Section of Endocrinology

President G I M Swyer DM

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Special Lecture

The Effect of Œstrogens, Androgens and Corticoids on Skeletal Kinetics in Man

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When œstrogens are given to women with postmenopausal osteoporosis, patients usually report relief of pain in approximately three weeks. Before that time, positive calcium and phosphate balances are achieved, urinary calcium excretion falls, but in most cases fæcal calcium excretion is not changed. Fractures no longer appear on long-term administration of œstrogens. In 220 women treated for 1,200 patient-years since 1948, biennial röntgen examination has disclosed no further vertebral fractures and semi-annual physical examination has shown no further loss of height, although fractures and loss of height were prominent features of the disease before treatment. Androgens produce similar effects on calcium and phosphate balance, but we have no data on long-term administration of these compounds to women because of their undesirable masculinizing qualities.

In attempting to ascertain the efficacy of various treatments proposed for osteoporosis, it is apparent that the subjective relief of pain is not a valid end-point since it can be obtained with inert placebos (Solomon *et al.* 1960). Balance studies are tedious, fraught with error, and difficult to perform in series of patients large enough for statistical analysis. Absence of fractures and maintenance of height take years and large numbers of patients to establish; otherwise, these criteria would be the most useful and convincing.

Tracer techniques make possible short-term evaluation of the ability of a compound to produce positive calcium balance. In addition, they demonstrate the mechanism by which positive balance is achieved: increased anabolism or decreased catabolism. Similarly, these kinetic methods make possible the prediction whether a corticoid will produce calcium loss, and presumably osteoporosis, as well as indicating the mechanism by which this loss is produced. While radioactive tracers have the advantage that they can be counted externally and analysed in blood and urine for very long periods of time, the use of non-radioactive tracers seemed more suitable for our purposes. These compounds can be administered repeatedly to large numbers of young, healthy persons without the hazards of radioactivity. It is therefore feasible to obtain statistically adequate data on normality, reproducibility, and the effect of various disease states, as well as to test the efficacy and mode of action of various steroids. For these purposes we have used stable strontium intravenously, following the lead of Fraser *et al.* (1960) but using the modifications and mathematical analysis of Eisenberg & Gordan (1961). Stable strontium is analysed flame spectrophotometrically at 461 mμ in a Beckman flame photometer with reversed acetylene and oxygen attachments to the sprayer burner; this is MacIntyre's (1957) method as modified by Loken, Teal & Eisenberg (1963). Serum and urine calcium and strontium levels are determined every twenty-four hours for four to six days. After thirty hours, equilibration has occurred and subsequently serum and urine levels decline as a mono-exponential function because of excretion and deposition in bone. The slope of the serum clearance curve gives us the rate of 'turnover'. Dividing the serum value at zero time into the administered dose (corrected for loss during equilibrium) gives the theoretical volume of distribution or rapidly miscible pool. The bone deposition or accretion rate is calculated as the difference between the total turnover rate and the rate of urinary excretion. The bone deposition rate is overestimated by approximately 9–16% because fæcal excretion

Table 1
Skeletal kinetics in normal subjects and conditions associated with osteoporosis

Condition	*No.*	*Pool size (l.)*	*Turnover (l/day)*	*Urinary excretion (l./day)*	*Bone deposition (l./day)*
Normal	31	42·7±1·1	13·5±0·6	3·9±0·2	9·6±0·4
Athletes	14	56·9±2·3	20·7±1·0	5·8±0·2	15·0±1·0
Hyperthyroidism	7	48·9±3·6	23·8±3·5	7·0±2·2	16·7±2·0
Acromegaly	9	73·8±6·2	23·7±2·6	5·7±0·5	18·2±2·3
Cushing's disease	3	23·9	11·7	7·2	4·5
Postmenopausal osteoporosis	40	33·0±0·9	8·7±0·3	2·3±0·2	6·4±0·3

Figures are means ± standard errors. from Eisenberg & Gordan (1961)

is ignored. It does not, however, affect comparison between groups (in the absence of diarrhœa), or before and after steroid treatment.

In normal subjects, the pool size is approximately 40 1. (Table 1). Since each litre of serum contains 5 milliequivalents (100 mg) of calcium, the rapidly exchangeable calcium is approximately only 200 mEq or 4 grams, compared to the much larger volume of about 1,000 g of calcium in the deep osteons of the skeleton. Normally, 10 litres or 1 gram is deposited in the skeleton daily, a rather sluggish rate. Athletes (the San Quentin penitentiary football team) have larger pools and deposit 50% more calcium in their bones every day. Kinetically, two kinds of osteoporosis are recognized: one with small pools and low bone accretion rates and another in which pools are expanded and accretion is rapid. The type with low rates is found in Cushing's disease and, to a lesser degree, in postmenopausal osteoporosis. Correction for body size or surface area does not abolish the significant difference between these low rates and those of normal subjects. The rapid bone accretion rates of thyrotoxicosis and of acromegaly can only mean that the osteopenia or reduced bone volume when present in these conditions must be due to excessive breakdown or resorption. Unfortunately, no direct measure of resorption is available.

The low values for bone accretion in postmenopausal osteoporosis require some comment since Heaney & Whedon (1958), Fraser *et al.* (1960) and Nordin (1959) found no difference between these rates in osteoporotic and normal subjects. Curiously, the discrepancy lies not with the values in osteoporosis but in normal subjects. This is in part explained by insufficient comparable normal controls (Heaney & Whedon 1958) or a different method of calculation (Fraser *et al.* 1960). In fact, Nordin's values obtained in normal young people are similar to our normal values as are those compiled from various sources by Bauer and his associates (Bauer *et al.* 1958). It is unfortunate that different unitage, inclusion of the 'mixing portion' of the serum curve, and different criteria for osteoporosis make comparison of various reported data difficult. Taking these factors into account, it appears that the low rate of bone accretion we have found characterizes far advanced postmenopausal osteoporosis. Naturally, only cases of undoubted osteoporosis were used for our early studies. In these, bone volume is certainly reduced by more than 40 or 50%, as judged by radiolucency of the skeleton. Yet the bone accretion rate is reduced by only one-third, suggesting that what bone is left is turning over more rapidly than normal. The low bone accretion rate cannot be taken to confirm Albright's hypothesis that postmenopausal osteoporosis is due to decreased bone formation. We shall show later that the end-result of naturally occurring endogenous corticoid excess (Cushing's disease) is an even greater decrease in bone formation than is found in postmenopausal osteoporosis although the primary effect of corticoids is *not* to reduce bone formation.

Table 2
Effect of 'anabolic' agents on skeletal kinetics in man

No.	*Steroid*	*Dose*	*Pool size (l.)*	*Urinary excretion (l./day)*	*Bone accretion (l./day)*
40	Base-line		33·0	2·3	6·4
28	Placebo		+1·9	−0·08	−0·26
7	Premarin	2·5 mg/day	−1·7	−1·6	−2·0
9	Vallestril	6·0 mg/day	−1·1	−0·4	−0·9
4	Anvene	20 mg/day	+3·0	−0·4	−2·6
8	Testosterone enanthate	200 mg/week	+3·2	−1·3	−0·5
4	Nilevar	10 mg/day	0	−0·6	−1·2
11	Fluoxymesterone	10 mg/day	+1·9	−0·5	−0·12
2	Oxandrolone	20 mg/day	0	−1·1	−1·5
4	Durabolin	25 mg/week	−1·7	−2·3	−1·3
11	Oxymetholone	7·5 mg/day	+1·3	−2·2	0

Table 3
Effect of corticoids on skeletal kinetics in man

No.	*Steroid*	*Dose*	*Pool size (l.)*	*Urinary excretion (l./day)*	*Bone accretion (l./day)*
31	Base-line		42·7	3·9	9·6
28	Placebo		+1·9	−0·08	−0·26
5	Cortisol	120 mg/day	+4·6	+1·1	+2·9
3	Prednisone	30 mg/day	−2·7	+1·9	+1·7
7	Dexamethasone	3 mg/day	−1·4	+4·6	+1·8
5	Dexamethasone	1·5 mg/day	+3·0	+2·6	+1·8
8	Triamcinolone	30 mg/day	+6·4	+2·3	+1·0
8	6-α-fluorotriamcinolone	12 mg/day	−0·9	+0·8	−0·5
5	6-α-fluoroprednisolone	12 mg/day	−3·2	+4·7	+1·1

The effects of administration of so-called 'anabolic' agents, œstrogens, androgens and synthetic derivatives, are shown in Table 2. In all cases, these were administered to women with postmenopausal osteoporosis for three to six weeks after base-line data had been obtained. The figures reported are the difference between the data after treatment and those before, a plus sign (+) indicating an increase during treatment, and a minus (−) a decrease. No consistent or significant decrease is observed in pool size during treatment. If the agents were truly 'anabolic' and new bone were being formed, one would expect more of the tracer to be diverted to the skeleton. In fact, the bone deposition rate does not rise but falls in every case.

There is also a fall in the rate of urinary excretion of the bone-seeking tracer. Whether this effect is exerted at the level of the osteoclast or the renal tubule is under investigation. In any event, it is apparent that these agents exert their effects not by stimulating anabolism but by reducing osteolysis so that they should properly be characterized as 'anticatabolic'. Growth hormone, by contrast, is anabolic; it increases the bone accretion rate by 50%. It also increases urinary excretion, as has been well shown previously.

Table 3 shows the effects of various corticoids administered to normal volunteers. Again, there is no consistent effect on pool size. That the agents are not primarily 'antianabolic' is shown by the fact that the bone accretion rate does not fall but actually rises. This is probably compensatory to the primary catabolic effect shown in the increased urinary excretion of the bone-seeking tracer. We have screened a rather large number of new experimental corticoids in hope of finding one devoid of the calcium-wasting propensity. Presumably, such a compound would be less likely to cause osteoporosis than agents now available. One di-fluorinated compound, 6-α-fluorotriamcinolone, having roughly twice the anti-inflammatory activity of prednisone or triamcinolone, has significantly less of the undesirable calcium-wasting effect than any other corticoid we have tested. Because of its potential importance, this compound has been tested further, compared with other corticoids, and fæcal excretion determined. Corticoid effects on fæcal excretion of the strontium tracer were variable, increasing it in some subjects but not decreasing it in any. Changes in stool excretion of tracer after 6-α-fluorotriamcinolone treatment were similar to those seen with other corticoids. The 6-α-fluorine configuration alone is not enough to prevent calcium wasting, for 6-α-fluoroprednisolone is as potent a calcium loser as any corticoid thus far tested.

We believe that kinetic techniques can be used to ascertain whether steroids possess anticatabolic activity and therefore are rational for use in the treatment of osteoporosis; to show in relatively short order the adverse effect of corticoids on the bone; and hopefully, to find corticoids devoid of this undesirable action. Correlation with long-term observation is necessary to ascertain whether the short-term kinetic data do, in fact, accurately predict these actions. Such information is available in the case of œstrogens, which do prevent further fractures in women with postmenopausal osteoporosis. Similar studies with the promising corticoid are now in progress.

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Solomon G F, Dickerson W & Eisenberg E (1960) *Geriatrics* 15, 46

Meeting June 26 1963

A combined meeting with the Society of Endocrinology and the Diabetic Association was held on the subject of **Protein Binding of Hormones**

The following papers were read:

Thyroxine Binding
Dr J A Tata

The Supply of Thyroid Hormone to the Fœtus
Dr N B Myant

Insulin Binding
Dr K W Taylor

Insulin and its Binding to Antibodies
Dr P H Wright

Cortisol Binding
Professor Ivor Mills

Determination of Corticosteroid Binding Protein Capacity
Dr D L Schatz and Dr C Osorio

Discussion on the Physiological Significance of Protein Binding
Professor F T G Prunty and Dr D Bellamy

Abstracts of these papers will be published in the *Journal of Endocrinology*

Books recently presented and placed in the Society's Library

(*Continued from p 798*)

Thomson A McC W
The history of the Glasgow Eye Infirmary 1824–1962
pp 102 17s 6d
Glasgow: John Smith 1963

Tomatis A
L'Oreille et le langage
pp 192
Paris: Éditions du Seuil 1963

Trevor-Roper P D
Ophthalmology: a textbook for diploma students
2nd ed pp 668 90s
London: Lloyd-Luke 1962

Ungar G
Excitation
pp 437 £5 8s
Springfield, Ill.: Thomas 1963

U.S. War Department. Surgeon General's Office
United States Army Veterinary Service in World War II
pp 779 $7 (£2 16s)
Washington: Office of S.G. Department Army 1961

Wade O L & Bishop J M
Cardiac output and regional blood flow
pp 268 45s
Oxford: Blackwell 1962

Wall C, Cameron H C & Underwood E A
A history of the Worshipful Society of Apothecaries of London
Vol. 1 pp 450 55s
London, New York, &c.: Oxford University Press 1963

Wheeler A & Jack W R
Wheeler and Jack's handbook of medicine
12th ed pp 722 22s 6d
Edinburgh & London: Livingstone 1963

Whipple A O
The story of wound healing and wound repair
pp 135 46s
Springfield, Ill.: Thomas 1963

Widdess J D H
A history of the Royal College of Physicians of Ireland 1654–1963
pp 255 40s
Edinburgh & London: Livingstone 1963

Wilhelmj C M & McCarthy H H
Dietary and neural factors in hypertension
pp 153 54s
Springfield, Ill.: Thomas 1963

Woodrow C E
The export and import of dogs and cats
pp 107 35s
Oxford: Pergamon Press 1962