Heberden Society

Clinical Meeting, Manchester, September 12, 1974

At a clinical meeting held in Manchester on September 12, 1974, the following papers were given.

Joint formation in culture. By K. T. RAJAN and H. J. MERKER (*MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, 11, Anatomisches Institut der Freien Universität Berlin, 1 Berlin 33*)

The object of the research was to shed light on the factors concerned in the formation of synovial joints, with specific reference to the possible role of movement.

The digits of human embryos (8–12 weeks) were maintained in organ culture (Trowell, 1954; Dingle, Fell and Lucy, 1966; Rajan and Hopkins, 1970) in the presence of synthetic medium BGJ (Biggers, Gwatkin, and Heuner, 1961) containing 15 % fetal calf serum (Flow Laboratories) and 150 µg/ml ascorbic acid (Reynolds, 1966).

In the young fetus, 10 weeks and under, the digits consist of a cartilagenous bar. After 10 days in culture the joint cavity appeared and this was in the absence of movement. There was evidence of material in the joint cavity, which was acid mucopolysaccharide in nature, with occasional cells showing pyknotic nuclei lining the cavity. Under the electron microscope there was single cell necrosis with evidence of loss of glycosaminoglycans, some areas showing aggregation of this material as seen with ruthenium red stained sections.

So far 76 explants have been examined and the evidence suggests that the following factors may be responsible in part, if not wholly, in the formation of joints: (a) Necrosis of cells in areas undergoing joint differentiation. (b) Alterations in glycosaminoglycans.

As the organ cultures are devoid of blood supply, nervous control, and movement, these factors may not be essential to the formation and differentiation of joint cavity.

References

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DINGLE, J. T., FELL, H. B., AND LUCY, J. A. (1966) *Biochem. J.*, **98**, 173 (Synthesis of connective-tissue components. The effect of retinol and hydrocortisone on cultured limb-bone rudiments)
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REYNOLDS, J. J. (1966) *Exp. Cell. Res.*, **42**, 178 (The effect of hydrocortisone on the growth of chick bone rudiments in chemically defined medium)
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The actions of some anti-inflammatory and antirheumatic drugs on hexosamine biosynthesis. By P. J. WINTERBURN and C. F. PHELPS (*Department of Biochemistry, University College, Cardiff and Department of Biochemistry, The Medical School, Bristol*)

Many compounds which possess anti-inflammatory or antirheumatic properties inhibit the production of hexosamine-containing polymers, e.g. mucopolysaccha-

rides and gastrointestinal tract mucins. The key regulatory enzyme of the hexosamine biosynthetic pathway is glucosamine 6-phosphate synthetase which catalyses the amination of fructose 6-phosphate and thus deflects substrates from glycolytic consumption and into polymer production. The synthetase is subject to feedback inhibition by UDP-N-acetylglucosamine and this is modulated by glucose 6-phosphate, AMP, and UTP (Winterburn and Phelps, 1971b). In the present studies the action of a wide variety of anti-inflammatory and antirheumatic agents on a semipurified synthetase preparation from rat liver (Winterburn and Phelps, 1971a) was investigated to ascertain the effects on the catalytic reaction and whether there was any interference with the natural control mechanisms.

The synthetase activity and the effects of UDP-N-acetylglucosamine, glucose 6-phosphate, AMP, and UTP were measured according to Winterburn and Phelps (1971c). The concentrations of the agents which gave 50% inhibition were: flufenamic acid (0.5 mmol/l), indomethacin (1.9 mmol/l), phenylbutazone (3.0 mmol/l), ibuprofen (6.5 mmol/l), and ibufenac (11.5 mmol/l). Salicylate was considerably less potent, yielding 18% inhibition at 20 mmol/l and acetylsalicylate was non-inhibitory. Ibuprofen, flufenamic acid, and phenylbutazone were noncompetitive inhibitors towards fructose 6-phosphate. Ibuprofen was also a noncompetitive inhibitor to the second substrate, glutamine, while the latter two agents tested exhibited a complex inhibition which closely resembled that created by 1,10-phenanthroline—a chelating agent specific for first transition series metal ions. Many of the agents tested possessed functional groupings spaced approximately 2.5 Å apart, the same separation as in 1,10-phenanthroline. It is proposed that these compounds may inhibit polymer synthesis by metal ion chelation.

Phenylbutazone (2.5 mmol/l) and ibuprofen (5 mmol/l) did not affect either the potency of the UDP-N-acetylglucosamine regulation or the activating influence of UTP. In contrast, the potentiation of the UDP-N-acetylglucosamine inhibition by glucose 6-phosphate or AMP was alleviated by these two antirheumatic agents. Thus phenylbutazone and ibuprofen exert a complex effect on the activity of this key enzyme in which the inhibitory action at the catalytic site is to a certain extent offset by a relief of the inhibition due to the natural regulators, glucose 6-phosphate and AMP.

References

- WINTERBURN, P. J., AND PHELPS, C. F. (1971a) *Biochem. J.*, **121**, 701 (Purification and some kinetic properties of rat liver glucosamine synthetase)
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—, —, (1971c) *Ibid.*, **121**, 721 (The binding on substrates and modifiers to glucosamine synthetase)