ANIMAL BIOCHEMISTRY

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THE DYNAMIC STEADY STATE OF THE BODY

UNTIL RECENTLY, the physiological chemist described the animal organism as an engine with a relatively static structure, in which the food was the fuel. The view was that a small fraction of the food was used to replace the wear and tear losses of the engine's structure. The working parts of the engine were composed of what was called "protoplasm."

This concept was typical of the era in which thermodynamics with its satellite, Newtonian statistical mechanics, reigned as the newly enthroned queen of the physical and chemical sciences. It was the era of combustion engines, the era of Helmholtz.

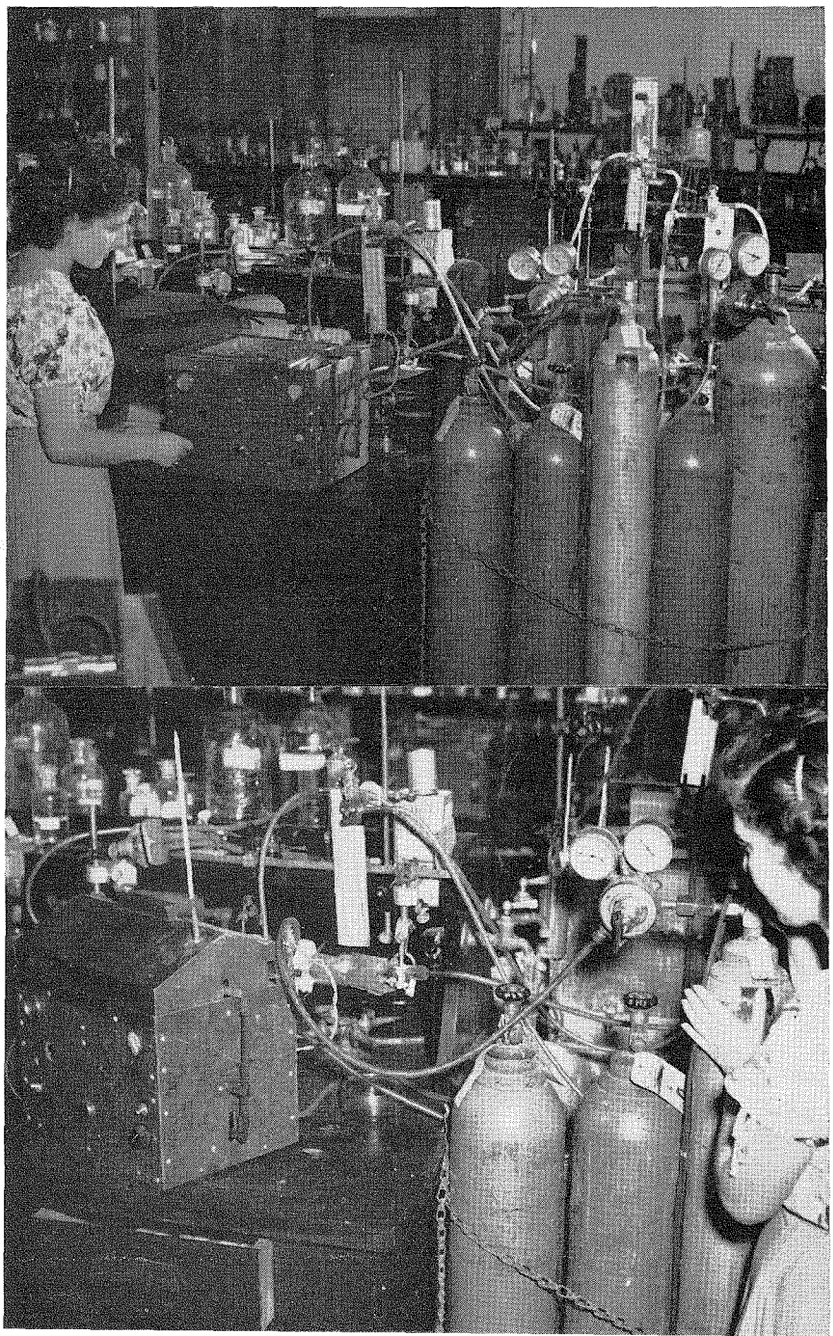
The simplicity of the concept of the organism as a combustion engine was, no doubt, one of its attractions. Another was that it helped the physiologists and physicians who were its devotees to feel respectable in the company of chemists and especially the physicists who were the rigorous pukka sahibs of natural philosophy of the nineteenth century.

The concept of the organism as a machine was not a biological concept at all; and we now see that it is not in accord with the facts. The organism is a dynamic steady state. Structural substance and fuel substance are continually interchanging on a large scale and very rapidly. There is in many instances little utility in attempting to distinguish between the chemical changes in the structure of the biological engine and its fuel. The animal organism is a chemical system in which protein, fat, carbohydrate, minerals, vitamins, and water are continually and rapidly interacting and yet maintain the chemical composition of the body nearly constant. A small shift in the steady state marks the difference between growth and old age, between health and disease.

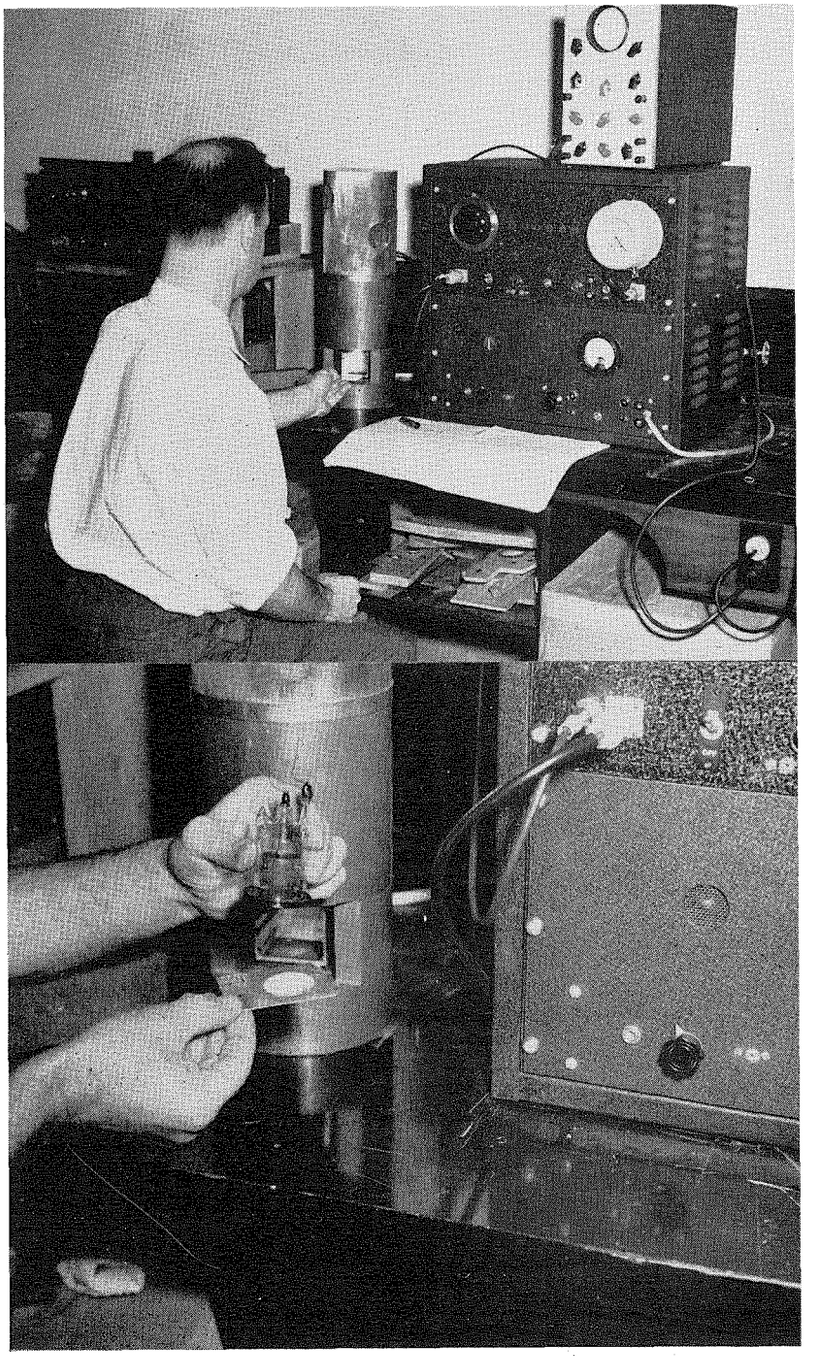
The group in Animal Biochemistry is studying this chemical steady state with special reference to proteins and their derivatives and to those reactions in which one component gains free energy from an oxidation; in other words, building-up processes. The formation of protein, creatine, and urea are representative.

These reactions can be viewed as a class of organic syntheses peculiar to living organisms. The mechanisms familiar to the organic chemist rarely operate in the body. Biological catalysts (enzymes) promote reactions which otherwise require high temperatures and pressures, strong acids or alkalies; and the yields are often higher than the latter conditions.

We are accustomed now in biochemistry to the transfer from one molecule to another of large radicals such as —C(:NH)NH_2, PO_4, and —CH_3. Knowledge gained in recent years on the mechanisms of methyl transfer reactions has elucidated some of the chemical aspects of growth and some diseases of the liver, muscle and heart. For example, cirrhosis of the liver is the result of a cumulative deficiency over years of labile methyl groups. Although many food components contain methyl groups, in only a few are the methyl groups physiologically labile **i.e.,** transferable from one compound to another. The



This tissue culture apparatus, designed by Dr. J. W. Dubnoff and demonstrated by assistant Ingelore Silberbach, maintains up to 30 tissue slices or homogenate samples in equilibrium with a physiological gas mixture at constant temperature. The apparatus, shown uncovered in the top picture, and in action in the lower, consists of a thermostatically controlled water bath, a shaking mechanism, and a removable vessel container. Individual handling is obviated since the container with its vessels is brought to temperature and equilibrated with gas as a single unit.



UPPER: Dr. Geoffrey Keighley with some of the apparatus for measuring the activity of radioactive materials used in physiological and biochemical experiments. Material containing the active tracer isotope is placed on a shelf in the lead shield, under the Geiger counter tube. On the right is the apparatus for counting the impulses from the counter. LOWER: Aluminum shelf bearing a sample of radioactive C^{14}. Above it is an end-window Geiger counter. The window is of mica thin enough to pass the weak radiations from the C^{14}. When in use both shelf and counter are held in similar relationship inside the lead shield.

most important food constituents with labile methyl groups are the amino acid methionine and the nitrogenous base choline. The methyl group of methionine is used in the body to make creatine which is essential for muscular contraction. Choline participates in the transfer of fatty acids from the liver to other tissues. The methyl radicals of methionine and of choline are interchangeable. Both substances are essential for growth principally because of their labile methyl groups.

Many reactions participate in the dynamic steady state; their participation is coordinated or there would be no steady state. As food supply and bodily activity vary, some reactions slow down, others become faster. When there is an abundance of carbohydrate in the diet, some is converted to fat; when muscular activity is increased, some protein is converted to carbohydrate.

ISOTOPES AS TRACERS

In a system so complicated and consisting of so many reactions, it has been extremely difficult to follow the course of any one compound through its transformations and disintegration. This study has been greatly helped by the availability of isotopes and their use as tracers. A molecule can be labelled by incorporating an isotope into its structure, N^{15}, for example, in place of normal N^{14}, or C^{14} instead of C^{12}, or H^2 instead of H^1. An isotope is identical in its chemical behavior with its normal counterpart; i.e., N^{15} behaves chemically the same as N^{14}, C^{14} the same as C^{12}, H^2 the same as H^1. It is possible, however, by physical methods to locate and measure isotopes. By following the label (the isotope) one can thus follow the molecule to which it is attached; by identifying the compound in which the isotope is found one learns what has happened to the compound into which the isotope was originally incorporated.

The Biochemistry Department is using isotopes as tracers. Large organic molecules are synthesized, incorporating as a tag the isotope C^{14} in place of the normal C^{12}. The whole molecule can then be followed into and out of protein molecules. And when it is disintegrated, identification of the fragment in which the C^{14} is found tells the manner of its disintegration. The C^{14} is measured by a Geiger-Müller counter, and by this method as little as 0.005 milligrams of an isotope-labeled amino acid can be located and measured.

In studies such as the foregoing it is rarely possible, and always cumbersome and inconvenient, to use a whole, living animal. Much more, and more precise, information is gained by using small amounts of tissue, 1 gram or less, or extracts of tissues. Consequently, it was necessary to develop specific and accurate micro methods for the determination of milligrams or even micrograms of specific substances. One of the most useful general methods for this purpose is chromotography, which is essentially separation of compounds by specific adsorption on selected adsorbents. By this means 2 milligrams of protein have been fractionated semi-quantitatively into 18 different amino acid fractions and each of the fractions identified.

The foregoing uses of physical and chemical methods in our biochemical work are characteristic of contemporary biological research. Spectrophotometers, electrophoresis and ultra-centrifuge equipment are commonplace now in biological laboratories.

The use of physico-chemical methods in Biology carries with it an implication of fundamental importance. It is the preference for physico-chemical and quantitative interpretations of biological phenomena. This trend in Biology may be said to have begun at about the middle of the nineteenth century. Now it is in full flood and dominates the field.