that a considerable part of the darkness of the lower side of strato-cumulus clouds consists of veil clouds, and that their dark colour indicates that their chemical composition differs very much from water. I suggest that veil clouds associated with alto- and strato-cumulus clouds are accumulations and condensations of the blue haze and therefore that they consist of particles of bituminous or asphaltic materials.

At the size the particles reach in veil clouds they may be effective condensation nuclei, and thus may play a part in cloud formation and even in rain production. They might thus partially replace sodium chloride particles as cloud- and rain-forming nuclei.

Only over vegetated areas are blue hazes and veil clouds observed. Over oceans one can see dilute white hazes, apparently consisting of sodium chloride particles, produced by evaporation of droplets of sea water, splattered by rain or splashing droplets into the air. Over absolute deserts no blue hazes occur (usually only yellow dust layers occur there); but over the creosote-covered California semi-deserts, and over the aromatic Australian interior, blue hazes, of perhaps lower concentration than the hazes hanging over Europe or the Mid-West of the United States, are typical. Blue hazes are typical of all tropical vegetated areas, pine forests, and forests in general.

About the fate of the bituminous and asphaltic particles in the veil clouds and grey or black inversion layers, little can be said. It is almost certain that they return to the Earth's surface with rain and snow, become attached to clay and other soil particles, and thus are either washed down with rivers into delta areas, where they might be the source materials for petroleum deposits, or they become incorporated in

humus and peat, and provide the basis for much of the heat content of bituminous coals.

I suggest that the blue hazes and veil clouds play a very important part in the heat balance of the Earth and specifically of the atmosphere. They probably absorb about 20 per cent of all the Sun's radiation reaching the Earth. Therefore they should be considered when calculating the heat conversions near the Earth's surface.

There is another interesting aspect of the veil clouds. They must have a considerable potential heat content. Perhaps they provide not only part of the ions needed to build up the enormous potential differences required for lightning, but they might also provide part of the energy for maintaining the multi-mile long lightning shafts. One of the reasons for suggesting this is that the denser the vegetation and the more terpenes volatilize (due to high temperatures), the more intense the thunderstorms are. Over oceans, over deserts, and over the Arctic, they are absent.

Thus it was suggested that volatilization of terpenes and other plant products results in the production of, first, blue haze, then veil clouds, and finally, bituminous materials which, when returned to the Earth by rain and snow, produce the source materials for petroleum formation. The quantities produced are of the right order of magnitude, and all facts seem to fit into a consistent picture, giving a balanced point of view of many, until recently, unrelated facts.

We need proof of many of the statements and suggestions made here before they can be accepted as any more than a hypothesis. But the hypothesis may serve as a basis for further observations on the hitherto neglected blue hazes and veil clouds.

CYTOPLASMIC PARTICLES AND THEIR ROLE IN PROTEIN SYNTHESIS

THE Colloid and Biophysics Committee of the Faraday Society sponsored an informal discussion on this subject which took place under the chairmanship of Prof. J. A. V. Butler, at the University of Reading during March 28-29. The meeting was attended by approximately 150 people. Twenty-three communications were presented; a considerable number of participants contributed in an informal manner to a stimulating and fruitful discussion.

Studies in the field of protein synthesis, which are designed to elucidate some part of the mechanism by which amino-acids are linked to form peptide chains, have become numerous in the past five years. During this period research in this field reached the stage at which a concern with the development of sufficiently simple biochemical systems suitable for examination *in vitro* superseded the employment of whole organisms for investigations on the incorporation of amino-acids into tissue proteins. A similar trend has also occurred in the field of studies on micro-organisms, the cell components of which can also be fractionated and their metabolic behaviour afterwards examined. In view of the large extent to which amino-acids are incorporated into the proteins of the microsomes they are considered to be an important site of protein synthesis in the cell. In micro-organ-

isms the cell membrane and particles inside it are assumed to be functionally analogous to the microsomal structures found in cells of large organisms. Concurrently with the attention given to the problem of protein synthesis in and by microsomes some research workers have been able to make use of other cytoplasmic components, the mitochondria, to demonstrate that isolated mitochondria are capable of forming their own proteins.

The success of metabolic studies on preparations of cell particles and a correct interpretation of such results often depends on some knowledge of the structure of the cell components. Their examination by physico-chemical and microscopic techniques has yielded useful evidence on their structure as well as on the effect on it of different procedures applied for their isolation. It may here be said that one of the purposes of holding this informal discussion at this time was to provide an opportunity for investigators of the dynamic as well as the structural and morphological aspects of protein synthesis to meet and exchange views.

The opening speaker (C. de Duve, Louvain) rightly dwelt at some length on the limits of resolving power of techniques of cell fractionation. This problem is of particular importance in the examination of the

localization of single enzymes, or of enzyme systems. Prof. de Duve discussed the results achieved by his group in applying the technique of centrifugation in a density gradient to the localization of enzymes in tissues. He also reported on another approach, density equilibration in a gradient. This technique makes use of differences in density of particles to separate them in a suitable medium.

Electron microscopy has been of much help in formulating hypotheses which would try to explain some aspects of protein synthesis. It was pointed out by M. S. C. Birbeck and E. H. Mercer (London) that whereas cells such as hepatic cells which synthesize and secrete protein possess an endoplasmic reticulum, that is to say, membranes covered with particles, cells which retain the synthesized protein appear to contain free particles only. Efforts to correlate the site of synthesis with the location of particles or of the endoplasmic reticulum are made difficult by the existence of cell types in which these two points have been observed to be in different parts of the cell.

Many investigators have accepted the particles having a diameter of 15 μ seen by electron microscopy in the cytoplasm of cells fixed with osmium tetroxide as the structural basis of microsomal nucleoprotein. V. Hanzon (Uppsala) has obtained evidence that these particles may not exist in the living cell since they cannot be detected in pancreatic cells fixed by freeze-drying and subjected to the vapour of osmium tetroxide. These particles seemed to be formed rapidly by a change in the internal milieu of the cell as it is affected by, for example, homogenization or fixation with osmium tetroxide in aqueous medium. Should this observation be confirmed by examination of other cell types it would link up with the results of other investigations which have given evidence that there is no direct relation between the number of visible granules and the content of ribonucleic acid of the microsomes. These ribonucleoprotein particles, now generally referred to as ribosomes, can be examined in the ultracentrifuge as H. E. Huxley and G. Zubay (London) have shown in their studies using *E. coli*. Ribosomes from this organism were found to be able to exist in a number of different stable forms. They could be interconverted by changes in the composition of the medium, particularly in the concentration of free magnesium ions. In addition these workers were able to examine the effect of such changes under the electron microscope. Thus, particles of size 50 *S* have an 'active face' which becomes opposed to another 50 *S* or 30 *S* particle. Examination of 70 *S* particles showed them to be made of two sub-units which resemble 50 *S* and 30 *S* particles, respectively. The pattern shown by ribosomal ribonucleic acid in X-ray diffraction photographs was compared by A. Klug, J. T. Finch and K. C. Holmes (London) to that given by extracted ribonucleic acid. It was considered likely that ribonucleic acid from various ribosomes had its own characteristic configuration; in contrast, the configuration of spherical viruses was probably determined by that of the protein framework.

One effect of chloramphenicol on micro-organisms is to inhibit protein synthesis. S. Dagley and J. Sykes (Leeds) were able to show that in *E. coli* exposure of the cells to chloramphenicol resulted in an accumulation of abnormal species of ribosomes which are apparently useless for protein synthesis. If the antibiotic was displaced from such preparations

by magnesium ions, normal ribosomes of size 29 *S* were formed again. The results of an investigation of physical properties of ribosomes from the Jensen sarcoma were reported by M. L. Petermann (New York). The capacity of these ribonucleoprotein preparations to bind protein was found to depend on pH or the concentration of magnesium ions, and was observed to be sensitive to changes in ionic strength.

K. McQuillen (Cambridge) was able to report results of an extensive investigation which he and co-workers at the Carnegie Institution of Washington made on time relationships during protein synthesis in *E. coli*. Kinetic studies showed that the smaller ribosomes probably were precursors to the larger. During cell growth the ribonucleic acid and the structural protein of the ribosomes were found to be conserved. Regeneration of ribosomes occurred at an exponential rate in depleted cells; protein, however, was synthesized at a rate proportional to the ribosome content at any time. Evidence was given that radioactivity from amino-acids passed through the ribosomes where it had a transient existence as a protein before it appeared as a soluble protein. The use of ribosomes to study the mechanism of incorporation of amino-acids into proteins has been extended by A. Korner (Cambridge) to preparations obtained from rat liver. These ribosomes prepared by treatment of microsomes with deoxycholate were found capable of incorporating amino-acids on incubation in the presence of adenosine triphosphate and magnesium ions for more than 2 hr.

Most current theories on the mechanism of protein synthesis require the presence of ribonucleic acid from two cytological sources: from the microsomes and from the cell sap (*S*-ribonucleic acid). L. Bosch (Amsterdam) examined the metabolic behaviour of fractions obtained by passing ribonucleic acid through a column of 'Ecteola' resin. Only one of the four fractions obtained by stepwise elution of *S*-ribonucleic acid seemed to be involved in the transfer of amino-acids to microsomes and of radioactivity from phosphorus-32-labelled *S*-ribonucleic acid to microsomal ribonucleic acid. Conversely, only one of three fractions of microsomal ribonucleic acid was found to be capable of accepting radioactivity from phosphorus-32-labelled *S*-ribonucleic acid. The part which *S*-ribonucleic acid might play in protein synthesis was also discussed by V. V. Koningsberger (Utrecht), who reported on the existence in baker's yeast of small, carboxyl-activated peptides originating from activated amino-acids.

F. Gros (Paris) gave an account of experiments in which an alteration in the composition of ribonucleic acid led to modifications in the biosynthesis of proteins. When the uracil analogue 5-fluorouracil was added to growing cultures of *E. coli* some proteins produced were found to have an altered amino-acid composition. The synthesis of new specific protein could also be detected which differed markedly both in biological and physical properties from that produced under normal conditions. These effects appeared to be due to the changes in the ribonucleic acid alone because they also occurred in the presence of thymidine which would make unlikely the incorporation in place of thymine of 5-fluorouracil or its analogues. Another most promising development in the quest to shed light on the role of ribonucleic acid in protein synthesis was discussed by E. M. Martin (London). The use of encephalomyo-

carditis virus, a small ribonucleic acid virus, which can be propagated *in vitro* within Krebs II mouse ascites cells, may make it possible to demonstrate net synthesis of protein. Viral protein can easily be purified and is then available for further chemical study. This system may also allow the study of the effect of previously modified viral ribonucleic acid on protein synthesis. Preliminary experiments designed to examine the time course of events after infection of the host cell indicated that a marked inhibition of the turnover of ribonucleic acid, as measured by incorporation of 6-carbon-14 orotic acid, was followed by the appearance of most of the virus in the mitochondrial fraction.

The incorporation of amino-acids in the protein of whole microsomes requires the presence of the so-called *S*-ribonucleic acid and of certain enzymes from the cell sap. However, evidence has been produced to show that in some microsomal preparations the addition of these factors may be omitted whereas in others different enzymic extracts can replace these 'pH-5 enzymes' from the cell sap. M. Beljanski (Paris) showed that a soluble enzyme preparation purified from *Alcaligenes faecalis* stimulated the incorporation of amino-acids into the protein of a particulate fraction from the same microorganism. The same enzyme preparation was capable of replacing *S*-ribonucleic acid and 'pH-5 enzymes' in a system suitable for the incorporation of amino-acids into microsomal protein from rat liver. This soluble enzyme preparation did not contain those activating enzymes which catalysed the exchange of adenosine triphosphate with pyrophosphate. However, a transfer in the presence of this enzyme preparation of orthophosphate from nucleoside triphosphate to the homologous diphosphate could be observed for the nucleotides of adenine, guanine, cytosine and uracil. If amino-acids were added to this system the formation of peptides was detected. Although the link is not clear between the presence of enzymes which bring about the above-mentioned transfer of orthophosphate to nucleoside diphosphates, and the ability of this enzyme preparation to stimulate the incorporation of amino-acids into proteins, it was of interest to have a report from J.-P. Zalta (Paris) that he could detect this transfer in certain microsomal fractions from rat liver. They were obtained by the action on whole microsomes of detergents of the polyoxyethylene and arylalkylsulphonate type. These fractions were observed to be capable of incorporating amino-acids into their protein without addition of any enzymic system from the cell sap.

The composition of ribonucleoprotein fractions from the microsomes of rat liver has been investigated by P. Cohn and J. A. V. Butler (London). Extraction with acid of these ribonucleoprotein preparations revealed the presence of basic proteins which, like histones, contained about 25 per cent of basic amino-acid residues but possessed a higher content of acidic residues than histones. Some observations on the process of incorporation of amino-acids into various protein fractions of microsomes indicated that, in contrast to results obtained on the living animal, incorporation under conditions *in vitro* apparently occurred to only a small extent in protein fractions which were not associated with ribonucleic acid in the endoplasmic reticulum. Such a connexion between extent and pattern of incorporation into microsomal fractions and the structural integrity of the mem-

branes of the reticulum, and the possible enzymic inhibition resulting from damage to their lipoproteins were suggested by the experiments of A. von der Decken and T. Hultin (Stockholm). The mushroom poison phalloidin was observed to inhibit, under certain conditions, the incorporation into whole microsomes, but not into a ribonucleoprotein preparation which was derived from microsomes by removal of much lipoprotein material with the aid of a detergent. P. Emmelot and E. L. Bennedetti (Amsterdam) were also able to report an inhibition of the ability of whole microsomes to incorporate amino-acids if the rats had previously been injected with dimethylnitrosamine.

Differences in the requirements for the incorporation of amino-acids into proteins of mitochondria and of microsomes were indicated by the paper of D. B. Roodyn (London). The addition of cell sap or of 'pH-5 enzymes' was found not to be essential, nor was the process of incorporation sensitive to ribonuclease. The distribution of radioactivity in the protein of various fractions obtained by differential centrifugation of mitochondria disrupted by treatment with a non-ionic detergent was reported. M. V. Simpson (New Haven) has employed mitochondria of both rat liver and calf heart to study the incorporation of amino-acids into cytochrome *c*. Mitochondria from calf heart which maintained their ability to incorporate for periods up to 12 hr. permitted the demonstration of a net increase in cytochrome *c* of about 20 per cent during this time. This synthesis required the supply of energy and the presence of amino-acids. It was also observed that amino-acids were incorporated into total protein of a supernatant fluid obtained from mitochondria subjected to ultrasonic treatment.

Much of the available evidence shows that ribonucleic acid is in some manner concerned with protein synthesis. R. W. Hendler (Bethesda) discussed the possibility that complexes between amino-acids and lipids might be involved in the transport of amino-acids and in protein synthesis. Extraction with organic solvents of the oviducts of laying hens, or of the cell wall and ribosomes of *E. coli*, revealed the presence of amino-acids which were bound to lipids in some way. A possible connexion between protein synthesis and the occurrence of amino-acid-lipid complexes was suggested by the pattern of their chromatographic distribution which was altered by processes which inhibit protein synthesis. The existence of such complexes in *Bacillus megaterium* was reported by G. D. Hunter (London), who suggested that their properties would be consistent with those of a precursor of labelled protein. Other studies gave evidence that the primary site of protein synthesis was present in material which sedimented at comparatively low speeds with the cell membrane, after osmotic lysis of protoplasts. A different approach to the problem of the mechanism of protein synthesis in micro-organisms was reported by B. Nisman and H. Fukuhara (Garches, France), who examined the ability of cell fractions of *E. coli* to incorporate amino-acids. Protoplasts were lysed by treatment with digitonin and then subjected to differential centrifugation. Of the two particulate fractions produced, one contained little and the other no enzymes which catalysed the exchange between adenosine triphosphate and pyrophosphate. Both fractions, however, were capable of readily incorporating amino-acids into protein.

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