effects of freshwaters. It is at this stage that much of a limestone's original porosity (about 50–60 per cent) is filled in with calcite. This process requires a large amount of calcium carbonate.

STEROIDS

How ACTH Works

from a Correspondent

A TIMELY and interesting symposium on the mode of action of adrenocorticotrophin (ACTH) was organized by the Steroid Group of the Biochemical Society at the University of Aberdeen on June 2. In the first contribution, Dr M. Golder and his colleagues (Tenovus Institute for Cancer Research, Cardiff) described their combined histochemical and biochemical studies on the adrenal cortex. Dr Golder reviewed the vascular system of this gland and correlated certain aspects of structure and function. The cell migration theory was contrasted with the zonation theory and the morphological effects of ACTH in this gland were shown to be associated with a decrease in the fasciculata cells; this suggests that these cells respond to ACTH.

Dr Golder and his colleagues have applied the ultramicro techniques of Glick and associates to study the distribution of 11β-hydroxylase activity in rat adrenal. By using iodinated ACTH, it was possible to ascertain the distribution of this hormone in various guinea-pig tissues. In this species the uptake of labelled ACTH appeared to be greater in the outer zones of the adrenal cortex. It was shown that ACTH can activate *in vitro* the adenyl cyclase activity of guinea-pig adrenal cortical sections and, by coupling these histochemical methods to precise statistical analyses, Dr Golder demonstrated that ACTH affects the adenyl cyclase system in the fasciculata and the reticularis without influencing the activity of adenyl cyclase in the glomerulosa.

Dr D. Schulster and his colleagues (University of Sussex) reviewed current views on the mode of action of ACTH in the adrenal gland; these ideas evolved largely from the studies of the late Dr L. D. Garren. Dr Schulster went on to describe his studies using isolated adrenal cells: decapsulated rat adrenals are treated with collagenase, filtered through nylon mesh, washed, centrifuged and incubated. By the application of a radioimmuno-assay for corticosterone it has been possible to use this adrenal cell preparation to study the mode of action of ACTH. Dr Schulster then went on to discuss the apparent anomaly that when the cells are stimulated by ACTH the corticosteroidogenesis can be maximal at a concentration of ACTH at which the intracellular cyclic AMP concentration is still markedly sub-maximal. The time lag of 0–3 minutes before steroidogenesis is switched on is not caused by a lag in cyclic AMP production because the rise in cyclic AMP concentration on exposure to ACTH is almost instantaneous. Dr Schulster considered that his experiments confirmed the idea that this "time lag" may be the consequence of the time required for translation of the messenger RNA for a specific protein.

Dr Schulster showed that there is a good correlation between the dose of cycloheximide required to give 50 per cent inhibition of steroidogenesis with the dose of cycloheximide required to inhibit protein synthesis in these isolated cells. He has found that at certain concentrations of cycloheximide, the time lag for steroidogenesis to commence doubled and the rate of steroidogenesis was halved. Dr Schulster suggested that the labile protein produced as a consequence of the ACTH stimulation may have a half-life of about 4 minutes.

Dr L. R. Johnson (University of Utah) discussed the effect of ACTH on the early sterol and steroidal events in the adrenal cortex—namely, the mitochondrial conversion of cholesterol to pregnenolone. He described the development in his laboratory of a radioimmuno-assay of pregnenolone and discussed the sensitivity and specificity of this radioimmuno-assay. He then reviewed the various phases of the electron transport system in adrenal mitochondria associated with the steroid hydroxylation reactions in the conversion of cholesterol to pregnenolone. It has been suggested that ACTH might facilitate the efflux of pregnenolone from mitochondria. Dr Johnson has found, however, that there seems to be no inhibition of secretion of pregnenolone by adrenal cortical mitochondria and that it seems unlikely that ACTH is involved in the mitochondrial pregnenolone efflux phase of steroidogenesis.

Dr E. R. Simpson (University of Edinburgh) reviewed the electron transport system in adrenal cortical mitochondria and discussed the lipid content of adrenals with special reference to the cholesterol ester content of these glands. He reviewed the evidence which implicates cholesterol esters as the precursors of the free cholesterol required in steroidogenesis. He has confirmed that cycloheximide administered to rats *in vivo* fails to block the ACTH-induced cholesterol esterase activity of the adrenal glands. He showed that in bovine adrenal cortex tissue the cholesterol esterase in the cell supernatant in the presence of a protein kinase and cyclic AMP together with ATP gave a three-fold increase in the specific cholesterol esterase activity. Dr Simpson then discussed the effect of ether anaesthesia as a means of increasing the plasma ACTH concentration in rats. Under ether anaesthesia, the protein kinase activity of rat adrenal cortical tissue was significantly increased. In rat adrenal mitochondria there is a biphasic response in pregnenolone formation and Dr Simpson went on to

Genetic Heterogeneity of Xeroderma Pigmentosum

XERODERMA pigmentosum is one of those fortunately rare genetic defect diseases which have captured the interest of human geneticists and provided them with useful experimental material. The chief symptom of the syndrome is an extreme hypersensitivity of the skin to ultraviolet radiation resulting apparently from an autosomal recessive mutation that impairs a cell's DNA repair machinery. Patients with the classic symptoms develop severe skin lesions, but a few suffer from the De Sanctis-Cacchione syndrome which includes neurological and mental deficiency. This heterogeneity of symptoms suggests, of course, that the genetic defects resulting in xeroderma pigmentosum might involve more than one gene; that this is almost certainly the case has now been established by Bootsma's group in Rotterdam.

Writing in next Wednesday's *Nature New Biology* (July 19), Weerd-Kastelein, Keijzer and Bootsma report measurements of DNA repair capacity of heterokaryons produced by fusing in pairs cultivated fibroplasts from men and women suffering from either classic xeroderma pigmentosum or the De Sanctis-Cacchione syndrome. Their data indicate that mixed heterokaryons obtained by fusing xeroderma pigmentosum cells with De Sanctis-Cacchione cells marked by sex chromosomes are better able to repair DNA damaged by radiation than are heterokaryons obtained by fusing pairs of xeroderma or De Sanctis cells. Furthermore, both the nuclei in the mixed heterokaryons incorporate ^{3}H-thymidine after irradiation.

The simplest and obvious interpretation of these findings is that two different genes each specifying different proteins are required for repair of radiation damaged DNA, and that in classic xeroderma pigmentosum one gene is inactivated and in the De Sanctis-Cacchione syndrome the other gene is inactivated. In the mixed heterokaryons, intergenic complementation would restore the repair function. By isolating and characterizing the properties of the repair enzyme it should be possible to confirm this.