The Internal Mechanism of Life.

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Until rather recently, in fact less than 200 years ago, it was considered sinful—at least in the Western World—to pry into the intricacy of life. We may recall here an old solemn warning: Do not tempt, mere man, the wrath of gods. Of special interest is also the conviction, expressed by Kant, possibly the greatest western philosopher and leading sage of his time (in the 18th century), that the limitations for study are drawn at the outer borderline of life and that the understanding of its mechanism would likely for ever be closed to the human mind.

But man would not be stayed, neither in dreams nor in action, in his attempts at least to understand,—if not to master—, life and its mechanism. With the realization and comprehension, that blood flows in most animated forms of life, the gate was opened for further inquiry into the nature and working of body organs and tissues.

Physical Means.

The greatest single contribution, permitting internal visibility of cell structures, is the application of X-rays to medicine. (I like to think back to a thrilling experience of my younger days, when my right hand, by a lucky coincidence, served the discoverer of the rays, Professor Roentgen, in one of his lectures to demonstrate the penetrating character of these rays to the student body). In spite of all attempts, however, the visibility of internal tissues remains but a contrast of shadows, which requires—in disease—the interpretation of the experienced specialist. The advantage of X-rays is greatest in locating bone fractures, foreign bodies, as pins in stomach or lung, and calculi such as kidney or gall stones in bladders or ducts. Its use is always limited by the danger of tissue destruction through over exposure, and by the high cost of the special equipment needed. With strong lights,

suitably mounted, some tissues, such as fingers, ears and especially also embryonal units, will disclose a certain amount of internal organisation such as capillaries and bloodflow, (as in the toe membranes of frogs). Especially photography with infra-red and ultra violet light has been a helpful tool in increasing the visibility of some internal living structures.

As a further aid in observing internal organs or tissues directly, a flexible spyglass, called "gastroscope" has recently been developed by Dr. Schindler,[1] Professor of gastroscopy at the University of Chicago. This new instrument is said to permit the close inspection of the inner stomach wall, to assure positive diagnosis of the most common stomach disease, gastritis, and to assist in the detection of certain lesions, as well as of cancer of the stomach (the latter held responsible for the death of annually 30,000 people in the United States of America alone. The gastroscope consists essentially of a special flexible, 2 1/2 feet long tube with a tiny electric bulb, inserted above a finger-like rubber tip; the tube is inserted through the anesthesized throat into the stomach, after its contents are siphoned out. A front lens, called eyelet, collects the images, reflected from the wall of the air-inflated stomach, and transmits the images by means of 48 additional lenses, set at short intervals in the tube, to an eye piece for study.

Chemical Means.

Organs and tissues may be made visible by means, such as the one elaborated by Professor Spalteholz, University of Leipzig, who worked out the most successful procedure as follows: The animal, if alive, is chloroformed; the skin with hair is removed, unless a young embryo without hair development is used. The tissue is hardened in formaldehyde; if necessary, freed from the lime of the bones with dilute acid; then bleached with hydrogen peroxide; dehydrated with alcohol of increasing concentration; then placed in anhydrous benzol, and finally in a clearing mixture of wintergreen oil and benzylbenzoate. These liquids, with the refractive index of the animal tissues, permit permanent mounting of any animal tissue

and organ of so-called higher animals, as well as especially of whole embryos and of older animals of all stages of development. For quick temporary effects of clearing of animal and vegetable material anilin and phenolic acid have proven useful.

But, alas, by these chemical means,—as life is extinguished, only the location of organs, and the distribution, as well as certain structural differences, may be observed and demonstrated,—never the actual mechanism.

Physiological Means.

Plasma Motion.

Hoping to find a living unit in nature, suitable for the study and demonstration of the internal mechanism of life, I began a rather elaborate search, recorded in a survey on "Transparent Life," published almost 10 years ago [(2)]. I found no ideal representative, thus far in plant life. Waterplants of simple internal structure permit visibility of certain functions such as eelgrass (Valisneria), showing protoplasma movement best in the wide elongated internal cells of the leaf. Elodea shows this movement of plasma and of the chlorophyll-grains and the nuclei, carried along with the stream, most distinctly in the elongated cells of the midrib leaf area.

Blood Motion.

The streaming of blood may readily be observed in the webb of frog legs and in the tails of many fish (e. g. gold fish), disclosing in favorable specimens even the corpuscles moving by.

Windows in Living Tissues.

Dr. and Mrs. Clark, anatomists of Philadelphia, Pa (U. S. A.) succeeded in making windows in rabbit ears for the observation, (—though restricted in scope—) of certain dynamic living reactions in living tissue [(3)]. As a result of well over 1,000 experiments I finally succeeded in preparing windows in leaves of ornamental house plants. Thus the process of tissue regeneration was made visible.

Daphnia magna, Remarkable Transparent Animal.

In my search for a living animal creature, comparatively highly organized, and yet with a visible internal mechanism, I found very

few transparent forms, that might well be called freaks of nature. Among these the crustacean Daphnia, and among them the species magna, the largest known, stands out as a unique representative.[4] Increased familiarity through breeding and study for a period of 15 years has strengthened my confidence in the usefulness of this organism as a reagent in following the internal mechanism of life.[5] (See Plate I).

Breeding: Daphnia magna, with its body 3-4 millimeters long, and, with outstretched antennae or swimming arms, even approaching 1/2 centimeter in length, is a scavenger. It abounds in water, rich in organic matter and polluted with protozoa, algae and bacteria, serving it as food. It can be readily bred on the principle of a moderate but steady and suitable food-supply of organisms, living in a culture medium-like environment. A drop of blood or serum added daily to a quart of chlorine free-water, kept alkaline with marble, and inoculated with the desired flora of the above organisms or of another daphnia culture, will suffice 100-200 daphnia a day and permit their growth and reproduction. Equally soybean, cottonseed meal or dilute suspensions (0.1%) of manures will, under careful control, permit the propagation, always providing that 1. an excess of gases as chlorine, hydrogen sulphide and carbon dioxide is removed, that 2. oxygen is available and that 3. the reaction remains slightly alkaline, 4. the osmotic pressure of the medium is kept low, 5. other water animals such as hydra and worms are removed, should they occur in the water-supply, 6. that the strain used is adjusted to the temperature, selected for standardized cultures, and 7. that the daphnia are frequently (at least once a month) transferred into a freshly made culture medium.

By the process of parthenogenesis we can assure the availability, every 6-7 days, at the temperature of 30° av. prevailing here, of new young, reaching motherhood, and bearing from about 10 to over 50 (in the older animals) of young, followed within 2-3 days by another brood, until, after approximately 18 broods, life is terminated. Daphnia provides us thus with an unusual number of uniform experimental units of uniform age, sex, size, and vitality.[6,7,8]

Organs and Functions: Daphnia possesses well developed muscular, nervous, and glandular systems, permitting the visualization of body functions, as the entire animal is transparent. Qualitative and quantitative reactions may be observed in vivo, as produced by the minutest amounts, by therapeutic, toxic, critical, and fatal doses of medicinal products. The physiological mechanism of their action may be studied, as well as the influence of temperature, oxygen pressure, light and other biophysical influences. The effect of such biochemical factors as food elements, minerals, vitamins, hormones, and enzymes, may be observed directly or shortly after introduction into the culture medium or the animal by injection. As apparatus have been developed and adapted to permit great magnification of the image of organs, a close and comprehensive, as well as a detailed view of the mechanism, many times enlarged, is readily available.

Records: With Micro-motion pictures we have recorded the normal, intricate life functions,[9] as well as the striking effects to specific causes, to which all higher biological units, including man, are subject. Thus convulsion of the muscular organs as swimming arms in daphnia, accompanied by a progressive depression of the respiratory and circulatory systems, and the final paralysis of the gastro-intestinal tract may be observed as stages in the progressive debility resulting from strychnine poison.[10,11] Certain death may be prevented by the administration of barbiturates, now also found effective in human cases of poisoning with strychnine.[12]

The usually filled food canal can be quickly evacuated, the speed of evacuation depending obviously upon the efficiency and concentration of evacuants[13] as cascara[14], aloes,[15] rhubarb,[16,17] podophyllum,[18] bitter salts and phenolphtalein. The heart depression caused by substances as veratrum,[19] yohimbine and chloroform, can be prevented or removed by administration of digitalis and [21, 22, 23] and its preparations, and their efficiency judged by this non-toxic method.[24] The sexual system has been studied with the fertility vitamin E,[25] responsible for the reproduction, and with aphrodisiacs or local irritants such as cantharidin and others, exciting the male sex

organs, to a varying degree.[20] The nervous system has been studied with the use of anesthetics,[26] hypnotics, alkaloids, and narcotics as morphine and cannabis,[27] toxin and venins; the breathing system with nicotine etc.; the glandular system, other than the sexual, with adrenalin and others. A host of additional substances has been tested, qualitatively and quantitatively, e. g. glucosides like amygdalin,[28] enzymes, [28] adsorbents, disinfectants, stimulants and insecticides,[29] foods, like calocasia or puak, feeds and products of unknown physiological value.[30]

Remarkable also are the results from actions of differential vital stains, of antagonists, and antidotes to poisons and narcotics, with the typical manifestation, in many instances corresponding to those in cold-and warm-blooded animals of the vertebrates.[19, 30-35]

Observations made by other workers on Daphnia and its internal mechanism, as recorded in world literature, in general, agree with or confirm our findings. While an extended critical survey will be published subsequently, it may be of interest to point out here, that daphnids were probably first observed almost 400 years ago by the Dutch naturalist Jan Swammerdam (1637-80), who illustrated some of the internal organs in action. [36] The Russian physiologist Metschnikoff (1884) observed the injestion of fungus species in the intestine of daphnia and based on this discovery his classical views of "phagocytosis" as the basis of disease resistance and long life, [37] The surgeon de Fouché recently concluded from preliminary experiments that human carcinoma can be transplanted into Daphnia magna and encourages their further use in the study of cancer. [38] Anderson used Daphnia magna in observing regeneration of body tissue and swimming organs. [39-40] He and other American and Canadian biologists such as Banta, Ingle, Wood, and MacArthur and Baillie used Daphnia magna and related species in genetic studies and in observing the effect of food on growth, reproduction, vitality and longevity. [42-4] Most recently the zoologist Obreshkove found the eggs deposited in the brood sac self-sufficient in nutrients and thus could raise the isolated embryos on tissue culture slides, and minutely study their growth to the fully developed animals.

Outlook.

Our results have further been checked with the transparent prawn (Palaemonetes), also found by me in the local klongs of Bangkok, and certain translucent[36], so-called scaleless fish with visible gall-bladder, heart and intestines (see Plate 2), such as Carassius auratus and Carnegiella stregata, or transparent fish like Gobiella, which we have thus far studied physiologically only in comparatively limited numbers. The fullest possible utilization of these, esp. also of the transparent fish for the observation and study of internal life reactions, will come with our success, now sought, of breeding them in sufficient numbers and in sufficient speed. With our stethographone, (a device recording the heart impulses and breathing rate of vertebrates, including man), we can now check many observations made on the simpler mechanism of transparent organisms as Daphia magna.[19, 24, 46]

Viewing closely the transparent creatures of nature, we gain an inside look; crossing thus the outer borderline of life, we envision (without disturbing it) the intricate mechanism; this appears ever ready to respond to inner and outer influences, unto—and even beyond—the last beat of its heart.

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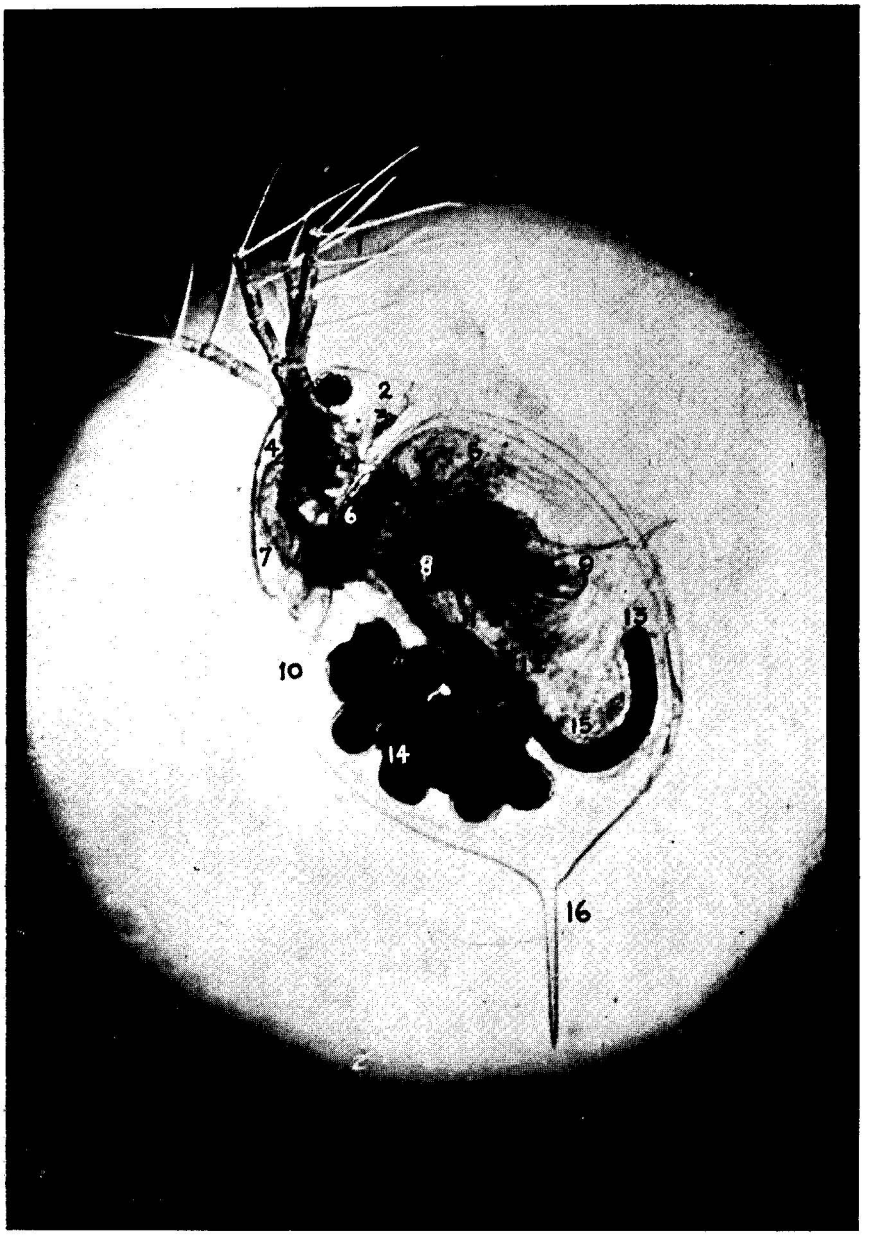
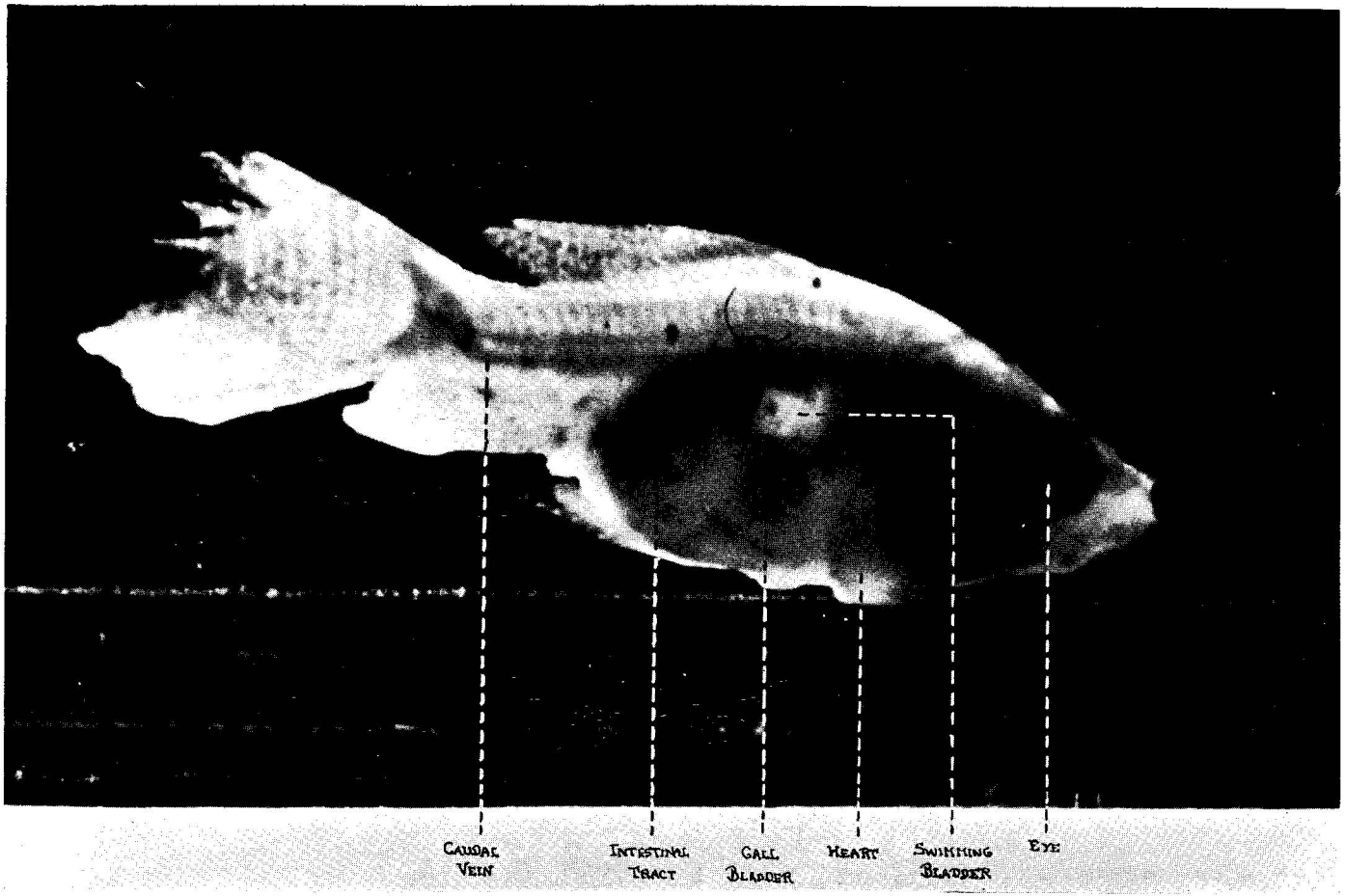


Daphnia magna Strauss, (Crustaceae).

1. Swimming Organs
2. Eye with Lenses
3. Muscles with Optical Nerves
4. Liver Glands
5. Nephridial Bands (Kidney)
6. Green (Shell) Gland
7. Stomach (without Food)
8. Food Pouch
9. Breathing Organs
10. Heart with Valve
11. Upper Intestines (with Food)
12. Ovaries
13. Cleansing Hook between Double Shell
14. Brood Sac with Embryos
15. Lower Intestines (with Waste)
16. Supporting Stilt (Spine).



Scaleless Telescope Fish.
Carassius Auratus, Varietas Macrophthalmus Duerigen..