TISSUE GROWTH

Chalones after 15 Years

from a Correspondent

TISSUE specific proliferation control in multicellular organisms is *sine qua non*, but the elucidation of such control mechanisms depends on a combination of satisfactory assay methods and analytical biochemical techniques of sufficient resolution. The concept of a negative feedback system was elaborated some 15 years ago by Weiss and Kavanau, and Bullough, who demonstrated mitotic inhibition in the epidermis by an epidermal extract, adopted the term "chalone" to denote such proliferation inhibiting feedback substances.

In the hope of clarifying both conceptual and methodological questions, the first international chalone conference was held at the Upjohn Company's Brook Lodge, Augusta, Michigan, on June 5–7, under the auspices of the National Cancer Institute and Office of Naval Research. The first day was devoted primarily to epidermal chalones, the second to the lymphocytic and granulocytic chalones and the third to miscellaneous (fibroblast, liver, lung, kidney, melanocyte) chalones. It was soon evident that although the chalone concept involves the physiological control of cell population sizes, the operational definitions vary (at present) from inhibition of the G_2 or G_1 or S phases of the cell cycle to inhibition of growth of cell lines and to depression of the cellular immune reaction.

Extracts from skin epidermis, for example, produce a G_2 inhibition as reported by Bullough, Lawrence, Iversen, and others. Dr E. Lawrence (University of London) explained that the previously reported potentiation of this effect by stress hormones does not seem to result from an interaction between chalone and, for example, adrenaline. These extracts contain, however, a G_1 inhibitor too, and Dr K. Elgjo (Rikshopitalet, Oslo) and Dr F. Marks (Deutches Krebsforschungzentrum, Heidelberg) reported that this does not seem to be identical with the G_2 inhibitor. Although the molecular sizes are both in the range of 10,000–30,000, the G_1 inhibitor seems to be heat stable, insensitive to proteolytic enzymes and metabolically more stable than the G_2 inhibitor.

The biological assay of lymphoid tissue extracts (such as spleen and lymph nodes) is more satisfactory. Dr J. C. Houck (Children's Hospital, Washington), for example, reported a "chalone" cencentrate which inhibits the onset of DNA synthesis in stimulated lymphocytes without, however, affecting their phenylalanine uptake. This means that it is not merely cytotoxic. The concentrate also inhibits growth of lymphoid cell lines *in vitro*, without affecting fibroblast growth. The inhibition of the immune system by purified and concentrated extracts has been demonstrated by Drs E. Garcia-Giralt and M. Kiger (Hôpital Paul-Brousse, Villejuif) using the GVH, graft rejection and MLC reactions and the formation of 19S cells. The lack of cytotoxicity was shown by inhibition of ^{3}H-thymidine uptake by such preparations, without affecting RNA or protein precursor uptake.

The granulocytic "chalone", reported on by Dr T. Rytomäa (University of Helsinki) and Dr W. R. Paukovits (University of Vienna), is about one-tenth the size of its lymphocytic counterpart. This small polypeptide inhibits DNA synthesis in the bone marrow cells and has some marked inhibitory effects on experimental myeloid leukaemia in rats. Its biological specificity is, however, not yet as clearly established as, for example, that of the lymphocytic "chalone". Indeed, cell group specificity rather than precise cell population specificity cannot be excluded until further purification of the respective tissue extracts.

A specific fibroblast growth inhibiting factor of molecular weight about 30–50,000 with similar chemical properties to the lymphocytic chalone was reported by Dr Houck, but this does not inhibit the PHA reaction. Apart from its broader aspects, the possibility of depressing fibroblast overgrowth in primary explants of epithelial tissue should make such a substance very attractive. A byproduct of this work was the finding of a serum factor which enables diploid fibroblast to grow in media which are otherwise serum free. Experimental evidence for a liver chalone was discussed by Dr M. P. Stack-Dunne (National Institute for Medical Research, London) and Professor W. G. Verly (University of Montreal). This inhibitory compound is unusual in that it is a very small molecule (about 1,000 daltons) and inhibits ^{3}H-thymidine and amino-acid uptake by liver slices but not, apparently, by kidney slices. Evidence for chalones was also reported in lung and kidney organ cultures by Dr. J. Simnett (Royal Victoria Infirmary, Newcastle) and Dr D. P. Chopra (Temple University, Philadelphia). The role of the flow rate of the blood in the regeneration of lungs was emphasized.

Apparently, even tumour cells do not escape some feedback control of growth—and again this may be specific. Dr P. Bichel (Cancer Research Institute, Aarhus) described a specific effect in a mixture of two different kinds of ascites tumour cells *in vivo*. The

Subunit Interactions in Haemoglobin

Two articles in next Wednesday's *Nature New Biology* (June 28) deal with structural and spectroscopic properties of an abnormal haemoglobin, and draw some inferences that relate to conformational equilibria in haemoglobin generally. The abnormal species is haemoglobin M Milwaukee-1, which has a mutation in the β-chain, leading to an abnormally high reduction potential, such that both β-chain haems are normally in the ferric state. The substitution occurs in the haem pocket, on the side normally occupied by ligands, and is of a glutamic acid for a valine.

The oxygenation properties of the normal α-chains are surprisingly different from those in the apparently comparable hybrid of normal ferrous α-chains and normal cyanmet-β-chains. Unlike the last species, the Milwaukee variant shows haem-haem interactions and a considerable Bohr effect. This is explained, as Perutz, Pulsinelli and Ranney now find, by the crystal structure, which is isomorphous with that of deoxyhaemoglobin, not, as in all other ferric species so far known, with oxyhaemoglobin. When the α-haems are oxygenated or oxidized, the structure becomes similar to normal met- (or oxy-) haemoglobin.

That this finding signifies a change in the β-chain from the deoxy- to the oxy-type conformation is confirmed by a change in absorption bands of the ferric haem, when the α-chains are oxygenated, in wavelength regions known to reflect the spin state, and hence the conformation of the derivatives. Thus, as with the oxygenation reaction, the conformational change in the abnormal ferric β-chains is linked to the movement of the iron atom out of the plane of the haem. In the methaemoglobin subunits the out-of-plane displacement is small, indicating that the conformational equilibrium is less strongly biased towards the oxyhaemoglobin-type state, and the molecule can exist in either form.

In an accompanying article, Lindstrom, Ho and Pisciotta have examined the characteristic hyperfine shifts in the NMR spectrum that arise from the haem groups. The magnitudes of the shifts in the α-chain haem spectrum are identical with those in normal deoxyhaemoglobin. Six β-chain resonances are observed, and when the α-chain iron atoms are liganded with carbon monoxide, one of these peaks undergoes a considerable shift, indicating that events at the α-haem affect steric features in the ferric β-haem, as the results of Perutz *et al.* demand.