BIOLOGY OF DAPHNIA

by

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HISTORICAL REVIEW

MORPHOLOGY

Swammerdam, the Dutch scientist, was the first author who took notice of any of the Daphniae. In his Historia Insectorum Generalis, 1669, he gave a description of a species of Daphnia, which resembled Daphnia pulex and which he named Pulex arboreus or arborencens. He stated that its beak was slender and pointed, and was used for drawing up food. He called the second antennae arms and described their motion as three-fold--rectilinear, unequal, and gyratory. He asserted that there were two eyes which seemed to be joined together.

Merrett mentioned Daphnia in his Pinax rerum Britannicarum, published in 1677.

Redi (1684) gave three figures of a species which he called animaletti aquatici but which Muller quoted as pulex. Bradley (1739) described certain animals in the Thames River which were evidently Daphnia pulex.

Trembley (1744) published a description of a species which he named Puceron branchi. His observations and figures were again published by Adams in 1746. Linnaeus (1744), in the fourth edition of his Systema Naturae, described the same species under the name of monoculus pulex arborecens.

The first naturalist who made an attempt to distinguish the different species was Schoeffer (1755). He illustrated two species, Daphnia pulex and Daphnia sima, and gave a sketch of the head only of a third, which when provided with a body was later cited by Muller and Straus as Daphnia longispina. In 1766 Schoeffer described Daphnia pulex but called it Branchipus conchiformus primus. He was the first to notice the first antennae, which he considered to be palpi for distinguishing the food. He showed the true position of the mouth and described the liver. He did not agree with Swammerdam that there were two eyes.

Muller (1771) enumerated several species of Daphnia found in Norway and Denmark. He established the generic name of Daphne. He later changed it to Daphnia which was adopted by succeeding workers.

DeGeer (1778) was instrumental in correcting errors made by Swammerdam in his descriptions.

Ramdohn (1805) published a detailed account of the anatomy of two species: Daphnia sima (Vetula) and Daphnia longispina (Muller). Previous to this time, Schoeffer, DeGeer, and Muller were the only naturalists who had attempted any particular anatomical details.

In 1820 Jurine's investigations added information

about the anatomy and habits of Daphnia. Among other things he stated that the first pair of legs were used to direct particles of food to the mouth. Straus, who included Daphnia in his studies of insects, believed that the first and second pairs of legs were organs of prehension. He described the mouth as consisting of a labrum, two mandibles and one pair of jaws.

Herreck (1883), who studied Daphnia in pools in Tuscaloosa, Alabama, applied the name Daphnia longispina to the young only; as the mature females that he found were not spined.

Wesenberg-Lund (1926), in his extensive account of Daphnia, classified the species and pointed out the main characteristics of each. He stated that Daphnia magna can be distinguished by the very conspicuous excavation of the posterior edges of the abdomen and by the almost total want of any variation combined with season, locality and age. Daphnia pulex is differentiated mainly by its being smaller than Daphnia magna and less globular in shape. Daphnia longispina is characterized by the length of its spine and is similar to Daphnia cucullata, which differs in that the pigment spot is commonly invisible and the antennae are always placed upon the tip of the rounded rostrum instead of on the ventral side

of the head.

Gicklhorn and Keller (1926) used vital staining to detect the minute details of the chemoreceptors, or setae, in Daphnia magna.

Hanstrom (1927) stated that the eyes as well as the optic centres of Daphnia are fused medially.

Herwerden (1928) treated living muscles of Daphnia pulex with acid and found that regular rows of vesicles appeared in the sarcoplasm. He believed these to be swollen mitochondria.

Dejdar (1930) advocated the use of vital stains in the morphological investigation of the organs of Daphnia. He studied the small coelomic pouches of the nephridia of the antennae and the larger ones of the maxillae of Daphnia magna, Daphnia pulex, and Daphnia longispina.

Anderson and Brown (1930) proved that the formation of new chitin for any given adult instar begins approximately at a period which sixty per cent of the previous instar has passed.

In 1931 Binder studied living and fixed specimens of Daphnia magna and gave a description of the musculature. She divided the muscles into three groups: one included the muscles of the five pairs of legs; another the muscles

of the body; and the other those of the head and its appendages. She pointed out that in the male there are two additional strong bands of muscle in the first pair of legs.

Jager (1935) located the fat body of Daphnia magna and described its structure. He stated that they were composed of plates situated at various definite places in the abdomen.

Viehoever (1938) used vital stains to illustrate the nerves in the muscle fibres leading from the brain to the retina of the eye. In 1942 Heberdey and Kupka studied the eye in detail. According to these investigators, Daphnia has an appositional eye of nearly spherical form and consisting of twenty-two ommatidia. There are no corneal nor cone cells, and no pigment migration.

Deller (1947) used fluorescent dyes in ultra-violet light to extend the work of Dejdar on the nephridial structure of Daphnia.

PHYSIOLOGY

Owing to the transparency of Daphnia, the activities of its intact organs have been subjected to study and

experimentation. To a great extent the reactions of its heart and intestine have been used as indices of the effectiveness of certain pharmacological products.

Hykes (1926) investigated the effects of endocrine substances on the heart rate. Adrenaline increased the rate. Thyroxine had a similar effect while pituitrin decreased the rate.

MacArthur and Baillie (1927) established the fact that the same total number of heart beats, averaging 5,410,000, occur no matter what the temperature of the environment or the sex of the Daphnia was. An elevation of temperature from eight degrees Centigrade to twenty degrees Centigrade, however, increased the heart rate of both sexes, being more noticeable in the male. These workers varied the density of the population and found that overcrowding diminished the heart rate in both sexes.

Levy (1927) discovered a method of partially disconnecting the heart of Daphnia and keeping it beating in Ringer's solution for ten to twelve hours. He treated the disconnected heart with a solution very rich in potassium. This at first altered the rhythm then increased it, causing the heart to be reduced to a small compact and trembling pocket which ceased moving in about ten

to twenty minutes. In a solution rich in calcium the heart rate was decreased. When put in a solution containing both potassium and calcium, the heart, after a few irregularities, became normal and the rhythm regular. Adrenaline had a more powerful effect on the animal on which Levy had operated than on the intact animal.

In 1929 Berger treated Daphnia magna with single salts and compared their effect on the heart rate with that of a solution of artificial sea water and with that of isotonic sodium chloride solution. Sodium chloride at first quickened the heart rate and then caused an irreversible heart stoppage. Calcium chloride produced similar effects. Potassium chloride had a short but very pronounced stimulating effect before the animal became inert.

Fox (1932) irrigated Daphnia pulex under a compressorium with carbon dioxide saturated tap water, causing the heart to stop within a minute in diastole. Neither water brought to pH 5.0 with hydrochloric acid nor water saturated with nitrogen stopped the heart beat.

Snider and Kersten (1935) studied the action of soft X-rays on Daphnia magna. They found that the time of heart stoppage was about half as long when the whole animal was subjected to the rays as it was when only half

of the animal was treated. The time required for heart stoppage was longer when the posterior half of the body was irradiated than when the anterior half was exposed. When the heart was shielded and the anterior and posterior halves exposed to the rays, the time required for cessation of the heart beat was longer than when the animals were otherwise treated.

According to Ingle, Wood and Banta (1937) the heart rate of Daphnia averages 4.68 beats per second at birth; it reaches a maximum of 5.46 beats per second at the seventh instar in well-fed animals. The rate gradually and then rapidly falls off until it is 3.4 just preceding death.

Viehoever (1937, 1938, 1939) treated Daphnia with several pharmacological products. Strychnine caused a drop in its heart rate. The heart became convulsive and then paralyzed. Yohimbine, capsaicin, and piperine each progressively depressed the heart, producing dilatation. Digitalis leaf exhibited a protective action against such depressants as yohimbine.

Sollman (1941) confirmed Hykes' finding that adrenaline accelerated the heart rate. Atropine caused a moderate slowing. Mecholyl and pilocarpine had no effect.

Quinidine caused a slow irregular arrhythmia with incomplete systoles and dilated diastoles. Quinine, magnesium, ether and alcohol each slowed the heart rate.

Obreshkove carried out a series of experiments in which the action of acetylcholine, atropine and physostigmine on Daphnia magna was considered. In 1942 he studied their effect on the heart. Acetylcholine caused a powerful and regularly rhythmic beat in formerly arrested hearts. Physostigmine intensified and prolonged the effects of acetylcholine. He suggested that the heart of Daphnia may be controlled by cholinergic nerves.

Baylor (1942), contrary to Obreshkove, found acetylcholine to inhibit the heart rate; its effect was reversible. Eserine and potassium caused inhibition which was not reversible. Nicotine showed a stimulation in weaker concentrations and an inhibition in stronger concentrations; the latter was irreversible.

Swammerdam described the colour of Daphnia as being "like that of beef, which has been some time steeped in water". This colour is due to the haemoglobin, which was seen first by Lankester in 1871. It was, however, Verne (1923) who recognized that the red colour of Daphnia was due to this blood pigment.

Fox (1945, 1946, 1948, 1949) discovered that the species of Daphnia have different haemoglobins, the wave length of the alpha-band axis for the oxyhaemoglobins of Daphnia magna, Daphnia pulex, and Daphnia obtusa being respectively 5766, 5764, and 5761 Angstrom units.

Fox found that the intensity of the red colour of some populations varied conversely with the oxygen content of the water; and that Daphnia synthesized blood haemoglobin in poorly aerated natural water. The haemoglobin content of blood varied within each instar, having been least when the eggs were laid by the parthenogenetic females in her brood chamber, and greatest when the eggs had developed into late stage embryos ready for release. In the synthesis of haemoglobin the minimal nutrition was required and the degree of synthesis increased with the amount of food up to the optimal value of the latter.

He de-oxygenated the haemoglobin in the blood of Daphnia and noticed that there was no immediately fatal effect. He concluded from his studies that this blood pigment was apparently unimportant in respiration and perhaps functionless in the blood.

Dresel (1948) traced the passage of haemoglobin from Daphnia into her eggs. He saw that the ovaries became

progressively pinker as they enlarged during the last few hours preceding egg laying. The haemoglobin passed into the eggs as they developed in the ovary. After egg laying, the haemoglobin of the mother gradually regenerated to its original level.

Fox tested the importance of haemoglobin in the development of the embryo. According to his results, the presence or absence of haemoglobin up to the stage where the embryo has two red eyes and the antennae are not free made no difference in the rate of development. In the last stages of development, however, embryos without the haemoglobin lagged behind. No trace of haemoglobin was found in fertilized eggs taken out of the ephippium; but they did contain abundant haem.

Gastro-Intestinal System.

In 1935 Cannon published a comprehensive account on the feeding mechanism of Daphnia magna. The median food groove is along the mid-ventral line on the top of the wall on which are attached the limbs. These move with a metachronial rhythm, giving the impression of waves passing from behind forward. The third and fourth pairs of limbs function to suck water into the cavity and their filter combs serve to strain out the food particles.

The lower part of the food groove, just behind the maxillules, is a region of comparative quiet. Here the labral glands extrude secretions which entangle the food particles. These are passed forward by the maxillules at irregular intervals on to the mandibles.

Hasler (1935) was the first to report on a biochemical assay of the digestive enzymes of Daphnia. He stated that the mechanism which exists is capable of utilizing proteins, carbohydrates, and fat; and that the pH varies from 6.8 in the anterior end of the tract to 7.2 in the caudal end.

Rogers (1938) gave a detailed account of the movements of the intestine of Daphnia during digestion. He studied the course of stained food particles and found a pH of about 8.2 at the anterior end, a pH of about 6.8 in the tract just below the heart, and of about 8.2 near the anus.

Viehoever (1936) treated Daphnia with poisonous substances and these were retained by the liver. In 1937 he treated Daphnia magna with strychnine and found that it caused paralysis of the intestine. He studied the degree and speed of the evacuation of the intestine with the use of rhubarb and rhaponticum in particular and

laxative drugs in general. The intestine was emptied in about ten to twenty minutes.

Gallisten (1938) carried out a histochemical study of the musculature, the intestinal epithelium, the epithelium of the shell glands and the shell lamellae. There was considerable storage of glycogen in all above-named structures. In addition small droplets of fat were seen in the intestinal epithelium. According to Hardy (1938), within ten to twelve hours after the intestinal epithelium absorbs the fat, the latter is found in the blood cells. Carmine is also taken in by the intestinal epithelium.

In 1938 Metropolitanskaja demonstrated the presence of an acetylcholine-like substance in Daphnia. In 1941 Obreshkove treated Daphnia with acetylcholine and caused the muscular peristaltic and antiperistaltic contractions of the intestine to become extremely violent. Atropine antagonized the action of acetylcholine; in certain cases completely abolishing the contractions. Physostigmine heightened the effect of acetylcholine.

Sollman (1941) observed that adrenalin inhibited peristalsis of the intestine. Adrenalin antagonized peristalsis started by mecholyl but did not relax the intestine. Pilocarpine produced a marked increase in peristalsis.

Physostigmine tended to cause spasms which were diminished by atropine. Magnesium caused a slowing of peristalsis. Such asphyxiants as sodium cyanide and tannic acid prevented peristalsis. Carbon dioxide had no effect. Nicotine caused some increase in peristalsis but this was followed by arrest.

Genital System.

Investigations into the nature of the genital system of Daphnia were initiated in the late eighteenth century. Muller (1785) stated that the large first antennae of the male were organs of generation. He observed one copulation only in his culture, and with others, believed that this was sufficient to make the mother productive for life and all her female descendants productive for several generations. Muller was the first to name the "ephippium"; but Jurine was the first to assign any cause or use to it. He believed that it was caused by disease, the effect of which was to arrest fecundity. Straus proved it to be a substance containing two eggs destined for future generations of species in the spring; these eggs resisted cold which was fatal to perfect animals.

McClendon (1910) concluded from his experiments which showed that heat and starving hastened sexual maturity

in Daphnia, that conditions which were adverse to the growth of body cells either failed to retard the development of germ cells or stimulated their development. In either case Daphnia became sexually mature at a less developed stage.

Ashworth (1913) examined abnormal specimens of Daphnia pulex and detected the occurrence of male antennules in females. He concluded that this was indicative of the presence of male characters in females which in normal cases are in a completely latent state. These findings also indicated to him that the secondary sexual characters are not necessarily linked with primary ones. These abnormalities were not transmitted.

Banta (1914) reared one hundred parthenogenetic generations of Daphnia pulex without sexual forms. He had removed the animals from a pond in which no males had been found. There was no evidence of decreased vigour nor any loss of vitality in the lines. He concluded that the sexual cycle was not necessary in Daphnia pulex.

Treillard (1925) postulated that bacteria in a culture is necessary for the appearance of sexual forms.

Banta and Satina (1925) performed a biochemical test for sexual differences in Daphnia pulex. Applying al-

cohol and other reagents they discovered that sexual forms developed a pronounced violet colour, parthenogenetic females showed the same colour but with much less intensity. Males showed no colour.

MacArthur and Baillie (1927, 1929) proved that the metabolic rate of males of Daphnia is higher than that of females. Males survive excessive crowding longer than females.

Banta and Brown (1928) investigated a possible control of sex in Daphnia. Overcrowding, low temperatures, and other conditions that retard development are known to be responsible for the appearance of the male offspring. They added to this knowledge the fact that it is not until the eggs have been laid for approximately four hours that the sex is fixed. Their findings suggest the probability that sex control operates through the control of a sex-determining mechanism.

Wood (1933) endeavoured to induce hatching of the sexual eggs of Daphnia longispina and found that the repeated aeration of wet eggs was the most successful of all methods tested. Working with Banta, Wood (1936) found that in Daphnia longispina the animals which were larger at birth and had unlimited food, produced more young in

their first clutch than did their smaller sisters. With limited food, however, the larger animals did not produce a greater number of young during that period.

Snider and Kersten (1936) irradiated adult females carrying eggs in the brood chamber. The dosage given caused the destruction of the eggs but had no apparent effect on the ovaries. The eggs were most susceptible to the lethal effects of soft X-rays when they were in the segmentation stage. Consequent young produced by the mothers tested were not affected by the rays.

Viehoever (1936, 1937) speeded up the time taken for the development of the eggs of Daphnia by adding pituitrin to the culture. Strychnine interfered with the normal function of the ovaries by suppressing the release of the embryos into the brood chamber.

Ingle, Wood, and Banta (1937) starved the animals and reduced the reproductive rate. They then fed these animals which consequently increased their rate and exercised their potential reproductive capacity.

Harvey and Schoepfle (1939) centrifuged single oil drops in several eggs of Daphnia pulex and studied their interfacial tension.

Obreshkove and Frazer (1940) made observations on

the growth and differentiation of Daphnia magna eggs in vitro. They developed in the same time, approximately forty-six hours, and as well as those in the brood chamber. These workers established that sufficient nutrition is already stored in each egg at the time of laying, and that lower temperatures reduce the rate of embryonic development.

In 1945 Haget tried to prove the existence of a secretion in the brood chamber necessary for the development of the embryos. He exchanged eggs at various stages of their development from one mother to another and noted the effects. The only difference observed was that the "larval envelope" was dropped sooner than usual.

Sanford (1947) worked with intersex clones of Daphnia longispina. He tested the influence of the temperature upon the expression of the intersex character. Animals reared at low temperatures produced a larger proportion of intersex offspring which exhibited a greater expression of male secondary sex characters than their siblings cultured at higher temperatures. There was complete inhibition of intersex expression at thirty-two degrees Centigrade.

Respiratory System

In 1778 DeGeer in studying the anatomy of Daphnia discovered the respiratory function of the third and fourth pairs of thoracic appendages. Banta referred to these in his Carnegie Institute Publication as leaf-like appendages which characterized Daphnia.

Viehoever (1936, 1937) in testing drugs found that ammonia affected the respiratory organs, thereby causing death. Yohimbine, capsaicin, and piperine depressed them progressively. In 1938 Viehoever evaluated the activity of benzedrine sulphate and its derivatives on Daphnia magna. These chemicals also depressed the respiratory system. Strychnine decreased the rate of movement.

Sollman (1941) tested the drugs, which he had used in his experiments on the intestine of Daphnia, on the striated muscles of the respiratory appendages and obtained similar results. However, the stimulant phase of nicotine, cyanide, and strychnine was absent; these depressed respiration directly.

Responses.

According to the American Microscopic Journal (1884), Sir John Lubbach showed that Daphnia was sensitive to

ultra-violet rays. Mereskhouski had formerly stated that Daphnia was not attracted by the colour of the light but by its brightness. Sir John investigated and found that Daphnia had a preference for the green over the red end of the spectrum; for coloured light rather than white; and for the more brightly lighted environment.

Yerkes (1900) stated that Daphnia pulex showed a marked increase in the rate of movement toward the light when the intensity of the light was increased.

Rose (1910) used violet, blue, and slightly green lights and caused negative Daphnia to become positive. Urea and calcium chloride when added to the medium increased the positivity of the animals while potassium chloride diminished the reaction.

Moore (1912) elaborated upon experiments performed by Lubbach. He showed that ultra-violet rays caused Daphnia to become negatively phototropic; light of wave length less than 3341 Angstrom units causing an instant negative response. The latter was cancelled when acids, especially carbon dioxide or hydrochloric acid, were added to the culture medium during the experiment.

Robertson (1925) stimulated Daphnia simultaneously with light and an electric current. Large individuals

showed a resultant reaction by going to the lighted and cathodic corner of the dish.

Schulz (1928) confirmed that Daphnia preferred the yellow and green part of the spectrum. He found that Daphnia utilized both light and gravity for orientation in space; and that reduction in light influenced the quantity and quality of eggs unfavourably. He also found that the animals behaved differently when exposed to light from above instead of from the side. In his experiments, ultra-violet light produced a negative phototropic action on eyeless Daphnia.

Bidder (1929) suggested that the actual stimulus for geotropy might be an increase in the tone of the lower muscles of the antennae; or a decrease in the tone of the upper muscles.

Lumer (1932) exposed Daphnia magna and Daphnia pulex to thirteen coloured lights of equal intensity. Contrary to Lubbach's findings, his experiments showed that the orange light had the maximum stimulating effect and the blue the second maximum. His data indicated that photosensitive substances are specific for each species of Daphnia.

Clarke (1932) discovered that the response of Daphnia to light could be temporarily reversed by abruptly

changing the intensity of light falling on the animal. He introduced strychnine sulfate in the pond water and Daphnia magna, which was originally negatively phototropic became constantly positively phototropic to all light intensities after six to ten minutes in the solution. Animals which were originally primarily photopositive remained constantly photopositive. The usual responses to abrupt changes in illumination were abolished. Clarke also tested the effects of an electrical current in the medium of Daphnia magna. Strong anodic galvanotropism was exhibited. After being in the strychnine solution, however, Daphnia became strongly cathodic. The orientation mechanism was unaffected by strychnine, and as a result Daphnia always faced the anode.

Viaud's studies (1933) of the phototropic response of Daphnia indicated that the animal moved toward the light with a certain speed that increased uniformly and then decreased progressively. He postulated that tropism manifested by a population was similar to a physical force.

Heberdey (1936) tested the sensitivity to colour of light-adapted Daphnia which were strongly photopositive. He used blue, green, yellow, and red lights. The animals

exhibited almost no reaction to the red colour. With the other colours used, however, the animals always accumulated on the side toward the light of shorter wave length, as shown by former investigators.

Foxon (1938) pointed out that orientation of Daphnia to gravity appeared to be due to a combination of bodily shape and posture, and not due to the activities of other appendages.

GROWTH AND DEVELOPMENT

In 1883 Herreck pointed out that the common species, Daphnia pulex, was subject to perplexing variations.

Warren (1903) made an attempt to ascertain the relationship between the size of the cell and the size of the body of Daphnia magna.

Berger (1929), in treating Daphnia with single salts, observed that the longest life length occurred when sodium chloride was added to the medium.

Volterra (1926) studied growth and variability in Daphnia longispina. Growth was less between moults at higher temperatures, but the animals moulted more rapidly. Sexual maturity was reached sooner, but after the

same number of moults.

Anderson (1930) found that the number of pre-adult instars in Daphnia was not correlated with the size of the individual during the first instar. His results indicated that the number of pre-adult instars was dependent upon the food supply available; and that a minimum number of pre-adult instars existed for each species. In 1932 he presented data on the life history of Daphnia magna. Relative growth changed at sexual maturity; five pre-adult instars were accounted for; and growth was greatest during these pre-adult instars. The number of young produced increased with each instar until the sixth adult instar and then dropped gradually.

Breukelman (1932) found that as Daphnia developed, its resistance to poisons increased. The resistance of females was greater than that of males.

Wood and Banta (1936) worked out an unbiased mathematical evaluation of the interdependence of many pairs of variables involved in growth in Daphnia longispina. Among other things, they concluded that early growth is, in general, independent of birth length; animals which grow more during juvenile instars tend to grow less in the following two instars; and in any given instar the growth

increment is positively correlated with the number of eggs simultaneously produced. This fact indicates that egg production does not occur at the expense of growth.

In 1937 Anderson, with Lumen and Zupancic, extended his previous investigations so that he might establish the events in the life span of Daphnia pulex. The probable minimum number of pre-adult instars in this species was four. The number of young produced increased until the tenth instar and was then followed by a gradual decrease.

Ingle, Wood, and Banta (1937) reported that starvation induced a slow rate of growth; but the rate was increased when the animals were returned to a normal medium. Each instar lasted longer in starved animals.

Dunham (1938) semi-starved animals from birth until various periods after maturity, and then fed them abundantly for the rest of their lives. The rate of growth was lower and the instars longer in the semi-starved state. The normal capacity for active growth, however, had not been lost but was suppressed; for when the animals were fed abundantly they grew rapidly.

Banta (1939) studied growth and development in Daphnia longispina. Growth was very rapid in the first four instars, well-cultured animals approximately doubling their

size after each moult. The animals continued to increase in size for seventy-one per cent of their lives.

In 1942 Anderson and Jenkins presented a comprehensive review on Daphnia magna. The average longevity was approximately seventeen instars or nine hundred and sixty hours. In general, the duration of the instars increased with age. The brooding periods varied directly with the duration of the instars.

REGENERATION

Various experiments have been undertaken to estimate the extent of regeneration possible in Daphnia.

In 1930 Agar made a statistical study of the regeneration of the setae of the antennae of Daphnia. He proved that the number of setae regenerated did not bear any close relation to the number removed. The vigour of regeneration did not show any decline with age. The extent of regeneration was influenced by the general complex of external conditions but not by simultaneous regeneration nor by previous regeneration. In 1931 Agar performed a Lamarckian experiment involving one hundred generations. He amputated the dorsal branch of the second

antennae of Daphnia carinata. Regeneration consisted essentially of the formation of setae, the missing segments of the axis never being reproduced. The regenerated setae did not attain the length of normal setae. Regeneration did not become more perfect nor was the normal growth of the antennae affected.

Anderson (1933) experimented with the carapace of Daphnia magna. The results showed that the amount of regeneration during any instar varied directly with the size of the wound; it increased with each instar until the adult condition was reached and then decreased with the age of the animal; and during any adult instar the amount of regeneration decreased with the age of the wound.

In 1935 Anderson worked on the antennae of Daphnia magna. He amputated at various levels during the early part of the first instar. The amount of regeneration varied inversely with the level of the injury, except when the injury reached the base of the segment. Anderson believed that the length of the uninjured portion and not the length of the segment left after the operation (Agar, 1930) was probably the most important factor in determining the amount of regeneration.

Anderson and Busch (1941) found that the normal and regenerating antennal segments followed the law of Allometry. As long as the level of injury was distal to the critical level, growth rate was such that the regenerating segments tended to approach the length of the normal segment simultaneously as the full growth of the animal was attained.

GENETICS

Elman (1904) stated that all Daphnia races are derived from the pond and pool forms of Daphnia longispina variety rosea.

In 1910 McClendon stated that nutrition was the most important factor that could influence the hereditary tendency of the life cycle of Daphnia.

Banta (1913, 1918) selected two strains, with a pure line of Daphnia, on the basis of a purely physiological character. This character was the reaction time of young Daphnia to a definite intensity of light. The strain which showed the lesser reactiveness to the light had the higher reaction time.

In 1926 Banta, with Snider and Wood, carried out a

study on the occurrence of mutations and their inheritance in Daphnia longispina. The characteristics involved were "excavated" heads and sex intergrades. In parthenogenetic young the inheritance was complete. These characteristics were found in the sexual offspring from the mutant strains, indicating that they are definitely inheritable and behave in bi-parental inheritance in Daphnia like the characteristics in bi-parental inheritance in other organisms.

Dunham and Banta (1940) produced mutant clones by treating parthenogenetic eggs with dilute solutions of colcheine. Half of these clones effected a perfect and complete reversion to normality after several generations in the mutant condition. These experiments failed to reveal the nature of genetic changes involved in mutations in Daphnia.

ECOLOGY

Daphnia has been found in all parts of the world in various bodies of fresh water. The diversities in environment have been noted to affect the structure and the behaviour of populations.

In 1890 Sars made known the nature of the phenomenon of cyclomorphosis in Daphnia. This was also studied by Zacharias (1893) and Wesenberg-Lund (1900).

Stingelin (1897) showed that there was a regular seasonal dimorphism in Daphnia pulex.

Warren (1900) subjected Daphnia magna to a confined volume of water and found that its power of reproduction decreased.

In 1918 Olufsen observed that the eye of northern races of Daphnia pulex was smaller than that of southern races.

Decksbach (1926) reviewed former literature and pointed out that Daphnia carinata is eurythermal and eurytropic in small bodies of water and is limited ecologically rather than geographically.

Wesenberg-Lund (1926) made a survey of the fresh water bodies in Denmark and observed that Daphnia magna was always found in very small pools and ditches where water is extremely rich in organic matter. He found Daphnia pulex in ponds with clear and often peaty water. These were also common in the littoral region of smaller lakes among the vegetation.

The habitat of the various forms of Daphnia longispina

ranged from small ever-drying up pools to the central parts of ponds and lakes. The Daphnia cucullata had developed from littoral forms to a full planctonic life. Wesenberg-Lund found that the type was altered morphologically and biologically in accordance with alteration of life conditions. For example, the more the type accustomed itself to life under pelagic life conditions, the more the clumsy form of body was altered into an elegant slender one, the more the spinosity of the tail was reduced, and the more the size was reduced. He further noticed that the tendency to form variation, especially seasonal variation, increased with tendency to pelagic life.

Volterra (1926) studied the Daphnia of Italy. Daphnia longispina is indigenous to Lake Nemi and Albanio. Daphnia cucullata, which is limited to Northern Europe and Northern Asia was introduced into Lake Nemi from Denmark. Daphnia cucullata survived for eleven months and maintained its morphological characteristics and its characteristic autumnal sexual period in spite of a change of environment.

Woltereck (1914) performed a similar operation when he introduced Daphnia cucullata from Danish lakes into these Italian lakes. Daphnia cucullata became established

and the descendants developed into a giant race in fourteen years. Woltereck stated that change in size was due chiefly to selection. Woltereck (1930) noted that the helmetless form of Daphnia cucullata was limited to unstratified water of two to four metres in depth but the helmeted form was found throughout all levels of water.

MacArthur and Baillie (1927) investigated the environmental effects on the duration of life in Daphnia and found that life was lengthened at lower temperatures.

Ancona (1927) made observations on Daphnia cucullata which had been introduced into Lake Nemi. This species showed a marked increase in variation in spring. Ancona concluded that seasonal variation responded in part to the external environment and in part revealed the influence of hereditary factors.

Volterra (1927) studied the effects of higher temperatures on Daphnia. They accelerated moults, resulting in a quicker life cycle.

In 1927 Schubert made a quantitative analysis of the effects of temperature and seasonal changes on Daphnia cucullata. He saw that growth continued during six ecdyses in spring and four in the winter, and found out that races depended largely on the temperature factor.

MacArthur and Baillie (1929) presented an extensive paper on the influence of temperature on longevity of Daphnia magna. They confirmed and elaborated on the work of Volterra. The males were more sensitive to temperature alterations. The contrast in size between males and females was greatest in the cold and least in the heat.

Fowler (1931) studied the relationship of numbers of animals to survival in toxic concentrations of electrolytes and found that groups of Daphnia survived longer than single individuals in the stronger solutions. Single individuals survived longer than groups in weaker concentrations of the same solutions.

Hutchinson (1933), in his experimental studies in ecology, considered the magnesium tolerance of Daphnia and its ecological significance. He proved that Daphnia magna was unaffected by the electrolyte content of the water made up to contain the same quantity of electrolytes as Lake Tanganyika. Zinc was markedly toxic. Hutchinson concluded that the absence of Cladocera in Lake Tanganyika was in all likelihood due to chemical factors.

Coker (1939) recognized cyclomorphosis to be a function of a combination of internal and external conditions.

Dunham (1938) stated that only animals subjected to

environments poor in food from the beginning of the third or sixth instar were young enough to be able to adjust themselves to the lowered metabolic rate enforced by such an environment.

Brehm and Woltereck (1939) made known the fact that all the principal types of Daphnids are capable of cyclomorphosis in warm waters.

Ingle (1939) changed the environment of Daphnia by conditioning the medium with certain fish extracts. At the time of completion of five growth increments, the animal in the treated medium was larger than its respective control.

Pratt (1943) studied the effect of temperature upon the rate of increase and its influence upon subsequent changes in the numerical strength of the population. The range of pH--6.9 to 7.1--did not appear to exert an important influence upon population growth. He stated that the influence of the temperature upon the mean population size was indirect. It exerted its effect only by modifying the action of population density.

INTRODUCTION

It is evident from the foregoing historical review that the biology of Daphnia has been extensively investigated since the time of Swammerdam, Straus, and Muller. There exists, however, controversial data concerning certain processes. For example, when Hasler (1935) studied the digestion of Daphnia he found the pH to vary from 6.2 in the cephalic end of the tract to 8.4 in the caudal end. On the other hand, Rogers (1938) states that the pH is alkaline in the caudal as well as in the cephalic regions, and acidic in the mid-region just below the heart. I have undertaken in this research to study the digestion of carbohydrates, proteins, and fats in Daphnia; and to establish in which region the food is digested as indicated by the change in pH in the tract.

The young of Daphnia are positively phototropic and as they mature they gradually acquire a negative phototropic sign. Biologists have changed and strengthened the phototropic sign of the young and the adult Daphnia by such methods as varying the intensity of the light and by introducing drugs. I have varied environmental conditions by introducing two factors; namely, the hydrogen-ion concentration and the temperature of the culture medium;

and have studied their separate effects on the phototropic sign of Daphnia.

The study of the biology of Daphnia would be incomplete without taking into account the response of vital organs to external stimuli. I have investigated such phenomena by using adrenaline, ephedrine, and cocaine--drugs which have characteristic reactions.

MATERIAL

The first culture of Daphnia was secured from an aquarium maintained by Dr. Berrill for class use at McGill University. These animals were introduced into an aquarium containing tap water which had been standing for at least three months, and were reared on Fleischmann's Royal dry yeast. A few grains of yeast were placed in the water once a week, and one package of yeast lasted two months. The utilization of yeast as food for Daphnia was initiated by Bond (1934), who used the cake yeast. He states that air should be bubbled through the medium. However, a healthy culture was maintained throughout this research without keeping the water well aerated.

Tap water which had been standing for a week was

used to replace the water lost by evaporation. The temperature of the culture medium remained between twenty and twenty-two degrees Centigrade. Animals in the first instar were used in all experiments unless otherwise stated.

I. DIGESTION

Procedure. Finely ground casein, on which the indicators neutral red and bromocresol purple had adsorbed, was fed to Daphnia. The colour change of the indicators was observed in progress through the intestine and the pH approximated. The animals were also fed finely ground corn starch which was stained with iodine. In order to detect the change of pH in the digestion of fats, emulsified oil was added to the culture medium. When the animals were permitted to remain in this medium, the oil prevented respiratory movements. The animals were therefore placed in a medium containing the stained emulsified oil until oil droplets were seen in the food groove. The animals were then removed to a medium without oil and observations were made.

Results. During the digestion of the casein, which is said to remain in the digestive tract from fifteen to

thirty minutes, the pH was 6.8 in the anterior part of the tract extending as far as the region immediately below the heart. Posterior to this the tract showed a pH of 7.2.

The dark blue colour of the stained starch grains changed to a pink below the region of the heart. Bromothymol blue had been added to the oil emulsion and a bright green colour (pH 6.9 to 7.2) was observed in the anterior end of the tract. This colour faded quickly, and in the posterior end of the tract only the characteristic colour of the faeces persisted.

Discussion. The distribution of food in the intestinal tract of Daphnia is brought about by the peristaltic and anti-peristaltic movements of the musculature of the wall. The food passes down to the region below the heart, where most of the digestion takes place. The waste is forced down by the intestinal movements and is passed out by sphincter-like motions of the anal opening.

The pH range found in the digestion of casein is in accordance with that of Hasler (1935), who states that the activity of the proteinase present in Daphnia permits considerable digestion. He also found present in the digestive tract an amylase. Its activity was demonstrated

on the iodine-stained starch grains.

Although the oil emulsion became a bright green in the anterior part of the tract the colour faded quickly and faeces excretion was rapid. Hasler stated that a lipase is present in Daphnia. Its action, however, could not be observed in this study. The oil-droplets were either too large and resistent to be broken down by the enzyme, or the action of the enzyme was too rapid to be indicated by a colour change.

Conclusion. As Hasler pointed out, the digestive tract of Daphnia can break down carbohydrates and proteins. There is no evidence, on the basis of this experiment, to indicate the breakdown of fats.

II. PHOTOTROPISM

Procedure. The experiments on phototropism were carried out in a dark room. An oblong pyrex dish, measuring thirty centimetres by eighteen centimetres, was used for the experiments. Light was provided by a 25-watt bulb covered with a concave metal shade in such a way that the rays of the light were directed on one half of the dish.

Two environmental factors were varied and the effect on the phototropic sign studied. In the first experiment the pH of the medium was altered; and in the second the temperature was changed.

In studying the effect of pH on the phototropic sign, tap water, that had been standing, was used as the first medium. The pH of this was determined by testing with Universal Indicator, bromothymol blue and phenol red. It was found to be between 7.0 and 7.5.

Ten animals were introduced into the medium and their reactions studied. This was repeated nine times. The bottom of the dish was then calibrated in centimetres, as shown in Plate I. Ninety animals were introduced, one at a time, at the one centimetre mark, and the time that each took to reach the lighted area was recorded. The time was taken for the first four centimetres covered, the next four, and the next, until sixteen centimetres were reached. The ten-centimetre line marked the boundary between the dark and lighted areas.

The pH of the tap water was reduced to a range of 6.0 to 6.5 with dilute acetic acid in the second part of the experiment; and in the third, dilute sodium hydroxide was added to the tap water until the pH was between

8.0 and 8.5. The same method of testing was followed, and the same animals were used in each medium. Observations were made to determine whether the pH of the medium affected the response and the rate of response to the light.

In the second experiment on phototropism, the response of Daphnia was studied at three temperatures, namely, 22°C, 16°C, and 9°C. The pH of the culture medium was between 7.0 and 7.5.

The reactions of the animals were first studied at twenty degrees Centigrade. Then the temperature of the medium was gradually lowered by adding ice water to a trough that had been placed under the experimental dish. When the temperature reached sixteen degrees the response of the animals was noted. More ice was added to the trough until the temperature remained at nine degrees, at which point the response was again studied. The same number of animals were used as in the first experiment on phototropism. The rate of response at different temperatures could not be conveniently measured.

Results. (i) The Effect Of pH On Phototropism. In studying the effect of pH on groups of animals, slight differences in response were observed. When the pH range

was 7.0 to 7.5, all animals, when introduced into the dark half of the dish, moved into the lighted area and remained there. Only three animals of the one hundred and eighty used returned to the dark half of the dish when the range of pH was 6.0 to 6.5. However, in the medium in which the pH was between 8.0 and 8.5, twenty returned to the dark half in the first experiment. When the experiment was repeated with another set of animals, many returned to the dark half and several persisted in swimming in and out of the lighted area.

The use of individual animals proved more indicative of the effects of pH on the phototropic sign. In the pH range of 7.0 to 7.5, the time taken to cover the first four centimetres averaged 9.4 and 10.8 seconds, and the next four centimetres 6.4 and 7.0 seconds. From eight centimetres to twelve centimetres the time taken decreased to 3.0 and 4.8 seconds respectively. From twelve to sixteen centimetres, which was well within the lighted area, the time averaged 6.0 seconds and 4.4 seconds.

In the more acidic medium of a range of pH 6.0 to 6.5 the animals were slower in responding. The time taken to travel the first four centimetres averaged 18.6 and 20.1 seconds. The speed averaged 6.0 and 6.9 seconds when

travelling from the eight-centimetre to the twelve-centimetre mark. Four animals reached the lighted area and returned to the dark half of the dish. These results are seen in Graphs I and II.

Graphs III and IV indicate the results obtained when the animals were placed in a medium in which the pH range was 8.0 to 8.5. Graph III gives the average time for a distance of sixteen centimetres, taken by seventy animals, and Graph IV shows the average time for a distance of eight centimetres only. The animals in this more basic medium were inconsistent in their behaviour.

The curve of Graph III shows that the time taken to cover the first four centimetres averaged fourteen seconds. It decreased to eleven seconds in the next four centimetres, and to six seconds in the last four. In the next group of animals, however, as seen in Graph IV, the average time taken to travel the first four centimetres was twenty seconds, and to travel the next four forty seconds. The animals then swam haphazardly around, and in and out of the lighted half of the dish.

(ii) The Effect Of Temperature On Phototropism. The animals tested were all positively phototropic at room temperature, twenty-two degrees Centigrade. When intro-

duced into the dark half of the experimental dish the animals swam directly into the lighted area until they reached either of the two corners of the dish. No difference in the phototropic sign was observed as the temperature was gradually lowered to sixteen degrees, and then to nine degrees. The animals were less active in the water , but no change in sign or in the strength of the sign was observed at the lower temperatures.

Discussion. Daphnia did not move immediately towards the light when introduced into the experimental dish. There was a period, called the latent period by Clarke (1932), during which each animal appeared to adjust itself and to co-ordinate its movements. As soon as the movements of the antennae were co-ordinated and the animal was adjusted, it moved definitely in a positive direction and swam forward with its dorsal side facing the light source.

The times given as indicative of the rate of response in media of different hydrogen-ion concentrations do not include the latent period.

The average time taken to cover the first four centimetres was greater than that taken to cover the other four-centimetre lengths in each of the three media. This was

probably due to the fact that each animal, although it was timed after the latent period and had indicated its positive reaction, was not completely adjusted to the environment. Another factor could be the greater distance from the light source; as a result of which there would be less effect on the light receptors and a slower response.

The animals were quickest in responding when the pH of the medium was between 7.0 and 7.5; which range approximates the pH of the normal culture medium. The time taken to respond in the medium whose pH was between 6.0 and 6.5 did not differ greatly from that taken in the medium of pH 8.0 to 8.5. In the former, the average times were 18.6 and 20.1 seconds; in the latter, 14.0 and 20.2 seconds.

The speed increased over the second four centimetres (four centimetres to eight centimetres) and the times taken in a pH of 7.0 to 7.5 were 6.4 and 7.0 seconds. The animals moved quicker as they neared the lighted area, and once in the lighted area they slowed down. This was also observed in a pH of 6.0 to 6.5. In the pH range of 8.0 to 8.5 the time taken after the first four centimetres decreased in one testing and increased in the other.

The animals took less time to cover the second four centimetres in a pH of 6.0 to 6.5 than in that of 7.0 to 7.5 or of 8.0 to 8.5. However, as seen in the Graphs, the animals were slower in crossing the boundary line (ten centimetres) in a pH of 6.0 to 6.5 than in that of 7.0 to 7.5. They took approximately the same time in a pH of 6.0 to 6.5 and the pH of 8.0 to 8.5.

The foregoing facts suggest that any variation from the normal hydrogen-ion concentration of the culture medium of Daphnia affects the response of the animals to light. Except for the second four centimetres covered the animals responded less quickly when not in a medium of pH 7.0 to 7.5. The more basic medium, for which complete recordings could not be made, had a greater effect on the response than the more acidic environment.

In general, either variation from the normal affected the response adversely, by slowing down the rate and in the pH of 8.0 to 8.5 causing uncertain and haphazard movements. The condition of the hydrogen-ion concentration of the medium might act on the musculature of the antennae to modify the rate, and on the nerve centre to influence the response.

The temperature of the medium was gradually lowered

and, as stated above, no change was observed in the primarily positive sign of Daphnia. A sudden decrease in the temperature reverses the primary sign (Clarke 1932), and a rapid rise in temperature strengthens the negative sign (Groom & Loch 1890) of Daphnia.

In this research, the slow rate of fall in temperature could account for the phototropic sign of Daphnia remaining stable, and the lower temperatures for the lessened activity. The light receptors and musculature became accustomed gradually to the lower temperatures and were therefore unaffected.

Conclusions. (a) A change in the hydrogen-ion concentration of the culture medium alters the phototropic response of Daphnia. (b) A medium that is more basic than the normal culture medium modifies the response and rate of response more than a medium that is more acidic. The latter, however, has a noticeably slowing effect on the rate of response. (c) A gradual fall in temperature does not change the primarily positive sign of Daphnia; nor does it affect the strength of the sign.

III. THE RESPONSE TO EXTERNAL STIMULI.

Procedure. The animals were studied individually, ninety being used for the experiments. Each animal was placed by means of a pipette into sufficient culture medium so that its activity was confined to the field of the microscope. The movements of the intestine were studied and the heart rate was recorded. The latter was performed in the following manner: The heart was closely observed and beats were recorded with a coloured crayon used on a white sheet of paper. A stop watch was employed to measure the time.

An animal to be tested was carefully transferred to the adrenaline solution (1:10,000), and after fifteen seconds the heart rate was recorded. The animal was returned to the culture medium and then placed in a solution of adrenaline (1:10,000) and ephedrine (1:10,000). The heart rate was again recorded after fifteen seconds. This procedure was repeated and the intestinal movements were studied.

The same method was used in studying the effects of ephedrine (1:10,000), and of ephedrine (1:10,000) and cocaine (1:10,000).

Results. (i) Adrenaline And Ephedrine. The normal heart rate of the young of Daphnia varied from 4.12 beats per second to 5.26 beats per second at room temperature. The heart rate changed in the adrenaline solution, and also in the solution of adrenaline and ephedrine. These results are recorded in Tables one, two, and three. In the first thirty animals, as seen in Table one, the average heart rate was 4.748 beats per second. In adrenaline it became 5.261; and in the adrenaline and ephedrine solution it increased to 5.40 per second. In Table two the rate increased from 4.625 to 5.154 and then to 5.278 beats per second. And, as seen in Table three, 4.881 rose to 5.297 and then to 5.432 beats per second.

The movements of the intestine of untreated animals are rhythmic in nature. There is a regular wave-like motion of the muscular wall of the tract and a gentle but pronounced surging back and forth of the material in the tract. When each animal was placed in the adrenaline the wave-like motions of the wall were not as deep, and they followed each other in slower succession. The intestinal contents moved slower and faeces excretion was less frequent.

In adrenaline and ephedrine the intestinal movement

was slower than normal and approximated that observed in adrenaline. Complete inhibition of the intestinal movement was not brought about by the adrenaline and ephedrine solution.

(ii) Ephedrine And Cocaine. Tables four, five, and six indicate the effects of ephedrine, and a solution of ephedrine and cocaine on the heart rate. The averages for the three groups of thirty untreated animals were 4.835, 4.782, and 4.796 beats per second. In ephedrine these averages increased to 5.089, 4.959, and 4.974 beats per second respectively.

When the animals were placed in the solution of ephedrine and cocaine the individual results were erratic, as can be seen from the Tables. Table four shows an average of 5.109 over 5.089 beats per second. In Table five the rate of 4.959 decreased to 4.930 beats per second.

The pronounced movements of the intestine changed when the animals were placed in ephedrine. There was a reduction in the depth of the waves and a slower surging of the contents. However the partial inhibition of the movements was not as pronounced as that seen with adrenaline. As in the case of the heart rate, the effects of ephedrine and cocaine on the intestine were variable.

TABLE ONE

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In adrenaline (1:10,000)	In adrenaline and ephedrine (1:10,000)
3	4.25	5.09	5.25
3	4.45	5.20	5.28
3	4.70	5.05	5.10
3	5.25	5.64	5.70
3	5.26	5.60	5.74
3	4.83	5.30	5.60
3	4.76	5.13	5.33
3	4.55	5.30	5.51
3	4.43	4.90	5.00
3	5.00	5.40	5.49
Average of 30 animals	4.748	5.261	5.400

TABLE TWO

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In adrenaline (1:10,000)	In adrenaline and ephedrine (1:10,000)
3	5.19	5.49	5.59
3	4.37	4.60	4.81
3	4.50	5.06	5.19
3	4.68	5.29	5.35
3	4.88	5.20	5.40
3	4.40	5.18	5.27
3	5.18	5.60	5.69
3	4.16	5.00	5.15
3	4.12	4.90	5.00
3	4.77	5.22	5.35
Average of 30 animals	4.625	5.154	5.278

TABLE THREE

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In adrenaline (1:10,000)	In adrenaline and ephedrine (1:10,000)
3	5.13	5.70	5.81
3	5.09	5.29	5.43
3	5.00	5.80	5.95
3	5.00	5.58	5.72
3	5.20	5.60	5.80
3	4.80	5.30	5.45
3	5.20	5.50	5.60
3	4.35	4.52	4.67
3	4.79	5.12	5.19
3	4.25	4.53	4.70
Average of 30 animals	4.881	5.297	5.432

TABLE FOUR

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In ephedrine (1:10,000)	In cocaine and ephedrine (1:10,000)
3	5.09	5.30	5.30
3	4.18	4.25	4.30
3	4.72	4.90	5.00
3	4.90	5.15	5.14
3	5.25	5.30	5.26
3	4.85	5.20	5.20
3	4.60	4.95	5.00
3	4.50	5.00	5.05
3	5.18	5.54	5.54
3	5.12	5.30	5.30
Average of 30 animals	4.835	5.089	5.109

TABLE FIVE

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In ephedrine (1:10,000)	In cocaine and ephedrine(1:10,000)
3	5.00	5.29	5.15
3	4.75	4.85	4.90
3	5.25	5.25	5.20
3	4.83	5.10	5.10
3	4.66	4.87	4.70
3	4.79	5.00	4.83
3	4.28	4.28	4.26
3	4.60	4.90	4.80
3	4.66	4.95	4.91
3	5.00	5.10	5.05
Average of 30 animals	4.782	4.959	4.890

TABLE SIX

No. of animals	Average Number of Heart Beats per Second		
	Untreated animals	In ephedrine (1:10,000)	In cocaine and ephedrine (1:10,000)
3	4.16	4.28	4.25
3	4.72	4.95	4.80
3	4.83	5.10	5.16
3	4.72	4.95	4.85
3	4.23	4.43	4.40
3	5.05	5.25	5.20
3	5.10	5.23	5.18
3	4.95	5.07	5.00
3	5.20	5.30	5.35
3	5.00	5.18	5.12
Average of 30 animals	4.796	4.974	4.930

In some animals the intestinal movements approximated those of untreated animals; in others the movements were greater; and in others there was a slight inhibition.

Discussion. The results of this experiment show that when the animals were placed in adrenaline the heart rate increased and the peristaltic and anti-peristaltic movements of the muscular wall of the intestine were invariable inhibited. There was a persistent rise in the heart rate. The increase ranged from 0.17 to 0.84 beats per second. This was brought about by a shorter systolic beat. Diastole appeared to remain unchanged.

The adrenaline, which was commercially prepared by Parke & Davis, contained sodium bisulfite, which minimizes the total effect of adrenaline. It is therefore probable that pure crystalline adrenaline would completely abolish intestinal movement and stop the heart beat.

The solution of adrenaline and ephedrine produced a greater increase in the heart rate of Daphnia than did a solution of adrenaline only. An additional rise ranging from 0.06 to 0.39 beats per second was observed. The ephedrine, which is an alkaloid similar to adrenaline in its action, increased the heart rate by 0.05 to 0.51 beats per second. The effect of ephedrine on the

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intestine, like that on the heart rate, was not as pronounced as that of adrenaline. As stated previously the effects of cocaine varied.

Such investigators as Viehoever (1936) and Baylor (1942) established the fact that most drugs produce in Daphnia their characteristic reactions. Adrenaline, ephedrine, and cocaine acted likewise in this study.

In 1914 Gaskell studied the giant chromaffin cells present in certain Annelids and Crustacea and put forth the theory that these cells may be the progenitors of the sympathetic nervous and adrenal systems in vertebrates. In 1939 Lancaster used precipation tests on giant nerve cells of H. medicinalis, Cambarus and other invertebrates, and proved that these did not contain any adrenaline-like substance. The giant chromaffin nerve cells, however, gave a positive reaction to various chromatin tests for adrenaline; and the results were similar to those obtained for the medullary cells of the rat and rabbit adrenals. Her findings supported Gaskell's theory.

No record of there being giant chromaffin cells in Daphnia could be found. The body of Daphnia does contain an acetylcholine-like substance (Artemov 1938, Metropoli-

tanskaja 1938). It is probable that Daphnia also has an adrenaline-like substance in its body. Baylor states that the heart of Daphnia resembles closely the vertebrate heart rather than the higher Crustacea heart in its reaction to drugs. In vertebrates adrenaline is broken down by an enzyme, amine oxidase, which also acts on ephedrine. As a result the presence of ephedrine tends to preserve adrenaline from destruction and to enhance its effect.

As seen from Tables one, two, and three, ephedrine did potentiate the effect of adrenaline. This lends further support to Baylor's statement.

Conclusions. (a) Adrenaline accelerates the heart rate and inhibits the intestinal movements of Daphnia. (b) Adrenaline in the presence of ephedrine produces a greater acceleration than when used alone. (c) Ephedrine accelerates the heart rate and inhibits the intestinal movements of Daphnia, but to a lesser degree than adrenaline. (d) Cocaine antagonizes the effect of ephedrine and at times enhances it. In general, its effects are erratic.

SUMMARY

1. A historical review of the biology of Daphnia has been presented. Studies of the morphology, physiology, and ecology have been surveyed; and the experiments to determine the regenerative powers and the hereditary traits have been taken into account.

2. A series of experiments have been carried out to study the change of pH in the intestine during digestion, and to find out the effects of pH and temperature on the phototropic sign of Daphnia. In addition, the responses of Daphnia to such external stimuli as adrenaline, ephedrine, and cocaine have been determined.

3. The pH in the intestine of Daphnia varies from 6.8 in the anterior end to 7.2 in the posterior end during digestion. A change in the pH of the culture medium alters the phototropic response of Daphnia; in that a medium that is either more acidic or more basic than the normal culture medium slows the rate of response. A more basic medium (pH 8.0 to 8.5), in addition, causes random movements, and in some cases, it changes a positive sign to a negative one. A gradual fall in the temperature of the culture medium does not alter the phototropic sign

of Daphnia. Adrenaline and ephedrine increase the heart rate and inhibit the intestinal movements of Daphnia. The effects of cocaine are variable.

PLATE I. Diagram of apparatus used in experiments in phototropism. Discussion in text (page 39).

GRAPHS I & II. Representations of results obtained in experiments in phototropism showing differences in response of Daphnia in culture media of pH 7.0 to 7.5 and pH 6.0 to 6.5.

GRAPHS III & IV. Representations of results obtained in experiments in phototropism showing differences in response of Daphnia in culture media of pH 7.0 to 7.5 and pH 8.0 to 8.5.

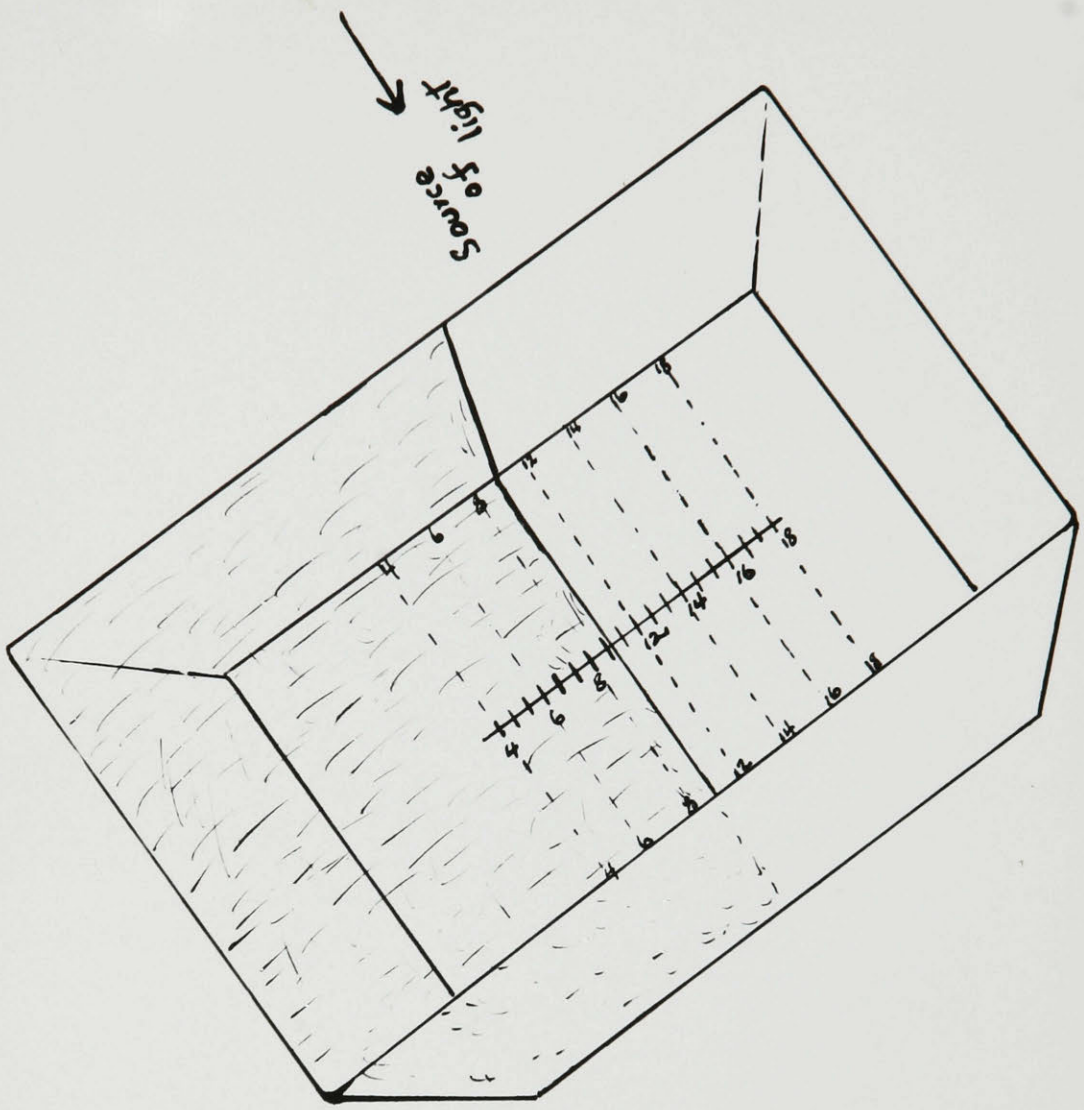
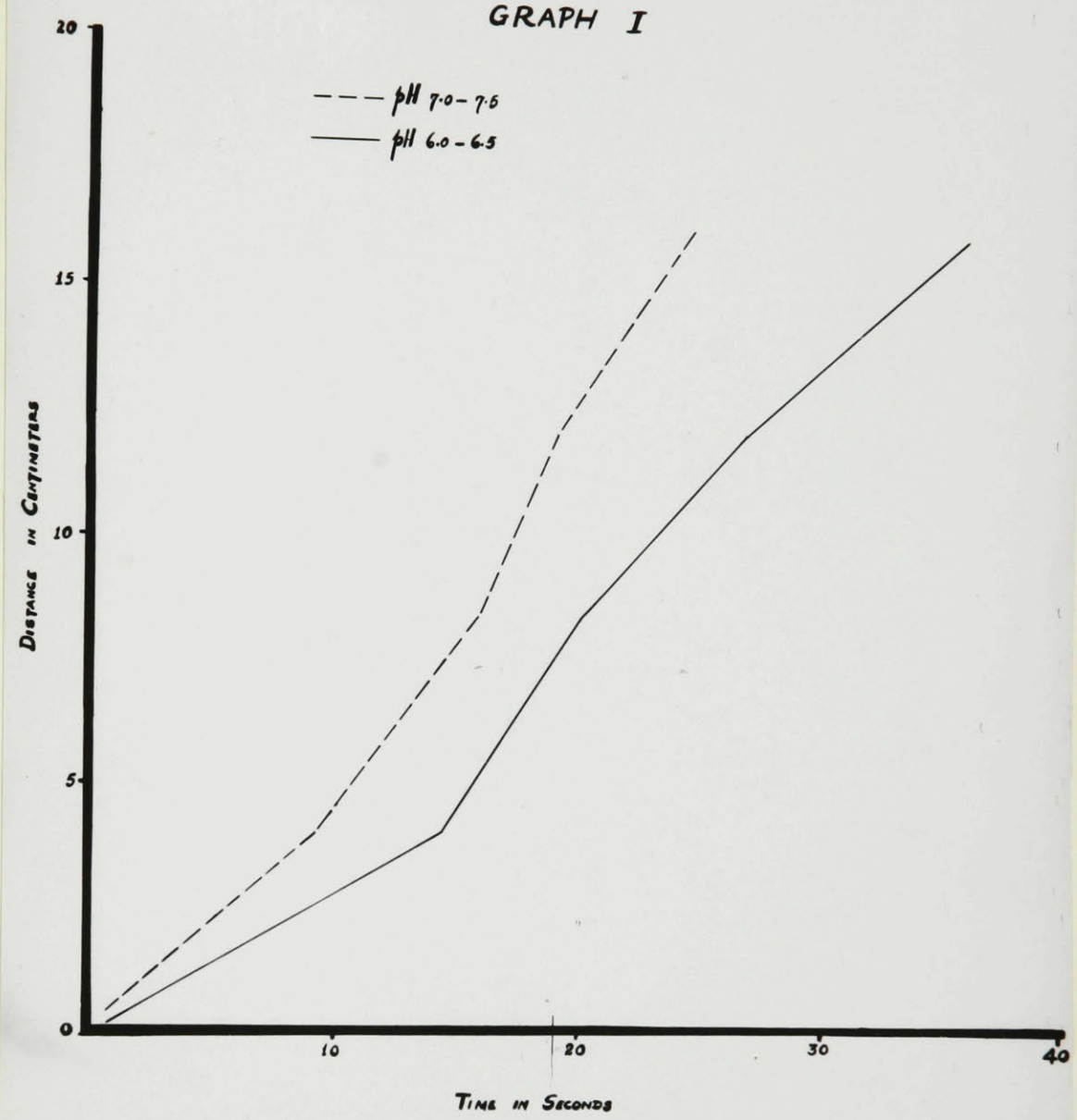
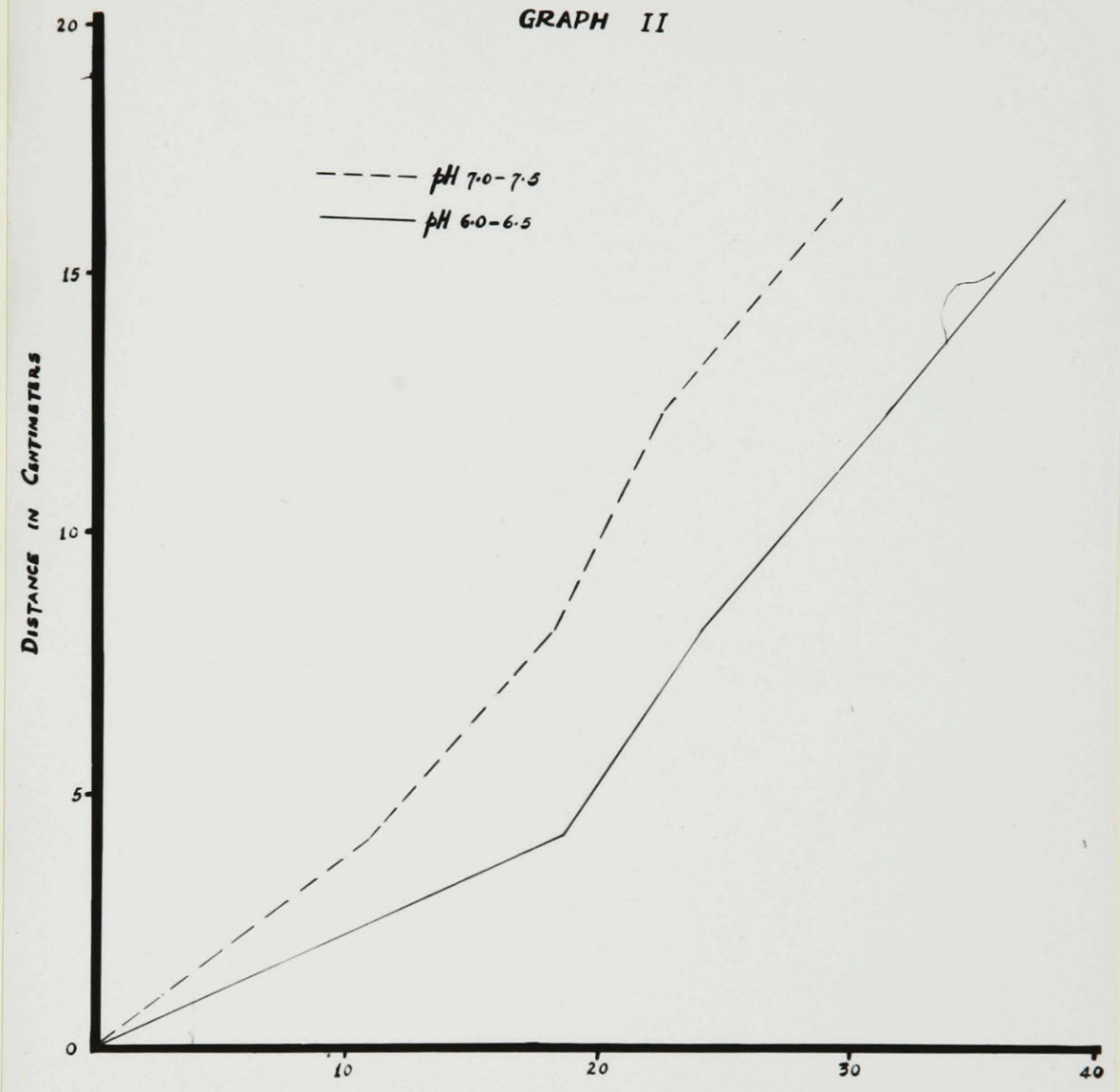


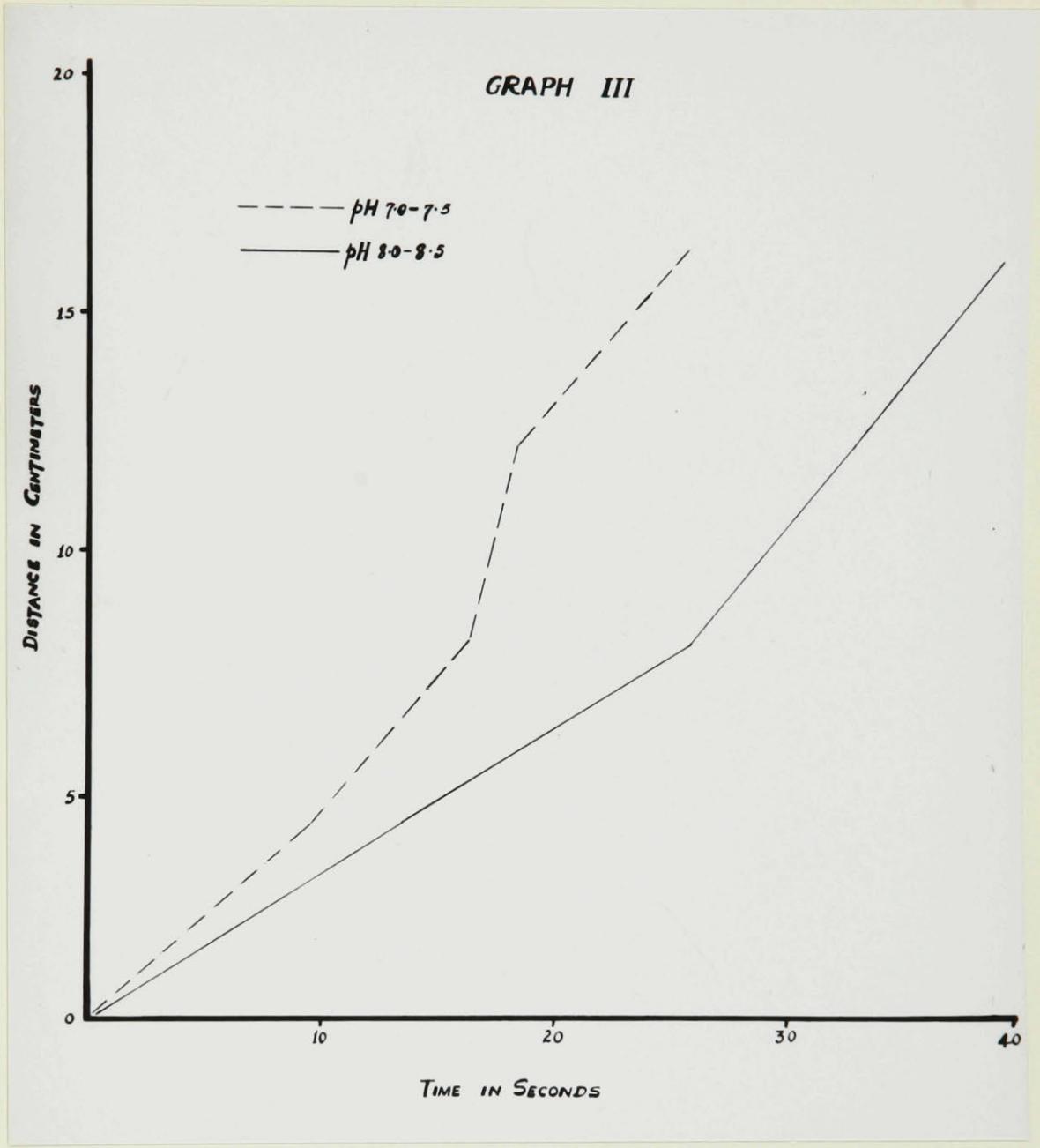
PLATE I

GRAPH I

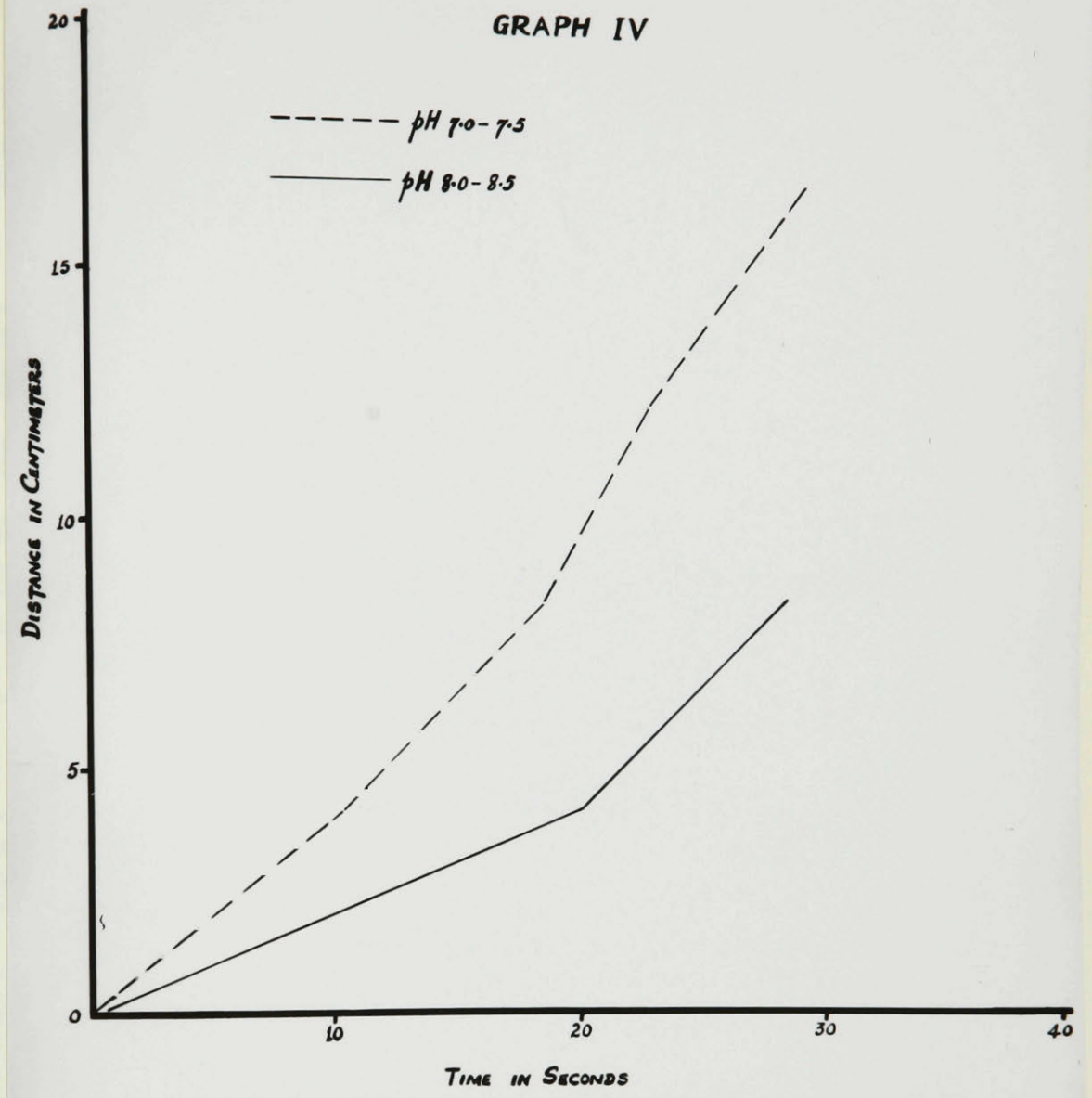

pH 7.0 - 7.5
pH 6.0 - 6.5
20
15
10
5
0
Distance in Centimeters
10
20
30
40
Time in Seconds

GRAPH II


pH 7.0-7.5
pH 6.0-6.5
Distance in Centimeters
20
15
10
5
0
10
20
30
40
Time in Seconds


GRAPH III
pH 7·0-7·5
pH 8·0-8·5
DISTANCE IN CENTIMETERS
20
15
10
5
0
10
20
30
40
TIME IN SECONDS

GRAPH IV


pH 7.0 - 7.5
pH 8.0 - 8.5
Distance in Centimeters
20
15
10
5
0
10
20
30
40
Time in Seconds

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