writer knows, to obtain more definite information as to the reality of this function.

But first let us see to what organs a respiratory function has been attributed. In the starfishes we find the so called skin-gills (papulae), lacking only in one genus, Brisinga, which are supposed to have respiratory importance. On their rôle in excretion we spoke in the preceding chapter. They are little blind sacs of the body wall, in which the coelomic fluid, with its corpuscles, is kept in constant motion by the cilia of the epithelial cells of the coelom. Peritoneum and epidermis are here in contact according to K. C. Schneider; Delage and Hérouard state (p. 48) that occasionally muscle fibres are found in the wall. Probably the ambulacral feet and therewith the whole water-vascular system have a certain importance in respiration, as later on we shall see. The constant movements of these tube feet makes them very suitable for such function, and in the chapter on excretion we have mentioned other facts which make this still more probable. Their walls are easily permeable.

In many sea urchins the peristome is continued into branched outgrowths, called internal gills or organs of Stewart, two in number, situated in each interradius. Every tube foot is moreover connected by two canals with its ampulla, and cilia cause a current up on one side, down on the other side of the tube foot, thus allowing O_2 to be taken up in that way and CO_2 to be given off by the perivisceral fluid. I have already stated that some authors attribute respiratory function to the accessory intestine of the urchins, but that this did not seem to me very probable.

In the brittle stars the so called bursa is supposed to be an

organ of respiration, in which a constant current is kept up by means of cilia. In the third part of this chapter we shall speak of the occurrence of hemoglobin in Ophiactis virens.

In the Holothurians we have very definite organs of respiration, the so called water-lungs [1]) or respiratory trees in which an alternating in- and outflow of water through the cloaca can be observed. They have a more or less tree-like appearance, the stem of the tree being represented by the two main tubes opening into the cloaca. The finer branches of these gills end in rounded, thin-walled swellings, termed ampullae. The rhythm of the process of in- and outflow appears to comform to van 't Hoff's rule 18[a]). The inflow seems to take place intermittently, the outflow however in one strong current (Peach. Ann. Mag. Nat. Hist. Vol. XIII. 1881. p. 418; Ayres (Thyone). Proc. Boston Soc. Nat. Hist. Vol. IV. 1851—1854; Sluiter. Natuurk. Tydschr. v. Nederl.-Indie. Bd. XL. Batavia. 1880). They are not the chief organs of excretion, as Hérouard supposed, but phagocytes loaded with granules (excretion products) or artificially injected substances, leave the body through their wall.

Since however as we have pointed out in the preceding chapter, these „nephrocytes" leave the body by almost any route provided that oxygen is present. this is not a specific function of the respiratory trees.

One observation certainly is an argument for their respiratory function. The animals which I used were kept in a very shallow aquarium with a standing overflow. Now, occasionally several specimens could be seen to move their cloaca upward above the surface of the water and to open it to the air. This attitude is frequently kept up for a considerable length of time and has apparently some importance for respiration.

The same behavior was observed carefully by Winterstein 133), who found that it occurred especially in water which had been polluted „durch den Abgang des Exkremente".

It is not a pure air-breathing act. The lungs are kept full of water and this water is even changed every now and then — the cloaca again being moved under the surface —. The gaseous exchange between the water and the air is greatly facilitated by this procedure, however, as analogous cases show (compare e. g. the larvae of Aeschnidae and the buccal respiration of fishes. their „Notatmung"). In case of lack of oxygen the animals first contract, then they relax and faint, finally they lose all tonus and „Reaktionsfähigkeit".

One thing which certainly proves that they are not the only organ concerned with respiration, is the fact that they are so

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[1]) The water-lungs have also frequently been supposed to be lymph glands (e. g. by Hérouard) and organs of excretion (see the chapter on this topic).

frequently discharged from the body which as a rule is not done with important organs. The same thing holds true for the so called Cuvierian organs, situated in their immediate neighborhood, the function of which is as yet unknown except for their importance as organs of defence. For these organs Mines 85 and 86), has shown that their discharge takes place simply by an increased internal pressure, not by any intrinsic action of the tubes themselves.

Winterstein has studied the relative importance of the water-lungs in the gaseous exchange. He estimated the exchange in normal conditions and also when the water-lungs are put out of commission by means of a rubber condom, using Winkler's method for the estimation of O_2 50 % of the total respiratory exchange appears to go through the lungs. The fore-gut and tentacles play no important rôle, as similar experiments in which the head was covered, show.

Crozier 19) has calculated the amount of water pumped through by the large Bermudian species, Stichopus moebii. This was done by determining the amount of water pumped through in one pumping and figuring out the average number of pumpings in one day.

He concludes that 20—21 L. is pumped through per day by a large Stichopus 24.5 c.M. in length. Compared with the figures for some other animals this is fairly low. In the sponge Spinosella Parker 97) found, that by a single „finger" as much as 78 L. a day is pumped through, and for Ascidia atra Hecht 52) found as much as 173 L. a day. This, however, is not strange if we remember that in these animals the watercurrent has the double function of carrying oxygen and food, whereas in Holothurians only the oxygen is needed.

1. In this big species Crozier found a difference in P_H between coelomic and sea water, (contrary to my findings in the starfish, mentioned in the chapter on the perivisceral fluid) due to CO_2 of course. The P_H of sea water is 8.2; that of the perivisceral fluid is 7.6. The water expelled during spouting has a $P_H = 7.8$; this shows: 1. that actually respiration takes place through the lungs, 2. that the short stay of the water in the lung is sufficient for the respiratory exchange.

2. Frequently small fishes are found in the cloaca of large Holothurian species, f.i. Fierasfer in the cloaca of Holothuria.

Summarising these remarks on the organs of respiration in Echinoderms, we may quote the words of Delage and Hérouard: „L'animal respire par tous les points où une membrane mince sépare les liquides de l'économie d'une eau de mer plus ou moins renouvelée".

b. Are there catalases and peroxydases in the perivisceral fluid.

We must in the first place try to get some information as to whether the perivisceral fluid may be considered as a medium

of respiration, The studies of the last decennia have revealed the great importance of catalases and peroxidases in respiratory and metabolic processes and the question arises whether these enzymes can also be demonstrated in forms as low as the Echinoderms. The reader of course knows that, according to Schönbein (J.f. prakt. Chem. Bd. 75. 1858 and Bd. 89. 1863), their discoverer, their properties were common to all enzymes, but that at present we have distinguished them as separate enzymes.

Perivisceral fluid was secured from Asterias, Arbacia and Thyone and filtered. On these liquids the four following tests were made:

1. To a few c.c. of the perivisceral fluid some hydrogen peroxide was added. The three samples appeared to be fairly active and even after a few minutes many bubbles of oxygen were seen. This shows the presence of a catalase acting directly upon hydrogen peroxide with the formation of oxygen.

For the demonstration of peroxydases, phenolic substances are used as a substrate together with hydrogen peroxide, at least when no natural peroxide or oxygenase is present. The peroxydase produces „active" oxygen -oxygen in statu nascendi- which oxydises the substrate.

2. A freshly prepared one per cent solution of guiacum in alcohol was colored blue in contact with the perivisceral fluid and hydrogen peroxide. It took some time for the color to develop, but a very dark color resulted finally.

3. A one per cent solution of α-naphthol in equal parts of water and alcohol was colored dark lavender.

4. A one per cent solution of the same kind of benzidine became blue. A brown precipitate was formed, but only after about 16 hours.

These four tests showed convincingly the presence of both catalases and peroxidases in the three species investigated. The benzidine test gave the least striking results, it took a rather long time before the brown color appeared, but the next day a brown precipitate was always seen. In Thyone all the three tests gave a very strongly positive result.

A paper by Portier 101) in which, according to Kobert 73) many valuable data concerning the occurrence of oxidases may be found, could not be secured. Kobert found catalases to be present in a great many insects, spiders etc., even in their eggs and in alcoholic specimens etc., also in living Ascarids, and he is of opinion that they occur „in allen lebenden funktionsfähigen Zellen". In the blood of fishes, some worms, Sipunculus, Octopus and Eledone he also found them present, in Eledone and Octopus even if the corpuscles were removed. Oxidases also occur in the blood and organs of a great many invertebrates, as Giard (C.R. Soc. Biol. T. 48. 1896. p. 483), Pieri and Portier (C.R.

Acad. Sc. Paris. T. 123. 1896. p. 1314), Abelous and Biarnès (C.R. Soc. Biol. T. 49. 1897. p. 175 and 249) and Portier 101) have shown. In Kobert's paper I found an indication, that the latter author had found them in Echinoderm blood; unfortunately I was not able to consult the original paper.

What their exact function is in all these groups, nobody can tell. We can not say, whether the catalases serve only as immune bodies against the highly toxic hydrogen peroxyde — toxicity due to the fact that it causes air embolisms —, as Oskar Loew supposed (Catalase. U.S. Deptm. of Agriculture. 1901. Rep. No. 68. After Kobert), or whether the peroxide-theory of A. Bach (Du rôle des peroxydes dans les phénomènes d'oxydation lente. C.R. Acad. Sc. Paris. T. 124. 1897. p. 251) and of Engler and Wild (Ueber Sauerstoff-activierung. Chem. Ber. Jahrg. 30. 1897. p. 1669. Both after Kobert) holds true. The latter theory assumes the formation of peroxides as the primary stage in every combustion of organic substances in the living organism and their destruction by catalase. It is certainly not a measure for metabolic activity, according to Morgulis 88).

Note. 1. The so called melanosis in the blood of catterpillars etc. has also been supposed to be due to peroxydases. Here we surely have to do with a tyrosinase according to Biedermann 8).

2. The results of Portier who found oxidases to be present nearly everywhere have partly been denied by Kobert. For this reason a repetition of his work does not seem superfluous.

c. Contents of the Polian vesicles of Thyone. Importance of the water vascular system in respiration.

The writer 60) was struck by the brilliant red color of the Polian vesicles of Thyone each time that he dissected a specimen. Curiosity drove him to examine this phenomenon a little more closely though it did not seem to have any direct bearing on the problem in hand.

In Thyone briareus these vesicles which form as we know a part of the water-vascular system, are present in numbers varying from one to four. Scott 118) has studied their anatomical relations a little more closely and just in this particular species. He finds that usually one is present, often two, occasionally three and rarely four. The general tendency for them is to go to the left side, and thus to disturb the bilateral symmetry. The retractor muscles of course vary with the number of the Polian vesicles. In size as well as in number there is a considerable variety.

The Polian vesicles form, as mentioned above, a part of the water vascular system and therefore they are on the one hand in connection with the ambulacral feet, which are found all over the outside of the body. At the inside of the muscular body

wall these podia are represented by their ampullae and remarkably enough, all these little ampullae can be seen on dissection to contain the same colored material. On the other hand this system communicates freely with the coelomic fluid. The stone-canal stiffened by carbonate of lime, which in other Echinoderms and in larval Holothurians communicates freely with the sea water, forms here the so called „internal madreporites", of which there may be one or even five, as in Holothuria tubulosa. Furthermore the water vascular system stands in connection with the tentacles, the tree-like feelers, which by their active movements may assist greatly in respiration.

The Polian vesicles are generally supposed to be the regulators of the internal pressure of the water-vascular system. Their wall contains muscle fibres and by a slight contraction of these fibres the tentacles may be extended very easily.

It seems however that it also plays a rôle in respiration. The red material namely appears to be *hemoglobin*. The color is due to the contents; if one cuts a little hole in the wall of the vesicle, the color „flows away". It can be centrifuged off and a microscopical examination of the fluid reveals that the color is bound to corpuscles.

These corpuscles look under the microscope very much like mammalian blood-corpuscles. They do not exhibit any active movements as far as could be observed, have the same yellowish color that is characteristic for the erythrocytes and appear to be perfectly spherical. A nucleus is in most cases clearly visible, the protoplasm has a granular structure and a cell membrane gives them a perfectly sharp outline.

Hemoglobin is known to occur in many invertebrates. Though it was originally thought to be characteristic for the vertebrates, it has since been found in almost all groups of the animal kingdom. Even in animals as low in the scale of phylogenesis as the worms, we find it in many forms, either in the coelomic liquid (common earthworm) or in corpuscles (Glycera, Capitella, Phoronis, etc.). In molluscs and arthropods it is generally found in solution. It is chiefly found in mud-dwellers or, to put it more generaly, in animals, living in a medium where oxygen is scarce. The larva of Chironomus is a classical example.

Our knowledge of the occurrence of respiratory pigments in Echinoderms is very incomplete. In one case, in the brittle star, Ophiactis virens, hemoglobin has been found by Foettinger 39). Here it is also present in the tubes of the water vascular system and in the Polian vesicles, two of these being present in each interradius here. It is bound to spheroidal corpuscles, which are sometimes flattened and eventually may have the form of discs. By means of different chemical reactions, Foettinger demonstrates that we have to do here with hemoglobin The same pigment perhaps is also found in a species of Ophiolepis.

The presence of these corpuscles in the water-vascular system in both these species, clearly demonstrates the respiratory importance of the latter. Probably this is due to the fact that it connects all the tube-feet which surely have respiratory importance.

Other statements with regard to respiratory pigments in Echinoderms, e.g. the assumption of such a function for the so-called echinochrome (Mc. Munn. 1885. 92)) in Echinus and Strongylocentrotus, are to be accepted with some reservation, Winterstein e.g. could demonstrate, that a solution of echinochrome did not take up any more oxygen than the same amount of sea water.

The red pigment in Thyone seems to be hemoglobin. The following tests have led the author to this conclusion:

1. A small quantity of the liquid was dried on a slide, heated a few times under cover-glass, until bubbles appeared, then cooled. Blue black prismatic crystals could be seen which though not absolutely congruent with the figures of the text-books of biochemistry, were doubtless hemin crystals.
2. To a solution of one or two drops of 1 % benzidine in a large quantity of hydrogen peroxide a few drops of the „blood" were added. A dark blue color developped at once and a foaming to way up on top of the test tube could be seen (peroxidase action).
3. Laked and centrifuged „blood" showed the typical absorption spectrum of oxy-hemoglobin—one broad band in concentrated solution, two on dilution, one of which can easily be identified with the D-sodium line.
4. On reduction with Stokes' reagent the single band of hemoglobin could be seen. After continued vigorous shaking, the double band of oxyhemoglobin could be produced again.
5. A small quantity of the material gave a positive prussian-blue test for iron.
6. Material treated with KCN and kept in the 37° incubator for some time, became orange-yellow and showed the absorption band in the green which characterises cyanhemoglobin.

From this evidence, I have concluded that hemoglobin is present in these corpuscles. This fact is very interesting in connection with the fact that Thyone is a mud-dweller and thus lives in a medium, in which oxygen is scarce. Though there is still a diversity of opinions as to whether oxygen acts as a *store* of oxygen or not, it is beyond all doubt that the presence of a respiratory pigment like hemoglobin is a great help in the struggle for oxygen.

In how far the Polian vesicles act as „movers" of the corpuscles, as a kind of very primitive heart in that way, or as formers — these organs have frequently been called „lymph-glands" (Cuénot) —, could not be investigated. The same cor-

puscles occur however not only in the water vascular system, but also in the wall of the water-lungs e. g. In a preparation of the tip of one of these organs between cover-glass and slide, cells were seen which seemed to be identical with the cells in the Polian vesicles.

Thus they might also occur in the „blood-system"; and so in all the three fluid systems, viz. the water vascular system, the perivisceral fluid and the blood system.

25. RESERVE SUBSTANCES IN ECHINODERMS.

It has frequently been assumed in the previous chapters that the Echinoderms live from day to day, so to say, resorbing with surprising rapidity everything that comes into their perivisceral fluid and constantly starving and hunting for food. For this reason it seemed desirable to make sure whether they are perhaps in the possession of many reserve substances available under such circumstances.

As far as the fatty reserves are concerned, I made some experiments in order to ascertain whether fats do occur at all in the starfish and, if so, in what quantities. I made these experiments for a double purpose, in the first place for the reason mentioned above, in the second place however in order to elucidate the question of the digestion of fats, discussed from another angle in a special chapter. The presence of fats in certain animals of course, as stated before, does not necessarily prove the presence in them of steatolytic enzymes, these may be derived from other sources, from carbohydrates or even from proteins. But nevertheless it makes their presence more probable.

In order to determine the quantity of fats present in one starfish, I extracted two specimens with a mixture of alcohol-ether. The arms of one specimen and its disc suspended in a filter-paper cone, were extracted in a soxhlet till the extraction fluid was watery clear, after this another specimen was treated in the same way.

The liquid thus obtained appeared to contain a large quantity of pigment. Not all the pigment was dissolved away from the animal though, a small quantity seemed to be deposited in some way in the calcarous skeleton. In to get rid of the pigment, the liquid was boiled for some time with charcoal, then filtered. The charcoal was again extracted with cold ether, this ether was added to the rest of the extract; the treatment was then repeated, again with a fresh quantity of charcoal.

By repeating this procedure numerous times I succeeded in getting rid of all the pigment. Whether or not I lost any fat, I do not dare to decide, but it does not seem to me probable.

The liquid was now gradually evaporated in a large watch-glass over a water-bath. The residue which did not seem to

contain much pigment, was dried in an exsiccator and weighed. The quantity of fat present in two large, freshly caught specimens, proved to be 103 mgr. But 51.5 mgr. of fat per starfish seems to be an exceedingly small quantity, especially when we take into consideration that part of it may have been pigment.

In Holothurians also Pütter 105) found a very small quantity of fats. According to his figures 0,014 % of the wet weight of Cucumaria grubei consists of fats and lecithins. This means that in an average specimen of this fairly large species 203 mgr. of these substances are present. This figure is also very low and might represent only the lecithins of the nervous system and some pigment. It need not be mentioned that in our figures the lecithins, of course, are also included.

A second group of reserve substances are the carbohydrates, generally represented in the animal kingdom by glycogen. This substance present in almost all invertebrate groups, is wanting in starfishes. To make sure of this I treated several sections of the radial sacs of these animals, made in the usual way — alcohols, paraffin — with J in KJ, after the paraffin had been dissolved away with xylol, the xylol replaced with alcohol and the alcohol with water. Whereas in many other invertebrate groups, as f. i. in snails — see Biedermann and Moritz 9) —, the „liver" contains an abundance of glycogen, so that every section immediately shows the typical glycogen reaction, nothing of the kind occurs here, the reagent failing to give any indication of the presence of glycogen. Krukenberg also searched in vain for glycogen in the starfishes [1]).

A similar test was made on the guts of Thyone fixed in several different ways. Here every section gave a strongly positive test. This explains at the same time the nature of the „insoluble carbohydrates" found by Pütter 105) in his Holothurians. Not less than 15.8 % of the dry weight of his Cucumaria is insoluble carbohydrate.

A third class of reserve substances are the proteins. We find protein crystalloids in many invertebrates of different groups, from the flat worms (Saint-Hilaire 113)) up to the highest and in many cases they have been considered as reserves. These protein crystalloids are also found in Echinoderms. They occur in the amibocytes (Wanderzellen) of the sea-urchins, where they were discovered by Cuénot 23). In the same cells we find numerous grains of pigment. These pigments are produced, accor-

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[1]) Stone 125) also failed to find glycogen in the starfish radial sac; she extracted them by means of Pflüger's method and did not obtain any evidence of its presence.

ding to the general opinion by accumulation or transmutation of waste-products. The origin and fate of these protein-crystalloids has been studied in detail by List 81). They are very regular crystals, generally hexaeders or rhomboeders, which show the typical reactions with J in KJ, with eosin, Millon's reagent and picric acid. They are found in the nuclei of the corpuscles, also in those of the cells of the middle-gut, as in many other cases (see [1]), and occur especially in the „amibocytes incolores" of Cuénot. In the middle-gut of Tenebrio they are found not only in the nucleus, but also in special inclusions of the cell-body, reminding one of the aleuron-grains in plants and even free in the cell protoplasm. More than one crystal is never found in our urchins, they are preceded by small, round bodies (protein vacuoles?) which later on unite to form larger ones. Whether or not these crystalloids actually are reserves, is not clear from this research; it is the opinion of the author that „nach den am lebenden und conservierten Materiale gemachten Beobachtungen, es sehr wahrscheinlich ist, dasz die Krystalloide sich schliesslich in Pigmentkörner umwandeln".

All the evidence collected in this chapter shows clearly that large amounts of reserves are not present in our group. Lack of time has prevented me from investigating this point more in detail.

26. GENERAL METABOLISM.

Though I do not have to offer any experiments of my own in this chapter, it has been added because as I said in the introduction, this paper aims to give not only original work, but also a review of the literature on the subject. Moreover some critical remarks must be made on one of the most paradoxical modern theories on nutrition, on Pütter's theory of the nutrition of marine animals.

Two papers in which an attempt is made to study the metabolism of the Echinoderms have come to my attention, one by Cohnheim 17) and one by Pütter 105). Both concern themselves with dendrochirote Holothurians, Cohnheim works on Holothuria tubulosa, Pütter on Cucumaria grubei.

Cohnheim's paper is of a very preliminary nature, in fact, it does not contain much more than a few experiments on the CO_2 cutput. His experimental procedure is of the simplest kind; he puts some animals in a stoppered bottle having two outlets. O_2, purified by NaOH, is bubbled through the apparatus. The outcoming air is led through baryta and the amount of CO_2 produced estimated by titration.

The output per K.G. hour appears to be somewhere in the neighborhood of 0.147 gr. In the case of Ophioderma longi-

[1]) See e.g. Joh. Frenzel. Berl. Ent. Ztg. Bd. 26. 1882. p. 267—361.
C. Rengel. Zs. wiss. Zool. Bd. 28. 1896. p. 1—60.
P. Mangazzini. Mitt. Zool. Stat. Neapel. Bd. 9. 1889. p. 1—60.

cauda a somewhat larger amount is found: 0.228 gr. The relatively low CO_2 output in the Holothurians is doubtless due to their lack of extensive and rapid movements. In the experiments the animals did not move at all and even in their natural environment they are entremely sluggish. Cohnheim also warns against comparing the metabolism, measured by the CO_2 output, of representatives of different groups of the animal kingdom. It may be justified to compare two mammals e. g., but it is absolutely impossible to compare an animal with a calcareous or chitinous skeleton with a jelly fish which has only 0.24 % dry weight or a Holothurian the major part of whose weight is formed by the perivisceral fluid.

The paper by Pütter is doubtlessly more „gründlich", but I regret to say that in my opinion it contains some very serious errors, which I will discuss briefly. I believe that it is justifiable to give serious attention to the work of this author because his conceptions on the nutrition of sea animals are so excedingly strange that they are either fundamentally wrong or will revolutionise all our conceptions on nutrition.

Serious criticism has also been made against his methods and the „superstructure" built by him on a very weak experimental basis. While his whole theory tries to emphasise the importance of dissolved C- and N-compounds as a source of food for most marine animals, in all his papers we only seldom find estimates of the amounts of such substances present. And the few estimates he has given contain a very serious methodical error as the work of Moore 87) and others has shown.

He digests sea water with sulfuric acid for the determination of the dissolved carbon. The hydrochloric acid which is set free in heating sea water with sulfuric acid, is passed through a wash-bottle containing lead acetate for the purpose of absorbing it. This is the serious error; the acid is not only bound and kept back by the lead acetate, but also sets free acetic acid which after combustion is interpreted as „dissolved organic carbon" of the sea water. In his O_2 determinations an error of the same kind is made in which the chlorides present in the sea water are not taken into account.

But for the present we shall take the methods of Pütter as methodically justified and shall confine ourselves to a criticism of his theoretical considerations.

The paper as I have said, deals with Cucumaria grubei, a dendrochirote Holothurian, rather closely related in that way to our Thyone. Pütter first determines the general composition of the animal by means of a simple, general analytical procedure. The water content is 78,8 % of the total weight. 5 % of the dry weight is extractive materials (inorganic salts etc.), 17.1 % soluble carbohydrates, 5.6 % fats, 1.2 % lecithins, 15.8 % insoluble carbohydrates and 55.2 % proteins.

The same determinations are made with animals that have been starving for six months. All dry components have dimished, except for the „insoluble carbohydrates"; the ratio of C : N which was 1 : 5.1 at the beginning, appears to be 1 : 5.6, so that the nitrogenous constituents have diminished more than the carbon compounds.

The loss of weight is determined for every month and in combination with the above mentioned data he finds that the animal loses on the average 0.0155 mgr. C and 0.00327 mgr. N in an hour.

The author then determines the oxygen consumption. This appears to be dependent on three factors: light (!), temperature and physiological condition. The temperature relation is quite clear; not clear however is the influence of light and the fact that animals which have been starved for three month consume about twice as much oxygen as before starvation.

Carbon is ex-(se-)creted in four different ways:

1. As CO_2, partly dissolving in the sea water, partly disappearing in the air.
2. As a volatile hydrocarbon, probably CH_4 (methene).
3. As formed material, e.g. as mucus and cells.
4. As complex watersoluble carbon-compounds.

These four factors are all studied quantitatively. CO_2 and the hydrocarbon are very regular, the two other factors very irregular. The respiratory quotient is *very* high in the beginning (about 3.8), later on it is about 0.8.

The total C-production is now determined and the relative importance of the three groups. The fresh animals secrete 1.64 times as much C. The nitrogenous excretion is also studied. The production of nitrites and nitrates is quite regular, but the Kjeldahl-N shows no regularity whatsoever. Average values are used. The irregularity may partly be due to the excreted cells which come very irregularly.

Since in the combustible excreted gases more H is present than agrees with the formula CH_4, it is evident, that free H_2 is also excreted in small amounts.

The quantity of „volatile acids" and „volatile alkalies" excreted is also determined.

From all these data the author tries to get an impression of the „total metabolism". Here he enters into the field of speculation.

His „point de départ" is the total N-metabolism, figured from the N-excretion. On the assumption that this represents the protein consumption. he finds the corresponding C-production. The remaining C is now divided up in several ways, an ingenious and clever system, based on various assumptions and facts.

Let us first consider the fundamental figure of the whole

system and this in connection with other statements made by the same author a little further on.

The author postulates that *nine tenths* of the C which has been produced by the animals is derived from the soluble carbonaceous constituents of the sea water which have been used as food. This also seems to be the case for the nitrogen, but such substances account for only 60 % of the total N-metabolism however, 40 % comes from the proteins of the body.

Now here we are: at the very basis of his theoretical considerations the N-excretion is supposed to be the product of the protein consumption. On this basis figures on the C-metabolism are secured which finally after many detours lead to the above mentioned conclusions. This is apparently a circulus vitiosus, an escape from which does not seem to be very easy.

Confining ourselves for the present to the N-metabolism, we find another discrepancy. From the fact that in the metabolism experiments the ratio of C : N always remained within the range of 1 : 27 and 1 : 30, whereas in the figures on the composition (see above) this ratio is between 1 : 5 and 1 : 5.6, „folgt schon ohne weiteres dasz Stickstoff erspart wird, indem stickstoffhaltige Abbauprodukte mit Hilfe aufgenommener Kohlenstoffverbindingen wieder zu Körperstoffen umgearbeitet werden."

If this is true — I do not feel capable of forming a definite opinion on this subject — we see another fundamental mistake in Pütter's logic. In that case more C than the quantity corresponding with a certain amount of N according to the empirical protein formula, must be produced together with that amount of N.

Here are two errors in the fundamental figure. If actually nitrogenous water-soluble substances are taken up from the sea water in order to play a rôle in the N-metabolism, they may have any composition, but that of proteins. The amount of CO_2 resulting from the decomposition and combustion of these substances may have any desired ratio to that of the Kjeldahl-N and not that of the proteins. At the same time more CO_2 than one would expect is obtained by means of the N-saving system, postulated by Pütter.

From this evidence we see that the conclusion: „Es bleiben also 141.2 mgr. Kohlenstoff übrig, die nicht aus Eiweisz stammen", is excedingly questionable and that the speculations based on this assumption, are to be taken with the greatest reservation. It is of no particular use to go into details about them, it may only be mentioned that the assumption of a „Methangärung" and a „Buttersaüregärung" of sugar as a source for the observed CH_4 and H_2 is a rather arbitrary one and that their analogies in mammalian physiology are at their best very hypothetical, as I mentioned in the foot-note of p. 50.

Pütter's deductions are also weakened by the following con-

siderations. There is still another thing to be thought of, suggested by a paper of Bohn 10). This eminent French biologist shows that starfishes can produce O_2 in the daylight. The enormous quantity of pigments, present in Echinoderms (von Fürth 138), p. 518) might have one among them with respiratory importance. The same thing had previously (C.R. Acad. Sc. Paris. 19 Oct. 1908) been demonstrated by the same author for certain actinians (A. equina, Sagartia erythrochila), even when they do not have any symbiotic algae. It seems, according to him, to be a general property of lower animals. In oxygen-poor water the starfishes suffer in the dark, even as far as to autotomise their arms. In the same water in diffuse daylight there is produced in five hours as much as 1.4 mgr. of O_2 in excess of the quantity consumed by respiration; the animals are perfectly normal. In supersaturated sea water much more O_2 is consumed in the dark than in the light. Several controls have been made so as to exclude the action of chlorophyll-carrying microorganisms, of an incidental introduction of O_2 and of a sick condition of the animals used, but the same result invariably is obtained. Starfishes need much oxygen and suffer in water which does not contain more than 3 mgr. of oxygen, but they endure large quantities of potassium cyanide, a property which Bohn, Drzewina and others always found in animals with anoxybiotic respiration.

Unexpected as this result may seem, it is made highly probable by the few, but critical and sufficiently controled experiments given. In the beginning of this chapter I mentioned that Pütter finds in his Holothurians a big difference in CO_2 production in the light and the dark. This phenomenon has its minimum in the freshly caught animals, as we can see from the table 14. The figures in this table indicate the oxygen consumption.

Table 14. From Pütter.

	In dark.	In light.	Ratio.
1st month.	31	31	1 : 1
2d month.	61	38	1 : 1.6
3d month.	60	23	1 : 2.6

It is, as Pütter says, hard to imagine that the metabolic processes can be influenced to such an extent by light; analogies from other parts of the animal kingdom fail, unless we assume, that also by these Holothurians O_2 *is set free in the light in some way.*

The possibility of the presence of symbiotic algae does not seem to be very great. The only thing that is left, is the active

production of O_2 in some way by the cucumbers themselves. Whether this is a phenomenon of the metabolism of the species or whether respiratory pigments of the nature of chlorophyll and numerous others simply decompose CO_2, is a question into which we can not and do not have to enter. I could not perform any experiments on this question myself, because Pütter's work only came to my knowledge after my experimental work had been completed. The latter assumption however has its peculiar charms, especially when we remember that analogous facts have been established for the starfishes.

Pütter's calculations are based on CO_2 production, CO_2 is one of the most important sources of C in his calculation of the „Gesammtkohlenstoffausscheidung" and the total carbon metabolism. On these figures his whole system of analysis of the „total metabolism" of Cucumaria is based. If this second corner-stone of his building appears to be weakened, if in fact the O_2-production in the light is important enough to influence the respiratory quotient in a measurable degree — and the above mentioned evidence seems to make this probable —, the whole system falls down and the conclusions are no longer logical postulates. This is also true of his assumption of water-soluble C- en N-compounds in the sea water as a source of food. The work of Moore and his coworkers has shown this very clearly. In the beginning of this chapter I had occasion to call attention to one of Pütter's serious methodical mistakes.

But determinations even with these wrong methods are very scarce in the work of Pütter. Several facts are taken for granted from the work of other authors. One of the most striking examples is his assumption of the presence of a fatty acid, dissolved in the sea water. Here he quotes Natterer who in 1892 succeeded in destilling a „ganz geringe Menge" of a substance, resembling palmitin or stearin by its smell when heated, from 200 L. of sea water. Pütter quotes this, but does not try to repeat the experiment.

From destillation experiments Pütter concludes that 36 mgr. of such acids are present in one Liter of sea water. But he does not try to study the nature of this relatively enormous quantity of acids more in detail.

It is true that many circumstances make estimations of this kind particulary hard, chiefly the presence of a large quantity of chlorides and CO_2 which are normal constituents of the sea water. Henze (Pflüger's Arch. f. d. ges. Physiol. Bd. CXXIII. 1908. p. 487), however, using appropriate methods — taking a tube of metallic antimony and a glowing tube of lead chromate and copper oxide to prevent hydrochloric acid or other Cl-compounds from reaching the CO_2 apparatus, concludes that the amount of organic material present, falls within the limits of error.

The same results have been obtained by Moore c. s. with another method. Knowing the scarcety of Pütter's own experiments, we may conclude that there are many reasons to be doubtful with regard to his hypothesis, and that for the Holothurians we certainly have many reasons of doubt of various nature.

I do not flatter myself that these few remarks disprove as general an hypothesis as the one of Pütter. But I may have succeeded in pointing out some weak spots in his building.

Addendum after the paper had partly been printed:

Prof. Dr. Withrow Morse who had the kindness to look over the manuscript called my attention to the fact that the enzyme which I studied in the starfishes shows more similarity to *erepsin* than to trypsin. In fact, the former enzyme digests „native proteins", albumins and globulins to amino-acids. Trypsin, according to the modern conception, works only on lower peptides, proteoses and peptones, prepared by the gastric pepsin.

As far as the control experiment on p. 20 is concerned, we must remember the Merck's „pure trypsin" is made from extracts of the pancreas and activated by enterokinase from the gut. The latter is mixed with intestinal erepsin.

The P_H-range of really pure trypsin is very limited. Cf. the recent papers of Mc. Clendon and Dernby in the J. Biol. Chem. and the very recent work of Northrup, just published in the J. gen. Physiol. and the J. Biol. Chem.

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PRINCIPAL ERRORS IN THIS PAPER:

p. 1. line 20. we will . . . . must be: we shall. This error occurs on several places between p. 1 and p. 48.

p. 1. line 20. from where . . . must be: whence.

p. 1: line 22. till now . . . . must be: until now.

p. 2. line 8. f. i.. . . . . must be: e.g. This error occurs on several places between p. 1 and p. 48.

p. 3. line 7. came . . . . . must be: have come.

p. 6. line 14. reminding weakly. must be: reminding one slightly.

p. 6. line 28. they can close . , must be: they close.

p. 11. line 3 from below. fishers must be: fishermen.

p. 13. line 2. rifs . . . . . must be: reefs.

p. 13. line 27. Has a Thyone . . must be: If a Thyone has.

p. 13. line 36. pro . . . . . must be: per.

p. 18. line 26. which . . . . , must be: whom.

p. 28. line 26. alive . . . . must be: live.

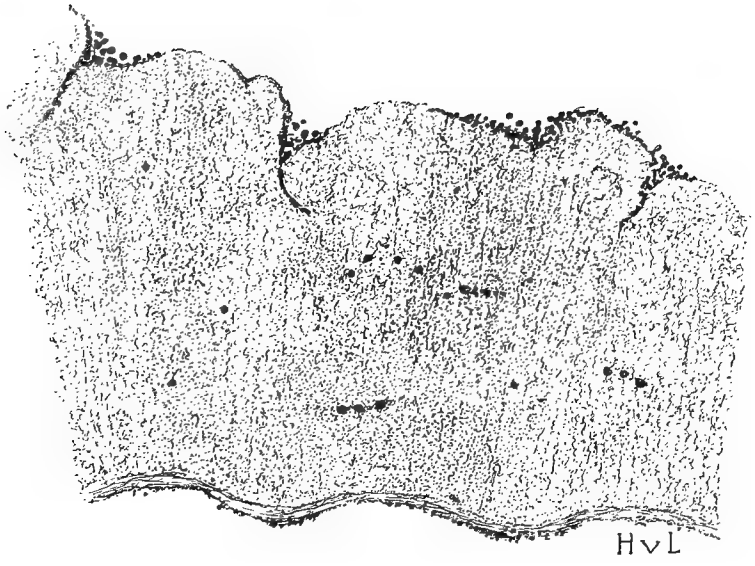

Fig. 3.

Phagocytosis in the gut-epithelium of Arbacia?

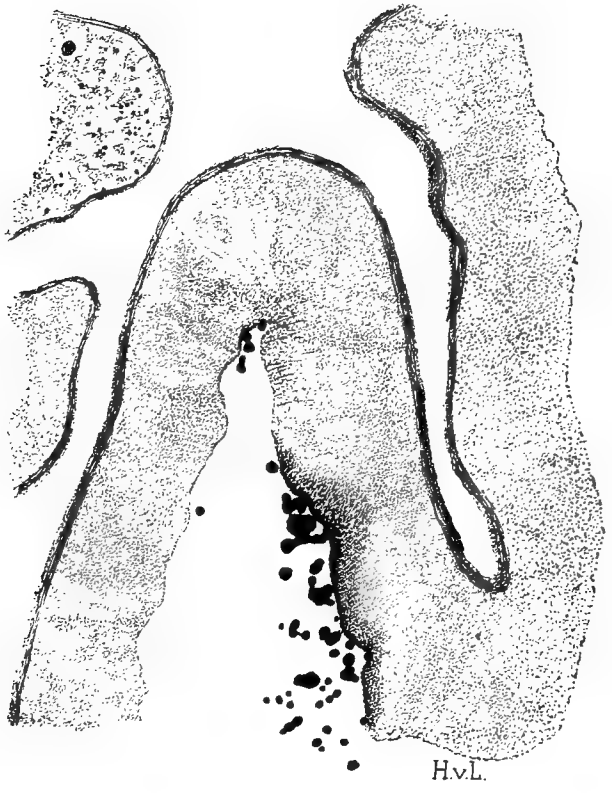

Fig. 5.

Resorption of iron in the radial sacs of Asterias.

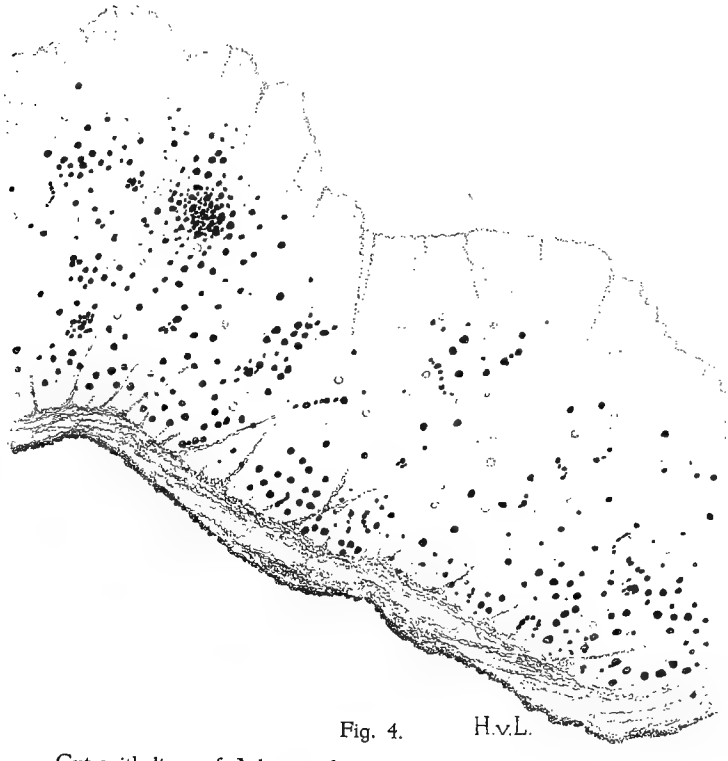

Fig. 4.

Gut-epithelium of Arbacia after fat-feeding. Flemming-paraffin.

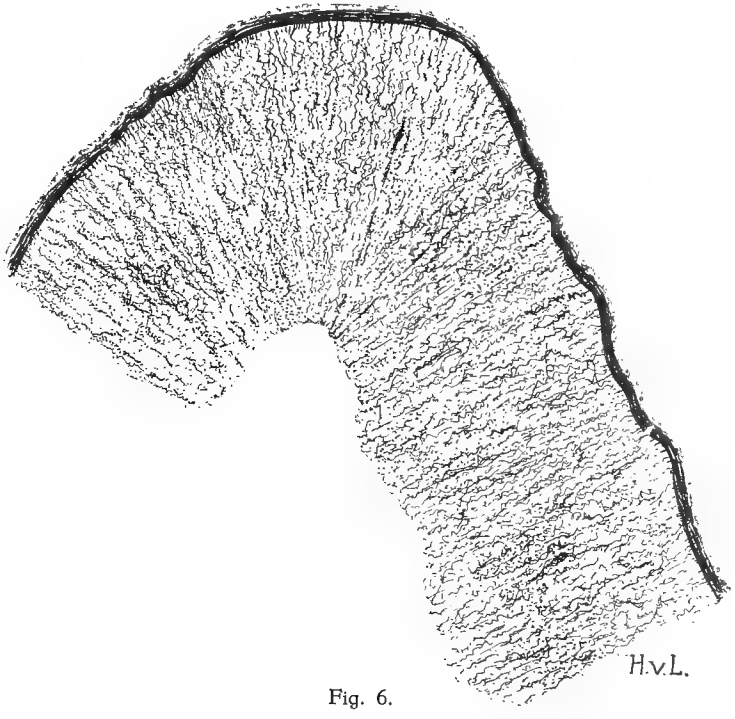

Fig. 6.

Resorption of ammonium carminate in the radial sacs of Asterias.

STELLINGEN

I

Men mag met Broman aannemen, dat het organon vomero-nasale Jacobsoni het voor het landleven gemodificeerde „Wassergeruchsorgan" der Anamniota is en slechts werkzaam kan zijn, als de reukstoffen door een waterig vloeibaar medium het zintuigepitheel ter plaatse bereiken.

II

De opvatting van Austin H. Clark, dat de Echinodermen aberrante Arthropoden zijn, is onjuist.

III

Bij de resorptie van de staart der kikkerlarven speelt autolyse een primaire rol.

IV

De natuurlijke immuniteit van het konijn voor atropine is niet te danken aan de eigenschap van het bloedserum van deze dieren om dit alkaloïd te binden en daardoor physiologisch onwerkzaam te maken.

V

Er zijn geen bewijzen, dat ideeënvorming voorkomt bij sub-anthropoïde dieren; door gewoontevorming kan wel een „Komplexqualität" in „Teilqualitäte" worden ontleed.

VI

De boleten behooren niet tot de Polyporeeën, maar zijn Agaricaceeën.

VII

De komplex-theorie van Renner geeft geen voldoende verklaring voor de resultaten van het Oenothera-onderzoek.

VIII

Vele planten-gallen vertoonen structuren, die duiden op „fremddienliche Zweckmässigkeit (Becher)".

IX

De reconstructie van den schedel van Eoanthropus dawsonii door Smith Woodward is onjuist.

X

De mammoeth was niet voldoende aangepast aan de kou en is onder den invloed van het ruwe klimaat van den ijstijd gedegenereerd en tenslotte uitgestorven.

XI

De zgn. wet-Limburg maakte als „nood-wet" een eind aan onrechtvaardige verhoudingen. Te betreuren is het echter, dat zij bewerkt heeft, dat een zij het dan ook elementaire klassieke opleiding niet langer een voorvereischte is voor universitaire examens en het is gewenscht, dat ten spoedigste een herziening plaats vinde.

XII

De vergelijkende physiologie dient ten spoedigste te worden ingevoerd als officieel leervak aan onze universiteiten.