Marked Molecules

The first international conference on methods of preparing and storing marked molecules was held in Brussels, 13–16 November 1963; 180 scientists representing 16 countries attended. The program was scheduled so that the participants could enjoy the widest international exchange of views.

Topics of the 7 general reviews, 63 original papers, and more than 100 oral contributions included original syntheses and improvements of already known methods of preparation of marked molecules and techniques of purification and storage. Particular emphasis was placed on the study of methods of autoradiolysis of marked compounds with high specific activity.

In the opening address of the conference P. De Groote (member, European Atomic Energy Community) gave examples of how Euratom is procuring, supplying, and acting as a clearing house for marked molecules. The first lecture was then delivered by J. Roche (rector, University of Paris) who reviewed the applications of radioisotopes in biochemistry.

Many papers were concerned with the preparation of carbon-14 and tritium-marked molecules by chemical, biological, and radiation methods. Others described the synthesis of molecules marked with such isotopes as hydrogen-2, carbon-11, nitrogen-15, oxygen-17, oxygen-18, phosphorus-32, sulfur-35, iodine-131, and uranium. Informative papers were also given on the decomposition of radioactive compounds on storage.

W. Stoffel (Germany) described the chemical synthesis of polyunsaturated fatty acids used to elucidate the biosynthetic mechanisms of chain longation and olefination. Oleic, linoleic, linolenic, and other polyunsaturated acids were marked with either carbon-14 or tritium. Thus, polyunsaturated fatty acids marked with carbon-14 in the methyl group, in the 1-position and in the 2-position, were synthesized starting from acetylenic precursors. In addition, tritiated polyunsaturated acids were prepared by reducing the appropriate acetylenic compounds catalytically in the presence of tritium gas.

B. Samuelsson (Sweden) discussed the synthesis of labeled stereoisomers of sterols and bile acids and their use in studies of biosynthetic mechanisms. Cholic and deoxycholic acids were labeled with tritium in positions 6α, 6β, 7β, and 8β. Also, cholesterol was tagged in positions 3α, 4α, 4β, and 6 with tritium. The biosynthesis of squalene-C^{14},H^3 and conversion to cholesterol-C^{14},H^3 was achieved in a single continuous two-stage incubation.

Farnesyl-C^{14} pyrophosphate was first enzymically synthesized from mevalonate-2-C^{14} and then incubated under nitrogen in the presence of a microsomal squalene-synthesizing system together with TPNT (tritiated triphosphopyridine nucleotide reduced form) as a cofactor. Then the bulk of the tritium was removed from the TPNT by equilibration with water before treatment with unlabeled TPNH and glucose-6-phosphate. Incubation under oxygen converted the radioactive squalene to cholesterol. The implications of the mechanisms were pointed out.

The labeling of sugars and some dicarboxylic acids with tritium by enzyme reactions or by the Wilzbach procedure was covered by H. Simon, G. Mullhofer, and H. D. Dorrer (Germany). Differences in the tritium distribution in a sugar and its corresponding phosphate prepared by exposure to tritium gas were described. For example, irradiated D-glucose contains about 90 percent of the incorporated tritium in the 3-position whereas the irradiated D-glucose-6-phosphate gave multiple labeling in positions 2 through 6. The tritium distribution in D-mannose, D-fructose, D-fructose-1,6-P, D-ribose, and *d*-ribose-5-P prepared by the Wilzbach method was reported.

K. Hempel (Germany) described the production and storage of tritium-marked amino acids of high millimolar radioactivity. Tritiated amino acids (1 c/mmole and above) were synthesized either by catalytic tritiation of appropriate unsaturated compounds or by catalytic tritiation of suitable organic halides. Virtually all the amino acids present in proteins were prepared. The decomposition of these highly radioactive amino acids during storage was investigated; the best storage condition was found to be refrigeration of an 80 percent ethanol solution of the tritium-marked amino acids at −15°C. Some of the radioactive impurities formed during storage were isolated and identified.

Concerning the iodination of proteins with a degree of radioactivity, R. Ph. Ekins (Great Britain) reported two methods for iodinating various protein hormones with iodine-131. The first method involved the use of Conway microdiffusion units where iodine diffuses into the protein with an efficiency of 30 to 40 percent and produces a protein with an iodine-131 content of 200 mc/mg. In the second method chloramine-T is used to oxidize iodide to iodine prior to iodination of protein. Insulin iodinated by this technique can have a radioactivity of up to 1.5 c/mg.

H. Elias (Germany) reported a unique procedure for labeling organic compounds in a gas chromatographic column. The labeling of volatile organic compounds with tritium and radioiodine is feasible.

Such a procedure has advantages over isotopic-exchange reactions in solution because (i) the labeled compound is obtained directly in a pure form; (ii) small quantities can be labeled and isolated; (iii) exchange is faster in gas chromatographic columns partly because of higher temperatures employed; and (iv) a higher specific activity is obtained by a single passage through the column than by a single exchange in solution if the initial specific activity is the same.

A gas chromatographic column containing about 10 percent sorbitol on kieselguhr was labeled with tritium water. Then organic compounds containing active hydrogen atoms were tritiated by passage through the column. The procedure can also be modified for deuteration.

In a similar manner organic iodides were labeled in a gas chromatographic column with a substrate containing potassium iodide-131 at a temperature between 154° to 184°C.

P. E. Schulze and M. Wenzel (Germany) presented a novel determination of the molecular weight of a compound by double labeling. The tritiated compound of interest was isolated in a radiochemically pure form and its radioactivity [disintegrations per minute (dpm) per microliter] was determined. Then the tritiated compound was allowed to react with a C^{14}-labeled reagent of known molar activity (dpm/μmole), and after isolation and purification of the derivative the radioactivity due to tritium and carbon-14 was measured. The molecular weight can then be calculated if the number of groups reacting per mole is known. When selected tritiated steroids and amino acids (5 to 20 μl) were used the molecular weight found was between 0.5 and 4.5 percent of the theoretical value.

D. Samuel (Israel) described recent syntheses of organic compounds labeled with the stable isotopes of oxygen (O^{17} and O^{18}). The main methods of synthesis of oxygen-labeled compounds with isotopic water or oxygen are oxidation, hydrolysis, and exchange. Labeled organic compounds are obtained by hydrolyzing nitriles, halides, or esters in acid or alkaline solutions that contain isotopic oxygen. Alcohols labeled with O^{17} and O^{18} are prepared from esters which hydrolyze with C-O bond fissions such as dialkyl sulfates and trialkylphosphates. Labeled phenols are best made by hydrolysis of diazonium salts.

By use of O^{18}-labeled alumina a new method has been devised for labeling alcohols and steroids. Compounds such as cholesterol, α- and β-cholestanol, and ethyl alcohol have been prepared by heating them with alumina-O^{18} in benzene or petroleum ether. Although the isotopic enrichments are not large (1 to 2 percent of O^{18}) there is complete retention of stereochemical configuration by the sterols.

H. A. Smith and M. Lindauer (United States) discussed tritium labeling by catalytic exchange. A rapid exchange takes place between isotopic hydrogen in the gas phase and the carboxyl hydrogen of an organic acid in the presence of a platinum or rhodium catalyst. The labile hydrogen can be stabilized by decarboxylation of the organic acid or the marked acid can be heated with certain organic compounds in the presence of a catalyst. An exchange takes place which allows labeling of the new molecule. Acetylene, ethylene, and *cis*- and *trans*-dichloroethylene have been labeled by this procedure. The method may be used with pure deuterium or tritium or with relatively low mole fractions of these in the hydrogen gas. There are virtually no side reactions.

B. M. Tolbert (United States) described the decomposition of solid amino acids by radiation. Under the influence of ionizing radiation, organic compounds undergo chemical changes that result in fragmentation, polymerization, and isomerization during the course of synthetic and degradative processes.

Decomposition and decarboxylation were combined with efr data to give a postulated decomposition mechanism. Specific decomposition pathways for DL-leucine were given together with yields for various products. The G ($-M$) values (number molecules of studied material destroyed per 100 ev) for a number of amino acids in solution and in solid state were also presented.

In his closing speech, E. Medi (vice president of the Euratom Commission) stated that the Brussels conference was a starting point for international exchange on matters concerning the preparation and storage of marked molecules.

In conclusion, it should be pointed out that the applications of labeled molecules are of great consequence for research. For example, in 1962, there were almost 2000 published papers dealing with the use of carbon-14 in chemistry, biology, and medicine. Many of these studies required a carbon-14–labeled compound other than radioactive carbon dioxide. During the next 10 years dramatic new syntheses based on a combination of chemical and biological methods should be developed. Also, unique and simple procedures are continually being devised for the preparation of labeled compounds in a radiochemically pure form and at high specific activities.

Therefore similar conferences with an international scope will be welcomed as an efficient method for the continued interchange of pertinent information between scientists with related interests and discoveries.

This conference was sponsored by the European Atomic Energy Community Commission.

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Ethology

Behavioral physiology, motivation, development, and descriptive and comparative ethology were the four main topics of discussion at the 8th International Ethological Conference held at The Hague, Netherlands, 12–22 September 1963.

The number of studies on behavioral physiology are increasing in ethology, because of the diversion of neurophysiologists toward behavioral problems and because of dissatisfaction among behaviorists with purely descriptive analysis. Hormonal studies begin to reveal in detail the intricate chain of interlocking events that underlie, for example, the sequences of actions in the reproductive behavior of birds and mammals. Knowledge of sensory physiology, particularly of invertebrates, is illuminating behavioral problems ranging from food selection and the responses of moths to the ultrasonic pulses of bats, to the accurate auditory localization of mice by owls in darkness and the role of auditory feedback in the control of vocal behavior in birds. General functions of the central nervous system were also considered, both in vertebrates and invertebrates. Peripheral and central contributions were tied together in an analysis of the control of the pattern of flight actions in the locust. This paper resolved the conflict between proponents of endogenous and exogenous (that is, proprioceptive chain reflex) mechanisms by demonstrating that the natural behavior is a result of both kinds of process interacting with each other.

Stimulus analysis and other problems of motivation were also approached from a behavioral rather than a physiological point of view. The nature of chemical stimuli controlling behavioral responses was explored in several cases, including the famous swimming sea anemone, *Stomphia*. Among situations involving visual stimuli one of the most dramatic examples was the use of polymorphic egg markings made by cliff-nesting guillemots for locating their own eggs on the nest ledge. Another was a study of the communicating function of various postures in gulls by the use of movable models. Some new data on orientation problems were also presented. Aside from stimulus analysis, quantitative studies of motivation, including the fine analysis of the temporal organization of behavioral sequences, were also noted. Unitary drives were the subject of conflicting comment; one study emphasized the "unitary" nature of feeding behavior in fishes, and another demonstrated the multiple motivation of aggressive behavior in fishes.

Descriptive studies of natural development, both with and without experimental evidence, and analyses of the role of learning in parent-young relationships in fish, birds, and mammals were presented. Visual and chemical stimuli are important factors in the development of bonds between young and parent in cichlid fishes. New evidence was presented on the control of the critical period for the following responses in domestic chicks. Another long-needed and carefully controlled