there was an initial stimulation in the uptake of ^3H leucine into the hippocampal region. Hydén described how, as training proceeds, this enhanced uptake shifts to the cerebral cortex, while labelling in the hippocampus returns to normal. His study of S13, S14 and S100 proteins has also shown that the S100 protein which, before learning, is isolated as one band on electrophoresis, is recovered as two bands after a learning experience.

Dr D. A. Booth (University of Sussex) discussed the effects of cyclohexamide, an inhibitor of protein synthesis, on memory consolidation. He argued that it was still open to question whether the disruption of long term memory by cyclohexamide was the result of the blockading of the consolidation process or was the result of negative reinforcement arising from aversive characteristics of the drug. Dr J. T. Rick (University of Birmingham) pointed out that the majority of studies concerned with biochemical correlates of learning make use of aversive conditioning stimuli in order to obtain rapid overlearning on the part of the animal. Stimulation, such as electric shock, is known to have significant effects on brain chemistry independent of any learning process. He argued that studies based on the extinction rather than the acquisition of a learned response, when such stimulation is absent, might overcome this difficulty.

The simpler nervous systems of invertebrates have been well exploited in this area of research as exemplified by the work described by Professor G. A. Kerkut and Dr G. W. O. Oliver (University of Southampton). Using the common garden snail (*Helix aspersa*) and the cockroach (*Periplaneta americana*) these workers described how, during learning, acetylcholinesterase activity in the nervous system increases in the former where the transmitter acetylcholine is chiefly inhibitory and decreases in the latter where it is only excitatory. Professor Kerkut went on to argue that one explanation of these data is that changes in interneuronal activity that must accompany learning may be affected by conformational changes in the enzyme rather than by differential rates of its turnover. The state of the enzyme, in other words, would act like a switch increasing or decreasing synaptic security.

The second session was concerned with modifications of brain chemistry in relation to behaviour. Dr J. Dobbing (University of Manchester) reviewed some of his studies on the effects of various early environmental conditions on future brain development and the extent to which such effects depend on just when these conditions occur relative to the normal time scale of maturation for the species. Dr G. Ungar (Texas Medical Center, Houston) dealt with the possibility of "learning" as a transferable phenomenon. He described the amino-acid sequence of a polypeptide extracted from the brain of a trained animal which, after injection, specifically aided the learning of a naive animal. Professor P. Mandel (Centre de Neurochimie, Strasbourg) was critical of such possibilities, and took the idea to the logical conclusion as he perceived it; that is, because the synthesis of RNA is only possible where the complementary DNA is already established, every unit of acquired information must already have a receptor-site in the brain.

REVERSE TRANSCRIPTASE

Transforms Initiated

from our Cell Biology Correspondent

THE extent to which research groups are falling over each other in the headling rush to get into the reverse transcriptase scene is reaching ludicrous proportions. In the circumstances, repetitious research is inevitable, but repetitious publication is not. Isn't it time for some sort of moratorium? For example, it might be agreed that after a first token publication—the claim to a stake—further publications should await the collection of more than the odd datum. But, of course, there is

Macromolecular Stereopathology

AN article by Greer and Perutz in next Wednesday's *New Biology* may suggest to the imaginative mind the birth of a new branch of clinical science—diagnosis by crystallography. The number of recognized human haemoglobin variants now runs well into three figures. Most are only structurally, and not functionally, distinguishable from the normal molecule, and do not give rise to clinical symptoms. Others do their possessors no good at all: the most famous, sickle-cell haemoglobin, has an anomalous tendency to aggregate in the deoxygenated state, and comes out of solution with disastrous consequences for the red cell. Some others are aberrant in their oxygen equilibria: one, Hb-H, is generated by an imbalance in the concentrations of α- and β-chains, and is a tetramer made up of four normal β-chains, which are incapable of generating haem–haem interactions, and the contingent sigmoidal oxygen uptake curves. In a further set of variants an amino-acid substitution in a position intimately related to the binding of the haem to protein leads to other types of functional failure, in some cases (the haemoglobin-M variants) the complete inability of the associated haem to bind oxygen.

The species that Greer and Perutz have now examined is haemoglobin Rainier, which is a β-chain mutant with its substitution at the important, C-terminal end, where there is strong sequence conservation between all vertebrate haemoglobins. The conversion of tyrosine-145 to cysteine brings with it a substantially decreased haem–haem affinity and Bohr effect, and an increase in oxygen affinity. The characteristic disorientation of the crystal lattice of the oxyhaemoglobin on deoxygenation was observed in haemoglobin Rainier crystals, which indicates that its abnormality does not arise from any inability of the quaternary structure to change from the oxy-pattern to the deoxy-pattern. A comparison of deoxygenated haemoglobin Rainier with the normal adult protein showed a clean difference Fourier map, except in the region of the β-chain C-termini. The missing tyrosine is apparent as a negative peak of electron density, and the cysteine sulphur shows up as a strong positive feature. Moreover, the normal cysteine-93, which normally resides in the F-helix, on the other side from the haem-liganded histidine, is rotated about the α-β carbon bond, so as to move the sulphydryl towards the pocket that normally contains tyrosine-145. The alignment of the normal with the abnormal cysteine side chains signifies the formation of a disulphide bond between them. This event causes a local conformational convulsion, in which the α-carboxyl group and the imidazole of the terminal histidine-146 are displaced, so as to break the ion-pairs in which they are normally involved, and the residue is drawn into the α-helix, in which it is found in myoglobin. Perutz and his co-workers previously demonstrated that one of the broken ion pairs is responsible under normal conditions for the alkaline Bohr effect.

Neither can the normal conformational change on deoxygenation described by Perutz, which involves the displacement of the tyr-145 side chain from its pocket, take its course. Moreover, model building suggests that in haemoglobin Rainier the α-carboxyl group can form an adventitious ion-pair with the α-amino group of the partner β-chain to provide a further source of stabilization of the oxy-form. The disulphide bond also presumably stabilizes the β-chain conformation, which may be the cause of the anomalous stability of haemoglobin Rainier towards alkaline denaturation. A "molecular disease" then, specified beyond Pauling's most optimistic predictions of more than twenty years ago.

always one thing to be said for a morass of mediocrity; it throws into sharpest relief anything which rises above it. The experiments reported by the Hanafusas in a short communication to *Virology* (**43**, 313; 1971) are very much a case in point. Instead of the minor variations on the themes of templates, inhibitors and distributions of reverse transcriptases these workers, by exploiting a variant Rous sarcoma virus (RSV(O)) they isolated in 1968, have provided the only direct evidence that this enzyme is required for the establishment of transformation of chick cells by RSV. And into the bargain their results suggest that the reverse transcriptase in RSV particles is not a preexisting host enzyme which is incorporated into virions as they mature.

The variant virus was isolated from chick cells transformed by the Bryan strain of RSV. Of the cells transformed by this virus 90 per cent liberated infectious progeny sarcoma virus, designated RSVβ(O), but 10 per cent of the transformants yielded physically normal progeny, designated RSVα(O), which are noninfectious and cannot transform susceptible cells. That this lack of infectivity is not simply the result of the failure of RSVα(O) particles to penetrate cells was shown by fusing the virus into its hosts with inactivated Sendai virus; the cells were not transformed. But after the 10 per cent of transformants which yield RSVα(O) are superinfected with an avian leucosis virus, about three-quarters of the cells start yielding pseudotypes, RSVα genomes enclosed in envelopes containing leucosis virus antigens (RSVα(ALV)), which are infectious and transform. But the progeny viruses produced by a cell transformed by an RSVα(ALV) pseudotype again are only noninfectious RSVα(O) particles.

Why are RSVα(O) particles noninfectious and unable to transform? One obvious possibility is that they lack reverse transcriptase. If this were the case, and if the virus could not use any putative reverse transcriptase indigenous to its host, double-stranded DNA proviruses of RSVα genomes would never be made, and the virus would not be infectious. Sure enough, when the Hanafusas assayed RSVα(O) for reverse transcriptase activity they failed to detect it. By contrast, the infectious pseudotypes, RSVα(ALV), possesses this activity. The obvious conclusion is that RSVα(O) is noninfectious because it lacks this enzyme and cannot make a provirus. Either the RSVα(O) particles contain a functional enzyme but also a potent inhibitor of its action or they carry a defective enzyme presumably because the gene which specifies it is mutated. It is at present difficult to distinguish between these two explanations. The observation, that when RSVα(O) particles are mixed with avian leucosis virions and then assayed, the detectable activity is less than that with the same amount of leucosis virus alone, is compatible with both alternatives. For defective enzyme, as the Hanafusas point out, might competitively inhibit an active enzyme.

The finding that the RSVα(ALV) pseudotypes possess transcriptase activity and are infectious suggests that these particles pick up active enzyme specified by the superinfecting leucosis virus. And the observation that cells transformed by RSVα(ALV) pseudotypes are indistinguishable from cells transformed by infectious RSVβ(O) indicates that reverse transcriptase plays a part in the establishment but not in the maintenance of transformation. Finally, a cell superinfected by leucosis virus produces in addition to pseudotypes, RSVα(ALV), some straight noninfectious RSVα(O). The former virus has transcriptase activity, the latter does not. This suggests perhaps that the reverse transcriptase in these viruses is not a pre-existing cellular enzyme.

SODIUM CHLORIDE

More Control Systems

from our Microbiology Correspondent

It has been shown recently that additions of sodium chloride to growing bacterial cultures cause major changes in the free amino-acid pools and especially in the concentrations of glutamate (Tempest, Meers and Brown, *J. Gen. Microbiol.*, **64**, 171; 1970). The free amino-acid pools of a range of Gram positive and Gram negative bacteria were significantly different when the organisms were grown on identical media and when all other environmental parameters were constant. The predominant amino-acid, glutamic acid, accounted for 52 to 89 per cent of the free pools in ammonia-limited cultures. Increasing the growth rate seven-fold induced a predictable rise in the concentration of free amino-acids from 5.5 to 14.7 mM, but these changes were small compared with those resulting from the additions of salt to the cultures. For example, 4 per cent NaCl caused a nine-fold increase in the free amino-acid pool

Nature of the M87 X-Ray Source

Although the orbiting Explorer 42 satellite is beginning to oust rocket and balloon borne X-ray experiments from their position of prime importance, the group of astronomers at the University of Leicester, who have been among the most successful X-ray rocketeers, are still producing valuable data, and with the aid of a colleague from the Institute of Theoretical Astronomy at Cambridge are carrying out the analysis without which observations are of very little significance. Their latest report, in the forthcoming issue of *Nature Physical Science*, concerns another flight by the apparently infallible Skylark rocket which has done so much to open up this new field of high energy astrophysics.

A. F. James, K. A. Pounds, M. J. Rickets and M. J. Rees have studied the data obtained from a flight carried out primarily to study the isotropy of the diffused background of X-radiation which seems to permeate space but, as the rocket scanned the X-ray sky, one of its detectors viewed M87 and found an X-ray flux at the 5σ significance level in the position of this source. The other detector, for which the line of sight passed rather below M87, did not detect any corresponding peak in the X-ray spectrum, and this ties the position of the source to that of the external galaxy fairly accurately. The evidence shows, however, that both the intensity and slope of the X-ray spectrum of this object has changed over the two years since it was first determined by several groups in 1968.

The variation of celestial objects over short periods of time is an important tool in determining their sizes. A source can only vary coherently in a time less than that taken for light to cross its shortest width—otherwise, variations would occur out of step and cancel each other out. This test is most familiar from its application to quasars. In the case of M87 it seems that the central region of intense X-ray activity must be smaller than one light year. This could mean that the source is concentrated in the nucleus of M87, or it may be in the jet (some 10 light years long) being emitted from this giant elliptical galaxy.

Partly from the evidence of radio observations of the jet and galaxy, James *et al.* favour the alternative that the energy source is in the galaxy nucleus. It is unlikely (although not impossible) that this compact source is radiating by synchroton emission and so a choice must be made between an inverse Compton mechanism in which low energy photons are increased in frequency as they gain energy in a very active nucleus (for example, scattering of infrared photons by energetic electrons), and a thermal bremsstrahlung mechanism in which the gravitational energy of matter falling on to a very massive point mass or black hole is converted into heat and thus produces intense radiation at all frequencies.