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.

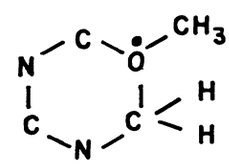
A short summary of the ESR technique was presented by Ralph Livingston (Oak Ridge) for the benefit of those participants not well familiar with it. Livingston then discussed ESR signals from radiation-produced radicals in liquid systems. Often the hyperfine structures of the signals are much better resolved 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

in the radical concentration, not second order as would be expected for radical recombination. He suggested that the radicals might combine either with hydrogen atoms moving through the lattice or with electrons. In the ensuing discussion, no really satisfactory alternative to this hypothesis was presented.

Mueller also discussed the difficulties in measuring absolute radical yields by electron-spin-resonance. In 1961 a number of laboratories interchanged standards and found that there were differences of a factor of two in the absolute numbers measured by various laboratories. The source of this discrepancy is under active investigation at Karlsruhe and Utrecht, two laboratories which were far apart on the initial exchange.

Robert Schulman (Bell Telephone Laboratories) talked about the work which is being done at the Bell Telephone Laboratories on identifying the radicals produced in DNA by ultraviolet light. Irradiation of dry DNA with ultraviolet light at 77°K gives a strong singlet, and a much smaller component showing a number of lines which can be unequivocally linked to thymine. If the sample is irradiated in the presence of moisture, the thymine signal can be made to dominate the observed signal. The Bell Telephone Laboratories group postulates that this radical is



They have also identified a phosphorescent emission from thymidine which is quenched when the thymidine is in DNA in the native state. The level giving rise to the phosphorescence was unequivocally identified as a triplet state. There were speculations on the possible connection between these results and the formation of the thymine dimer in ultraviolet-irradiated DNA.

Thormod Henriksen (Oslo) reviewed the introduction of ESR signals into dry protein by ionizing radiations. At very low temperatures a broad, featureless signal is produced which is best interpreted as the superposition of many different free radicals. On warming to room temperature, the observed pattern goes through a series of changes until it gradually resolves into

two relatively distinct signals—a broad signal having a *g*-factor somewhat more than 2, associated with sulfur atoms, and a doublet having a *g*-factor equal to that of the free electron. In several special cases, including crystals of various amino acids and in silk fibers, this doublet has been identified as a radical on the alpha carbon atom located in the polypeptide backbone and bonded to a single hydrogen atom. This might suggest that the glycines were being preferentially attacked.

The connection between radiation-induced ESR signals and biological activity, as shown in studies on trypsin irradiated in the dry state, was discussed by Peter Alexander (London). He suggested that a major inactivating event in the case of proteins could be the removal of an amino acid residue from the polypeptide chain. This concept is certainly in good agreement with the doublet signals described by Henriksen. It was, however, pointed out that the postulated breakage of the bond between the alpha and beta carbons is unexpected.

Tolbert (Colorado) summarized information on the radiation chemistry and radiation biochemistry of proteins irradiated in the dry state. From the chemical data, Tolbert concluded that there is only a difference of a factor of 2 or 3 in radiosensitivity for destruction of various amino acids in irradiated proteins, and that there was no selective breakage of sulfur-sulfur bonds. There also does not seem to be any real evidence for breakage of the main polypeptide chain in most proteins. Warren Garrison (Berkeley) briefly reviewed his mechanism for chain breakage following the production of a free radical on the alpha carbon in the polypeptide backbone and gave evidence for this process in irradiated gelatin. No suggestion was advanced as to why this breakage apparently does not occur in other proteins, except that the free ends may sometimes recombine with each other.

Comprehensive studies on irradiated dry spores of *Bacillus megaterium* were presented by E. L. Powers (Argonne). These studies show excellent correlations between spore viability and a free-radical mechanism within the spore, as deduced from the effects of such reagents as oxygen, nitric oxide, or hydrogen sulfide, all of which have well known reactions with free radicals. Powers preferred to define free radicals on the basis of their chemical reactivity with such reagents. Other participants thought that definition of a free radical in terms of an unpaired electron was more satisfactory. The variations in spore survival could also be correlated with ESR measurements, although Powers was careful to point out that a large number of free radicals are created in a spore before it loses its ability to multiply, and that the free radicals responsible for spore inactivation probably consitute only a very minute fraction of those which can be measured by the ESR technique.

In the final session, Peter Alexander suggested that electron-spin-resonance was rather unlikely to give much of a clue as to what events were significant in causing the loss of biological activity of irradiated cells. On the other hand, he felt that the ESR technique had great potential for the understanding of details of events taking place once the key materials involved in the biological inactivation process had been identified. Alan Conger (Temple) echoed a rather similar idea when he said that he did not really care about the detailed interpretation of ESR signals. What concerned him was the location in which the ESR signals were found.

In summing up, E. C. Pollard (Pennsylvania State), chairman of the Subcommittee on Radiobiology, felt that it was clear that evidence of physical damage by radiation was plentiful, but that relating it to biological effects still seemed difficult.

The conference was sponsored by the subcommittee on radiobiology of the National Research Council. The complete proceedings, including the extensive discussion after each paper, will appear soon as an NRC publication.

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Forthcoming Events

November

7. American College of **Dentists**, Las Vegas, Nev. (O. W. Brandhorst, 4236 Lindell Blvd., St. Louis, Mo.)

7–9. American **Science Film** Assoc., annual, Washington, D.C. (ASFA, 1319 F St., NW, Washington 20004)

7–11. American Soc. of **Mechanical Engineers**, winter annual mtg., Chicago, Ill. (ASME, 345 East 47 St., New York)

7–12. **Anatomical Pathology**, 5th Latin American congr., Lima, Peru. (J. J. Andujar, P.O. Box 118, Fort Worth, Tex.)

7–13. **Paediatrics**, 11th intern. congr.,