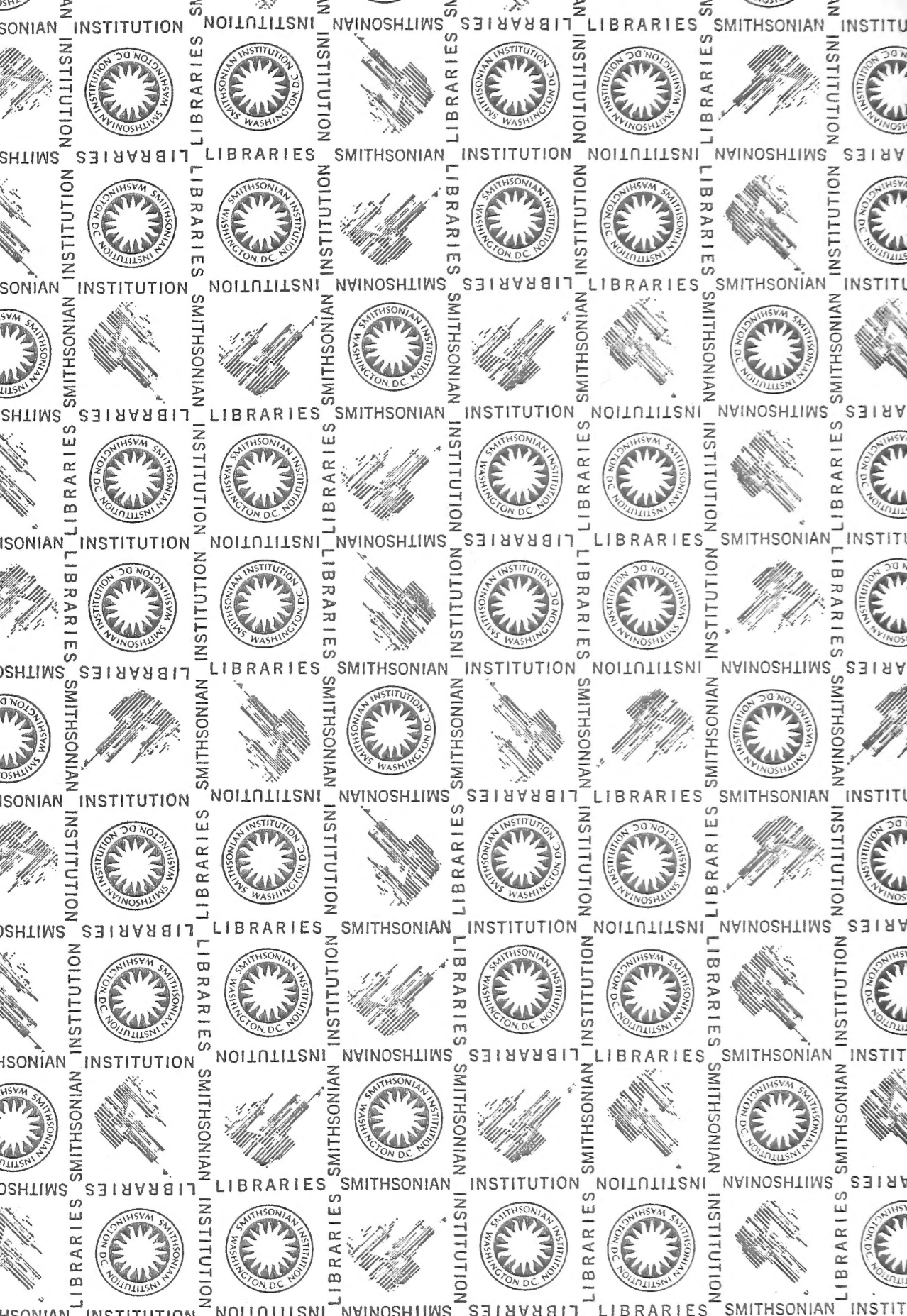
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INTERMEDIARY METABOLISM OF FISHES AND OTHER AQUATIC ANIMALS

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by

M. Gumbmann, W. Duane Brown, and A. L. Tappel

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ABSTRACT

This paper is a review of current knowledge of intermediary metabolism in fish (teleosts and elasmobranchs), shellfish (mollusks and crustaceans), and certain other aquatic animals. All important research relative to the topic is mentioned, although the paper is not a totally exhaustive review. Numerous references to the original literature are included.

The review is organized around the major well-known metabolic divisions: carbohydrate metabolism, including pathways of glycolysis and the phenomenon of rigor mortis; respiratory metabolism and the tricarboxylic acid cycle; electron transport systems; phosphorus metabolism; protein metabolism; and lipid metabolism. The information is summarized in figures wherever feasible.

A section concerning the needs for research is included, and specific suggestions for needed investigations are given.

INTERMEDIARY METABOLISM OF FISHES AND OTHER AQUATIC ANIMALS

by M. Gumbmann[1/], W. Duane Brown[2/], and A. L. Tappel[3/]

INTRODUCTION

A sizable body of knowledge dealing with composition and physiology of fishes and other aquatic animals is available. There have been but few studies, however, of intermediary metabolism in these organisms. This paper is a review of the studies that have appeared in the literature.

Physiology and composition are discussed where their relationship to intermediary metabolism is indicated. The major differences in composition that distinguish aquatic animals from land animals is pointed out, since such differences suggest deviations in metabolic routes and pathways.

The animals discussed are limited to fish (teleosts and elasmobranchs), shellfish (mollusks and crustaceans), sea urchin, and aquatic mammals (dolphins and whales). Metabolic studies of the sea urchin have been included because of the large amount of study given this animal and because the metabolism of the sea urchin appears to resemble that of other marine invertebrates more closely than it does that of higher marine forms. The review is organized around the major well-known metabolic divisions: carbohydrate metabolism, including the Embden-Meyerhof pathway, the phenomenon of rigor mortis, and the hexosemonophosphate shunt; respiratory metabolism and the tricarboxylic acid cycle; electron-transport systems of terminal oxidation, including various cofactors;

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phosphorus metabolism and the processes of storage and transfer of energy; protein and nitrogen metabolism, including nitrogenous compounds characteristic of aquatic animals; and lipid metabolism, including fatty acid composition. Where possible, the information is summarized in figures along the lines of conventional metabolic maps.

The presentation of compositional analyses and physiological data of aquatic animals is not the concern of this review except in a general sense where it indicates metabolic pathways and possible unique processes occurring in aquatic animals; tables and charts giving such information have not been included. Many excellent reviews are available where this type of information has been collected, and a few of them might be mentioned here. A recent book giving extensive coverage of the subject, for example, is The Physiology of Fishes by Brown (1957). In a symposium on the biochemistry of fish (Williams 1951) are reviews of "The Proteins of Fish" by Hamoir, "The Chemistry and Metabolism of the Nitrogenous Extractives in Fish" by Shewan, and "The Chemistry and Metabolism of Fats in Fish" by Lovern. Hamoir (1955 a) has made an intensive review of fish proteins, including enzymes, respiratory pigments, and contractile proteins.

This review is similar to those in an Annual Review of Biochemistry. It is exhaustive to the point of not missing important research on the intermediary metabolism of aquatic animals, but it does not cite all the work done in this field. By limiting the review to appropriate enzyme researches, it is found that very few enzyme researches of major interest in the present problem were completed before 1945. The survey of literature pertaining to this review was completed in February 1958.

CARBOHYDRATE METABOLISM

The study of carbohydrate metabolism in fish has been confined mainly to compositional analyses of fish tissues and eggs. Evidence defining the processes involved in this phase of metabolism is indirect and, for the most part, has been inferred from changes in amounts of total carbohydrate, glycogen, and glucose in relation to various physiological and environmental factors. Little has been done to characterize the specific enzyme systems and the pathways relating the intermediate products of carbohydrate metabolism.

Immers (1953) followed glycogen metabolism in the stickleback and minnow by measuring changes in glycogen distribution in the liver, gonads, and skin, with regard to sex differentiation and the sexual cycle. In the prespawning salmon, the examination of glycogen mobilization indicates that gluconeogensis (the transformation of endogenous fat and protein to carbohydrate) must occur in order to meet the energy demand required during migration (Fontaine and Hatey 1953). This process must occur extensively in order to account for the relatively high level of sugar in the blood and the more or less constant reserve of glycogen maintained by these fish during the period of fasting incident to migration. In mammals, the process of gluconeogensis is dependent upon the pituitary growth hormone (Bishop 1954), and evidence indicates that the same holds true for fish (Brown 1957). In the fasting eel that has been previously hypophysectomized,

glycogen reserves progressively decline (Hatey 1951 a,b). Upon injection of glucose, however, the hypophysectomized eel is able to synthesize glycogen. In the hypophysectomized, but feeding, fundulus, normal glycogen reserves are found (Pickford 1953).

Glycolysis in the red-colored swim bladder gland of the scup has been demonstrated by Strittmatter et al. (1952). He obtained significant increases in production of lactic acid after addition of glucose and other hexoses to the bisected gland, under both anaerobic and aerobic conditions. De Vincentiis (1952) has measured rates of respiration and glycolysis of retinas of teleosts and of octopuses by manometric measurements, with glucose as a substrate. The rate for retinas of octopuses was similar to that found for retinas of teleosts and generally resembled the small values obtained with cold-blooded vertebrates rather than the higher values obtained with birds and with mammals. Hishida and Nakano (1954) measured the uptake of glycogen and of oxygen in developing Oryzias eggs. Determination of the respiratory quotient showed that metabolism of carbohydrate probably begins after the gastrula stage; such a scheme agrees with the glycogen analyses made over this period. Anaerobic glycolysis and lactic acid production also were observed.

Evidence of glycolysis occurring in the developing fundulus embryo in relation to regulation of osmotic pressure has been shown by Shanklin (1954). That glycolysis supplies the energy necessary for maintaining normal ionic transport and development of the egg was shown by treatment with fluoride and with iodoacetate. These two known glycolytic inhibitors disrupted the normal ionic gradient established across the egg membrane. The enzyme catalyzing the conversion of phosphopyruvate to pyruvate has been shown to occur in the muscle of several species of fish (Boyer 1953). The presence of adenosine diphosphate (ADP) and potassium ion is essential for the reaction. The presence of this enzyme in all the species tested indicates the probability that other enzymes of the glycolytic system also are present and functioning.

Hexokinase is a glycolytic enzyme that is responsible for the initial activation of a hexose sugar molecule, thus allowing phosphorylation and the subsequent sequence of intermediary steps of glycolysis to proceed. Kerly and Leaback (1957) found this enzyme to be active in brain homogenates of squids (Sepia officinalis), elasmobranchs (Scyllium canicula and Raia brachyura), teleosts (Seophthalmus maximus and Gadus merangulus), and frogs (Rana temporaria). Glucose and fructose were utilized at a high rate in all of the species examined, adenosine triphosphate (ATP) being required for activity. The hexokinase of these aquatic animals resembled the nonspecific type found in mammalian brain in that it attacks both aldoses and ketoses and, in contrast to the hexokinase of yeast, is inhibited by its reaction product.

The hexosemonophosphate shunt is an important oxidative pathway in carbohydrate metabolism. For fertilized eggs of the sea urchin, Arbacia,

it may be considered a short-cut method by which the embryo obtains energy while awaiting the development of other glycolytic and tricarboxylic acid cycle enzymes (Krahl 1956). The existence and relative importance of this pathway in fish has yet to be investigated. A compound formed by the hexosemonophosphate pathway, ribose, is liberated from various ribose-containing compounds by enzymes present in muscle tissue of fish.

Tarr (1952, 1953, and 1954) and Tarr and Bissett (1954) found that ribose was cleaved from ribonucleic acid, ATP, ribonucleosides, ribonucleotides, and ribose-5-phosphate added to the muscle of certain fish. More recently, using lingcod muscle, Tarr (1955) was able to purify a nonphosphorolytic nucleosidase capable of hydrolyzing ribonucleosides, such as adenosine, inosine, guanosine, xanthosine, and cytidine. It appears that fish muscle contains a ribonuclease and other enzymes that hydrolyze ribonucleic acid to constituent mononucleosides. These, in turn, may be attacked by a non-phosphorolytic riboside hydrolase enzyme, with the liberation of ribose and a base. This degradation process appears to be more rapid in fish than in warm-blooded animals (Hamoir 1955 b).

Alcohol dehydrogenase, which in mammals is found almost exclusively in the liver, has been isolated and purified from fish liver (Boeri et al. 1954).

ATP is the key compound for trapping the energy released during carbohydrate oxidation in all forms of life and serves as the immediate source of energy for muscular contraction. It therefore is not surprising that considerable interest has been shown the presence and utilization of ATP in fish. Contraction and rigor mortis in fish muscle have been related to post-mortem changes in amounts of glycogen, ATP, and free sulfhydryl groups (Noguchi and Yamomoto 1955 a,b; Partmann 1953). These compounds decrease rapidly, reaching a minimum after 9 hours with full rigor and a decrease in pH. Somewhat similar studies of fish muscle were made by Fujimaki and Kojo (1953 a,b), in which glycogen, lactic acid, ammonia, amide nitrogen, and pH were determined in frigate mackerel killed by various methods. Fish that were left to die underwent a rapid decrease of ATP, with a corresponding increase of adenylic acid and inosinic acid within 4 hours. Reay and Shewan (1949) have also reviewed the immediate post-mortem changes in glycogen and lactic acid content and pH of fish muscle. Compared to mammalian muscle, fish muscle usually contained less glycogen, owing to exhaustion before death, and had a higher pH; these factors increase the speed of onset of rigor mortis.

Saito and Hidaka (1955 a,b; 1956 a,b), using purified myosin fractions isolated from carp muscle, have studied ATP-ase activity and stability and the post-mortem changes of myosin fraction nitrogen, ATP, and free sulfhydryl groups in carp muscle. In general, ATP-ase activity of the myosin fractions was high and showed a temperature optimum similar to that of rabbit muscle. The decrease of this activity at 25° C. over a period of 10 hours coincided with the decrease in sulfhydryl groups. Rigor mortis also paralleled the decrease of ATP and sulfhydryl groups and was complete after 6 hours, at which time all ATP had disappeared.

The main differences of fish muscle as compared with muscle of land animals are lower glycogen content, initially higher lactic acid content, and greater ATP-ase activity (Hamoir 1955 b). Steinbach (1949) compared ATP-ase activity of muscle-tissue homogenates of swordtail, bluegill, minnow, frog, turtle, mouse, and bird. Measured at 30° C., ATP is split at nearly the same rate by all the homogenates, but at 0° C., wide differences occur, with the activity of the fish preparations remaining fairly high. Based on the Q_{10} values obtained, the animals fell into three groups: fish, 1.4-1.7; bird, mouse, and turtle, 2.0; and frog, intermediate. The relative insensitivity of fish ATP-ase to changes in temperature suggests that low Q_{10} values for critical reactions may be an adaption of cold-blooded animals that must survive variable temperatures. Apparently, however, no correlation exists relating ability to survive in cold temperatures with Q_{10} values, since the turtle, minnow, frog, and bluegill all can live at cold temperatures, whereas fish from warmer water (for example, swordtail) can withstand only a very small decrease in temperature. Davidson and Richards (1954) have calculated activation energies for muscle ATP-ase of the crayfish, minnow, and cockroach. At low temperature, the quantitative level of ATP-ase activities is in the same order as is the activity of the whole animal in nature. They suggested that ATP-ase activity could be a limiting factor governing activity of these species.

Glycolysis in the oyster has been given considerable attention. As with fish, general composition (fat, protein, and carbohydrate) of this marine invertebrate has been studied (Lee and Pepper 1956). Humphrey (1950 b), working with vitreous and nacreous muscle homogenates and extracts of the oyster, demonstrated pyruvic and lactic acid formation from glycogen, glucose, glucose-1-phosphate, glucose-6-phosphate, and fructose-1,6-diphosphate added as substrates. He stated that the following intermediates associated with glycolysis were present: ATP, ADP, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, triose phosphate, phosphoglyceric acid, and phosphopyruvic acid. In addition, both vitreous and nacreous muscle are able to synthesize glucose-6-phosphate from glucose-1-phosphate and, in the opposite direction, glycogen from glucose-1-phosphate. The formation of fructose-6-phosphate and fructose-1,6-diphosphate from glucose-6-phosphate also occurs. The glycolytic ability of oyster muscle is several hundred times less powerful than is that of rabbit muscle. It may be that glycolysis in the oyster is a slow, continuous process in contrast to glycolysis in mammals, where glycolytic activity is temporary and is brought into operation after muscular contraction (Humphrey 1950 b). Usuki (1956) investigated the effect of the inhibitors of glycolysis--iodoacetic acid and sodium fluoride--on the activity of oyster gills. The suppression of ciliary action that occurs can be overcome partially by addition of pyruvate and succinate, but not by addition of glucose. This fact suggests that the Embden-Meyerhof pathway is blocked by these inhibitors.

Also studying the effects of inhibitors, Humphrey (1950 a) demonstrated a decrease in respiration of oyster spermatozoa upon treatment with sulfhydryl-group reactants, such as iodoacetate and phenylmercuric acetate. Compared to glycolytic rates of the sperm of land animals, however, the glycolytic rates observed are very low, as was shown by the limited production of lactic acid from the breakdown of various hexoses that were added as substrates. Since consumption of oxygen is higher than

is that found in other tissues of this animal, the sperm probably depends upon oxidative metabolism almost completely. The respiration of homogenates of oyster eggs is stimulated by some of the intermediates of glycolysis, such as glycogen, fructose, glucose, glycerol, and glycerophosphate (Cleland 1950).

Rather extensive investigation of the metabolism of the eggs of Arbacia punctulata and other sea urchins has been carried out over a number of years. The emphasis of these studies is on the chemical events taking place with fertilization and the processes by which energy is liberated for cleavage and development. A review of this information covering the period from 1932 to 1949 has been presented by Krahl (1950) and is mainly concerned with the nature and interrelationships of the catalysts by which the Arbacia egg derives energy from foodstuffs. At that time, none of the enzymes of glycolysis had been studied in this species. The carbohydrate and glycogen content was known (Hutchens et al. 1942); however, oxidation of glucose, lactate, 2-glycerophosphate, succinate, or butyrate could not be demonstrated with cytolysates of Arbacia eggs (Ballentine 1940).

The observations of Cleland and Rothschild (1952) from their study of carbohydrate oxidation of the eggs of the sea urchin, Echinus esculentus, were consistent with the assumption that the glycolytic pathway is operative in these animals. Oxygen uptake and lactic acid production of homogenized eggs were increased by adding glycogen, glucose, and fructose and were stimulated by addition of diphosphopyridine nucleotide (DPN). The phosphorylated intermediates of glycolysis such as glucose-1-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate also stimulate the uptake of oxygen. Pyruvate and lactate were shown to accumulate in homogenized eggs under anaerobic conditions and in the presence of hydrogen cyanide. Other evidence presented by Cleland and Rothschild for glycolysis includes inhibition of oxygen uptake by fluoride ion and its reversal by pyruvate, and the accumulation of pyruvate after addition of glucose-6-phosphate or hexose-diphosphate. Ycas (1950) reported the existence of phosphoglucomutase, enolphosphopyruvic acid, phosphodihydroxyacetone, enolase, aldolase, and oxoisomerase in eggs of sea urchins.

Krahl et al. (1954 a,b) have reported the occurrence of hexokinase, phosphofructokinase, and aldolase in the supernatant and homogenates of Arbacia eggs. Evidence that the hexosemonophosphate shunt also is operative was shown by the fact that triphosphopyridine nucleotide (TPN) was reduced and pentose was formed from glucose-6-phosphate and 6-phosphogluconate by these egg preparations. Indications are that glucose-6-phosphate can be metabolized by the glycolytic pathway at only about 5 percent of the rate it can be oxidized by the TPN system. Krahl et al. suggest that the latter system is probably the major pathway for utilization of carbohydrate in Arbacia eggs. Other glycolytic enzymes present in Arbacia eggs, as shown by TPN reduction of the corresponding substrates, are glucose-1-phosphate, phosphoglucomutase; fructose-6-phosphate, hexose isomerase; and fructose-1,6-diphosphate, a fructose-1,6-diphosphatase

(Krahl et al. 1954 b). It is assumed that each of these substrates is connected to glucose-6-phosphate and that its direct oxidation does not occur.

Rothschild (1951) has reviewed the evidence both for Embden-Meyerhof and hexosemonophosphate paths in eggs of sea urchins. Inactivity of the glycolytic pathway in Arbacia eggs also has been suggested by the work of Hutchens et al. (1942) and Keltch et al. (1951) in which very limited production of lactate following consumption of carbohydrate was shown to occur. Neither the production nor consumption of lactic acid appeared to be important as an energy-yielding process for the first 24 hours. Recent work by Krahl (1956) establishes more conclusively the relative importance of the hexosemonophosphate shunt to the Embden-Meyerhof pathway in Arbacia. By determining the ratios of $C^{14}O_2$ formed by developing eggs and embryos in the presence of the substrates glucose-1-C^{14}, glucose-2-C^{14}, and glucose-6-C^{14}, he found that glucose appears to oxidize by means of the hexosemonophosphate shunt during the early stages of cleavage, the Embden-Meyerhof pathway becoming more important during later development.

Fragmentary information on the carbohydrate metabolism of other aquatic animals is available from studies of a similar nature. ATP-ase activity in heart, skeletal muscle, liver, brain, and kidney tissue of the dolphin also has been exhibited (DuBois et al. 1948). Marsh (1952) has investigated rigor mortis in the baleen whale in relation to pH, buffering capacity, formation of lactic acid, and dephosphorylation of ATP. In most respects, post-mortem behavior differed little from that of other animals. The high buffering capacity found may be an adaption to extended anaerobic activity. Attempting to estimate the reserves for anaerobic activity in muscle tissue of a variety of animals, Manery (1935) determined the maximum formation of lactic acid that could be produced. Muscle of seal showed no appreciable difference from muscle of cat, dog, rabbit, frog, and tortoise, in this respect.

That glycolysis proceeds through the Embden-Meyerhof pathway in various tissues of the dolphin has been shown by DuBois et al. (1948). Skeletal muscle, brain, liver, kidney, and heart contain the following phosphorylated intermediates of glycolysis: glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, triose phosphate, pentosephosphate, phosphopyruvic acid, and phosphoglyceric acid.

A positive assay for a specific enzyme of glycolysis, glycerophosphate dehydrogenase, has been obtained from isolated frog skin (Hunter and Hunter 1957).

The mechanism of ATP-ase activity of lobster muscle has been investigated by Clarke and Koshland (1953). Using H_2O^{18}, they found that muscle phosphatase hydrolyzes with nucleophilic displacement on the terminal phosphorous atom.

In summarizing present knowledge of carbohydrate metabolism in aquatic animals, we must say that our knowledge is meager. Since the pathways of glycolysis have been known for many years, however, our

knowledge of the metabolism of carbohydrates in aquatic animals is more developed than is our knowledge for the other biochemical entities of great importance in metabolism. It seems desirable to summarize present knowledge in the form of diagrams of metabolic pathways, which have found popular use in textbooks of biochemistry such as Outlines of Enzyme Chemistry (Nieland and Stumpf 1955) and Dynamic Aspects of Biochemistry (Baldwin 1952). From these and other sources the reader can see the detailed information that is available on land animals, plants, and bacteria. In figure 1 is summarized the known intermediary metabolism of carbohydrates in teleosts and elasmobranchs. Considering the importance of carbohydrate metabolism in supplying energy from carbohydrate foods, it can be seen that our knowledge is very fragmentary. There is no real information on the many metabolic transformations in glycolysis except for that on the formation of pyruvate from phosphopyruvate by pyruvate phosphoferase. Evidence on the occurrence of the hexosemonophosphate shunt is completely lacking. The known intermediary metabolism of carbohydrates in aquatic mammals is shown in figure 2. The identification of five of the important Embden-Meyerhof intermediates suggests that this pathway of metabolism is operative in aquatic mammals, but much more detailed information is needed for confirmation. There is no information on the hexosemonophosphate shunt. The known intermediary metabolism of carbohydrates in oysters is shown in figure 3. Here also, the positive identification of seven important intermediates suggests that the Embden-Meyerhof pathway of glycolysis is operative in the oyster. Much more detailed information, especially identification of the enzymes catalyzing the transformations in glycolysis, is needed. Again, there is no evidence of the hexosemonophosphate shunt. The known intermediary metabolism of carbohydrates in the sea urchin is shown in figure 4. The identification of five important enzymes catalyzing reactions in the Embden-Meyerhof pathway suggests that this pathway is operative in the sea urchin. Even though the sea urchin has been an aquatic animal of choice for many biochemical studies, our evidence for the existence of this important metabolic pathway is far from complete. It would be very desirable to have positive identification of the occurrence of the intermediates of glycolysis in the sea urchin. The identification of two enzymes of the hexosemonophosphate shunt suggests that this shunt is operative in the sea urchin, but much more information is needed for a definite conclusion.

TRICARBOXYLIC ACID CYCLE AND TERMINAL OXIDATION

The process of glycolysis, which apparently functions in marine animals, usually results in the formation of pyruvate, which is completely oxidized by the series of enzymatic reactions comprising the tricarboxylic acid cycle. In animals, plants, and bacteria that have been more extensively studied, the acetyl coenzyme A, formed by oxidative decarboxylation of pyruvate, enters the TCA cycle by reacting with oxalacetate; and upon completion of one cycle, three molecules of carbon dioxide are produced with the regeneration of oxalacetate. Since the TCA cycle establishes a link between the metabolism of carbohydrate and that of lipids and protein and thereby provides a means for their interconversion and complete oxidation for production of energy, it is important that we have knowledge of this metabolic cycle in marine animals.

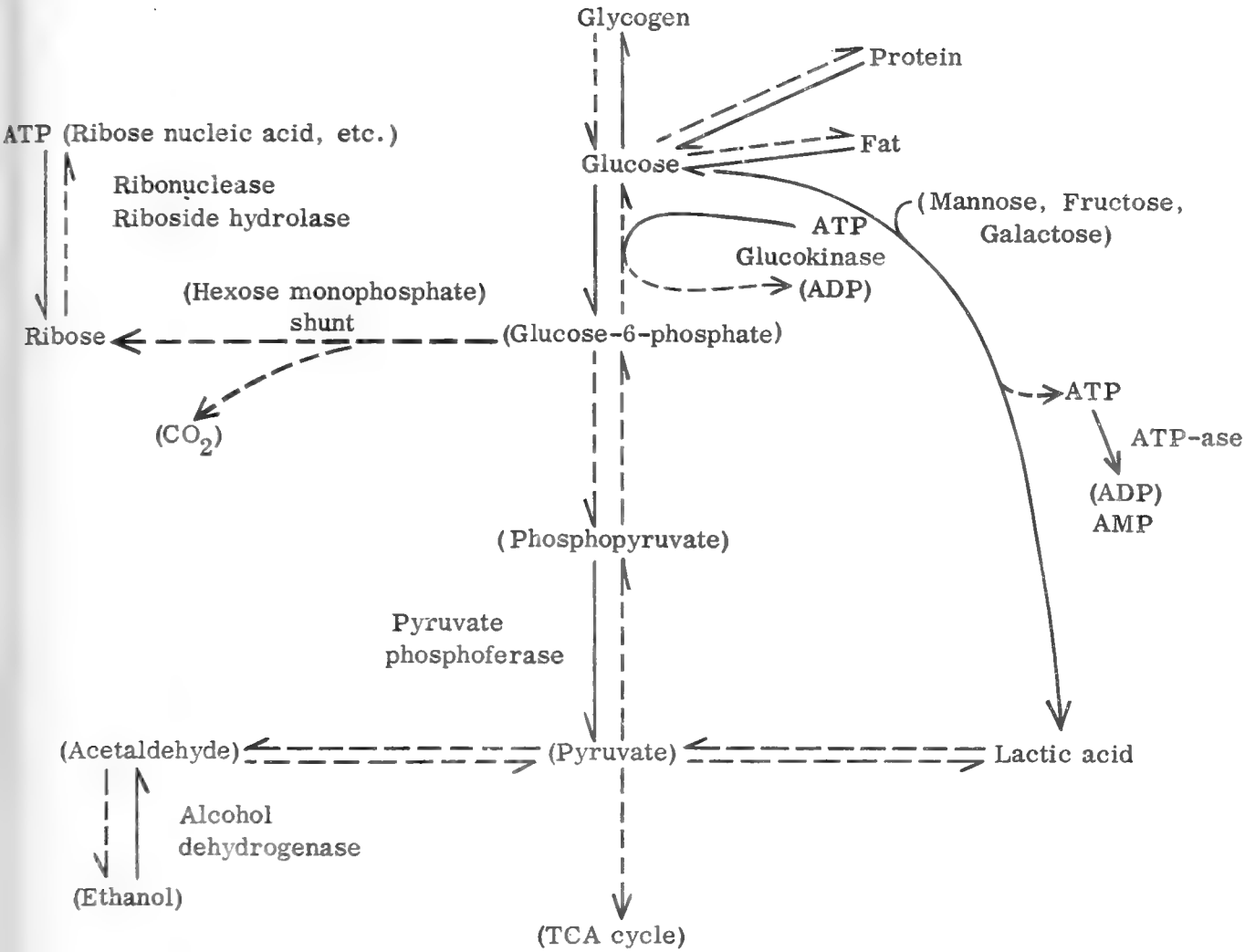

Figure 1. Known intermediary metabolism of carbohydrates in teleosts and elasmobranchs.

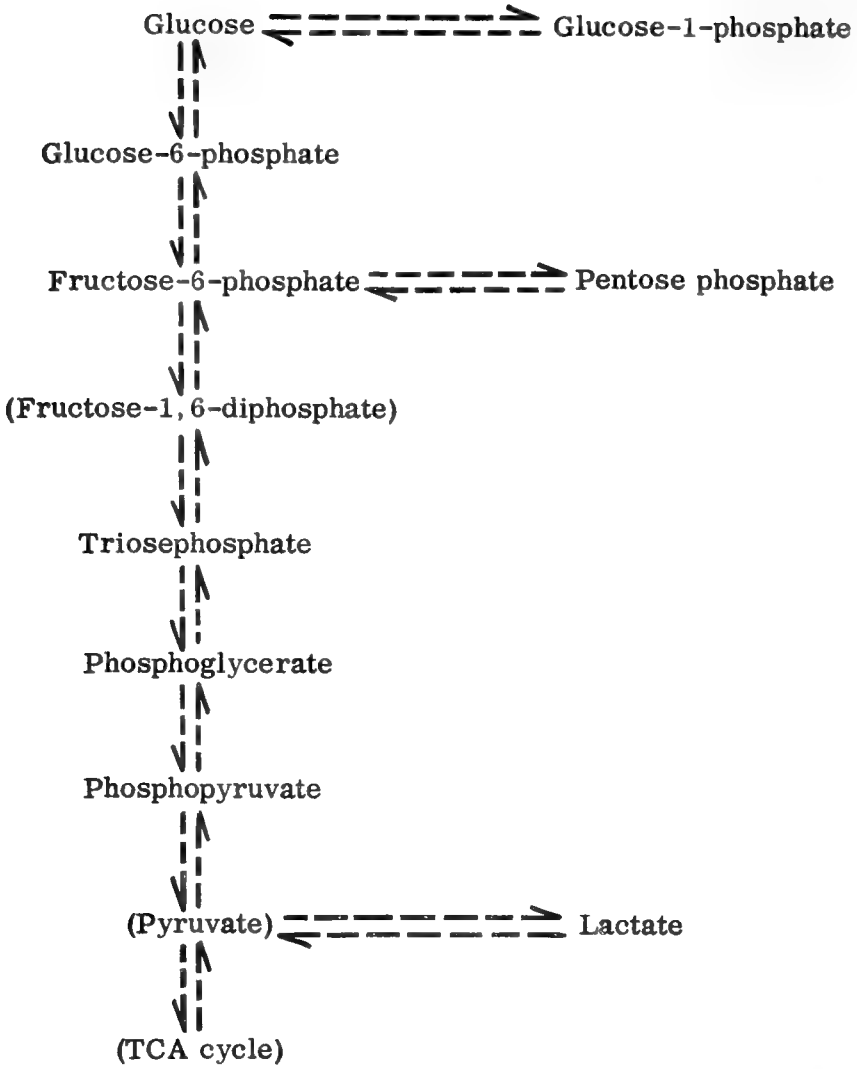

Figure 2. Known intermediary metabolism of carbohydrates in aquatic mammals.

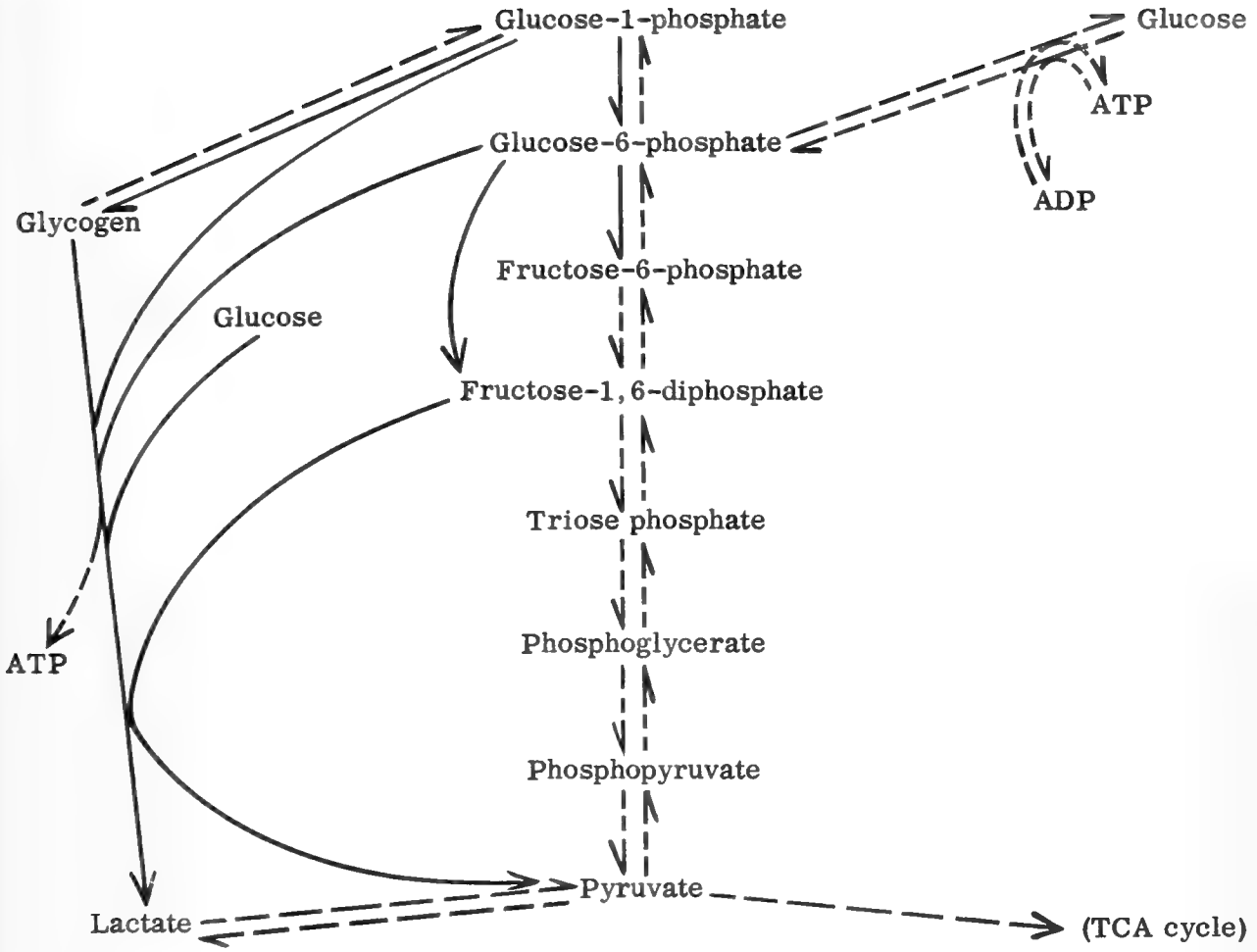

Figure 3. Known intermediary metabolism of carbohydrates in oysters.

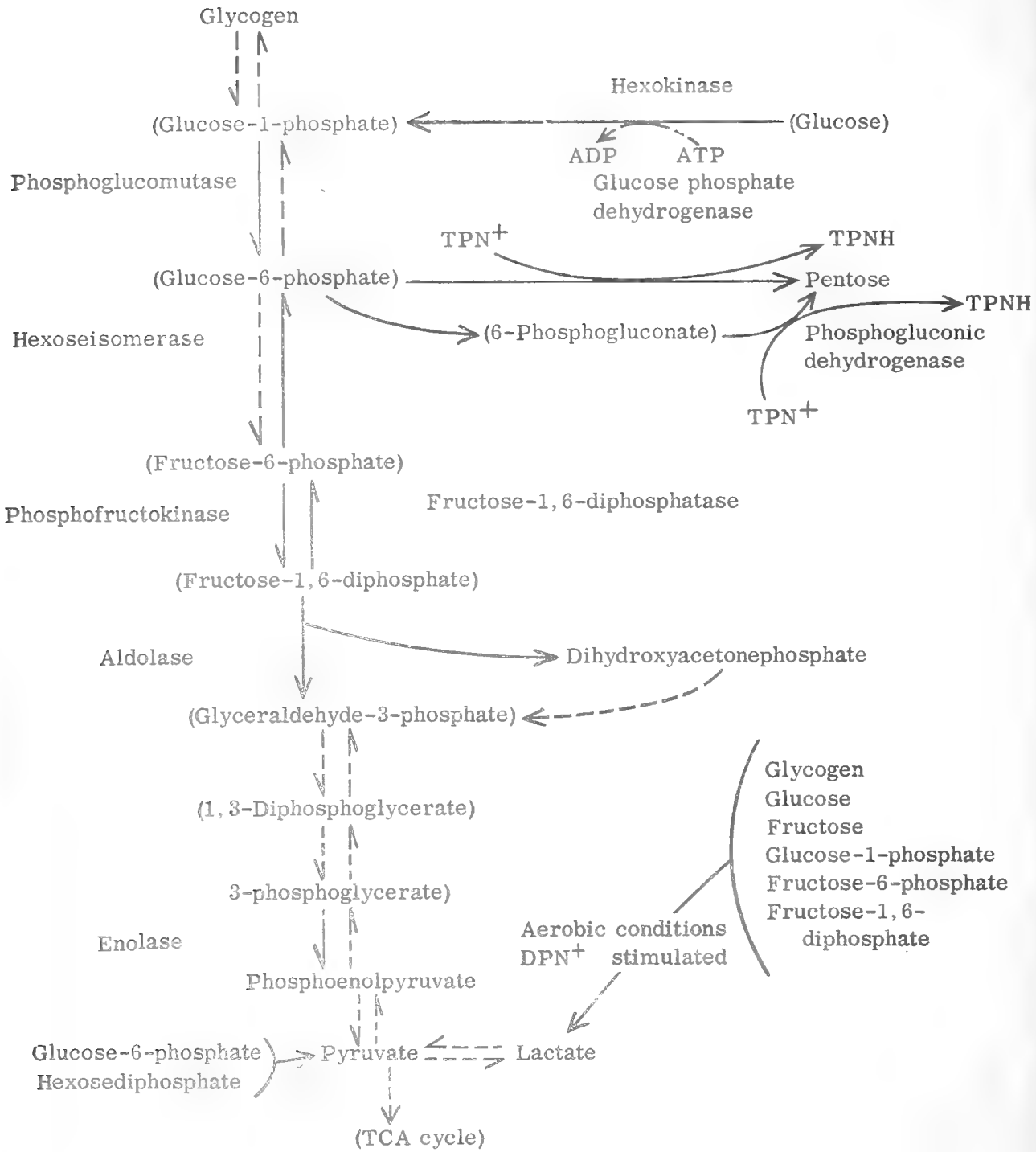

Figure 4. Known intermediary metabolism of carbohydrates in the sea urchin.

Because most of the oxygen used by respiring organisms possessing the TCA cycle is for the oxidation of TCA-cycle intermediates, uptake of oxygen may be used as a measure of the activity of this cycle. Numerous investigations have been undertaken to relate overall oxygen consumption of fish tissues to various physiological factors.

Vernberg and Gray (1953), working with 17 species of marine teleosts, found a positive correlation between the activity of each species and the oxygen consumption of excised brain tissue. Based on consumption of oxygen, these fish could be arranged into three groups that related to their activity: active species, characterized by constant swimming movements; species of intermediate activity; and sluggish, bottom-dwelling species. The brain tissue of more active fishes, such as menhaden, had a higher Q_{O_2} than did bottom dwellers such as the toadfish. In addition, these Q_{O_2} values could be correlated to various physiological indices of activity such as concentration of hemoglobin, level of blood sugar (Hall and Gray 1929 and Gray and Hall 1930) and ratio of gill area to body weight (Gray 1947). Menhaden, for example, has higher concentration of hemoglobin and level of blood sugar and has 10 times more gill area per gram of body weight than has toadfish. Menhaden and other active fish also have a greater number of immature circulating erythrocytes than have less active species (Dawson 1953). A later comparison of Q_{O_2} values of brain, liver, and muscle of the toadfish, scup, and menhaden again showed a definite correlation between activity of the species and consumption of oxygen for brain tissue; however, this relationship did not hold for liver and muscle tissue (Vernberg 1954). No positive relationship of activity to body size was noted. The oxygen consumption of excised brain of largemouth bass was found to be less than was that of slices of rat cortex (Fuhrman et al. 1944).

Other workers have investigated consumption of oxygen with respect to acclimatization to temperature (Freeman 1950 and Peiss and Field 1950). Freeman held goldfish at temperatures ranging from 4° to 37.5° C. for 1 to 2 weeks, after which time the influence of this thermal acclimatization on the rate of oxygen consumption of excised brain tissue was noted. Uptake of oxygen measured at a given temperature was highest for fish acclimatized at the lowest temperatures. This finding supports the idea that the brain probably plays a major role in determining the level of oxygen consumption of fish, as indicated above by the work of Vernberg (1954). Peiss and Field demonstrated adaptation to cold by comparing the respiratory activity of minced or sliced brain and liver preparations of Arctic cod, which lives normally at -1.5° to 2.0° C., to that of golden orfe, which lives at 25° C. Uptake of oxygen, measured over a temperature range of 0° to 25°, increased with temperature. As was found by Freeman (1950), however, respiratory activity showed an inverse relationship to the environmental temperature to which the animal is acclimatized; that is, uptake of oxygen at any given temperature was greater for the preparations of Arctic cod than for those of golden orfe. Similar results were obtained with the mud sucker Gillichthys mirabilis acclimatized to high and low temperature (Wells 1935). In addition, the temperature coefficient of brain and liver of Arctic cod remained essentially constant over the entire experimental range of temperatures, whereas it increased sharply in the orfe tissues from 0° to 10° C. Novikov (1954) found a low activation energy for the dehydrogenation system of minced carp muscle. By means of the Thundberg technique of methylene blue reduction, he compared the

succinic dehydrogenating ability of muscle tissue from carp and frog over a temperature range of 10° to 30° C. The activity of the fish muscle was constant within this range, whereas that of the frog muscle showed an average Q_{10} of approximately 1.7. It appears that the two factors--a relatively high oxygen consumption and a low temperature coefficient for respiratory enzymes of fish, especially at low temperatures--are of value in metabolic adaptation to temperature and also indicate an important difference in these enzymes of fish compared to those of warm-blooded animals. Such adaptation also has been noted by Steinbach (1949) for ATP-ase activity of cold- and warm-blooded animals.

Of the enzymes of the TCA cycle involved in the uptake of oxygen observed in these experiments, the succinoxidase system probably predominates, owing to its relative stability; however, numerous other enzymatic pathways account for some of this activity. Succinoxidase activity has been observed in homogenized goldfish gill by noting the increase in consumption of oxygen following the addition of succinate. Inhibition studies with mercuric chloride showed this activity to be dependent upon the presence of sulfhydryl groups (Sexton and Russel 1955). Umemura (1951 a,b, and c) investigated the respiratory enzymes of carp by comparing the dehydrogenase activities of lateral red and white muscle, liver, and kidney homogenates. Using the Thunberg technique and succinate or malate as substrates, he found that red muscle showed five to eight times the activity of ordinary white muscle; the activities of liver and kidney were unaffected by these substrates. Succinic and cytochrome oxidases also demonstrated in these tissues were found to be most active in red muscle. Thus the oxidative and reductive activity of the lateral red muscle of fish far surpasses that of ordinary muscle and resembles that of heart muscle in these respects. Measurements of succinic dehydrogenase and cytochrome oxidase also have been made on a variety of homogenized tissues of the toadfish (Lazarow and Cooperstein 1951). Heart muscle was by far the most active, followed by liver and kidney tissue. Compared with corresponding preparations of rat tissues, approximately the same order of activity was shown; however, the absolute activity of rat heart was three times that of toadfish heart.

Homogenized eggs of the fish, Oryzias latipes, can oxidize several substrates of the TCA-cycle (Hishida and Nakano 1954). The rate of uptake of oxygen is stimulated by addition of citrate, succinate, malate, glutamate, and pyruvate. ATP was found to have a stimulatory effect on the oxidation of any of these substrates other than succinate. Endogenous oxygen uptake and succinoxidase activity increase as development progresses.

The citric acid content of fish muscle decreases after death and is found to accumulate upon addition of pyruvic or oxalacetic acids (Yamada and Suzuki 1950). As their work indicated, this is probably not the result of microbial action but of the presence of the condensing enzyme system. Yamada and Suzuki (1951) also reported a malonic acid decomposing enzyme, present in carp-muscle homogenate, that decomposes malonic acid into carbon dioxide and acetic acid.

The respiration of other aquatic animals, including marine invertebrates, also has been investigated to some extent. Oxygen consumption of seven species of marine invertebrates, scallops, and crustacea was compared with regard to their environmental temperature (Fox 1936 and Fox and Wingfield 1937). Measurements of oxygen consumption have also been made on the fiddler crab (Démeusy 1957) and on numerous species of other crustaceans, including the kelp crab (Weymouth et al 1944). By means of staining techniques, using neotetrazolium and tetrazolium, Nayar and Parameswaran (1955) detected succinic dehydrogenase in neurosecretory cells of the thoracic ganglion of the crab.

Humphrey (1947) has studied endogenous respiration and succinoxidase activity of homogenates of oyster muscle. The large increase in respiration observed when succinate was added indicates that oyster muscle possesses an active system for succinate metabolism. The inhibitory effects of certain narcotics on respiration of oyster-muscle homogenate also were studied (Humphrey 1948).

Jodrey and Wilbur (1955) have demonstrated that a major portion of the TCA cycle is present in the oyster. Specific assays for aconitase, isocitric dehydrogenase, succinic dehydrogenase, malic dehydrogenase, cytochrome oxidase, and fumarase were made, using homogenates or acetone powders of oyster tissue. With the exception of aconitase, positive results were obtained for these TCA-cycle enzymes; however, their activities were considerably lower than in mammalian tissue. Of possible special significance in the process of carbonate deposition is oxalacetate decarboxylase activity, which was found to be more than 100 times greater in oyster mantel than in mouse liver (Jodrey and Wilbur 1955). The effects of several TCA-cycle acids on the respiration of strips of mantel tissue also were studied. Succinate, malate, and oxalacetate brought about significant increases in consumption of oxygen; pyruvate had only slight effect; and citrate was without effect. Heavy-metal catalysis in oyster mantel is indicated by strong inhibition of respiration by cyanide; however, the inability of methylene blue to reverse this inhibition suggests that the cytochrome system may not be of major importance in respiration of this tissue. Inhibition of oxygen uptake by the metal-complexing agent, diethyldithiocarbamate, indicates the presence of a metal respiratory catalyst, perhaps copper (Jodrey and Wilbur 1955).

Evidence for the occurrence of the TCA cycle in oyster eggs was shown by Cleland (1950); preparations of homogenized oyster eggs could bring about the complete oxidation of pyruvate. Uptake of oxygen was also stimulated by glutamate and various TCA intermediates: succinate, citrate, α-keto-glutarate, fumarate, and malate (Cleland 1951).

Consumption of oxygen by oyster spermatozoa is increased by the addition of certain α-keto acids such as α-ketoglutaric, oxalacetic, pyruvic and isocitric acids and amino acetic acid (Humphrey 1950 a and Humphrey and Jeffrey 1954). No significant effect on respiration was noted, however, when other TCA-cycle intermediates were used as substrates. To overcome possible problems of permeability, esters of these acids were tried. The

ethyl esters of succinate, malate, citrate, and butyrate; glucose pentaacetate; glucosamine; and hippuric acid all increased consumption of oxygen. Apparently, esters are able to penetrate the cell more easily. It would appear that the TCA cycle is operative in oyster spermatozoa, since most of the intermediates of the TCA cycle are able to stimulate respiration. Malonate, mainly considered to be an inhibitor of succinate dehydrogenase, was found to cause a significant increase in uptake of oxygen. There is evidence in other animals (Glass 1951) that malonate may serve as an uncoupling agent for oxidative phosphorylation. If this effect of malonate also occurs with oyster spermatozoa, a reduction in the efficiency of oxidative phosphorylation would account for the increased consumption of oxygen. A small amount of information about the TCA cycle in a few other aquatic animals is also available; this information is summarized below.

The demonstration of the TCA cycle in sea-urchin eggs (Arbacia) has been confined to the study of their ability to oxidize pyruvate, oxalacetate, α-ketoglutarate, and succinate and to the study of the occurrence of oxidative phosphorylation (Krahl 1950).

Goldinger and Barron (1946) determined the rate of disappearance of preformed pyruvate from intact unfertilized and fertilized eggs of Arbacia. Fertilized eggs were over six times as active as were unfertilized eggs. Krahl et al. (1942) observed a similar pattern for the rate of disappearance of added pyruvate to cytolysates of unfertilized and fertilized eggs except that the breakdown of the egg structure in preparing the cytolysates raised the rate for unfertilized eggs closer to that of the level of fertilized eggs. The fate of pyruvate in these studies was not determined. Changes in succinic dehydrogenase activity of sea-urchin eggs in relation to the process of fertilization also were studied (Hida 1957).

Evidence presented by Cleland and Rothschild (1952) for the occurrence of the enzymes of the TCA cycle in the sea-urchin egg (Echinus esculentus) includes: (1) significant stimulation of uptake of oxygen by an addition of citrate, α-ketoglutarate, succinate, fumarate, malate, and glutamate; (2) inhibition of endogenous uptake of oxygen by malonate and fluoroacetate; and (3) complete oxidation of added pyruvate. Uptake of oxygen of sea-urchin spermatozoa has been measured (Wicklund 1954).

Succinoxidase activity has been demonstrated in mitochondrial preparations of lobster leg and claw nerves (Foster 1956).

Baldwin (1938), in a study of the respiratory metabolism of Helix pomatia, has found the hepatopancreas to resemble liver tissue of vertebrates closely. Succinate, lactate, α-glycerophosphate, and alanine increased the rate of respiration. Succinic acid from the marine snail was isolated and identified (Ackermann 1955).

Even though the frog is not strictly an aquatic animal, metabolic studies made on the frog are of interest here. Hunter and Hunter (1957) obtained positive assays for succinic, malic, citric (isocitric), and α-ketoglutaric dehydrogenases and fumarase. A comparison of the activity

of respiratory enzymes in tissues of dolphin and rat was made by DuBois et al. (1948), using homogenized liver, kidney, brain, skeletal muscle, and cardiac muscle. Assays for cytochrome oxidase, succinic dehydrogenase, and malic dehydrogenase demonstrated the presence of these enzymes in dolphin tissues, although their activities were found to be lower than were those in corresponding rat tissues. A slower rate of metabolism in dolphin is indicated.

Our knowledge of the tricarboxylic acid cycle in aquatic animals is very incomplete. The presently available information can be summarized in two figures. Figure 5 summarizes the known information on the tricarboxylic acid cycle in teleosts and elasmobranchs. For the teleosts and elasmobranchs, identification of three important enzymes also suggests that this cycle may be operative. In figure 6 is summarized our present knowledge of the tricarboxylic acid cycle in oysters. For the oyster, there is sufficient information in the form of the identification of four of the important enzymes catalyzing the transformation of intermediates of the tricarboxylic acid cycle to suggest that this cycle is operative in these aquatic animals. In the oyster and in the large group, teleosts and elasmobranchs, much more detailed knowledge is required before we will know if they have a tricarboxylic acid cycle that is the same as that of land animals.

ELECTRON TRANSPORT SYSTEMS, COENZYMES AND VITAMINS

The aerobic transport of hydrogen and electrons to oxygen is accomplished by a chain of several carrier systems, beginning with dehydrogenases, which act directly on their substrates, followed by flavoproteins, and finally, the cytochromes. Some dehydrogenases are able to catalyze the reaction of hydrogen removed from the substrate with molecular oxygen. Other dehydrogenases, however, are linked to oxygen through the cytochrome system. Many require pyridine nucleotides, such as DPN and TPN, as coenzymes, and for others, the nature of the coenzyme is still unknown. Also included in this chain are flavoproteins, some of which are capable of dehydrogenating substrates directly, whereas others transport electrons from pyridine nucleotides to the cytochrome system. These enzymes require the prosthetic group, flavin adenine dinucleotide.

The probability that the coenzymes of the hydrogen-transport system are in fish is indicated by the results of numerous vitamin analyses of fish tissues, since many of the vitamins have been found to be coenzymes or precursors of coenzymes. Nicotinamide, which is incorporated in the pyridine nucleotides, has been measured in various tissues of fish (Higashi and Hirai 1946 and Ghosh et al. 1951). Assays of commercially important marine species for riboflavin, the precursor of flavin nucleotides and flavin adenine dinucleotide, have been made by Sautier (1946) and by Higashi and Iseki (1942 and 1948). The distribution of these B vitamins in fish also has been reported by Umemura (1951 c), Stansby (1953), Karrick (1955), Joshi et al. (1953), Braekkan et al. (1955), and Braekkan (1956). DPN and flavin have been determined in the eggs of Orizias (Hishida and Nakano 1954) and in the eggs of the sea urchin, Arbacia (Krahl 1950).

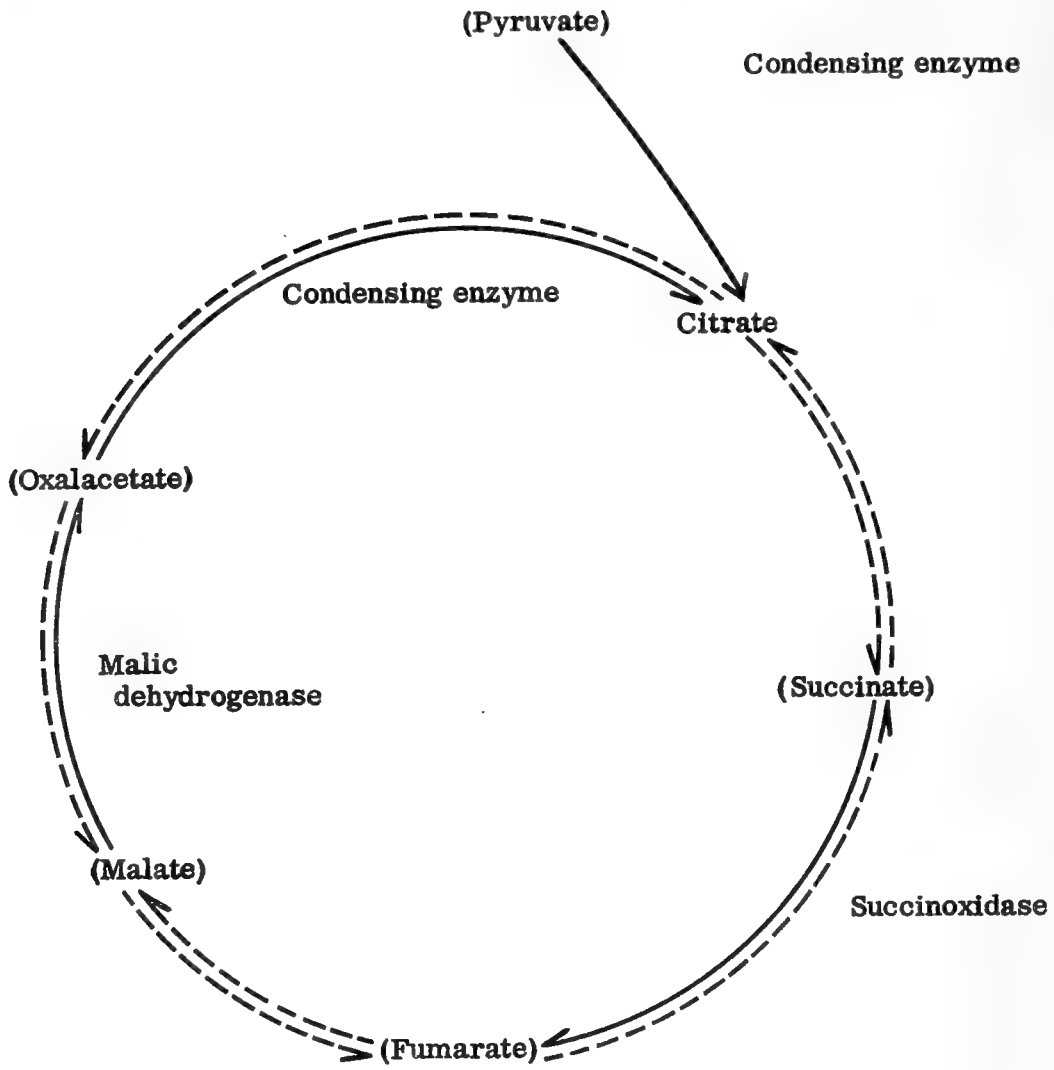

Figure 5. Known tricarboxylic acid cycle in teleosts and elasmobranchs.

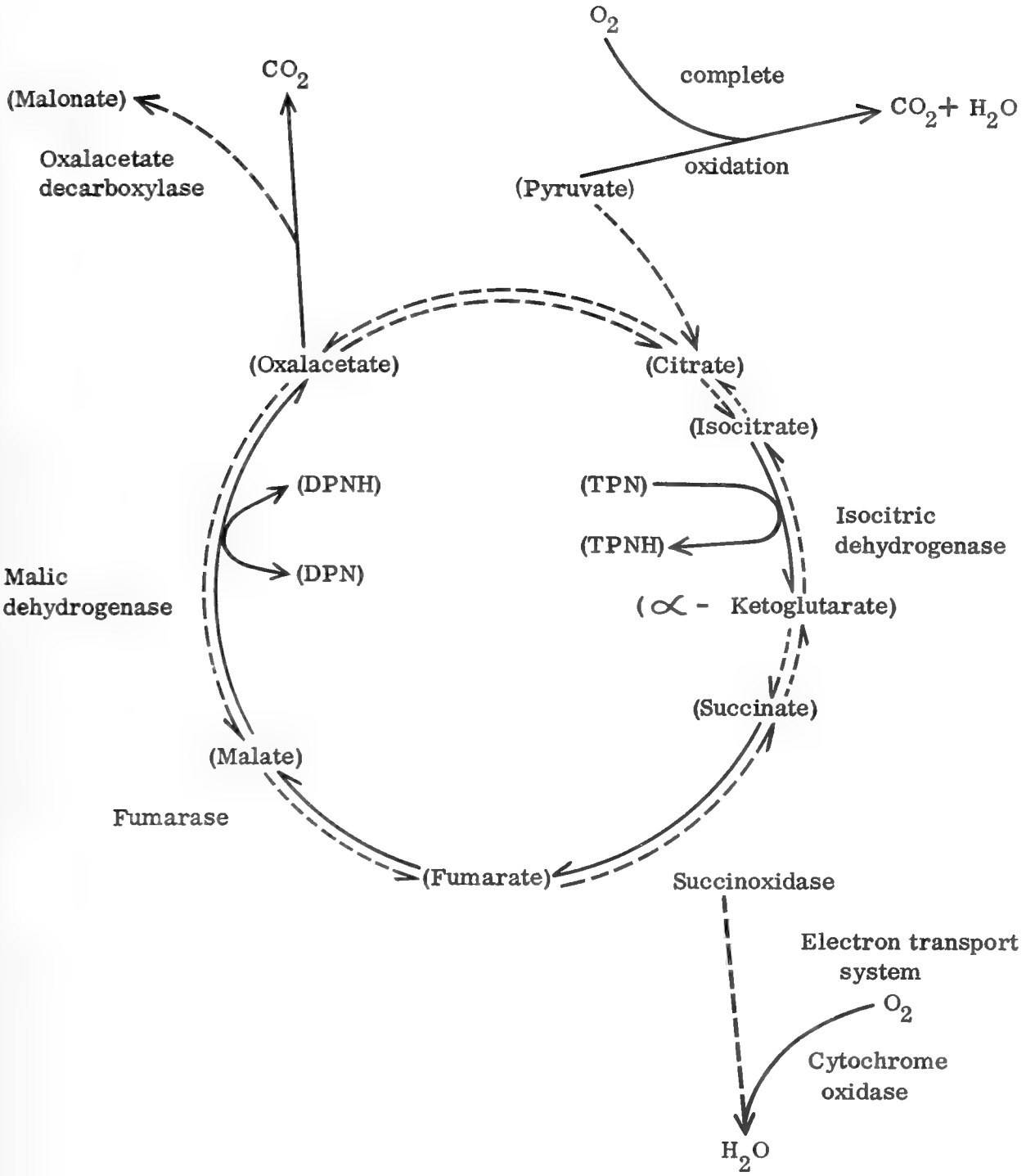

Figure 6. Known tricarboxylic acid cycle in oysters.

The cytochrome system is widespread in nature--occurring in microorganisms, higher plants, and animals--and has been studied a great deal in fish. Comparison of the amounts of cytochrome c in red and white muscle of several species of fish shows red muscle to be a much richer source (Matsuura and Hashimoto 1954 and Huys 1954). Crystalline cytochrome c from heart muscle of the bonito (Katsuwonus vagans, Lesson) and tunny (Thynnus alalunga, Gmelin) has been prepared (Hagihara et al. 1957 and Matsuura and Hashimoto 1955). Several cytochromes (a, a_3, b, and c) from the octopus (Octopus vulgaris Lam.) have been identified (Ghiretti-Magaldi et al. 1957). Paléus (1954) compared the amino acid composition of cytochrome c from salmon, beef, and chicken. He found it to be similar for these three cytochromes except for the presence of three histidine groups per mole in beef and chicken cytochrome c and only two per mole in that of salmon. Attempts to isolate cytochrome c from oyster muscle (Humphrey 1947) and sea-urchin (Arbacia) eggs (Ycas 1950 and Krahl 1950) have not been successful, indicating that it is present in extremely small amounts, if at all. In contrast to the eggs, sea urchin sperm does contain considerable cytochrome c (Ball and Meyerhof 1940). Ycas (1950) presents spectral evidence for the presence of cytochrome a and b in Arbacia eggs. Other marine invertebrates containing cytochromes are horseshoe crab, squid, and lobster (Ball and Meyerhof 1940). Baldwin (1938) was unable to detect any cytochrome in Helix pomatia by spectroscopic means.

Terminal oxidative processes occurring in the oyster have been investigated extensively by Humphrey (1946 and 1947). A study of inhibitors showed that the respiration of whole homogenate is strongly inhibited by cyanide, selenite, and arsenate. Azide, arsenite, pyrophosphate, and iodoacetate also inhibit. Inhibitions by cyanide and azide suggested the presence of cytochrome oxidase, which was confirmed by an assay using ascorbic acid as a reductant. A correlation may be made between the very low concentration of cytochrome found in oyster muscle and the lack of oxygen-transporting pigment in the blood with the low metabolism of the organism. Umemura (1951 b and c) found red muscle of fish to contain greater cytochrome oxidase activity than did liver, kidney, and ordinary muscle. In the toadfish, the concentration of cytochrome oxidase is highest in the heart, kidney, and liver. Although this distribution is similar to that found in the rat, the absolute activity of cytochrome oxidase in the toadfish is only one-third as much (Lazarow and Cooperstein 1951). During development of Oryzias eggs, cytochrome oxidase activity was found to increase over an 8-day period (Hishida and Nakano 1954). An enzyme similar to cytochrome oxidase of other animal tissues is present in Arbacia eggs (Krahl 1950 and Ycas 1950). The presence of cytochrome oxidase has been demonstrated in particles isolated from octopus muscle (Ghiretti-Magaldi et al. 1957), in oyster muscle (Humphrey 1947), and in horseshoe crab, lobster, and squid (Ball and Meyerhof 1940). Foster (1956) found cytochromeoxidase to be concentrated in mitochondria from lobster leg and claw nerves and from squid giant fiber nerves.

The vitamin, thiamine, is the precursor of thiamine pyrophosphate or cocarboxylase which is the coenzyme for decarboxylation reactions such as those catalyzed by pyruvic and α-ketoglutaric dehydrogenases. Thiamine assays of fish tissue often have been included in surveys of the vitamin content of fish (Higashi 1948, Stansby 1953, Joshi et al. 1953, Hishida and Nakano 1954, Braekkan et al. 1955, and Braekkan 1956).

An enzyme found only in fish and invertebrates, thiaminase, splits thiamine into pyrimidine and thiazole moieties (Krampitz and Woolley 1944 and Hamoir 1955 b). The distribution of thiaminase among marine invertebrates and marine vertebrates (Tatarskaya et al. 1954) and fresh-water fishes (Chaet and Bishop 1952) has been studied. Sealock and White (1949) have made inhibition studies of this enzyme obtained from carp viscera. Deolalkar and Sohonie (1954) found the thiaminase in fresh-water fish to be remarkably different from that found in brackish- or salt-water species with respect to activity measured over a wide range of pH. At least two thiaminases are indicated in brackish- and salt-water fish: one with an optimum activity in the alkaline range and the other with an optimum activity in the acid range. Attempts to fractionate these enzymes were unsuccesful, although the presence of a dialyzable coenzyme was demonstrated. Kaminishi (1951) found thiaminase of shellfish and that of fish to be similar and the respective apo- and coenzyme components to be interchangeable. Tatarskaya et al. (1955) have made quantitative estimations of co-thiaminase and have investigated some co-thiaminase-like compounds (Tatarskaya 1952); however, the presence or absence of specific thiaminase coenzymes in living cells is not known. A rather complete review of thiaminase has been made by Lee (1948) and Yudkin (1949). Harris (1951) has tabulated the occurrence of this enzyme in many fish, mollusks, mussels, clams, lobsters, shrimp, starfish, frogs, toads, and some warm-blooded animals and has listed the tissues in which it is found.

Fish possess an enzyme system capable of hydrolyzing folic acid, a vitamin that has coenzymatic function (Jacobsohn and Deodata de Azevedo 1949). The cobalt-containing vitamin, vitamin B_{12}, which is required for many reactions of intermediary metabolism, has been reported to occur in shellfish and in red and white fish muscle by Hashimoto et al. (1953). Other workers reporting vitamin B_{12} in fish tissue include Peeler et al. (1951), Hausmann and Mulli (1952), Karrick (1955), Braekkan et al. (1955), and Braekkan (1956). Pantothenic acid, which is a precursor of the important acetyl group carrier, coenzyme A, has been determined in red and white muscle of tunafish by Braekkan et al. (1955).

Reference to these and other vitamins found in fish is given by Stansby (1953) and Brown (1957). A definite coenzymatic role has not as yet been established for many of these vitamins.

Many enzymes are metal containing or metal activated. Iron, for example, is found in the porphyrin moiety of the cytochromes. Magnesium is involved in various kinase systems where phosphate is transferred from ATP to substrate. Some of the common metals directly involved in intermediary metabolism--such as iron, copper, magnesium, potassium, calcium, and cobalt--have been measured in fish and marine invertebrate tissues (Coulson et al. 1934, Umemura 1951 c, Kruchakova 1952, Hausmann and Mulli 1952, and Stansby 1953). A general discussion of the mineral composition of fishes may be found in The Physiology of Fishes (Brown 1957).

PHOSPHORUS METABOLISM

In considering phosphorus metabolism in fish, attention will be focused on the occurrence of acid-soluble phosphate esters on enzymes such as phosphatases and phosphorylases, and on the process of oxidative phosphorylation. Since phosphorus is of primary importance in carbohydrate metabolism and in energy production and storage through the formation of acid-soluble esters, such as hexosephosphate, adenylic acid, and creatine phosphate, it is important to learn if these compounds of phosphorus occur in fish. Considering the overall intermediary metabolism of energy production in animals, glycolysis yields one-fourth of the energy made available by oxidation of glucose to carbon dioxide and water, and the rest is trapped in the TCA cycle and accompanying terminal oxidative pathways. The only means known by which this energy may be trapped is by phosphorylation reactions resulting in the formation of ATP. Since the site for nearly all such phosphorylation reactions is along the electron-oxygen transfer system that transfers hydrogen or electrons from substrate to molecular oxygen, it is most important to examine the evidence for the occurrence of oxidative phosphorylation in marine animals. Also, since some esterification of inorganic phosphates can take place on a substrate level, in which case oxygen is not required, evidence for the occurrence of this reaction is also of considerable importance. One example of this type of reaction is found in the TCA cycle, where 1 mole of ADP is esterified when 1 mole of succinic acid is formed from succinyl coenzyme A breakdown.

Indirect evidence for the occurrence of oxidative phosphorylation in developing fish eggs (Oryzias latipes) was reported by Ishida (1951). Inhibition of growth and development with a simultaneous increase of oxygen consumption occurred upon treatment with 2,4-dinitrophenol (DNP). Since this compound is a powerful decoupling agent of oxidative phosphorylation in other animals, it may be assumed that phosphoryation is an essential process for the activity of developing fish eggs. Later, oxidative phosphorylation was actually measured in homogenized eggs of developing Oryzias latipes. Complete inhibition of phosphorylation but not succinate oxidation with DNP addition was observed (Hishida and Nakano 1954).

Oxidative phosphorylation by a cell-free particulate system prepared from unfertilized eggs of the sea urchin (Arbacia) has been shown to be greatly stimulated by the addition of the TCA-cycle intermediates, α-ketoglutarate, oxalacetate and succinate. Apparently the generation of high-energy phosphate bonds in Arbacia eggs is coupled with the functioning of the TCA cycle as is the case in mammalian liver or kidney (Keltch et al. 1950). There is also evidence, however, of a second phosphorylating mechanism in unfertilized Arbacia eggs in addition to the phosphorylation coupled to the oxidation of TCA-cycle intermediates (Clowes et al. 1951 and Keltch et al. 1951). The uptake of phosphorus in cell-free homogenates with α-ketoglutarate or pyruvate as substrate is completely suppressed by 10^{-5}M dinitrocresol (DNC). But with hexose diphosphate as a substrate, phosphorylation is 63 percent resistant to DNC addition. Indications are that DNC-resistant phosphorylation is

apparently coupled with anaerobic disposal of hexose diphosphate, since phosphate uptake in a nitrogen atmosphere with or without DNC and with hexose diphosphate and pyruvate as substrate is approximately equal to that under aerobic conditions in 10^{-4}M DNC. Cleland (1950) reported the formation of high-energy phosphate during respiration of homogenized oyster eggs. Anaerobiosis, cyanide poisoning, or DNP depleted the eggs of high-energy phosphate. Lobster nerve mitochondrial preparations have been shown to be capable or carrying out oxidative phosphorylation (Foster 1956); P/O ratios in excess of 1.0 were obtained.

Browne et al. (1950) and later Longley (1955), using histochemical methods to study the presence and distribution of alkaline phosphatase in the kidney of various marine teleosts and of other aquatic animals, demonstrated the presence of this enzyme in aglomerular as well as glomerular fish. Alkaline phosphatase was believed to function in the kidneys of glomerular vertebrates in the process of glucose resorption; however, its presence in aglomerular fish constitutes evidence against this theory.

Phosphorylase, which catalyzes the reaction: glucose-1-phosphate $\rightleftharpoons$ glucose + $HOPO_3^=$, has been obtained in extracts of lobster tail muscle (Cowgill and Cori 1955). An inactive form of the enzyme was found that on incubation, changed to phosphorylase b (inactive in the absence of adenosine monophosphate) and then to phosphorylase a.

Two enzymes of phosphorus metabolism found in oyster eggs are acid phosphatase and apyrase (Cleland 1951). Compositional studies on these eggs showed the presence of fructose phosphates, phosphoglyceric acid, adenylic acid, and ATP (Cleland 1950). Suzuki (1954) compared the phosphorus compounds of red and white muscle of fish. Although red muscle contained more total phosphorus than did white, white muscle had a higher concentration of acid-soluble and inorganic phosphorus.

The possibility of assimilation of inorganic phosphorus from water by fish was investigated by Shekhanova (1956). Carp was found to take up $HP^{32}O_4^=$ with activity being located mainly in the gills and scales. Some P^{32} was found in all other organs including blood and digestive tract. The various tissues of the carp, arranged in descending order according to rate of uptake of P^{32} are gills, fins, heart, liver, bladder, vertebrae, and muscle (Kirpichnikov et al. 1956).

PROTEIN AND NITROGEN METABOLISM

The metabolism of proteins includes a vast number of diversified processes giving rise to a large array of nitrogenous substances. Cellular structure and organization are primarily dependent upon proteins as is the regulation of chemical activity of the cell by proteins in the form of enzymes. The overall scheme may be summarized as follows: an amino acid pool is formed through the breakdown of food proteins, tissue catabolism, and the synthesis of nonessential amino acids. From this pool,

new protein is synthesized for growth and repair as also are essential non-protein substances such as coenzymes, hormones, creatine, and choline --to mention a few. Excess amino acids in this pool are deaminated; some, the glucogenic amino acids, are converted to glucose, while others, the ketogenic amino acids, give rise to ketones. The end products of many are yet unknown. Thus in addition to synthetic functions, amino acids may serve as a source of energy. Hutchens et al. (1942) found that certain samples of eggs of the sea urchin, Arbacia, consumed little or no carbohydrate in early stages of development, during which cell division proceeds most rapidly. Processes involving the oxidation of protein or protein breakdown products could account for the observed uptake of oxygen. Indeed, in some cases, ammonia production agreed with oxygen consumed for complete oxidation of protein. Ammonia formed from the process of deamination is eliminated either unchanged or in less toxic forms as urea or uric acid. Protein metabolism, therefore, is essentially the metabolism of amino acids and includes the pathways connecting their interconversion and those leading to the synthesis of other nitrogenous compounds, including nitrogenous waste products excreted by the animal.

Detailed information of protein metabolism, as outlined above, is almost nonexistent for fish, being limited almost completely to warm-blooded animals and to microorganisms. By means of compositional studies, however, possible relationships of amino acids to other metabolites found in fish may be initially formulated, using information known for other animals. Geiger (1948) has reported the protein content of the muscle of many species of fish and has compared the various fractions to mammalian muscle. Included are proteins of fish blood, collagens, and proteins isolated from fish sperm and from the female reproductive organs of fish. Analyses of fish protein by many workers have shown over 20 of the common amino acids to be present (Baertich and Weber 1947, Geiger 1948, Dunn et al. 1949, Kakimoto et al. 1953, Goncalves 1952, Master and Magar 1954, Sugimura et al. 1954, and Eastoe 1957). Compared to beef muscle, fish muscle has higher amount of glutamic acid (Jarpa 1950). The nature and function of the red muscle of fish have been given much consideration. Although it resembles heart muscle in certain respects, it has essentially the same amino acid composition as has white muscle (Matsuura et al. 1955). Studies of the extractive nitrogen of certain shellfish and squid muscle have shown generally higher amounts of amino nitrogen to be present than are found in vertebrate muscle (Simidu et al. 1953 and Endo et al. 1954).

L-amino acid oxidase, which catalyzes the oxidation of amino acids to the corresponding α-keto acids and ammonia, occurs in marine invertebrates (Roche et al. 1952). Differing from the L-amino acid oxidase found in many vertebrates, however, this enzyme in invertebrates is capable of catalyzing the oxidation of basic amino acids, especially arginine. Tissue extracts of hepatopancreas and digestive tract of annelids, gephyriens, mollusks, crustaceans, and echinoderms were able to oxidize L-histidine, L-leucine, L-tryptophane, L-ornithine, and L-citrulline in addition to

arginine. Arginine metabolism occurs by a process different from that common to vertebrates in that α-keto- δ-guanidovaleric and γ-guanidobutyric acids are formed. Glycine, DL-serine, L-glutamic, L-aspartic, and L-proline failed to be metabolized by these extracts. Umemura (1951 b and c) determined amino acid oxidase in various tissue homogenates of carp. The activity of red muscle was generally lower than was that of liver and kidney; white muscle showed little or no activity. Of special significance was the oxidation of glutamic acid and alanine by red muscle, liver, and kidney tissue, since the resulting keto compounds, α-ketoglutaric and pyruvic acids, are able to enter into the TCA cycle for production of energy or for synthesis of fatty acid and of carbohydrate. Thus a link is established between these two important intermediary pathways and protein metabolism in fish.

In the areas of nitrogenous excretion and nitrogenous composition of tissues, the comparative aspects of protein metabolism for fish are best known. Three substances characterize most of the nitrogenous excretion of fish: ammonia, urea, and trimethylamine oxide (TMA oxide). For both marine and fresh-water teleosts and for aquatic invertebrates, the predominant end product of protein metabolism is ammonia; for elasmobranchs the predominant end product is urea (Baldwin 1952). Suyama and Tokuhiro (1954 a and b) determined the distribution of urea in various tissues of sharks and rays. For the general distribution of urea in fish and shellfish, refer to Shewan (1951). Although ammonia, which is constantly formed during deamination processes, is toxic and must be eliminated rapidly, a provision for the storage of it for future use in synthesis of amino acids is found in the formation of the amides of glutamic and aspartic acids--glutamine and asparagine, respectively. The presence of asparaginase, reported to exist in liver and other tissues of fish (Terroine 1943), indicates the possibility of such a store for amino groups in asparagine.

Although arginase, a key enzyme in the ornithine cycle, is found in fish liver, evidence indicates that this elaborate mechanism for production of urea from ammonia does not exist in teleosts (Baldwin 1952). The addition of ornithine and ammonia and a source of energy, such as lactate, to slices of fish liver causes no production of urea in teleosts, though it does in animals possessing the ornithine cycle. Since arginine occurs in considerable amounts in most proteins, the presence of arginase can account for the production of urea in fish. Matsuura et al. (1953) found that white muscle of various kinds of fish contained little or no arginase. It was present, however, in red muscle. Similar studies with elasmobranch tissues (Connell 1955) showed arginase activity to be absent in the skeletal muscle of the common skate, thornback skate, and cuckoo ray but not in the white skeletal and red lateral band muscle of dogfish (Scyliorhinus caniculus). The dogfish (Squalus suckleyi) has also been reported to contain arginase in voluntary muscle (Hunter and Dauphinee 1924). Apparently, for rays, extrahepatic regulation of urea cannot occur in the muscle as it does in other elasmobranchs (Connell 1955).

Urease, commonly found in plant and invertebrate tissue (Baldwin 1952), has also been detected in the blood and muscle of shark (Ferguson-Wood 1950). Part of the ammonia excreted by fish may arise from action of this enzyme on urea. Urease activity of the sea urchin was followed

during development from eggs to adults by Brookbank and Whiteley (1954). Xanthine oxidase, uricase, allantoinase, and allantoicase also were found in the eggs.

Much speculation exists concerning the origin and significance of TMA oxide in fish muscle and blood. Formerly believed to be present only in marine fish (Kutscher and Ackermann 1933 and Baldwin 1952), it has been shown to occur in significant but smaller amounts in fresh-water species (Cook 1931, Reay 1938, and Lintzel et al. 1939). Reay found that elasmobranchs contain more TMA oxide than do teleosts. Suyama and Tokuhiro (1954 b) also studied the distribution of TMA oxide in elasmobranchs.

In contrast to the blood of elasmobranchs, the blood of marine teleosts has no appreciable TMA oxide (Norris and Benoit 1945). Norris and Benoit also reported the oxide to be of general occurrence in marine Crustacea, which includes the lobster (Reay 1938). For a more detailed comparison between various fresh- and salt-water fish, see the review on nitrogenous extractives in fish by Shewan (1951). As was pointed out by Shewan, environment, seasonal variation, size, and age all can affect the TMA oxide content. His work shows that different muscles also have different TMA oxide content; the tissue forming the dark lateral line of both herring and tunny have about half the content of the rest of the skeletal muscle. Similar differences also were found between the white and red muscles of fish by Endo and Shimidu (1955). The content of TMA and TMA oxide of numerous species of fish occurring near Bombay has been reported by Joshi et al. (1953). TMA oxide has been found in the skin of the octopus (Asano and Sato 1954), but in shellfish muscles, the amounts are relatively low (Simidu et al. 1953).

TMA oxide may be the end product of protein metabolism, but enzymes capable of oxidizing TMA in TMA oxide, such as occur to mammals, are apparently lacking (Shewan 1951). The difference in TMA oxide content between marine and fresh-water species is reflected in other organisms used as food by these animals. Fresh-water zooplankton contain a very low concentration of the oxide, whereas marine plankton have levels comparable to other marine species (Reay 1938). Feeding experiments carried out with young salmon (Benoit and Norris 1945) indicate that the origin of TMA oxide is exogenous in this species. When diets free from TMA oxide were fed, little or no TMA oxide was found, but some retention occurred when the oxide was included in the diet. TMA oxide reductase activity in the dark muscle of albacore and frigate mackerel has been demonstrated by the reduction of aseptically added TMA oxide (Kawabata 1953). Immediately after catch, large amounts of free trimethylamine were found in the dark muscle; these amounts increased on incubation. No increase in free trimethylamine occurred in white muscle, nor was there any reduction of added TMA oxide. The reaction was found to be sensitive to both cyanide and heat. Nickerson et al. (1950) found only slight increases in TMA content of sterilized fish fillets, indicating very low amount of any naturally occurring TMA oxide reductase. Since elasmobranchs show especially high concentrations of TMA oxide in all body fluids and tissues, the base probably serves as an osmoregulatory factor (Reay 1938 and Shewan 1951). TMA oxide therefore may not be entirely of exogenous origin and

may play an important role in ammonia detoxification and transmethylation reactions (Nikkila 1951 and Suyama and Tokuhiro 1954 b).

Both non-protein arginine and creatine may serve as phosphate acceptors; these amino acids are found in muscle tissue mainly in the form of phosphate esters (phosphagens). Just as arginine phosphate appears to be limited to invertebrates, only a trace of creatine being found in Crustacea and other shellfish (Kutscher and Ackermann 1933), creatine phosphate is limited to the vertebrates. In loach muscle and eggs, creatine was the major nitrogenous compound extracted (Marushima 1944).

The synthesis of creatine in mammals is believed to be associated with arginine from which an amidine group is furnished. This association is shown by the work of Bloch and Schoenheimer (1941), who fed rats arginine labelled isotopically in the amidine nitrogen and found a high concentration of labelled nitrogen in the amidine group of creatine. Whether or not this amidine transfer occurs in fish is not known.

Other guanidine compounds found in fish muscle--such as octopine, methyl guanidine, arcaine, agmatine, and glycocyamine (Kutscher and Ackermann 1933 and Kakimoto 1954)--are of unknown significance. Bacterial action may be responsible for the presence of some of these products measured in fish tissue. Agmatine, for example, can be formed by an arginine decarboxylase of bacterial origin (Baldwin 1952).

Two widely distributed imidazole bases in vertebrates, anserine and carnosine, have been found in the muscle tissue of numerous fish (Reay et al. 1943, Marushima 1944, and Shewan 1951). Yudaev (1950), however, found neither carnosine nor anserine in fresh-water species, although histidine was present in considerable amounts. Since the fish containing histidine had no β-alanine, histidine did not arise from carnosine or anserine in this case.

Jones (1954 and 1955) has reported an enzyme, anserinase, occurring in codling muscle, that is capable of splitting anserine into β-alanine and 1-methylhistidine. Analyses of extracts of codling muscle showed that before spoilage had occurred, 1-methylhistidine and β-alanine increased. The same results were obtained with sterile homogenates and cell-free aqueous muscle extracts with added anserine. Later, a partially purified anserinase was obtained by dialysis and acetone fractionation of cell-free muscle extracts; it underwent complete heat inactivation and apparently was Zn^{++} activated.

Various betaines have been found in fish muscle; however, little is known of their formation or function (Shewan 1951). It is possible that they take part in biological methylations. Since betaines are widespread among plants, many of them may originate directly from the food supply of an animal.

Ackermann (1955) has identified by means of paper chromatography various nitrogenous compounds such as taurocyamine, homarine, choline, large amounts of lysine, and other amino acids in the marine snail.

The enzyme cholinesterase, which is closely associated with the propagation of brain and nerve impulses, has been measured in homogenates of the central nervous system and other organs of fish (Laurent 1952 a and b).

LIPID METABOLISM

Consideration of the lipid metabolism of aquatic animals must include the metabolism of triglycerides, fatty acids derived from the triglycerides, the compound lipids such as phospholipids, and other lipid-soluble compounds such as hydrocarbons and sterols. Our present knowledge of lipid metabolism in animals is that free fatty acids are first converted into acyl coenzyme A derivatives, which then are rapidly degraded, two carbon atoms at a time, into acetyl CoA. Acetyl CoA enters the metabolic pool, and it may be utilized in a large number of synthetic reactions. The metabolism of carbohydrates and certain amino acids also may contribute to the metabolic pool of acetyl CoA. Since each step in the oxidation of fatty acids is reversible, their synthesis may proceed by the condensation of two carbon units. In general, our knowledge of lipid metabolism is still far from complete. This lack of knowledge is especially true for fish and other aquatic animals. There is essentially no information available on specific intermediary pathways of lipid synthesis and oxidation in aquatic animals. Studies to date on these animals have been chiefly concerned with compositional analyses and selective modifications of dietary fat. A review of this information on fish has been presented by Lovern (1951).

The fatty acids of fish, like those of land animals, are all straight-chain, are monocarboxylic, and contain an even number of carbon atoms. Although the entire series is not present in any given species, a wide range of homologous fatty acids containing 12 to 28 carbon atoms are found in fish fats, whereas the majority of fatty acids of land animals and plants contain 16 to 18 carbon atoms. The maximum possible degree of unsaturation for any given fatty acid increases with chain length. Mixtures of 22 carbon atoms, for example, have been found containing one, five, and six double bonds (Lovern 1942) and two, three, and four double bonds (Baudart 1948).

Cod-liver oil has a composition that may be regarded as being typical or average for marine species of fish (Lovern 1942). It is approximately 84 percent unsaturated, the unsaturation occurring mainly in fatty acids containing 18 and 20 carbon atoms and in some containing 16 and 22 carbon atoms. The saturated portion is composed primarily of palmitic, with smaller amounts of myristic and stearic acids. Since triglycerides in fish tend to be completely heterogenous, and since a large number of individual fatty acids are present—for example, about 20 in cod-liver oil (Baudart 1948)—the triglyceride composition of fish fat is extraordinarily complex (Harper and Hilditch 1937 and Hilditch and Terleski 1937). No compositional investigation that is based on content of glyceride is practical with present analytical methods.

In addition to triglycerides, fatty acids esterified with such compounds as chimyl, batyl, and selachyl alcohols are found in shark-liver oils. These alcohols are ether esters formed from higher normal fatty alcohols with one of the α-hydroxyl groups of glycerol (Lovern 1951).

Baldwin and Parks (1943) have reported on the low-temperature crystalization of glycerides and the occurrence of some of the 18- and 20-carbon-atom acids in menhaden oil. The separation and characterization of several highly unsaturated fatty acids in sardine oil have been described by Toyama and Tsuchiya (1953 a, b, c, d and e) and Tutiya (1940). Toyama and Shimooka (1953 b and 1954) have made ultraviolet absorption measurements of conjugated acids obtained by alkali isomerization of highly unsaturated acids from sardine oil.

Many other investigations have been made of the composition of fats in fish. Pathak and Ojha (1957) have compared the fat of two species of Indian fresh-water fish to that of some British fresh-water fish and of the herring with regard to component fatty acids. A comparison was also made of the lipids of an Arctic fish to those of the guppy (Wilber 1949). The naturally occurring eicosapentaenoic acids have been shown considerable interest. Their presence has been reported by Toyama and Tsuchiya (1935 c and e), Tutiya (1940), and Toyama and Shimooka (1953 a) in sardine oil, Matsuda (1942) in bonito oil, Baldwin and Parks (1943) in menhaden oil, Tsuchiya and Kato (1950) in herring oil, Tsuchiya and Okubo (1952) in fish-liver oil, and Abu-Nasr and Holman (1954) in cod-liver oil. Whitcutt and Sutton (1956) have isolated and determined the structure of an eicosapentaenoic acid from South Africa pilchard oil. These acids are not restricted to lipids of fish, however, as their occurrence also has been reported in ox-liver lipid (Klenk and Dreike 1955), ox-adrenal lipid (Herb et al. 1951), and butter fat (Shorland and Johannesson 1951).

From the many studies of composition, it is apparent that all fats of aquatic animals possess an exceptional variety of fatty acids, mainly unsaturated. The question arises as to what extent the complex mixture of fatty acids found in most fish is due to the ingestion of fat of that type or to modifications of ingested fat by the fish itself. Fresh-water animals possess one broad type of fat, which distinctly differs from that of marine species (Hilditch and Lovern 1936 and Lovern 1937 and 1942). The difference between these two types of fats in fish is shown mainly in the frequency of distribution of the unsaturated fatty acids; that is, there is a shift from relatively greater amounts of the unsaturated acids of shorter chain length of 16 and 18 carbon atoms in fresh-water species to larger amounts of the longer chain acids of 20 and 22 carbon atoms in marine fish. The total amount of 16-carbon-atom acids is also found to be less in marine fish. This same pattern in composition of fat is found in the fat of marine and of fresh-water plankton crustaceans (Lovern 1935), which are the main food link between the aquatic plant and animal kingdoms. It thus would seem that the nature of the fat of fish is due almost exclusively to the ingestion of fat of the same type. Further supporting evidence for this view are the results of feeding experiments with the fresh-water eel (Lovern 1938). On a typically high-fat marine diet (herring), there was appreciable modification of eel fat in the direction of the dietary fat.

In addition to dietary effects on the composition of the fat of fish, Lovern (1951) has noted specific modifications of deposited fat by small groups of closely related species. This situation is not unlike that found in land animals in which the nature of the dietary fat is superimposed upon a characteristic fat deposited by the species. Two types of changes may occur before deposition: (1) selective hydrogenation or dehydrogenation and (2) alteration of the chain length of one or more fatty acids by shortening or lengthening processes.

A physiologically reversible process of saturation and desaturation of fats is known to occur in mammals, as demonstrated by feeding experiments with mice in which saturated and unsaturated fatty acids containing deuterium were used (Rittenberg and Schoenheimer 1937 and Schoenheimer and Rittenberg 1936). For fish, a detailed fatty acid analysis of herring made over periods of fasting, feeding, and spawning was compared to the fat of Calanus finmarchicus, the principal organism in the herring diet (Lovern 1951). By following changes in the degree of unsaturation of any individual fatty acid it was observed, for the most part, that the composition of herring fat tended to approach that of Calanus finmarchicus during the period of active feeding but gradually returned to the composition characteristic of the fasting state. The results of these studies indicate that fish probably possess enzyme systems capable of selective hydrogenation or dehydrogenation of different groups of fatty acids similar to those systems found in mammals.

Evidence of another specific modification of ingested fat by fish and by other aquatic animals--that of shortening or lengthening processes affecting fatty acids--also is based on studies of composition (Lovern 1951). Since the fats of most marine fish are made up of roughly equal amounts of 16-, 18-, and 20-carbon-atom acids, decreased amounts of 14- and 22-carbon-atom acids, and little or no 24-carbon-atom acids, it is possible to define an "average" composition. Large deviations from this "average" distribution of fatty acids appear to be an indication of a progressive shifting or alteration of some fatty acids, two carbon atoms at a time, into others of different length. Examples of such transformations are found in the toothed whale (Lovern 1942), in which chain shortening occurs, and in the liver fats of sharks and related fishes, in which chain lengthening occurs. Another example is found in the liver fatty acids of the catfish (Anarrhicas lupus), which bear evidence of simultaneous chain lengthening and chain shortening (Lovern 1937).

Ingested fats are absorbed either directly in a finely divided emulsified form or as the free fatty acids after hydrolysis by lipases. The effect of lipolysis on absorption of fat in fish is not known; however, fish do possess very active lipases in their gastro-intestinal tract. As was shown by Lovern and Morton (1939), rapid post-mortem build-up of free fatty acids from lipids in the intestinal tract of halibut occurs, the content of free fatty acid rising to over 20 percent in a period of 5 to 10 minutes.

Lipases are found rather widespread throughout other tissues and organs of fish, as in other forms of life. Falk et al. (1927) and Noyes et al. (1927) obtained extracts of whole trout and trout eggs that possess

lipase action toward various simple esters such as phenylacetate, glyceryl triacetate, ethyl acetate, and others. Lipase activity also has been found in the spleen, heart, kidney, testes and liver of carp (Kernot and Hills 1932). Schmidt-Nielsen and Stene (1938) found "lipase activity" in salmon muscle that was absent in boiled muscle but that could be restored upon treatment with sodium sulfate. It generally is held that before oxidation of fats can occur, the fat must be hydrolized to free fatty acids. Thus these lipases probably perform an essential operation preliminary to the oxidation of fats.

The phospholipids of several aquatic animals have been investigated: the lecithins, cephalins, and phosphatidic acids of the heart of a rorqual (Balaenoptera borealis); the brain cephalins of the sperm whale (Physter catodon) and of pollock (Theragra chalcogramma); the egg lecithins and cephalins of pollock (Theragra chalcogramma); and the cephalins, lecithins and phosphatidic acids of unfertilized and fertilized eggs of crab (Paralithodes camtschatica) (Igarashi et al. 1956, Zama and Igarashi 1956, and Zama and Katada 1956 and 1957).

Recent compositional studies by Lovern's group have included the fractionation of the various classes of lipids extracted from cod flesh (Garcia et al. 1956). Analysis of the various fractions showed the following lipids and amounts to be present: lecithin 35 percent, waxes and alcohols 13 percent, free cholesterol 8 percent, phosphatidyl ethanolamine 7 percent, free fatty acids 6 percent, cholesterol esters 5 percent, triglycerides 3 percent, inositol lipids 2 percent, and unidentified lipids 21 percent. Similar results were found for the lipids of haddock tissue (Olley and Lovern 1954). Triglycerides, hydrocarbons, cholesterol esters, free alcohols, and esterified alcohols have been separated by means of silica-gel chromatography from extracts of haddock flesh (Lovern 1956 a). Determination of the fatty acid components of the cholesterol ester, triglyceride, and total lecithin fractions showed them to be characteric of each class of lipids.

The phospholipid fractions from haddock and from cod flesh have been investigated in more detail by Olley (1956) and Lovern (1956 b). Studies of the distribution of lecithin in sardine revealed the brain to be the richest source, followed by heart muscle (Matsumato 1950). Red muscle in the sardine was found to contain more than twice the amount of lecithin present in white muscle.

The sterols, cholic acid and cholesterol, have been identified in the bile of the Spanish tunny-fish (Castells 1953). A new sterol, isolated by chromatographic techniques and comprising about 50 percent of the sterols found in the oyster and in the clam, has been identified to be 24-methylene-cholesterol (Idler and Fagerlund 1955). Cleland (1950) has determined the contents of phospholipid and neutral fat in unfertilized oyster eggs and has found lipase to be present (1951).

NEED FOR RESEARCH

As this review shows, there is little knowledge of intermediary metabolism of aquatic animals. This lack of information is very undesirable in studies in the scientific disciplines of biochemistry, biology,

and nutrition. Especially for comparative purposes, a thorough knowledge of the intermediary metabolism of aquatic animals would be invaluable. There would be great value in the application of this knowledge to better commercial use of these marine resources.

Research study of the intermediary metabolism of aquatic animals is a fertile field for further scientific effort. Almost none of the research reviewed herein has been directed toward the objective of determining the pathways of intermediary metabolism, and the small amount of information that is available applies to this objective only superficially. Future studies should be directed to two broad objectives: Firstly, extensive research will be required to map out the overall pathways of intermediary metabolism in aquatic animals, especially the fish. Secondly, intensive research will be required to determine the exact mechanism of each metabolic transformation.

We believe that the overall need for future research may be outlined as follows:

1. Enzymes known to occur in pathways of general intermediary metabolism should be identified and their activities measured. The identification of a particular enzyme in the homogenates of tissue of the aquatic animal under study will require specific assays in which the reaction system consists of known chemical compounds. It will be preferable to have the enzymes either fractionated from the major portion of the protein and interfering enzymes or purified until all extraneous material has been removed.

2. The intermediate compounds of metabolism should be studied in such a way as to show the sequence in which they are formed. Several ways of carrying out this study are known. Radiolabeled reaction components may be added to the whole aquatic animal, to a given tissue, or to a tissue preparation; and after reaction, the products may be separated on filter paper and an autoradiograph developed (Bassham et al. 1953). The sequential transformation of metabolites also may be established by adding a reactant to the tissue or tissue preparation of the aquatic animal and then separating the products by filter-paper chromatography to determine the next member in the metabolic sequence (Avron and Biale 1957).

3. After enzymes and the metabolic intermediates have been identified, the pathways of intermediary metabolism should be checked at the whole-animal level, using radioactive or mass isotope tracers. This research can best be patterned after that which has already been done with land animals, plants, and bacteria. The amount of carbohydrate metabolized by the Embden-Meyerhof pathway and the amount metabolized by the hexose-monophosphate shunt, for example, can be determined from studies of formation of $C^{14}O_2$ following glucose-1-C^{14} and glucose-6-C^{14} injection into fish.

4. As our knowledge of the pathways of intermediary metabolism develops, attempts should be made to integrate this knowledge with that of the nutrition and physiology of aquatic animals. There are a variety of important questions in the nutrition and physiology of aquatic animals that should be answered at the level of intermediary metabolism. How, for example, are the characteristic highly unsaturated fatty acids of fish

metabolized for energy conversion and to intermediates for synthetic reactions? Does the "red muscle" of fish function primarily as a muscle, or does it have some properties in common with liver and other organs (Braekkan 1956)?

Experience in the past has shown that sound fundamental research of this kind opens wide fields to applied research, which then leads to new industrial applications or aids in the solution of long-standing industrial problems.

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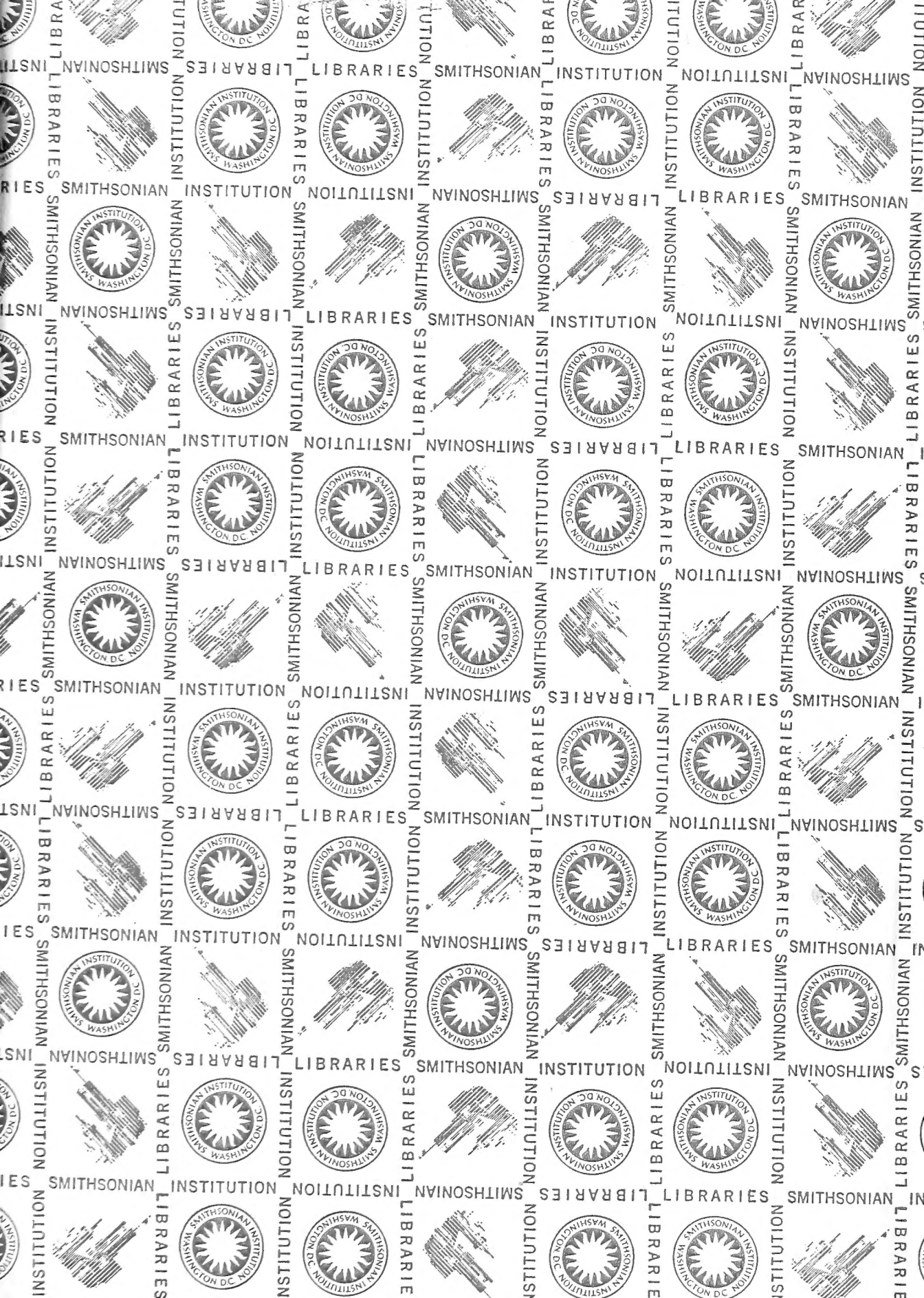
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