operon ; these cells therefore lacked the enzyme β galactosidase and so could not hydrolyse lactose to galactose and glucose (the principal reaction of the enzyme). The loss of β galactosidase is the only deficiency in the capacity to metabolize lactose of these cells ; so the mutants should be able to regain the ability to utilize lactose by producing this single enzymatic activity.

The ability of cells to grow on lactose can be tested by plating them on Tet-lac medium, which contains the tetrazolium-lactose indicator. Cells unable to metabolize lactose form deep red colonies ; but cells which can utilize the sugar grow white in colour. Another colorimetric reaction can be used to follow the activity of β galactosidase in extracts of the cell ; the enzyme releases *o*-nitrophenol from the reagent *o*-nitrophenol-β-galactoside in a reaction which can be followed spectrophotometrically by its development of a yellow colour.

When placed on Tet-lac plates, the deletion strain of *Escherichia coli* at first formed only red colonies. But about one month later white colonies began to grow. The white colony which grew most rapidly, representing the cells with the most effective ability to hydrolyse lactose, was isolated and then transferred to a fresh plate containing minimal medium supplemented with the lactose. These *ebg-1* cells, so called for evolved β-galactosidase activity, then gave rise to several colonies, the fastest growing of which was isolated and replated as the *ebg-2* cell line. After successive cycles of selection, the *ebg-5* cells were obtained.

This cell line grows as rapidly as wild type cells on the lactose minimal agar and produces pink colonies on Tet-lac plates, compared with the red of *lac*$^-$ cells and the white of *lac*$^+$ cells. Extracts possess a lactose hydrolysing activity which releases *o*-nitrophenol more rapidly than extracts of *ebg-1*—a visual demonstration of evolutionary improvement—but more slowly than extracts of wild type z^+ cells which possess the enzyme β galactosidase of the lactose operon.

The enzyme responsible for the recovery of β-galactosidase activity in the *ebg-5* cells differs both physically and in the conditions for optimum activity from the enzyme specified by the z^+ gene of the lactose operon. The *ebg-5* extracts contain an enzyme which is even larger than β galactosidase (itself unusually large with a sedimentation coefficient of 16S and a molecular weight of 5.4×10^5), sedimenting at 24.4S with a molecular weight of about a million. The *ebg-5* enzyme is extremely sensitive to inhibition by ammonium ions, has a much sharper *p*H optimum than β galactosidase, and a K_m for *o*-nitrophenol-β-galactoside four times greater. Immunological tests confirm the conclusion that the two enzymes are completely unrelated.

That five cycles of selection were used to isolate *ebg-5* implies that several different mutations must have contributed to the evolutionary revival of ability to hydrolyse lactose. The *ebg-5* characteristic behaves as a single gene in genetic crosses, implying that all these mutations must be located close together, presumably in the amino acid sequence of a single protein. The *ebg-5* locus maps very distant from the lactose operon on the *E. coli* chromosome and does not respond to any of the controls of the lactose operon, such as lactose repressor protein or induction by β galactosides.

The only other gene mapping in this region is a membrane determinant of tolerance to colicin E1 and so the *ebg-5* mutant identifies a new genetic locus. The mutation —or series of mutations— creating the lactose hydrolysing activity must presumably be located in an enzyme coded by this gene which is in wild type cells no relation of β galactosidase. One particularly intriguing question concerns the nature of the ancestral *ebg* enzyme in the deletion cells. What is its catalytic activity and how is this altered when it gains the ability to hydrolyse lactose? And, of course, it cannot but be interesting to isolate the enzymes coded by the successive *ebg* colonies and to determine their molecular changes. It is a long standing tenet of evolutionary theory that changes in protein activities lead a cell to gain the abilities appropriate for a new environment ; but it is not often that such evolution can be followed through changes in one enzyme.

ELECTRON MICROSCOPY

Wide Applications

from a Correspondent

THE scanning electron microscope (SEM) is a relatively new addition to the range of instruments available to the physicist and biologist. Nevertheless it has established itself in a short time as a major research tool because of its adaptability, and also because of its inherent suitability for the time-resolved and dynamic *in situ* experimentation favoured for much current research. In this role, scanning microscopy is treated primarily as a means of obtaining quantitative data rather than as merely a source of interesting pictures. This theme was stressed by many speakers at a conference held at the University of Newcastle on July 3–5. Professor T. E. Everhart (University of California, Berkeley) demonstrated how the SEM could be used as a direct source of input data for a computer, and he also showed how this interaction between the computer and the microscope could lead to the direct production of numerical information about a variety of effects in solid state physics. The use of the SEM as a means of obtaining crystallographic data was also described by many speakers.

A variety of methods are now being used for this purpose including the electron channelling technique (by Dr D. C. Joy, University of Oxford), the Kossel pattern technique (by Dr D. J. Dingley, University of Bristol) and a new approach using information contained in the angular distribution of the backscattered electrons (by Dr J. Venables, University of Sussex). Techniques for the study of the specimen chemistry, based on the analysis of the fluorescent X-ray spectrum stimulated by the electron beam, were reviewed by Dr J. V. P. Long (University of Cambridge) and a glimpse of the future was provided in the paper by Dr I. R. M. Wardell (Vacuum Generators Ltd, East Grinstead) describing the production of high resolution surface chemistry data by means of the Auger emission stimulated by the incident beam.

A major area of interest at this meeting was the use of the SEM in a scanning transmission mode of operation. The advantages of the SEM used in this way, compared to the conventional transmission electron microscope, were comprehensively reviewed for both physical and biological applications in papers by Professor L. Reimer (University of Munich) and Dr J. S. Wall (University of Chicago). Microscopes specifically designed for this application were described by several groups of workers including Dr A. N. Broers (IBM) who showed results from an instrument using a Lalharum Hexaboride electron gun. Much attention was also focused on field emission guns as possible electron sources for this (and other) applications, and developments in this area were described by several workers including Dr R. J. Taylor (Cambridge Scientific Instruments Ltd) and Dr J. R. Banbury (AEI Ltd).

A full range of papers on straightforward applications of the SEM were also presented covering topics as diverse as studies of metal working procedures (by Dr S. Ramalingam, University of New York, Buffalo) and applications to forensic science (by Dr M. E. Taylor, West Midlands Forensic Science Laboratory). A very complete commercial exhibition allowed conference participants the chance to examine and compare the wide range of instruments and accessories now available. The conference was attended by more than 250 participants including many from Europe and the United States. Mr D. Kynaston (Cambridge Scientific Instruments Ltd) and Mr E. H. Boult (University of Newcastle), the organizers, are to be congratulated for arranging a stimulating and timely meeting.