tissues (approx. $23\text{ }\mu\text{mol}/100\text{mg}$ dry wt.) are similar whether or not alkaloid is being produced.

The appearance of the first traces of purple pigment in superficial layers of the parasitic mycelium coincides with a sharp increase in the total lipid content of sphaelial cells from approx. 10% of the dry weight to approx. 30%. This increase represents the accumulation within new sclerotial cells of a complex triglyceride oil in which the principal fatty acid is ricinoleate. This substance is absent from the oil of sphaelial cells. Thus the appearance of ricinoleate is the first metabolic indicator of the morphogenesis of sclerotial cells. When alkaloid is synthesized it can be detected within 5 days of the differentiation of parasitic sclerotial cells under field conditions, and in the present studies reached 0.4% of the dry weight. Mycelium in fermentation may synthesize up to ten times this amount, but most of the product is found in the broth.

The differentiation of sphaelial hyphae into sclerotial-like cells during fermentation is an essential prerequisite for alkaloid biosynthesis, although, as in the parasitic state, this tissue does not always synthesize alkaloids. This morphogenesis is similarly accompanied by an accumulation (up to 40% of the dry weight) of triglyceride oil rich in ricinoleate, but, in contrast with parasitic tissue, the purple pigment is absent.

C. purpurea, both in the parasitic and in the saprophytic state, readily utilizes amino and ammonium nitrogen, and shows a protein content of sphaelial tissue (approx. 20% by weight) that falls to approx. 12% as developing sclerotial cells become filled with lipid reserves. Thus the proportion of amino acid metabolism that may also be involved in alkaloid biosynthesis is comparatively small. Changes in the concentrations of individual free amino acids throughout parasitic development on rye have been followed in two successive years. Fluctuations of both total amino acids and the relative proportions of individual components were observed, and these were compared with similar measurements during fermentations. Although specific incorporation of radioactive amino acids (alanine, phenylalanine, proline and tryptophan) into ergotamine has been demonstrated, no valid conclusions could be drawn about a consistent relationship between observed changes in amino acid concentrations and the accumulation of ergot alkaloids. However, this study has centred attention on certain amino acids that would merit investigation with respect to specific metabolic turnover. Aspects of the control of alkaloid biosynthesis, particularly involving the concentrations of tryptophan and its derivatives, may best be investigated in axenic culture, but parasitic growth is better suited to studies on the metabolic control of the change from sphaelial growth to sclerotial growth.

The most notable and consistent feature of amino acid analyses during parasitic development is the occurrence of an unidentified substance, the structure of which is being investigated. This occurs only as a minor component before alkaloid is detectable, but accumulates progressively, coincident with alkaloid synthesis, to become the principal free amino acid. This is only a feature of those strains of *C. purpurea* that produce ergoline alkaloids in their sclerotia. Accumulation of the unidentified amino acid in tissues is not observed in fermentations, but this may be due to the large volume of fluid in which the mycelium is suspended.

Viruses of *Penicillium* and *Aspergillus* Species

By D. J. BORDER, K. W. BUCK, E. B. CHAIN, G. F. KEMPSON-JONES, P. LHOAS and G. RATTI (*Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AY, U.K.*)

The discovery of viruses in several species of *Penicillium* was due, not to any effect on host morphology or growth rate, but to the antiviral activity of extracts of the infected moulds. Lampson *et al.* (1967) postulated that double-stranded RNA, an interferon inducer isolated from *Penicillium funiculosum*, may have arisen from a virus infection. Ellis & Kleinschmidt (1967) found that the antiviral activity of statolon, a fermentation product of *Penicillium stoloniferum*, was associated with polyhedral virus-like particles. Banks *et al.* (1968) isolated virus particles from *P. stoloniferum*, showed that the viral double-stranded RNA induced the formation of interferon in mice and also isolated morphologically similar particles from *P. funiculosum*. Later virus particles were also isolated from *Penicillium cyaneo-fulvum* (Banks *et al.*, 1969b), *Penicillium chrysogenum* (Banks *et al.*, 1969a; Buck *et al.*, 1971) and *Penicillium brevi-compactum* (Wood *et al.*, 1971). Borré *et al.* (1971) have found virus particles associated with lytic plaques in colonies of *Penicillium citrinum* and *Penicillium variable* grown in media of high sugar content.

Following the observation that a high content of cell-wall galactosamine seemed to be associated with the presence of virus particles in *P. stoloniferum* (Buck *et al.*, 1969), a number of fungi reported to contain cell-wall galactosamine were screened with the result that virus particles were found in *Aspergillus niger* and in a closely related species, *Aspergillus foetidus* (Banks *et al.*, 1970). A procedure for the isolation of viruses from *Penicillium* and *Aspergillus* species on a pilot-plant scale has been described (Banks *et al.*, 1971).

Examination of negatively stained preparations of all the known viruses of *Penicillium* and *Aspergillus* species by electron microscopy has shown poly-

hedral particles with diameters of 25–50 nm. Serological relationships were established between the viruses of *P. chrysogenum* and *P. brevi-compactum* (Wood *et al.*, 1971), and between the viruses of *A. niger* and *A. foetidus* (Banks *et al.*, 1970), but all the other viruses were serologically unrelated. The virus populations from *P. stoloniferum* and *A. foetidus* both contained two serologically distinct groups of particles (Buck & Kempson-Jones, 1970; Ratti & Buck, 1972); these are designated PsV-S and PsV-F, and AfV-S and AfV-F, respectively.

RNA extracted from the viruses of *Penicillium* and *Aspergillus* species so far examined has proved to be double-stranded and to contain multiple components. The molecular weights of the virus RNA components were determined by gel electrophoresis with reovirus RNA as standard and are as follows: *P. chrysogenum*, 2.17×10^6 , 2.00×10^6 and 1.87×10^6 ; *P. cyaneo-fulvum*, 2.23×10^6 , 2.06×10^6 , 1.96×10^6 and 1.86×10^6 ; *P. stoloniferum*, PsV-S, 1.11×10^6 and 0.95×10^6 , PsV-F, 1.05×10^6 , 0.88×10^6 and 0.21×10^6 ; *Asp. foetidus*, AfV-S, 2.76×10^6 and 2.24×10^6 , AfV-F, 2.31×10^6 , 1.87×10^6 , 1.70×10^6 and 1.44×10^6 . Bozarth *et al.* (1971) found similar values for the *P. stoloniferum* viral RNA components and Wood *et al.* (1971) found values of 2.18×10^6 , 1.99×10^6 and 1.89×10^6 for *P. brevi-compactum* virus RNA, closely similar to those of the RNA components from the serologically related *P. chrysogenum* virus. With the *A. foetidus* virus particles it has been shown that the multiple RNA components are all separately encapsidated in different particles (Ratti & Buck, 1972); preliminary results suggest that the same may be true for some of the *Penicillium* viruses. In contrast, the genome of reovirus and probably those of the other double-stranded RNA viruses of animals and higher plants, which are also characterized by multiple RNA components (Fuji-Kawata *et al.*, 1970), are contained in single virions (Shatkin *et al.*, 1968).

Lhoas (1971a) has shown that both PsV-S and PsV-F could be transmitted to virus-free strains of *P. stoloniferum* through heterokaryosis with the naturally infected strain (A.T.C.C. 14586) as the virus donor. The concentration of virus obtained after infection was only 10% of that obtained from the donor strain, and may be characteristic of the new host. A similar technique was successful in infecting virus-free strains of *A. niger* (Lhoas, 1970). Lhoas (1971b) has since shown that protoplasts from virus-free strains of *P. stoloniferum* could be infected with extracellular virus. After treatment of protoplasts with a virus preparation from strain A.T.C.C. 14586 only infection with PsV-S could be detected. In all the cases studied the originally virus-free strains showed the same growth rate and morphological growth pattern before and after infection. Recently infection of mating pairs of *Saccharomyces cerevisiae* has been achieved with viruses from *P.*

stoloniferum and *R. niger* (Lhoas, 1972), showing that these viruses are not host specific.

The distribution and ultrastructural appearance of virus particles within the mycelium of infected strains of *P. stoloniferum*, *P. chrysogenum* and *P. funiculosum*, fixed in a number of ways, have been examined by thin-section electron microscopy. Individual hyphae in the colonies have then been selected and sectioned throughout their length from their apices to the centre of the colonies.

Viruses were absent from the apical 50–100 μ m of the hyphae, but behind this region spherical structures 35–45 nm in diameter were found, slightly larger than the corresponding particles that appeared after isolation and negative staining. These particles occurred in aggregates of up to several hundred in the cytoplasm. Further back along the hyphae these virus aggregates were bound by a single membrane. In still older regions a lumen developed between the particles and the surrounding membrane, and small vacuoles were produced containing virus particles that were either randomly arranged or in regular crystalline arrays. These vacuoles fused until one large vacuole occupied almost the entire volume of the cell. The plasmalemma in autolytic cells near the centre of the colonies became detached from the cell wall and formed a membranous envelope surrounding aggregates of the viruses. The vacuolar membranes, which previously enveloped the particles, were apparently broken down. Work is in progress on the localization of sites of synthesis of viral RNA and protein by using electron-microscope radioautography.

- Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tyler, J. M., Hollings, M., Last, F. T. & Stone, O. M. (1968) *Nature (London)* **218**, 542
 Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E. & Himmelweit, F. (1969a) *Nature (London)* **222**, 89
 Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E. & Himmelweit, F. (1969b) *Nature (London)* **223**, 155
 Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., Himmelweit, F., Ratti, G., Sharpe, T. J. & Planterose, D. N. (1970) *Nature (London)* **227**, 505
 Banks, G. T., Buck, K. W., & Fleming, A. (1971) *Chem. Eng. (London)* **251**, 259
 Borré, E., Morgantini, L. E., Ortali, V. & Tonolo, A. (1971) *Nature (London)* **229**, 568
 Bozarth, R. F., Wood, H. A. & Mandelbrot, A. (1971) *Virology* **45**, 516
 Buck, K. W. & Kempson-Jones, G. F. (1970) *Nature (London)* **225**, 945
 Buck, K. W., Chain, E. B. & Darbyshire, J. E. (1969) *Nature (London)* **223**, 1273
 Buck, K. W., Chain, E. B. & Himmelweit, F. (1971) *J. Gen. Virol.* **12**, 131
 Ellis, L. F. & Kleinschmidt, W. J. (1967) *Nature (London)* **204**, 553
 Fuji-Kawata, I., Miura, K. & Fuke, M. (1970) *J. Mol. Biol.* **51**, 247

- Lampson, G. P., Tytell, A. A., Field, A. K., Nemes, M. M. & Hilleman, M. R. (1967) *Proc. Nat. Acad. Sci. U.S.A.* **52**, 741
 Lhoas, P. (1970) *Aspergillus News Lett.* **11**, 8
 Lhoas, P. (1971a) *Nature (London)* **230**, 248
 Lhoas, P. (1971b) *J. Gen. Virol.* **13**, 365

- Lhoas, P. (1972) *Nature (London)* in the press
 Ratti, G. & Buck, K. W. (1971) *J. Gen. Virol.* in the press
 Shatkin, A. J., Sipe, J. D. & Loh, P. (1968) *J. Virol.* **2**, 986
 Wood, H. A., Bozarth, R. F. & Mislivec, P. B. (1971) *Virology* **44**, 592

NEUROCHEMICAL GROUP COLLOQUIUM ON 'PROTEIN SYNTHESIS IN THE CENTRAL NERVOUS SYSTEM'

Mechanisms of Protein Synthesis in the Central Nervous System

By SIDNEY ROBERTS (*Department of Biological Chemistry, University of California at Los Angeles School of Medicine, Los Angeles, Calif. 90024, U.S.A.*)

The functional significance of protein-synthesizing systems localized in different cellular and subcellular regions of the central nervous system will be outlined. Physical, chemical and metabolic properties of cytoplasmic ribosomal systems of cerebral origin will be described, with emphasis on characteristics that appear to be unique compared with those of corresponding preparations from other mammalian tissues. These include mechanisms that regulate gene expression, turnover of mRNA, RNA components of polyribosomes, stability of mRNA-ribosome complexes and ribosomal state *in vivo*. Alterations that occur in these properties during cerebral maturation will be indicated. The relationship of these phenomena to specialized function in the central nervous system will be discussed.

Membrane-Bound and Free Ribosomes in Brain and other Tissues

By T. M. ANDREWS and J. R. TATA (*National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.*)

Membrane-bound and free ribosomes can be isolated from rat cerebral cortex, as from many other tissues. A rapid age-dependent decline in protein-synthetic activity in rat brain during the first 3 weeks of life is more markedly reflected in the membrane-bound fraction than in free ribosomes. This appears to involve some functional alteration in the ribosomes themselves rather than any change in soluble factors or endogenous mRNA content.

The role of membrane-bound ribosomes in brain will be discussed in relation to the known importance of ribosome-membrane attachment for secretion of newly synthesized protein in liver and other secretory tissues.

Polyamines and the Regulation of Protein Synthesis in Brain

By P. P. GIORGI (*Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE, U.K.*)

Analytical and experimental studies *in vivo* and *in vitro* show that spermine and spermidine are structural components of brain ribosomes and contribute to enhance their stability, aggregation and protein-synthetic activity.

Developmental changes of brain polyamines are well correlated with the corresponding changes in protein-synthetic activity, and both are typical of the brain.

About 50% of brain polyamines are associated with the microsomal fraction, and their half-life is similar to that of rRNA.

Some characteristics of the enzymes responsible for polyamine biosynthesis and their relationship to trophic hormones are such that changes in the concentration of brain polyamines could represent an effective mechanism for the regulation of protein synthesis in this organ.

Protein Synthesis by the Mitochondria of Neurons

By A. HERNANDEZ and T. S. WORK (*National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.*)

Brain-cortex cells are readily homogenized by standard techniques. Mitochondria of these cells purified by application of standard techniques of differential sedimentation in sucrose synthesize protein by two routes, one inhibited by cycloheximide and one inhibited by chloramphenicol. The cycloheximide-sensitive synthesis is due to 80S ribosomes enclosed in semipermeable vesicles, and the chloramphenicol-sensitive synthesis proceeds on the 55S mitoribosomes. The brain mitoribosomes synthesize ten or more proteins, and these are indistinguishable, in rate of migration on polyacrylamide, from the protein synthesized by isolated liver mitochondria.