

Plasma Immunoreactive Melanotrophic Hormones in Patients on Maintenance Haemodialysis

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Summary

Circulating levels of melanotrophic hormones and ACTH were determined in patients treated by maintenance dialysis for chronic renal failure. Plasma melanotrophic hormone levels were greatly increased in all patients studied (125-1100 ng/l as compared with 12-36 ng/l in normal adults) and were correlated with the duration of treatment. Skin pigmentation, especially in exposed areas, was notably increased, particularly in those patients with the highest plasma melanotrophic concentrations.

Plasma ACTH levels were normal or only slightly raised and circulating corticosteroid concentrations, as determined by a fluorimetric method, all lay within the physiological range. The dissociation between ACTH and melanotrophic hormone levels in these patients may have been the result of a slower metabolic clearance of the latter.

Introduction

The cause of skin pigmentation associated with renal failure is unknown. It has been attributed to the deposition of lipid-soluble pigments (lipochromes and carotenoids) in the epidermis and subcutaneous adipose tissue (Tsaltas, 1969), but the evidence is inconclusive. Recent studies by light and electron microscopy (R. A. J. Eady and J. J. H. Gilkes, unpublished observations) have confirmed a reported (Thiers *et al.*, 1958) increase in melanin in clinically pigmented skin.

Increased circulating levels of ACTH and melanotrophic hormones have been found in disorders associated with definite pigmentation of the skin, notably Addison's disease and Nelson's syndrome (Abe *et al.*, 1969). Initially it was thought that human β -melanocyte stimulating hormone (β_h -MSH)* (Harris, 1959) caused this increased pigmentation. Recent evidence based on immunological, chromatographic, and stability studies (Scott and Lowry, 1974; Bloomfield and Scott, 1974; Bloomfield *et al.*, 1974) suggests, however, that the hormones responsible are the larger β -lipotrophin (β_h -LPH) (Li *et al.*, 1965; Cseh *et al.*, 1972) and human γ -lipotrophin (γ_h -LPH), both of

which contain an identical sequence to β_h -MSH within their structures. Until this controversy is resolved we shall apply the general term melanotrophic hormone(s). Abe *et al.* (1969) showed that circulating levels of ACTH and the melanotrophic hormone closely parallel each other in a variety of clinical conditions, which suggested that both were controlled by the same mechanism.

We measured plasma levels of immunoreactive melanotrophic hormones in patients with chronic renal failure treated by maintenance dialysis to determine the hormones' possible role in the abnormal pigmentation often found in this group. In doing so we also discovered a dissociation between the circulating levels of ACTH and those of the melanotrophic hormones.

Patients and Methods

Twenty-four patients (14 men and 10 women, aged 17-54 years) from the renal unit, Royal Free Hospital, London, gave informed consent for their inclusion in the study. Twenty-two patients had been treated by haemodialysis for periods of from three months to 11 years using flat-plate dialysers (Kiil; Gambro) and warm single-pass dialysate supply systems (24-30 hours per week) (Moorhead *et al.*, 1969). The remaining two patients had started intermittent peritoneal dialysis (100-120 litres of dialysate per week) three weeks previously. The cause of renal failure was glomerulonephritis in all but six patients, and all had glomerular filtration rates of less than 1 ml/min.

In 17 patients blood samples were taken before dialysis and after a 15-minute rest period, and in the remaining seven they were taken two hours after the start of dialysis. Two patients had samples taken both immediately before and after haemodialysis. Except on three occasions when they were collected at 9 p.m. samples were collected between 9 a.m. and 10.30 a.m. using plastic syringes containing heparin. They were then centrifuged, separated within 10 minutes, and plasma-frozen immediately in dry ice and stored at -20°C until assayed. Plasma for corticosteroid estimations was kept at 4°C .

Radioimmunoassays using an extraction procedure with Vycor glass (Corning Glass Works, New York) were used to measure plasma ACTH (20 patients) and melanotrophic hormone (24 patients). The ACTH assay has been described by Rees *et al.* (1971) and the specificity of the N-terminal antisera by Orth (1974). The melanotrophic hormone (β_h -MSH) assay used synthetic β_h -MSH for iodination and standardization, and the antisera cross-reacted fully with synthetic β_h -MSH and natural β_h - and γ_h -LPH (Gilkes *et al.*, 1975). Both determinations were done simultaneously on the same sample. Plasma corticosteroids (24 patients) were measured by a fluorimetric technique (Mattingly, 1962).

Skin Pigmentation.—The patients were asked if they thought their skin had changed in colour since starting on dialysis, and the degree of skin pigmentation was assessed clinically by two independent observers. Reflectance meters were not used.

Results

Plasma Melanotrophic Hormone.—Greatly raised plasma concentrations (125-1100 ng/l) well above our normal range (12-36 ng/l; Gilkes *et al.*, 1975) were found in all patients (fig. 1). The highest levels were in patients who had been on haemodialysis for the longest periods, and plasma concentration and duration of treatment correlated well ($r=0.85$; $P<0.001$; fig. 2). In the two patients studied before and after haemodialysis there was no significant difference in the plasma concentrations. The two patients on peritoneal dialysis had plasma levels of 280 and 475 ng/l.

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*The nomenclature of Li (1959) is used.

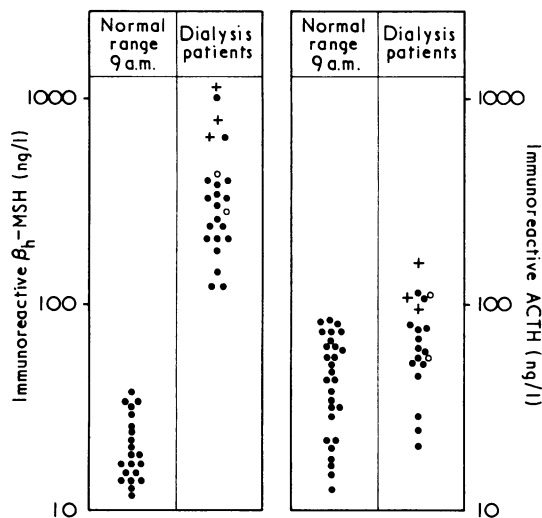


FIG. 1.—Plasma concentrations of immunoreactive β_h -MSH (melanotrophic hormone) and ACTH drawn on log scale in patients on dialysis compared to normal range at 9 a.m. + = 9.0 p.m. samples. O = Peritoneal dialysis patients.

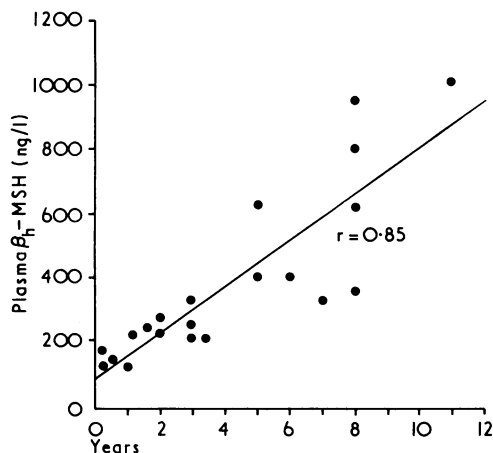


FIG. 2—Correlation between plasma β_h -MSH (melanotrophic hormone) and number of years on dialysis.

Plasma ACTH and Corticosteroids.—Fourteen patients had plasma ACTH concentrations within our normal range (15–85 ng/l) but six had slightly raised levels (93–165 ng/l). Though ACTH and melanotrophic hormone levels correlated well ($r=0.80$; $P<0.001$) the concentrations of ACTH were up to 20 times lower on a molar equivalent basis. Plasma fluorogenic corticosteroid levels were within the physiological range (6–26 mg/l).

Skin pigmentation was greatest in the four patients with the highest plasma concentrations of melanotrophic hormone, one of whom had vitiligo as well as hyperpigmentation. Two patients who had been treated by haemodialysis for over five years and had plasma levels above 350 ng/l, however, had pale sallow complexions. Three patients with concentrations below 250 ng/l had not noticed any change in skin colour, though another, with a value of 185 ng/l, was strikingly pigmented. Two patients with levels of 399 and 144 ng/l felt that their skin had become paler since starting haemodialysis. The pigmentation in those patients affected was largely limited to the sun-exposed areas. Several said that their skin tanned readily and that the tan would persist apparently unchanged or with little fading for up to a year.

Discussion

The raised plasma concentrations of immunoreactive melanotrophic hormone found in patients being treated with dialysis are within the same range as those found in Addison's disease

and Nelson's syndrome—both conditions associated with abnormal pigmentation. In other pathological and physiological conditions—for example, stress—in which raised plasma concentrations have been found there has been at least an equal increase in plasma ACTH (Abe *et al.*, 1969; Donald and Toth, 1973). All our patients had greatly raised immunoreactive melanotrophic activity without an equivalent increase in ACTH levels, and plasma fluorogenic corticosteroids were also all within the physiological range.

The dissociation between the circulating levels of the two pituitary peptides is difficult to explain. In-vitro studies have shown that endogenous melanotrophic hormone (Gilkes *et al.*, 1974) is more resistant to enzymatic breakdown than ACTH (Besser *et al.*, 1971) in fresh blood or plasma. This difference may be accentuated in patients on dialysis as values before and after dialysis were similar, indicating that haemodialysis does not seem to remove significant amounts of these peptides. The extra-renal metabolic clearance of the larger peptides (β_h - and γ_h -LPH) which are thought to constitute the immunoreactive melanotrophic hormone could be slower than that of the smaller molecule ACTH.

The progressive increase in the plasma melanotrophic hormone concentrations with length of treatment is also difficult to explain. Two patients on peritoneal dialysis had higher plasma concentrations than those who had recently started haemodialysis, and the paling of the skin noted by two other patients could be explained by an initial fall in plasma levels after starting treatment. We do not know whether patients with chronic renal failure have raised plasma levels of melanotrophic hormone before starting dialysis or whether these levels return to normal after transplantation. We intend to investigate further.

The slightly raised plasma immunoreactive ACTH concentrations in some of our patients is of interest because others (Varghese *et al.*, 1969; Asbach *et al.*, 1974) have shown a lack of the normal nyctohemeral rhythm of corticosteroids in dialysis patients. Such patients, however, do not develop the overt clinical features associated with Cushing's syndrome.

Our findings suggest that the abnormal pigmentation seen in patients on maintenance dialysis may be due to raised circulating levels of melanotrophic hormone. To have determined whether the immunoreactive hormone also had biological activity would have required large volumes of blood and would have been unethical as most patients were already anaemic.

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