Heberden Society

Clinical Meeting, Oxford, July 14, 1972

At a clinical meeting, held at the Oxford Polytechnic and the Nuffield Orthopaedic Centre, the following papers were given.

Effect of Some Adrenal Steroid Hormones on Skin Fibroblast Replication *in vitro*. By W. HARVEY and R. GRAHAME (Guy's Hospital Medical School, London)

We have previously drawn attention to the fact that, in patients suffering from rheumatoid arthritis, the familiar steroid-induced skin-thinning effect which occurs in those given long-term oral corticosteroid medication is absent in those treated with ACTH⁻ (Grahame, 1969). This observation is compatible with the earlier observations of Savage, Copeman, Chapman, Wells, and Treadwell (1962) and West (1961) that 'steroid bruising' is rare in ACTH-treated rheumatoid arthritis patients. This study was undertaken to investigate this apparent disparity of effect between ACTH and oral corticosteroids.

It is well established that *in vitro* corticosteroids (including synthetic analogues) inhibit fibroblast growth and replication (Berliner, Bartley, Kenner, and Jee, 1970). It follows that the 'protective' effect of ACTH could be due to a direct effect of ACTH itself, or perhaps to the effect of androgenic steroids released by the adrenal cortex in response to ACTH stimulation, since it has been shown that the skin of hirsute women is thicker and contains more collagen (Shuster, Black, and Bottoms, 1970) and that their urine contains increased amounts of testosterone (Nabarro, personal communication).

Controlled experiments were carried out to determine if such an effect occurred *in vitro*.

Human fibroblasts (Biocult BCL D2) were grown in 5 cm. disposable tissue-culture Petri dishes in an atmosphere of 95 per cent air and 5 per cent CO₂, using Eagles' Minimal Essential Medium reinforced with 10 per cent foetal bovine serum. The effects of varying concentrations of some adrenal androgens both with and without a standard concentration of cortisol were studied in terms of the rate of cell division over a 5-day incubation period.

It was demonstrated that the inhibition of growth caused by 10 µg/ml. cortisol is reversed by physiological concentrations of many of the commercially-available androgenic steroids, including testosterone, dihydroxy-testosterone, androstenedione, and dehydroepiandrosterone sulphate.

No direct effect of ACTH could be demonstrated.

Discussion

DR. J. GLYN (London) Have you used any of the non-virilizing anabolic hormones such as methandienone or nandrolone? It may be that a good case could be made out for giving these routinely to patients receiving long-term corticosteroids. Conversely, have you tried any of the other anti-inflammatory steroids such as triamcinolone, which has a slightly different effect on collagen?

DR. HARVEY We have not tried any of the other anti-inflammatory steroids, but have taken cortisol as the standard. We shall try others in due course. We are just about to start experimenting with other anabolic and non-virilizing steroids and I agree that there does seem to be a case for giving these concurrently with anti-inflammatory steroids.

DR. A. WHITE (Horsham) I find these results extremely interesting, but one thing that disturbs me slightly is that you have to go to such a high level of cortisol in the medium to reduce the growth of the fibroblasts. I should have thought that the level of cortisol *in vivo* was some two orders of magnitude less—something around 10⁻⁷ molar in water content of the fibroblasts of the skin—and this raises the possibility that, while you obviously are antagonizing something you still may not *in vivo* antagonize the effects of cortisol, which is responsible for the thinning of the skin. I say this with some feeling because we have shown in rats that anabolic steroids and testosterone do reverse many of the biochemical changes induced by a number of anti-inflammatory catabolic steroids in skeletal muscle (Bullock, Christian, Peters, and White, 1971), but they still do not change the rate of weight loss in the experimental animal.

DR. HARVEY Although the cortisol level used was higher than the *in vivo* level, I cannot agree that the inhibition we antagonized is unrelated to the thinning of skin. Current experiments, furthermore, suggest that the rates of DNA and collagen synthesis are affected by cortisol at 0.1 µg/ml., allowing direct comparison with the *in vivo* situation.

DR. M. J. O. FRANCIS (Oxford) In the last slide you stated that the levels of collagen were soluble collagen levels. I wonder what proportion of collagen in the medium was soluble?

DR. HARVEY I do not have that information.

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Properties of Skin Polymeric Collagen in Patients with Rheumatoid Arthritis and Normal Controls. By M. J. O. FRANCIS, A. G. MOWAT, J. ELLIS and D. C. MACMILLAN (Nuffield Orthopaedic Centre and Radcliffe Infirmary, Oxford)

The clinical impression that the skin of some patients with rheumatoid arthritis is thin and/or transparent has been

confirmed (McConkey, Fraser, and Bligh, 1965). Furthermore, the skin of many patients is friable, resists shear stresses poorly, and may produce problems in wound healing. Previous studies on skin collagen in these patients have been confined to measurements of soluble or total content. However, although total collagen is reduced, particularly in patients receiving corticosteroids, measurements on the small soluble fraction (<5 per cent) have been inconclusive. The major fraction of skin collagen is polymeric collagen, and having developed methods of measuring quantities and stability of this collagen in biopsies (85 sq. mm.) of human skin, we have applied them to patients with rheumatoid arthritis (Francis and Macmillan, 1971).

So far 19 biopsies have been performed, including repeat biopsies in two patients after 6 months' penicillamine therapy. Compared with matched normal controls, a modest reduction in total skin collagen, particularly in patients on prolonged corticosteroid therapy has been confirmed. However, the stability of the polymeric collagen was not clearly altered by these drugs but was reduced in patients with active disease. With penicillamine, skin thickness and total collagen content were dramatically reduced. In addition, there was a reduction in collagen stability. In patients on corticosteroids there was an increased proportion of soluble collagen.

It will be suggested that important changes in collagen metabolism occur in these patients which may influence skin strength, integrity and wound healing (and perhaps renew interest in the 'collagen diseases').

Discussion

DR. B. MCCONKEY (Birmingham) There is a distinction which is in danger of being lost between two different kinds of skin abnormality seen in patients with rheumatoid disease and sometimes in the elderly. One is the abnormality in which you get senile or steroid purpura and fragile skin, and the other is what we have called 'transparent skin' (McConkey, Fraser, Bligh, and Whiteley, 1963). Although patients with one tend also to have the other, I think the conditions are quite distinct and ought to be considered separately in studies of this sort.

DR. R. GRAHAME (London) One of your earlier slides suggested that there was no difference in total skin collagen in normal subjects and in patients who have not had steroids. This is different to the results of Shuster, Raffle, and Bottoms (1967).

DR. FRANCIS No, they showed in fact, that total skin collagen in patients with rheumatoid arthritis who had not been on steroid therapy was lower, but not significantly so.

DR. T. C. HIGHTON (New Zealand) Some years ago (Highton, 1963) I did some relevant work using the granuloma pouch system in rats and measuring the amount and weights of granulation tissue produced following injection of serum from rheumatoid and normal subjects. I also measured the strength of a standard wound. The results indicated that serum derived from patients with active rheumatoid arthritis, when injected into rats, leads to their producing significantly less new tissue in the granuloma pouches, and these rats had wounds of less tensile strength than those injected with normal serum or saline.

DR. L. E. GLYNN (Taplow) Would it not be valid to measure the stability of collagen at different temperatures?

DR. FRANCIS It could be done on isolated samples of collagen fibre, but we have not attempted this.

References

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Post-operative Wound Healing in Patients with Rheumatoid Arthritis. By R. W. GARNER, A. G. MOWAT, and B. L. HAZLEMAN (Nuffield Orthopaedic Centre, Oxford)

It is often supposed that surgical wounds are more liable to become infected or to heal poorly in patients with rheumatoid arthritis compared with controls and that corticosteroid therapy is likely to exaggerate these tendencies. There is considerable indirect evidence to support these concepts and this will be presented.

Since orthopaedic surgical procedures are being increasingly used in the management of rheumatoid arthritis, it seemed valuable to compare postoperative wound healing in these patients and control subjects.

In a retrospective study covering a period of 20 months, 100 patients with rheumatoid arthritis undergoing a variety of orthopaedic operations were matched with others undergoing operation for conditions other than rheumatoid arthritis and other inflammatory joint diseases. Of the patients with rheumatoid arthritis, 49 were receiving corticosteroids. Details of wound infection, wound haematoma formation, and wound healing were obtained from the medical and nursing records.

There were 13 wound infections, all superficial, in the patients with rheumatoid arthritis and eight superficial infections in the control group ($P > 0.10$). Eleven of the 13 patients with rheumatoid arthritis were receiving corticosteroids ($P < 0.02$). Eight of 26 rheumatoid patients undergoing Macintosh knee arthroplasty had wound infections. There were seven wound haematomas in the rheumatoid patients and five in the control group ($P > 0.10$), although in three of the latter anticoagulant therapy was a major cause. Anticoagulants were not given to patients with rheumatoid arthritis. Altogether 31 wounds failed to heal by primary intention in the rheumatoid group and 16 in the control group ($P < 0.02$). There was no difference in the mean number of days ± 1 S.D. to complete wound healing between the rheumatoid patients (16.6 ± 7.5) and the controls (15.2 ± 7.9). There was no correlation between the days to healing and activity of arthritis, duration of disease, or positive serological test for rheumatoid factor. However, patients receiving corticosteroids for more than 3 years took longer to heal their wounds (20.3 ± 11.0 days) compared to those receiving these drugs for 3 years or less (15.2 ± 4.9 days; $P < 0.05$). There was no correlation between haemoglobin concentrations and wound healing in either patient group.

It will be suggested that, although there are several theoretical and a number of practical reasons inherent in a study of this type which can never be truly comparative, which would lead one to expect problems with wound healing in patients with arthritis, only minor differences