

puppies, it was evident that there was a constant source of supply of these organisms. Therefore, the following investigations were undertaken. The hair from 5 normal adult dogs and from 4 puppies was cultured aerobically and anaerobically in digest meat broth. Positive cultures were obtained in each case. The gram-negative, spore-bearing bacillus so frequently found in the liver was demonstrated in each culture. Scrapings obtained from the breasts of the nursing mother were cultured, and the same spore-bearing, gram-negative bacillus, as described above, was found.

We believe the source of the organisms found in normal, healthy dogs' liver is obtained from the hair of the animals, the organism gaining entrance into the stomach by way of the mouth, resulting from licking of the body, passing into the duodenum and most probably up through the common duct into the liver. In only 1 case was an attempt made to identify the organisms found in the liver. This was a large, gram-positive organism, and cultural characteristics proved this organism to be the *B. welchii*.

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A Method for Determining Basal Metabolism of Fishes.

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Various methods used by other workers in metabolism experiments with fishes were considered inapplicable to studies in which physiological experimentation was to be extended over a considerable period of time. The constant flow method used by Hall¹ was modified for the present study. Hall measured the metabolism of many fishes over short periods of time. The present method is adapted for more detailed studies and provides for greater care of the experimental animals. Although Hall employed urethane to inhibit the movement of the fishes, Wieland (1915) states that the use of urethane causes the CO₂ threshold to be raised in the animals thus treated, and Winterstein (1914) concludes that O₂ consumption is decreased with the administration of urethane. Injection with urethane would have to be repeated after a 2-hour period, with consequent mechanical injury and metabolic disturbance to the fish; therefore Hall's method of controlling movement would not be applicable to protracted studies.

¹ Hall, F. G., *Am. J. Physiol.*, 1928, **88**, 212.

Twenty-eight fishes, 18 individuals of the large mouthed black bass, *Micropterus salmoides* (Lacepede), and 10 of the bluegill, *Lepomis incisor* (Mitchell), were used. Except in preliminary tests all experiments were carried out on fasting fishes, to obtain conditions more or less comparable with those prescribed for testing basal metabolism in humans. Twenty-four hours were allowed to elapse after the fishes were collected so that the intestine would be empty of fecal matter; each individual was then weighed, and placed in a fish jar in which it was to be tested (Fig. 1 B) for several hours before a series of determinations was begun.

Metabolic rate was measured in terms of cc. of oxygen consumed per kilo of body weight per hour. Oxygen determinations were made by the use of the Winkler method as described by Kemmerer, Bovard and Boorman (1924). According to these authors the regular Winkler method is regarded as satisfactory except when the water contains at least 0.1 part of nitrite nitrogen per million or considerable organic matter. It was considered that the small amount of organic matter that the fish, under the conditions of the test, would be discharging into the jar would be removed by the flow passing through the fish jar before the concentration became sufficient to affect oxygen determinations.

The set-up for the experiments is illustrated in Fig. 1. In the employment of constant flow a stream of water was allowed to pass through the fish jar. Water drawn through the sampling tube (C) would be representative of the inflow into the fish jar. The rate of flow was controlled by elevation for the outflow tube (D). A sample was taken from here simultaneously with that from (C). The difference in oxygen concentrations in these 2 samples represented the amount of oxygen being consumed by the fish.

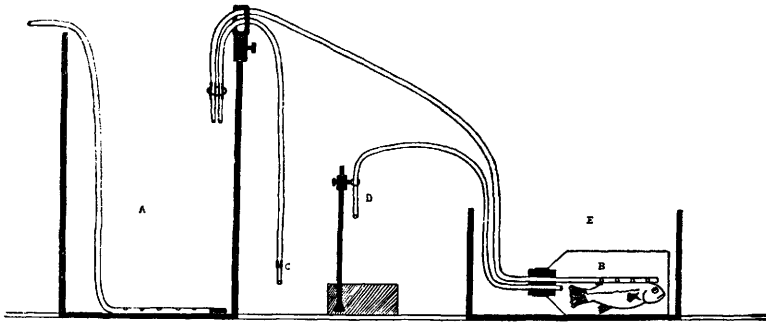


FIG. 1.

Diagram of apparatus used in metabolism experiments. (A) reservoir; (B) fish jar; (C) sampling tube from reservoir; (D) outflow tube from fish jar; (E) constant temperature bath.

In order that the water collected from the sampling tube represent the oxygen concentration of the water flowing into the fish jar the following conditions were observed. The reservoir was a 26 gallon tank, filled to overflowing from the laboratory tap, the end of the tube sealed so that all the inflow into the tank came through a number of comparatively small openings along the bottom of the tank. The upward currents thus produced circulation which prevented stratification or uneven distribution of oxygen. These precautions were necessary because early experiments showed wide hourly variations in oxygen content of the tap water. A mixing device in the fish jar similar to that installed in the reservoir provided that the inflow into the fish jar mixed freely with the water within. The rate of flow through the fish jar maintained in the jar at all times an abundant oxygen supply for normal metabolism. A constant rate of flow was maintained throughout each experiment.

The size of the fish jar was related to the size of the fish being tested. The fish was restricted to little more than respiratory movements of the fins and compensatory movement of the body. Constant temperature baths (E, Fig. 1) were 5-gallon crocks, the variation in temperature between inflow and outflow was at all times less than 0.5°C.

The glass fish jars with rubber stoppers were adapted in size to the fish being tested. One experimental animal, weighing 2100 gm. proved too large for convenient handling by this method. An aquarium was filled with water to within 3 inches of the top, and after the fish has been entered a layer of mineral oil, about an inch and a half in thickness, provided conditions approximating the air tightness of the fish jar. To guard against the fish getting into the oil a wooden lattice was secured just below the layer of oil. The rate of flow was regulated by running the water into a small container which could be raised and lowered at will. A constant level siphon provided for the maintenance of constant volume. During experimentation the sides of the aquarium were covered with an opaque screen.

In order to determine whether metabolic rate of fishes could be tested accurately by a method of intermittent flow the following procedure was devised. The apparatus of Figure 1 was used. Sample (1) was taken from the sampling tube simultaneously with Sample (2) from the fish jar. Then the flow of water into the jar was stopped. The determinations of (1)-(2) gave the oxygen consumption, with the use of constant flow. Fifteen minutes later

Sample (3) from the outflow tube of the fish jar was taken. The determinations of (2)-(3) gave the oxygen consumption with the use of intermittent flow.

The results of intermittent flow though themselves constant are noticeably lower than the results on the same individual using constant flow. This discrepancy might result from the following causes: 1. The oxygen tension of the medium in the fish jar varies with the use of intermittent flow. The fish, during the first 5 minutes of the test is extracting oxygen from a medium having an oxygen tension considerably different from the medium during the last 5 minutes of the test. According to Powers² oxygen consumption is affected by oxygen tension and carbon dioxide concentration of the media. 2. The carbon dioxide tension varies during the period of testing with intermittent flow. There is a resulting increase in hydrogen ion concentration which according to Powers might affect the oxygen consumption of the fish. 3. With the set-up regulated for simultaneous testing of continuous and intermittent flow, it was impractical to draw check samples for the intermittent flow determinations. The possibility of experimental error was thus increased.

The method of constant flow is therefore regarded as providing a greater degree of accuracy for the experiments at hand. Using constant flow 407 determinations were made on the 28 fishes tested. The time of testing an individual varied from 8 hours to 2 weeks, the number of determinations from 4 to 52. No attempt has yet been made to compile definite metabolic rates to be accepted as basal for fishes, even within the range of the species tested. The purpose was to develop a technique whereby fishes could be kept at a fairly constant rate of metabolism while undergoing physiological experimentation. Even the highly standardized procedure for humans admits considerable range of individual variation. The determination of the metabolic rate in fishes presents the added difficulties of control of a varying medium and the impossibility of completely controlling the movement of the fish. As yet little is known regarding the relation of age, sex, and physiological condition to respiratory metabolism of the fishes tested, and the purpose of the investigation was considered attained when results showed the fish to be metabolizing at a fairly constant rate. Duplicate determinations were taken and the results reported as a mean of the two. Deviations from the mean averaged less than 3%. When successive determinations of metabolic rate showed a varia-

² Powers, E. B., *J. Gen. Physiol.*, 1921, 4, 305.

tion within $\pm 15\%$ it was considered that a degree of constancy had been reached comparable with that expected in basal metabolism for humans.

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Serum Calcium, Inorganic Phosphorus and Plasma Proteins in Cardiac Edema and After Diuresis.*

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Peters¹ and his coworkers have demonstrated the interrelationship of serum calcium, inorganic phosphorus and the total plasma proteins with albumin and globulin fractions combined. They have analyzed previous reports and their results and have evaluated the relative effects of protein and phosphorus on serum calcium in the absence of any true defect in calcium metabolism. The relation of the components in this series was defined by the equation $Ca = 0.255 P + 0.566 \text{ protein} + 7$. An alignment chart was constructed to conform with this equation. The study of this blood chemical series in patients with edema of congestive heart failure was undertaken with the idea of establishing the presence or absence of any relationship between the series, but especially to determine the calcium level and the effectiveness or lack of the same on digitalization and other therapeutic measures.

The patients were all old white men and the normal levels for males established by Peters and his coworkers therefore are applied. The averages were: Total serum protein 6.93%; serum albumin 5.06%, and serum globulin 1.89%. The serum calcium normal is about 6 to 10 mgm. and the inorganic phosphorus is 3.5 to 15.6 mgm. per 100 cc. serum.

Only 4 of our cases are suitable for reporting now. All were arteriosclerotics, two, No. 1 and No. 3, with auricular fibrillation and two, No. 2 and No. 4, with normal sinus mechanism. Both of the latter died, while the fibrillators responded to digitalization with

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¹ Peters, J. P., and Eiserson, Leo, *J. Biol. Chem.*, 1929, **84**, 155.