Meetings

Insect Biochemistry

Strictly speaking, there is no such scientific discipline as insect biochemistry. However, biochemistry as applied to insects has long attracted a small number of investigators, among whom Japanese scientists have predominated. It would thus seem appropriate that a pioneering insect biochemistry seminar, held 20 June–3 July 1965, should have taken place at Chiba, Japan.

The biochemistry of the posterior silk gland of the silkworm *Bombyx mori* is of special interest—particularly to Japanese scientists—because of its unique biological function in biosynthesis of the silk fibroin of commerce. Y. Miura (University of Chiba Medical School) discussed the incorporation of C^{14}-orotate into silk gland cellular particulates. The pattern of labeling depended upon the age of the 5th-stage larvae, nuclear RNA being most radioactive at earlier periods. In more mature larvae three types of labeled RNA—low-molecular-weight, ribosomal, and DNA-like—were obtained from large (14,000*g*) subcellular particles. The DNA-like RNA, which was isolated in fibrous form, had a low turnover of C^{14}-orotate. H. Shigematsu (Sericultural Experiment Station) also considered a DNA-like RNA synthesized by the silk gland in the early 5th instar. However, in his experiments with P^{32} neither this nor any other RNA fraction had the properties of true messenger RNA. Employing fibroin antibody as a specific test for fibroin, Shigematsu found fibroin synthesized in vitro to be predominantly in the microsomal and soluble fractions. He also reported that blood concentrations of glycine and tyrosine, but not serine or alanine, could regulate the rate of fibroin formation in vivo. K. Shimura (Tohoku University) reported that electron-microscopic and sedimentation analysis indicated that, in vitro, polysomes (polyribosomes) of about 450*S* were the site of fibrion synthesis. Moreover, at least two enzymes, amino acid-activating and peptide-bond-synthesizing, were required in addition to a number of well-established components. The latter enzyme has been partially purified and characterized.

In a different approach, K. Maruyama (University of Tokyo) briefly reviewed the properties of proteins in insect muscle. In general, these properties are similar to those of their mammalian counterparts. However, examples were cited (for example, adenosine triphosphatase activity of myosin from developing flight muscle or cockroach muscle) of misleading results which have been obtained by assuming identical effects of the physicochemical environment on the respective muscle proteins.

L. Levenbook (National Institutes of Health) described the profiles of individual free amino acids throughout metamorphosis of the blowfly *Phormia regina*. The analytical picture was supplemented by kinetic data on the rates of oxidation, turnover, and incorporation into protein of labeled alanine and lysine during metamorphosis. The turnover and oxidation of alanine were much more rapid than those of lysine, but the two amino acids were incorporated into protein at about the same rate during metamorphosis. J. Corrigan (Tufts University School of Medicine) considered the occurrence of free D-amino acids in various insects. Young larvae of *B. mori* contain little or no D-serine. However, at the time of spinning or in the pupa, D-serine, which occurs in most lepidoptera, accounts for 15 to 70 percent of the total. The derivation of the blood D-enantiomorph from D(—)-2,3-diamino propionic acid (DAPA), isolated from silkworm digestive fluid by S. Wada and T. Toyota, was considered a most likely possibility. Preliminary experiments suggested that DAPA itself might be formed from glucose.

The end product of insect nitrogen metabolism is generally uric acid; some recent studies on the distribution and metabolism of this purine were presented by C. Hirano and S. Tojo (National Institute of Agriculture). In the diapausing rice stem borer (*Chilo suppressalis*), urate, located primarily in the fat body, was synthesized very slowly; the rate increased markedly in the post-diapause period of development as measured by the degree of incorporation of C^{14}-glycine into uric acid. In both *C. suppressalis* and *B. mori* labeled guanine and alanine were converted to uric acid, and a marked transport of the purine into the developing pupal ovary of *B. mori* was observed.

In discussions of insect enzymes, J. Mukai and S. Akune (Kyushu University) described the purification and properties of an unusual nuclease from digestive fluid of the silkworm. Apparently a single enzyme with a *p*H optimum of 11.2, this nuclease cleaves both RNA and DNA to a mixture of di-, tri-, and tetranucleotides. This enzyme is a potentially useful tool for the study of the structure of polynucleotides. Z. Ogita (Osaka University) gave an account of his extensive studies on the genetic control of insect isozymes and electrophoretic protein patterns. Genetic, immunological, and biochemical evidence led to the conclusion that isozymes can be classified into heteromorphic, allelomorphic, pleiotropic, and polygenic types. Superimposed upon this genetic control are developmental changes; thus, during the metamorphosis of houseflies the titers of amylase and acid phosphatase increase, whereas that of alkaline phosphatase decreases almost to zero. Concomitant changes occur in the isozyme patterns.

Lipids and steroids in insects have recently received considerable attention. E. Hodgson (North Carolina State University) discussed the relation between structure and function in dietary substitutes for choline and transmethylation reactions in *Phormia*. Several organic bases of the type $(CH_3)_2R{\cdot}NCH_2CH_2OH$, (where R is an alkyl substituent) promote growth, but the two methyl groups and terminal OH are essential. Phosphatidyl ethanolamine, in contrast to choline, predominates in this species, with only minor changes in composition of the phospholipids during the life cycle. Fat transport was discussed by H. Chino (University of Tokyo). In contrast to mammals where fat is transported mainly as triglycerides or free fatty acids or both, the form of transport in insects is principally diglycerides and free fatty acids complexed to blood protein. Diglyceride release from fat depots is an active process, inhibited by respiratory poisons and stimulated specifically by insect blood. Some experiments concerned with the fate of mobilized free fatty acids were described by E. Stevenson (Du Pont Company). Thus, in contrast to the work of Sacktor and others with flies, sarcosomes of flight muscle from the Southern armyworm (*Prodenia eridenia*) moth rapidly oxidized pyruvate plus malate with P:O ratios of about 2.5, and excellent respiratory control in the absence of any added cofactors or serum albumin. In the presence of a di- or tricarboxylic acid "sparker," such preparations also oxidized palmitate at surprisingly rapid rates. This reaction is in good agreement with the fact that these moths utilize fat as a fuel for flight.

In the field of insect steroids, R. B. Clayton (Stanford) has employed the roach *Eurycotis floridan* as the experimental subject. The roach was raised aseptically on a doubly labeled diet (H^3-cholestanol and C^{14}-cholesterol); analyses for labeled cholesterol, cholestanol, and Δ^7-cholestanol revealed three types of functional spaces of different structural specificity among the insect tissues. Together with studies on sterol distribution in subcellular fractions of individual tissues, the data supported the idea of a tissue-specific recurring, repeating structural unit common to all subcellular membranes. Sterol utilization was more simply invoked by S. Ishii and S. Kawahara (Kyoto University) to explain why the clothes moth *Tinea pellionella* feeds only on animal products, whereas the related *Tineola biselliella* feeds also on plant products. Larvae of the latter, similar to those of *Ephestia*, can utilize phytosterols from plants, whereas the former cannot. Unfortunately wool, being of animal origin, is eaten by both. N. Ikekawa and M. Saito-Suzuki (Institute of Physical and Chemical Research) applied modern analytical techniques to the silkworm. Throughout development the major steroid component is cholesterol, followed by β-sitosterol and campesterol. Larvae were much more active than pupae in converting H^3-β-sitosterol to cholesterol, while esterification of injected C^{14}-cholesterol was considerably depressed in brainless "dauer" pupae as compared with normal pupae.

Carbohydrates were discussed next. The major storage polysaccharide of insects is glycogen. However, as pointed out by G. R. Wyatt (Yale), insects exhibit some unusual biochemical features such as the conversion of glycogen to glycerol—a biological "anti-freeze"—during diapause and the disaccharide trehalose instead of glucose as the "blood sugar." The synthesis of the latter by way of trehalose phospate in the cecropia silkmoth fatbody is inhibited by free trehalose—an example of homeostasis through feedback control. However, the physiological roles of the hydrolytic soluble midgut trehalase or the membrane-bound muscle trehalase are still hypothetical. W. Chefurka (Science Service Laboratory, Ontario) discussed the difficulties and pitfalls in evaluating the relative importance of the pentose shunt and glycolytic pathways of glucose catabolism in insects. The extent to which glucose was catabolized by these alternative routes was influenced by numerous variables, for example, site of injection of labeled glucose, sex, species, stage of development, and O_2 tension.

The paper by Y. Umebachi (Kanazawa University) on the yellow pigments of papilionid butterflies was a remarkable achievement. He memorized the entire text of his presentation in what was to him a foreign language. As was shown by various painstaking experiments, these pigments are related to derivatives of kynurenine and of 3,4-dihydroxyphenylalanine (or tyrosine?) in contrast to the pterin wing pigments of pierid butterflies and the ommatin pigments of nymphalid butterflies. The chemical nature of the pigmented aminequinone complex is now under investigation. The chemistry and genetics of various pigmented chromoprotein granules in the hypodermis of silkworm larvae were discussed by M. Tsujita (National Institute of Genetics). Three types of granules were isolated—protein complexes of sepiapterin, isoxanthopterin, and uric acid. Twelve amino acids were found in the different proteins. Uric acid was the most abundant; its protein was particularly rich in glycine. Preliminary data suggest that this glycine is transferred to the silk gland to be incorporated into fibroin. The fibroin biosynthesis experts had no comments.

In conclusion, it was the general feeling that because of the informal atmosphere, the relatively small number of participants, and the goodwill on both sides, this seminar was marked by a particularly good exchange of views and information between the two groups of participants. The American party left Japan after a 6-day tour of laboratories in the Kyoto-Osaka-Nagoya-Tokyo area, enriched by new friendships and an understanding of Japanese contributions to insect biochemistry and endocrinology which could never have been achieved through a mere reading of the scientific literature.

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Electron-Spin-Resonance Signals and Biological Effects

Many laboratories in the United States and Europe are involved in research to discover correlations between radiation-induced electron-spin-resonance (ESR) signals and biological activity. In order to discuss recent work in this field, physicists and chemists from two continents met at Gatlinburg, Tennessee, 10–12 May 1965.

A short summary of the ESR technique was presented by Ralph Livingston (Oak Ridge) for the benefit of those participants not well familiar with it. Livingston then discussed ESR signals from radiation-produced radicals in liquid systems. Often the hyperfine structures of the signals are much better resolved because of the "averaging" effect of rapid motion in the liquid state, thus making it easier to identify the radicals involved. The possibility of studying signals produced in aqueous solutions presents considerable advantages to the radiation biologist. From the discussion it became clear that a number of different laboratories are working on the observation of ESR signals in aqueous solutions, and that there are advantages in using frequencies both higher and lower than those normally used today.

In a talk on irradiated nucleic acids, Adolph Mueller (Karlsruhe), pointed out that the number of spin-resonance signals at first increases linearly with radiation dose and then reaches saturation. From the shape of the saturation curve it may be deduced that the process removing the radicals is first order