cipitation by omitting a part, if not all, of the potassium or sodium bicarbonate. This is both illegal and detrimental to the therapeutic properties of the product. The potassium or sodium citrate formed by the added bicarbonate has therapeutic effect not possessed by magnesium citrate and its omission cannot be justified. The Pharmacopœia in permitting the use of CO_2 under pressure definitely states that this may be done in addition to the use of bicarbonate and not as an alternative process.

From our study it would appear that the present U. S. P. formula is unsatisfactory, inasmuch as, when made with many samples of U. S. P. magnesium carbonate on the market, the product is subject to precipitation. The pharmacist cannot be expected to assay his magnesium carbonate before using it, as would be necessary before a stable product could be made. The only means of preventing precipitation with the present formula is to withhold the bicarbonate until the solution is to be dispensed.

CONCLUSIONS.

1. Using 33 Gm. of citric acid per bottle a magnesium carbonate containing not more than 39.2 per cent oxide must be used to produce even a fairly stable solution of magnesium citrate.

2. Precipitation may be prevented using any official magnesium carbonate (39.2–41.5 per cent MgO) if the bicarbonate is withheld until the product is dispensed.

3. Sterilization in some manner retards precipitation observed in the product.

4. Many commercial samples give analytical results indicating that all or at least part of the bicarbonate is omitted from the product.

5. It is recommended that the U. S. P. permit a greater tolerance in the requirement for total citric acid.

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DAPHNIA—THE BIOLOGICAL REAGENT.*

BY ARNO VIEHOEVER.[1]

Daphnia (*magna*), the transparent crustacean, possesses well-developed muscular, nervous and glandular systems (1, 2). It thus may serve as a remarkable test-animal for the detection of the presence or absence of substances, affecting the cells or organs of these systems. Such active agents may either be obtained from the plant, animal and mineral kingdom, or prepared synthetically.

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As daphnia responds not only qualitatively but also quantitatively to medicinal products, added in therapeutic, toxic, critical and fatal doses, it permits the study of the physiological mechanism of drug action. It facilitates the recognition of the nature of a great variety of active substances and provides a basis for their evaluation.

From the multitude of stimulants, irritants and depressants, and chemicals of varied character, tested with this biological reagent, daphnia, the following are selected as representative. The grouping is based upon their outstanding physiological function.

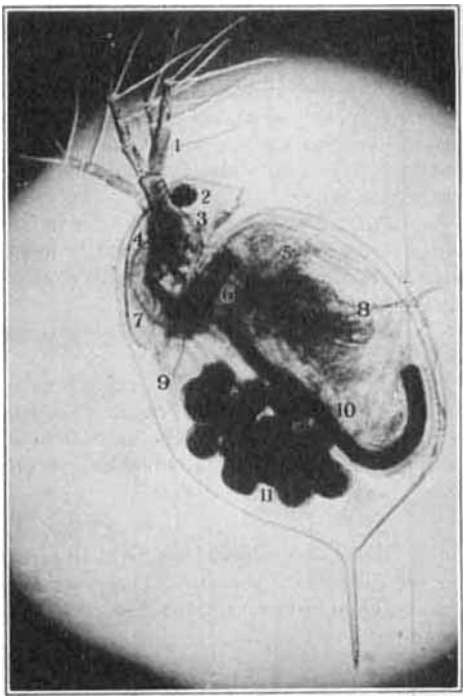

Fig. 1.—*Daphnia Magna:* 1—Swimming Arms. 2—Eye with Lenses. 3—Muscles with Optical Nerves. 4—Liver Glands. 5—Nephridial Bands. 6—Shell Gland. 7—Stomach (without Food). 8—Breathing Organs. 9—Heart with Valve. 10—Intestine with Food. 11—Brood Sac with Eggs.

I. Muscular System.

1. Locomotory Muscles.

Strychnine, used as the sulphate in various concentrations, causes in toxic doses a striking convulsion of the muscles of the swimming arms or antennæ. These convulsions resemble the tonic cramps so typical of strychnine poisoning of higher animals. Simultaneously the respiratory, and still more the circulatory systems, are greatly depressed (3). In this shift of body energy to the convulsing muscles of the swimming arms all other organs are made to suffer, even to the paralysis of the excretory system and final complete exhaustion (4). Death may, however, be prevented by the timely use of such physiological antidotes as ether and phenobarbital, mentioned later. Injection of strychnine into the blood stream of daphnia furnishes a very sensitive test for its presence, as amounts from as little as 1/50th to 1/100th of a milligram of strychnine sulphate caused the characteristic convulsions. Charts, lantern slides, photographic and photoelectrical records, as well as micro-movies, illustrate this effect.

Picrotoxin, too, produced the (clonic) cramps, the characteristic toxic effect known for vertebrate, or higher animals.

Excretory Organs.

Food Canal.—Of anthraquinone drugs, cascara has been studied, causing in a concentration of 1:15 (freed from alcohol) of the fluidextract progressive purgation and complete evacuation of the food canal within 19 minutes. Micro-movies illustrate this effect (5).

Aloe (*curacao*) and the resin (practically aloin-free) were found to be very active purgatives, causing complete evacuation in 0.05 per cent concentration in less than sixty minutes (6). The positive quantitative results obtained with many daphnia, standardized as to parentage, age,

sex and vitality may well represent a model for biological tests. The close agreement of the data is in contrast with the accepted view, expressed as late as 1933 by Clark (page 3) in his book on the action of drugs. "Physical and chemical tests are far more exact than are the biological tests; the information (provided by biological reactions) always tends to be vague and inexact."

The delayed action of aloin, considered three times more active than aloe, is of particular interest, as the finding agrees with that of early records of higher animal experiments, made even in the last century.

Rhubarb in low concentrations and its inferior substitute rhaponticum in like concentration, showed definite laxative effect (7).

Senna in the form of its fluidextract has been qualitatively tested with positive results of evacuating the food canal.

Phenolphthalein, in saturated aqueous solution, caused evacuation within thirty minutes.

Podophyllum studies, so far as they have progressed, permit the differentiation of toxic, laxative and inactive ingredients, all combined now in the crude drug and, more or less, in podophyllin. The studies promise a solution for the preparation of more suitable extracts and their evaluation (8).

Bitter salts as Glauber salt (sodium sulphate) and epsom salt (magnesium sulphate), the latter in concentration of 1 per cent or less caused speedy laxation; in higher concentrations, such as 3 per cent, it caused paralysis of the food canal and death through the dehydration of tissues.

Living organisms, yeast, added to daphnia in their normal culture medium, caused evacuation of the intestines (9).

Enzymes of the emulsin type, breaking down glucosidal substances as amygdalin, are evidently present in the food-canal. The enzymatic hydrolysis observed after ± sixteen hours, is speeded up according to the amount of emulsin added, causing the formation of the blood poison, hydrocyanic acid and benzaldehyde (10).

KIDNEY.

Aloin, as suspected from previous reports concerning its effect upon higher animals, affects the nephridia of daphnia (6). The toxic action becomes particularly marked in animals with low vitality, aloin producing a deep red vital stain upon retention and re-resorption in the nephridial bands.

RESPIRATORY ORGANS.

Insecticides, as rotenone, rotenone resin and cube extract containing rotenone, cause the paralysis of the breathing legs. If the rotenone is not oxidized in the upper food canal, then the digestive canal will also be paralyzed—a toxic action which has correspondingly been observed in higher animals, such as cats, rats and rabbits (11).

Ammonia in concentrations of 0.1 cc. (28 per cent) to 40 cc. culture water caused asphyxia and death to daphnia within fifty to sixty seconds, in higher concentrations much faster; in concentrations of 0.1 cc. (28 per cent) to 10 cc. culture water practically instantly. The symptoms corresponded to those characteristic for ammonia effect upon higher animals.

Benzaldehyde, when prevented from quick oxidation to benzoic acid, causes drowsiness, paralysis and asphyxia due, possibly, to its solvent action upon the cell lipoids. Similar results have been observed in rats and a dog (10).

CIRCULATORY ORGANS.

Digitalis and its constituents have been studied with interesting results.

Digitoxin.—Saturated aqueous solutions increased the tone of the heart muscle, the heart stopping in five to six hours. Digitoxin solutions lost their activity progressively on standing, even if kept in quartz containers to prevent alkali action of ordinary glass containers.

Digitonin.—A saturated solution of digitonin behaved in general like saponin solutions, a marked slowing of the heart was observed.

Digitalis (Whole Leaf).—Striking was the recovery of the heart of daphnia, depressed after chloroform administration, upon addition of a suspension of the leaf powder in culture water.

Gitalin and Bigitalin ("*Verodigen*").—The recovery of the chloroformed heart from its depression was much more speedy when these digitalis glucosides were added. A record in the form of motion pictures shows that, in the absence of digitoxin, accumulation of digitalis in the heart muscle is reduced. The speedy recovery is undoubtedly due to the rapid absorption of these glucosides, much more soluble in water than digitoxin.

Caffeine.—Tested qualitatively, caffeine markedly increases the force of the heart beat, simultaneously slowing the rate. The heart stopped in diastole, when toxic doses were given.

II. Nervous System.

Anesthetics.—Ether, alcohol and especially chloroform were studied in detail. Chloroform causes in 3 per cent of saturated aqueous solution a characteristic depression of the heart beat, passing through the stages of coupled beat, spasms and progressively increased rest-periods to complete paralysis and death. This effect is recorded photographically, photoelectrically and with motion pictures. The toxic effect may be overcome with digitalis and its glucosides, as mentioned.

Hypnotics.—Chloral hydrate and especially phenobarbital and sodium phenobarbital have been found effective. Particularly satisfactory was phenobarbital in saturated aqueous solution in overcoming the convulsive action of strychnine (which was also affected with aqueous ether solution—1 per cent).

Alkaloids.—Strychnine has been studied qualitatively and quantitatively, as already mentioned; of others qualitatively tested, morphine and nicotine may be mentioned as affecting markedly the nervous system and organs depending upon its normal function.

Toxins.—Podophyllotoxin causes paralysis and death, rather than laxation; picrotoxin, as mentioned, causes strychnine-like convulsions; the toxin produced by hydra, causes, when injected directly by the hydra, or upon injection of the hydra extract, paralysis and death of the daphnia, after brief initial cramps.

III. Glandular System.

Qualitatively tested, adrenalin exerted a markedly stimulating effect on the heart of a dying daphnia; pituitrin on the reproductive system, speeding birth of young in the brood-sac; cantharidin upon the generative organ of the male, causing the periodic ejection of the sperm.

Liver.—The general function of this gland to retain poisonous substances entering the digestive system, in order to render them harmless, marks the glandular structures, obviously representing the liver in daphnia. Vital stains, excessively used, are found in the liver; strychnine may also be detected there after administration causing a decided shrinking of the glandular tissue, corresponding to the effect upon the liver in higher animals.

IV. Blood.

The blood poison, hydrocyanic acid, obtained in the process of enzymatic hydrolysis of cyano-genetic glucosides, as amygdalin, effects speedy death with the symptoms characteristic of hydrocyanic acid in higher animals, laxation, cramps, paralysis, coma, death (10).

V. Miscellaneous.

Adsorbents.—Colloidal magnesium and aluminum compounds—as "Kaomagma," for instance, permit the demonstration of their adsorbent qualities for

poisons—as strychnine. This will not cause in their presence the usual convulsions unless the quantity of strychnine is greatly increased.

Antiseptics, Disinfectants.—Phenol, qualitatively tested, caused convulsion-like reactions of the swimming legs, followed quickly either by paralysis, or recovery when speedily diluted with culture water—in agreement with the irritant and toxic effect upon higher organisms.

Pyridium, as a specific antigonorrheal, quickly penetrated through the digestive canal, staining the intestinal wall and sex organs of the male in a differential manner, thus serving also as a vital stain.

Vital Stains.—The use of vital stains such as neutral red, alizarin and many others, permits the differentiation of cells, organs and tissues, the more adequate study of their morphology and physiological functions, normal and abnormal (16). Eosin brings out the nerves in the muscle fibres leading from the brain to the retina of the eye. The staining of vital tissues with fluorescing substances is particularly sensitive when seen under reflected light in the dark field microscope.

VI. Metabolism.

The effect of low-freezing temperature and progressive hydration on lowered body function and lowered metabolism can be readily observed, the circulatory system being evidently last affected (17). The continued specific study of toxic substances such as heavy metals, interfering with normal enzyme and tissue action, is in progress; as is the study of physiological antidotes; of vitamines and other food substances, affecting normal nutrition, growth and development of cells and organs; of additional hormones, assuring their normal function.

Methodical.—The methods of propagation of daphnia for experimental, biological purposes, have been worked out, assuring a uniform and ample supply of test animals (18). The testing by direct medication through the culture water or through injection into the blood stream has also been accomplished.

SUMMARY.

Daphnia (*magna*) with its major structures, and functions clearly visible—either directly or after vital staining, with its marked physiological responses to medical and other agents, not unlike those of higher animals, is truly a remarkable biological reagent.

Its use opens a new world for experimentation. It provides a new tool for identification, differentiation and evaluation of physiologically active substances. It furnishes a unique opportunity to study the intricate mechanism and balanced rhythm of life.

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ANTISEPTICS: A COMPARATIVE STUDY OF LABORATORY AND PRACTICAL TESTS.*

BY GEORGE F. REDDISH.[1]

Antiseptic substances were used for the preservation of food long before putrefaction and decay were shown to be caused by microörganisms. When Pasteur demonstrated the microbic origin of fermentation, he not only solved the problem of food spoilage but also took the first step in the scientific study of the cause of disease. The discovery of the cause of fermentation and disease led immediately to a study of the methods for preventing microbic activity pertaining to each. While Pasteur at first directed his efforts toward methods for controlling fermentation, a young English surgeon, Joseph Lister, made the first attempts to control the cause of infection.

Remembering the speculation of Robert Boyle two centuries earlier that the discovery of the cause of fermentation would lead to explanation of the cause of disease, Lister began his epochal studies on infection shortly after Pasteur announced results of his brilliant studies on fermentation. Without actually isolating the bacteria causing "hospital gangrene," Lister assumed that some kind of microorganism was the cause of these infections and in his effort to prevent post-operative "gangrene" he used a chemical which was known to be effective for preventing putrefaction. Carbolic acid in concentrations of 1–20 and 1–40 were employed for this purpose. After using this germicide for disinfecting surgical instruments, dressings, bandages and the operative field, infection following operations was greatly reduced. The use of this germicide in surgery formed the basis of Lister's system of antiseptic surgery.

From that day to this, antiseptics have been employed for two purposes (1) to preserve food by preventing the growth of bacteria causing putrefaction and decay, and (2) to kill or inhibit the bacteria which cause infections. This double meaning of the word "antiseptic" is still recognized. While the inhibitory meaning is emphasized in bacteriology textbooks, the germicidal meaning is most common among the medical profession and laity.

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[1] Professor of Bacteriology, St. Louis College of Pharmacy.