

From the Departments of Internal Medicine (Head: Professor H. Lagerlöf) and
Clinical Chemistry (Head: Professor B. Swedin), Karolinska Hospital,
Stockholm, Sweden.

CALCIUM RETENTION AND SKELETAL CALCIUM UPTAKE IN ASTHMATIC SUBJECTS TREATED WITH GLUCOCORTICIDS

By

EVA ARMAN, NILS SVANBORG, ANDERS CARLSTRÖM &
PETER REIZENSTEIN
with the technical assistance of
Mrs. K. HALLGÄRDE

Since Cushing presented his original cases of the disease that was to be called Cushing's syndrome (10), osteoporosis has been a wellknown complication of hypercortisonism. When cortisone was introduced as a therapy of severe rheumatologic, dermatologic and allergic diseases around 1950, it was soon recognized that also hypercortisonism of exogenous origin was complicated by osteoporosis.

Cortisone-induced osteoporosis mainly affects the spine, the pelvis and the ribs (1, 2, 58, 60, 63), but osteoporotic changes may be seen also in the skull and the long bones of the extremities (58). Cortisone-induced osteoporosis seems to be most common in those patients who are liable to get osteoporosis from other causes, e.g. postmenopausal women, old people and immobilized patients (51).

Histological studies have shown that cortex and trabeculae are thin, that there is usually a reduced number of osteoblasts and that the number of osteoclasts may be normal (57, 58, 60). Fracture healing seems to be delayed (58, 60). Osteomalacic changes have also been reported (41).

An increased incidence of fractures in vertebrae and ribs but also in the extremities affects cortisone-treated patients

and patients with Cushing's syndrome (9, 11, 51, 55, 60, 61, 63, 65, 67).

The mechanism by which cortisone induces osteoporosis is not fully known. Albright and Reifenstein introduced the anti-anabolic theory and also wanted to explain other types of osteoporosis by a relative excess of anti-anabolic hormones (1, 2, 51, 52). Balance studies have shown that many patients with Cushing's syndrome and many cortisone-treated patients are in a negative calcium balance (7, 8, 17, 41, 46, 47, 64). *Isaksson et al.* have shown that a cortisone-treated patient can remain in a positive calcium balance and also retain calcium from a high calcium intake (35).

A low intestinal absorption of calcium in hypercortisonism has been found by many investigators (7, 8, 46, 47, 49, 60, 64). The reduced intestinal absorption has been explained by some investigators as an anti-vitamin D effect (7, 22, 60).

The balance between accretion and resorption of calcium in the skeleton has been much studied, as osteoporosis might be the result of a low rate of accretion or a high rate of resorption or a combination of both. Both histologic and micro-radiographic studies have been made. Calcium kinetics have been explored by calcium and strontium isotopes. *Storey* found a low rate of bone formation while the rate of resorption seemed to be increased (60), which is in accordance with investigations by others (8, 36, 46, 47, 54). *Sissons* (57, 58) and *Klein et al.* (37) found mainly a low rate of bone formation, while *Eisenberg* (13-16) found varying rates of bone formation and concluded that the main mechanism was an acceleration of skeletal resorption. *Wajchenberg et al.* by giving dexamethasone to healthy individuals, found an increased rate of bone resorption while the rate of bone formation varied (64).

The finding of hypercalciuria in cortisone-treated patients and patients with Cushing's syndrome has been published in many reports (1, 14, 16, 39, 45, 51, 60, 64) but *Arnoldsson* (6) failed to discover significant differences in urinary calcium excretion between cortisone-treated asthmatic patients

and patients without cortisone therapy. The effect of cortisone on the kidneys may be an isolated decrease in tubular reabsorption of calcium (39) or it may be combined with an increase in the glomerular filtration of calcium (64).

Osteoporosis in old age and in patients with gastrointestinal disorders has been explained by some investigators as partly due to low calcium intake. *Isaksson* and *Lindholm* made a dietary analysis in cortisone-treated asthmatic patients (30). They could not find a significant difference in calcium intake between osteoporotics and non-osteoporotic patients. Neither could *Riggs et al.* (54) find any significant correlation between low calcium intake and the severity of osteoporosis in patients with Cushing's syndrome.

Urist introduced the hypothesis that osteoporosis in hypercortisonism depends on a suppression of the gonadal function (63).

While there is a general agreement of the high incidence of osteoporosis in patients with Cushing's syndrome—according to *Reifenstein* 90 per cent—investigations of the frequency of osteoporosis in cortisone-treated patients are complicated by the fact that many of these patients are rather old and thus liable to be affected by postmenopausal or senile osteoporosis and that many of the diseases necessitating cortisone therapy in themselves might give osteoporosis due to reduced physical activity. *Krokowski* and *Stressman* by studies of X-ray densitometry reported a figure of 30 per cent osteoporosis in long-standing asthma, whether the patients had been treated with cortisone or not (38), while *Arnoldsson* by studying X-ray of spines found a doubled frequency of osteoporosis in cortisone-treated asthmatic patients as compared to patients not treated with cortisone, 19.5 and 10.9 per cent, respectively (6). *McConkey et al.* found the same incidence of osteoporosis in patients with rheumatoid disease, whether cortisone therapy had been used or not (45). The lacking accordance in different reports could partly be explained by different age-groups and different interpretations of X-ray findings. There have also been different opinions as to whether the doses of

cortisone and the duration of the treatment are correlated to the degree and incidence of osteoporosis (40, 51, 55).

Many therapeutic agents have been tried to prevent or treat cortisone-induced osteoporosis. *Reifenstein* (51, 52), *Urist* (63), *Miravet et al.* (47), *Fischer & Hastrup* (17) and *Eisenberg* (15) have reported positive effects with anabolic steroids. However, *Rosenberg* (55), *Isaksson et al.* (35) and *Dymling et al.* (12) failed to discover any significant effects with this therapy, and *Lindholm* (42) reported development of osteoporosis during long-term treatment with a combination of corticoids and anabolic steroids in asthmatic subjects. Treatment with calcium tablets and vitamin D has also been recommended but disappointing results have been published (17, 55).

Oral phosphate supplementation has been used in the treatment of hypercalcaemia and hypercalciuria for several years. The effect of phosphate has been explained by the exceeding of the CaHPO_4 solubility product in serum with a resulting increase in calcium deposition in the skeleton or the soft tissues (24). Positive effects in the treatment of osteopenia of varying origin have been reported by *Goldsmith et al.* (18–21) with amelioration of skeletal pains, a reverse of negative calcium balance, a decrease in urinary calcium and hydroxyproline excretion and an accelerated healing of fractures. *Herrell* has suggested the use of phosphate supplementation in cortisone-induced osteopenia (25).

The disagreement in different reports on the incidence of osteoporosis and disturbances in calcium metabolism during long-term cortisone therapy and the varying effects of different therapeutic agents are probably due to different techniques of investigation, different criteria in interpreting the results as well as varying materials as regarding age, co-existent disorders, duration of corticoid treatment and compounds used.

Balance studies have been much criticized during the last few years by *Isaksson et al.* (28, 29, 31–34) and *Whedon* (66).

The diagnosis of osteoporosis has usually been a roentgenological one. However, several authors, e.g. *Lachman* (40), have shown that X-ray is a very insensitive method of judging osteoporosis.

Calcium kinetic studies after intravenous injection of calcium or strontium isotopes have given much valuable information. They necessitate, however, collections of excreta and are thus liable to some of the errors of balance studies. The results seem to vary almost as much as the mathematical models used and the approximations necessary to solve the equations (23). Based on such studies some investigators (8, 46, 47) found mainly a low rate of bone formation in cortisone-treated subjects, whereas others reported mainly an accelerated rate of bone resorption (13-16, 64).

During the last few years whole-body counting after oral and intravenous radiocalcium administration has been increasingly used, as it gives the opportunity of measuring calcium retention without necessitating collections of excreta.

The purpose of the present investigations was to determine calcium retention and skeletal calcium uptake in corticoid-treated patients by means of whole-body counting and to examine the effect of phosphate treatment in this material.

MATERIAL AND METHODS

Eight men and two women, 22-69 years of age, suffering from bronchial asthma were studied. One of the women had for the last year been treated with inhalations of deoxamethasone but before that she had been on oral triamcinolone therapy for a long time. All the others had continuous treatment with tablets of prednisolone, triamcinolone or betamethasone. Some of the patients also received regular injections of ACTH. The clinical features of the patients and the duration of the glucocorticoid therapy are presented in Table 1. Some of the patients were staying in the hospital during the examinations, the others were out-patients. A few of the patients received special diets to minimize allergic reactions. These diets were supplemented with tablets of calcium phosphate. Most of the patients were consuming ordinary, non-standardized meals. Except for the drugs mentioned in Table 1, the patients only received conventional, symptomatic treatment for their asthma, which was not supposed to interfere with the results of the present examinations.

Healthy volunteers, 20-86 years of age, were studied as controls.

Subject	Sex	Age yrs.	Duration		Daily dose of steroids		Skeletal pains	Fractures	X-ray signs of osteopenia	Comments
			of asthma yrs.	of continuous steroid yrs.	Average	Present				
EI	♀	69	39	9	Triamcinolone 6 mg	8 mg	No	No	Yes	
SD	♂	58	4	4	Prednisolone 10 mg	2.5 mg	Yes	Yes	Yes	ACTH 30 IU/day
GF	♂	51	6	2	Prednisolone 12.5 mg	15 mg	No	Yes	No	ACTH 60 IU/day
JH	♂	50	7	4.5	Prednisolone 6 mg	7.5 mg	No	No	No	ACTH 60 IU once or twice a week
EK	♂	48	13	10	Triamcinolone 6 mg	1.5 mg	No	No	No	ACTH 60 IU/week
KN	♂	48	20	1	Triamcinolone 8 mg	7 mg	No	No	No	ACTH occasionally
AK	♂	47	9	9	Betamethasone 1.5 mg	1 mg	No	No	No	ACTH 15 IU/day
HT	♂	42	0.5	0.1	Prednisolone 7.5 mg	7.5 mg	No	No	No	ACTH 60 IU/week
MS	♀	23	20	15	Triamcinolone 5 mg/day until 1968	Inhalations of dexamethasone	No	No	Yes	Cushing features
BN	♂	22	6	2.5	Prednisolone 10 mg	8 mg	No	No	No	

Serum calcium was analyzed according to Hill (26). Determinations of serum phosphate were made according to Simonsen *et al.* (56). A slight modification of the method originally described by Marsh *et al.* was used for determinations of serum alkaline phosphatases (43). Urinary calcium was analyzed by atomic absorption spectrophotometry (50).

The technical procedures of whole-body counting have been described in previous reports (53, 59). The retention of ^{47}Ca was measured in the whole-body counter 14 days after the administration of 2 μCi . The isotope was given orally in 30 ml of a solution of CaCl_2 containing 50 mg of stable calcium. The patients were fasting for ten hours before being given the isotope and for three to four hours thereafter. For four days before the administration of the isotope and for ten days after it, the patients were treated with calcium tablets, 2 mg of Ca daily (Calcium Sandoz, effervescent tablets, each tablet containing 0.5 gm of Ca). All urine was collected for 72 hours after the administration of the isotope and measured in the whole-body counter. This method of determining calcium retention has been described in previous reports (3, 4).

Skeletal uptake of calcium was determined by measurements of hand radioactivity after an intravenous injection of 2–8 μCi of carrier-free ^{47}Ca . One hand was measured between the crystals of the whole-body counter at 10, 60 and 240 minutes after the injection. More detailed descriptions of this technique has been published elsewhere (5, 27). The plasma elimination and the urinary excretion of the intravenously injected ^{47}Ca were also studied.

After the initial examinations some of the patients were treated with tablets of NaH_2PO_4 (effervescent tablets, each containing 0.5 gm of P). The patients received 1 gm of P daily in tablets for one to two months. During this treatment serum concentrations of calcium, phosphate and alkaline phosphatases and urinary excretion of calcium were followed regularly. In some patients a repeated study of calcium retention of skeletal calcium uptake was performed after one month of treatment with phosphate.

RESULTS

Serum calcium was significantly decreased in the corticoid-treated patients ($0.01 > p > 0.005$). *Serum phosphate, serum alkaline phosphatases and urinary calcium* were not significantly different from the normal (Table 2).

The *whole-body retention of ^{47}Ca* 14 days after the oral administration of the isotope was almost significantly decreased in the corticoid-treated patients compared to the healthy controls ($0.10 > p > 0.05$) (Table 3).

The *urinary excretion of orally administered ^{47}Ca* was not significantly different from the normal (Table 3).

TABLE 2

Serum Concentrations of Calcium, Phosphate and Alkaline Phosphatases and Urinary Excretion of Calcium. Means $\pm$ S.E. of Mean.

Subjects	No. of patients	Serum calcium mEq/l	Serum phosphate mg/100 ml	Serum alkaline phosphatases Buch units	Urinary calcium mg/24 hours
Patients treated with glucocorticoids	9	4.61 $\pm$ 0.07*	3.50 $\pm$ 0.18**	6.3 $\pm$ 0.7	215 $\pm$ 32.5
Healthy controls	32	4.84 $\pm$ 0.04	3.16 $\pm$ 0.09	5.4 $\pm$ 0.4	195 $\pm$ 12.7§

* n = 8.

** n = 7.

§ n = 20.

The sum of retention and urinary excretion of ^{47}Ca , expressed as per cent of given dose, was higher in healthy subjects than in the patients ($0.1 > p > 0.05$).

Hand uptake of ^{47}Ca measured four hours after the intravenous injection of the isotope did not differ significantly between the patients and the controls (Table 4). In the controls was found a high degree of correlation between hand uptake of ^{47}Ca and age ($r = 0.789$, $p < 0.001$). In the pa-

TABLE 3

Retention and Urinary Excretion of Orally Administered ^{47}Ca . Means $\pm$ S.E. of Mean.

Subjects	No. of subjects	Retention* per cent of dose	Urinary excretion§ per cent of dose
Patients treated with glucocorticoids, average age = 43 years	9	24.66 $\pm$ 1.79	7.29 $\pm$ 1.18
Healthy controls, average age = 40 years	20	30.00 $\pm$ 1.58	7.67 $\pm$ 0.64

* Retention at 14 days after the administration of the isotope.

§ Urinary excretion 0-72 hours after the administration of the isotope.

TABLE 4

Hand Uptake of Intravenously Injected ^{47}Ca , Expressed as Counts Per Second Per Given μCi . Means $\pm$ S.E. of Mean.

Subjects	No. of subjects	10 minutes	60 minutes	240 minutes
Patients treated with glucocorticoids, average age = 44 years				
	9	$38.0 \pm 1.8^*$	$50.3 \pm 1.1^*$	56.6 ± 1.4
Healthy controls, average age = 53 years				
	22	$33.8 \pm 1.8\text{§}$	48.8 ± 1.6	58.8 ± 1.5
* n = 8.		§ n = 17.		

tients there was no significant correlation ($r = 0.335$, $p > 0.1$).

There was no significant difference in the *plasma elimination of intravenously injected ^{47}Ca* between the patients and the healthy controls within four hours after the injection (Table 5).

Patients treated with glucocorticoids had an almost significantly increased *urinary excretion of intravenously injected ^{47}Ca* as compared to the healthy subjects ($0.05 > p > 0.025$) (Table 6). In these patients the urinary excretion of radiocalcium after the intravenous injection was significantly correlated to the amount of radiocalcium excreted in the urine after oral administration of the isotope ($r = 0.847$,

TABLE 5

Plasma Elimination of Intravenously Injected ^{47}Ca , Expressed As Per Cent of Injected Dose Per Liter Plasma. Means $\pm$ S.E. of Mean.

Subjects	No. of subjects	5 minutes	60 minutes	240 minutes
Patients treated with glucocorticoids, average age = 44 years				
	8	9.44 ± 0.77	4.00 ± 0.29	2.80 ± 0.18
Healthy controls, average age = 53 years				
	16	9.94 ± 0.66	4.49 ± 0.26	3.03 ± 0.16

TABLE 6

*Urinary Excretion of Intravenously Injected ^{47}Ca 0-72 Hours After the Injection.
Means $\pm$ S.E. of Mean.*

Subjects	No. of subjects	Urinary ^{47}Ca per cent per dose
Patients treated with glucocorticoids, average age = 44 years	9	12.10 $\pm$ 1.48
Healthy controls, average age = 53	21	8.07 $\pm$ 1.07

0.01 $>$ p $>$ 0.005). This correlation was not studied in healthy subjects.

The duration of the corticoid therapy was almost significantly correlated to plasma ^{47}Ca concentrations at five minutes ($r = 0.832$, 0.02 $>$ p $>$ 0.01) and at 60 minutes ($r = 0.794$, 0.02 $>$ p $>$ 0.01) after the injection of the isotope. After 240 minutes there was no significant correlation. We found no other significant correlation between the studied

TABLE 7

*Effects of Treatment with NaH_2PO_4 on Serum Concentrations of Calcium, Phosphate and Alkaline Phosphatases and Urinary Excretion of Calcium.
Means $\pm$ S.E. of Mean.*

Duration of treatment	Serum calcium mEq/l	Serum phosphate mg/100 ml	Serum alkaline phosphatases Buch units	Urinary calcium mg/24 hours
Before treatment	4.59 $\pm$ 0.07 n = 7	3.50 $\pm$ 0.18 n = 7	5.7 $\pm$ 0.8 n = 7	193.3 $\pm$ 45.1 n = 6
After 1 week	4.60 $\pm$ 0.06 n = 5	3.42 $\pm$ 0.43 n = 5	5.8 $\pm$ 0.4 n = 5	166.0 $\pm$ 37.4 n = 5
After 3-4 weeks	4.46 $\pm$ 0.12 n = 7	3.73 $\pm$ 0.15 n = 7	6.3 $\pm$ 0.6 n = 6	150.0 $\pm$ 21.8 n = 6
After 6-8 weeks	4.33 $\pm$ 0.28 n = 4	3.48 $\pm$ 0.50 n = 4	6.3 $\pm$ 1.7 n = 4	177.5 $\pm$ 11.1 n = 4

TABLE 8

Effect of Treatment with NaH_2PO_4 on Hand Uptake of Intravenously Injected ^{47}Ca in Asthmatic Patients Treated with Glucocorticoids. Results Expressed as Counts Per Second Per Injected μCi .

Subjects		Hand uptake of ^{47}Ca		
		60 minutes	10 minutes	240 minutes
HT	I	41	52	56
	II	35	42	49
KN	I	37	52	60
	II	33	44	55
EK	I	—	—	59
	II	50	62	66

I = Before treatment with phosphate. II = After treatment with phosphate for 1 month.

parameters of calcium metabolism and the duration of the therapy.

Effects of the treatment with NaH_2PO_4 : During treatment there was a slight decrease in serum calcium, which was not significant when the whole group of patients was regarded.

TABLE 9

Effect of Treatment with NaH_2PO_4 on Retention of ^{47}Ca as Measured in a Whole-body Counter 14 Days After the Oral Administration of the Isotope in One Asthmatic Patient Treated with Glucocorticoids. Retention Expressed as Per Cent of Given Dose. Urinary Excretion of ^{47}Ca Measured 0-72 Hours After the Administration of the Isotope and Expressed as Per Cent of Dose.

	Retention of ^{47}Ca per cent of dose	Urinary ^{47}Ca per cent of dose
Before treatment		
with phosphate	22.6	9.8
After treatment with		
phosphate for one month	24.6	7.9

However, the treatment had to be interrupted in three patients because of low serum calcium, 3.7–4.1 mEq./l. after four to six weeks of treatment without clinical symptoms of hypocalcaemia. There was also a slight decrease in urinary calcium and slight increase in serum alkaline phosphatases. None of these differences were significant. Nor was there a significant increase in serum phosphate during treatment (Table 7).

The effect of phosphate treatment on hand uptake of intravenously injected ^{47}Ca was studied in three patients (Table 8) and on whole-body retention and urinary excretion of orally administered ^{47}Ca in one patient (Table 9). No significant changes were observed.

DISCUSSION

The decreased serum calcium in corticoid-treated patients as compared to healthy individuals could be due to several factors. Low intestinal calcium absorption may contribute. A decrease in serum albumin would give a decrease serum calcium. Unfortunately, serum albumin was not regularly studied in these patients.

Whole-body retention of orally administered ^{47}Ca measured only the retention of the given radioactivity. Since the ingested ^{47}Ca is mixed with intestinally secreted calcium, it measures the retention of the amount of calcium mixed with the radioisotope.

The retention is the difference between absorption and losses via faeces, urine and sweat. Thus intestinal absorption is the retention plus the losses. Of these urinary losses are the most important and also the easiest to measure. We have regularly studied urinary radiocalcium excretion during the first 72 hours after the ingestion of the isotope. We have found in healthy individuals that about 60 per cent of the total amount of urinary ^{47}Ca excreted is eliminated during this period of time. Thus total urinary radiocalcium excretion during 14 days may be obtained from our figures.

Endogenous faecal losses have been studied by many investigators by giving intravenous injections of radiocalcium and measuring the radioactivity in faeces. These losses have usually been estimated to 100–150 mg/day. According to *Mautalen et al.* (44) this should correspond to about 3.3 per cent of the orally given dose of radiocalcium in normal individuals.

Dermal losses of calcium have often been neglected in studies of calcium absorption and retention. *Isaksson et al.* (28, 29, 32, 34) have found an average daily dermal loss of calcium of 120 mg/day, which is of about the same magnitude as the endogenous faecal calcium excretion.

Urinary losses amount to 7.5 per cent of given dose in 72 hours, which corresponds to about 12 per cent of given dose in 14 days. Endogenous faecal losses amount to about 3 per cent and dermal losses to another 3 per cent of given dose. Thus the retention values after 14 days in healthy subjects should equal absorption minus 18 per cent of the given amount of radiocalcium.

Retention of orally administered ^{47}Ca was significantly decreased in patients treated with glucocorticoids. As endogenous faecal losses have been reported to be less than normal in these patients (64), urinary radiocalcium excretion is normal and no evidence of increased dermal calcium losses has been reported, we conclude that absorption of radiocalcium is impaired in these patients.

There is no reason to believe that total intestinal calcium secretion should be increased in patients treated with glucocorticoids. On the contrary, *Wajchenberg et al.* (64) reported a significant decrease in intestinal secretion of calcium. The decreased absorption of radiocalcium in corticoid-treated patients should therefore also mean a decreased absorption of stable calcium.

Hand uptake of intravenously injected ^{47}Ca showed a significant regression to age in healthy controls, which has been interpreted as evidence of an increased rate of bone formation in aged individuals (5). Patients treated with gluco-

corticoids had a normal hand uptake of ^{45}Ca in comparison to age-matched controls. In accordance with *Eisenberg* (13-16) and *Wajchenberg et al.* (64) we therefore conclude that the rate of bone formation is not significantly affected in corticoid-treated patients. Though roentgenological findings have been used to suggest that the hand skeleton in cortisone-treated patients is not representative for calcium metabolism in the total skeleton, a comparison of our findings in various ages of normals and in these patients indicate a normal ability to accrete calcium in the latter material.

Studies by others have indicated an increased rate of bone resorption in corticoid-treated patients (8, 36, 46, 54, 60, 64). Our studies do not allow any conclusions in this respect, as long-term studies are needed. However, the low intestinal calcium absorption might give a secondary hyperparathyroidism, resulting in increased bone resorption.

The finding of significantly increased urinary radiocalcium excretion after intravenous injection of the isotope in corticoid-treated patients, is in accordance with the studies of *Wajchenberg et al.* (64). It could be due to an increased glomerular filtration of calcium (64) or a decrease in tubular reabsorption of calcium (39, 64). There might also exist a tubular secretion of calcium (62), which could be influenced by glucocorticoids.

According to *Goldsmith et al.* (18-21) oral phosphate supplement has no effect on serum concentrations of calcium and phosphate in normo-calcaemic individuals. We could not find a significant decrease in serum calcium during phosphate treatment in the patients as a group, but three of them developed hypocalcaemia. *Goldsmith et al.* found a constant decrease in urinary calcium, which was not observed in our patients. However, our number of patients treated with phosphate is very small and does not permit very extensive conclusions.

SUMMARY

Ten asthmatic patients treated with glucocorticoids were studied. With one exception they had all been treated for at least one year. One of them had skeletal pains, two had suffered from fractures and three had X-ray signs of osteopenia. Serum calcium was significantly decreased, while the concentrations of serum phosphate and serum alkaline phosphatases were within normal limits. Urinary calcium excretion was numerically but not significantly increased. Whole-body retention of orally administered ^{47}Ca was not quite significantly decreased. After intravenous injection of ^{47}Ca the radioactivity in one hand was not significantly different from the results in healthy individuals, while the urinary radiocalcium excretion was significantly increased.

The results of these studies are interpreted as signs of decreased intestinal absorption of calcium, increased urinary clearance of calcium and normal ability to deposit calcium in the skeleton in patients treated with glucocorticoids.

ACKNOWLEDGEMENTS

We want to thank dr K.O. Pegelöw, St. Göran's Hospital, Stockholm, Sweden, who allowed us to study his patients. Financial support was given by Sandoz AG, Osterman foundation and Karolinska Institute. Sandoz AG provided the calcium and phosphate tablets.

REFERENCES

1. Albright, F.: Cushing's Syndrome. The Harvey Lectures, 38, 123-186, 1943.
2. Albright, F. & Reifensstein, E. C.: The Parathyroid Glands and Metabolic Bone Disease. The Williams and Wilkins Company, Baltimore, 1948.
3. Arman, E. & Reizenstein, P.: Studies in the dumping syndrome IV. Effect of calcium therapy on calcium metabolism and osteopenia in gastrectomized patients. *Amer. J. Dig. Dis.* 14, 153-162, 1969.
4. Arman, E., Nilsson, L. H:son & Reizenstein, P.: Studies in the dumping syndrome VI. Calcium deficiency after partial gastrectomy. *Amer. J. Dig. Dis.* 15, 455-462, 1970.
5. Arman, E. & Reizenstein, P.: Hand radioactivity after intravenous injection of ^{47}Ca . Effect of age and femoral neck fracture. *Geriatrics* 1970.

6. *Arnoldsson, H.*: Long-term ACTH and corticosteroid therapy in bronchial asthma. A clinical evaluation. *Acta Allergol.* 12, suppl. 6, 1958.
7. *Bassett, S. H., Figueroa, W. G., Tuttle, S. G. & Jordan, T.*: Metabolic studies in Cushing's syndrome. *Clin. Orthop.* 17, 304-332, 1960.
8. *Bloch-Michel, H., Rosenbaum, H., Gorijs, A. & Dussart, N.*: L'Ostéoporose cortisonique. *Sem. Hôp. Paris* 42, 7-12, 1966.
9. *Curtiss, P. H. & Clark, W.*: Vertebral fractures resulting from prolonged cortisone and corticotrophin therapy. *JAMA* 156, 467-469, 1954.
10. *Cushing, H.*: The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull. Johns Hopkins Hospital* 50, 137-195, 1932.
11. *Demartini, F., Grokoest, A. W. & Ragan, C.*: Pathological fractures in patients with rheumatoid arthritis treated with cortisone. *JAMA* 140, 750-752, 1952.
12. *Dymling, J. F., Isaksson, B. & Sjögren, B.*: Anabolic steroids in the treatment of osteoporosis. In: *Protein Metabolism. Influence of Growth Hormone, Anabolic Steroids and Nutrition in Health and Disease. An International Symposium*, Leyden 1962, sponsored by Ciba. Ed. A. Gross, Springer Verlag, 412-423, 1962.
13. *Eisenberg, E.*: Effect of steroids on calcium dynamics. In: *Biological Activities of Steroids in Relation to Cancer*. Eds.: G. Pincus & E. P. Vollmer, Academic Press, New York, 189-196, 1960.
14. *Eisenberg, E.*: Effects of corticoids on bone. In: *Dynamic Studies of Metabolic Bone Disease*. Eds.: O. H. Pearson & G. F. Joplin, Blackwell Scientific Publications, Oxford, 119-122, 1964.
15. *Eisenberg, E.*: Effects of steroid hormones on strontium kinetics in man. In: *Proceedings of the Second International Congress of Endocrinology*. London 1964. International Congress Series no. 83. Excerpta Medica Foundation part II, 917-921, 1965.
16. *Eisenberg, E.*: Effects of androgens, estrogens and corticoids on strontium kinetics in man. *J. Clin. Endocrin.* 26, 566-572, 1966.
17. *Fischer, F. & Hastrup, B.*: Cortisone and calcium balance (effect of calcium, vitamin D and methylandrostenediol). *Acta Endocrin.* 16, 141-148, 1954.
18. *Goldsmith, R. S. & Ingbar, S. H.*: Effects of oral phosphate supplements in demineralizing disorders of bone. *J. Clin. Invest.* 45, 1014, 1966.
19. *Goldsmith, R. S., Woodhouse, C. F., Ingbar, S. H. & Senegal, D.*: Effect of phosphate supplements in patients with fractures. *Lancet* 1, 687-690, 1967.
20. *Goldsmith, R. S., Barton, H., Hulley, S. B., Ingbar, S. H. & Moloney, W. C.*: Phosphate supplementation as an adjunct in the therapy multiple myeloma. *Arch. Intern. Med.* 122, 128-133, 1968.
21. *Goldsmith, R. S., Killian, P., Ingbar, S. H. & Bass, D. E.*: Effect of phos-

- phate supplementation during immobilization of normal men. *Metabolism* 18, 349-368, 1969.
22. Harrison, H. E. & Harrison, H. C.: Transfer of Ca^{45} across intestinal wall in vitro in relation to action of vitamin D and cortisol. *Amer. J. Physiol.* 199, 265-271, 1960.
 23. Heaney, R. P.: Evaluation and interpretation of calcium-kinetic data in man. *Clin. Orthop.* 31, 153-183, 1963.
 24. Hebert, L. A., Lemann, J., Petersen, J. R. & Lemon, E. J.: Studies of the mechanism by which phosphate infusion lowers serum calcium. *J. Clin. Invest.* 45, 1886-1894, 1966.
 25. Herrell, W. E.: Demineralizing bone disorders treated with oral phosphate supplements. *Clin. Med.* 73, 13-14, 1966.
 26. Hill, J. B.: Automated fluorometric method for the determination of serum calcium. *Clin. Chem.* 11, 122-130, 1965.
 27. Höglund, S., Deshiri, K. & Reizenstein, P.: A method for measuring plasma clearance of iron without blood samples and of estimating the extravascular uptake of radiovitamin B_{12} . *Phys. in Med. Biol.* 12, 469-476, 1967.
 28. Isaksson, B., Lindholm, B. & Sjögren, B.: Dermal loss of nutrients and its significance to human metabolic balance studies. In: *Proceedings of the 6th International Congress of Nutrition, Edinburgh 1963*. E. & S. Livingstone Ltd, Edinburgh and London, 526, 1964.
 29. Isaksson, B. & Sjögren, B.: Metaboliska studier vid osteoporos. I. En kritik av den ortodoxa balanstekniken. *Nord. Med.* 71, 616-619, 1964.
 30. Isaksson, B. & Lindholm, B.: Studies on cortisone-induced osteoporosis. I. Estimation of daily calcium intake from diet history and seven day dietary record. *Amer. J. Clin. Nutr.* 16, 287-291, 1965.
 31. Isaksson, B. & Sjögren, B.: On the concept "constant diet" in metabolic balance studies. *Nutr. Dieta* 7, 175-185, 1965.
 32. Isaksson, B. & Sjögren, B.: Errors inherent in metabolic balance studies. In: *Proceedings of the 2nd International Congress of Endocrinology, London 1964*. Part II. International Congress Series no. 83. Excerpta Medica Foundation, 1041-1049, 1965.
 33. Isaksson, B. & Sjögren, B.: A critical evaluation of the calcium balance technique. I. Variations in fecal output. *Metabolism* 15, 295-302, 1967.
 34. Isaksson, B., Lindholm, B. & Sjögren, B.: A critical evaluation of the calcium balance technique. II. Dermal calcium losses. *Metabolism* 15, 303-313, 1967.
 35. Isaksson, B., Lindholm, B. & Sjögren, B.: Body composition during long-term administration of cortisone and anabolic steroids in an asthmatic subject. *Metabolism* 16, 162-175, 1967.
 36. Jowsey, J.: The mechanism of development of senile osteoporosis. In: *Aging of Connective and Skeletal Tissue, Thule International Sym-*

- posia 1968. Eds.: A. Engel and T. Larsson. Nordiska Bokhandelns Förlag, 270-279, 1969.
37. Klein, M., Villanueva, A. R. & Frost, H. M.: A quantitative histological study of rib from 18 patients treated with adrenal cortical steroids. *Acta Orthop. Scand.* 35, 171-184, 1965.
 38. Krokowski, E. & Streseman, E.: Röntgenologische Bestimmung des Mineralisationsgrades der Wirbelsäule von Kranken mit chronischem Bronchialasthma. *Klin. Wochenschrift* 45, 570-573, 1967.
 39. Laake, H.: The action of corticosteroids on the renal reabsorption of calcium. *Acta Endocrin.* 34, 60-64, 1960.
 40. Lachman, E.: Osteoporosis: The potentialities and limitations of its roentgenologic diagnosis. *Amer. J. Roentgenol.* 74, 712-715, 1955.
 41. Lichtwitz, A., de Sèze, S., Hioco, D., Parlier, R., Lanham, C. & Sfikakis, P.: Étude biochimique des décalcifications cortisoniques. *Sem. Hôp. Paris* 37, 682-687, 1961.
 42. Lindholm, B.: Body cell mass during long-term treatment with cortisone and anabolic steroids in asthmatic subjects. *Acta Endocrin.* 55, 222-239, 1967.
 43. Marsh, W. H., Fingerhut, B. & Kirsch, E.: Adaption of an alkaline phosphatase for automatic colorimetric analysis. *Clin. Chem.* 5, 119-126, 1959.
 44. Mautalen, C. A., Cabrejas, M. L. & Soto, R. J.: Isotopic determination of intestinal calcium absorption in normal subjects. *Metabolism* 18, 395-405, 1969.
 45. McConkey, B., Fraser, G. M. & Bligh, A. S.: Osteoporosis and purpura in rheumatoid disease: prevalence and relation to treatment with corticosteroids. *Quart. J. Med.* 31, 419-427, 1962.
 46. Miravet, L. & Landron, A.: Études cinétiques avec le Ca^{45} dans l'ostéoporose. In: *L'Ostéoporose*. Symposium publié par D. J. Hioco, Masson & Cie, Paris, 74-76, 1964.
 47. Miravet, L., Dryll, A., Hioco, D., Ryckewaert, A., Debeyre, N. & de Sèze, S.: La cinétique du calcium au cours de l'ostéoporose cortisonique. *Sem. Hôp. Paris* 42, 60-64, 1966.
 48. Molinatti, G. M., Camanni, F. & Olivetti, M.: A study on the metabolism of calcium in the hyperadrenocortical syndrome. *Acta Endocrin.* 34, 323-334, 1960.
 49. Nordin, B. E. C.: An isotope test of calcium absorption. In: *Medical Uses of Ca^{47}* . Technical Reports Series no. 10, IAEA Vienna, 44-46, 1962.
 50. Perkin-Elmer Corporation, Norwalk, Connecticut, U.S.A.: Analytic method for atomic absorption spectrophotometry.
 51. Reifstein, E. C.: Control of corticoid-induced protein depletion and osteoporosis by anabolic steroid therapy. *Metabolism* 7, 78-89, 1958.
 52. Reifstein, E. C.: Osteoporosis. In: *Bone as a Tissue*. Eds.: K. Rodahl,

- J. T. Nicholson and E. M. Brown. McGraw-Hill Book Company Inc., 83-90, 1960.
53. *Reizenstein, P. & Karlsson, H. Å.*: Clinical whole-body counting. I. Whole-body scanning with two crystals. *Acta Radiologica (Stockholm)* 4, 209-220, 1966.
 54. *Riggs, B., Jowsey, J. & Kelly, P. J.*: Quantitative microradiographic study of bone remodelling in Cushing's syndrome. *Metabolism* 15, 773-779, 1966.
 55. *Rosenberg, E. F.*: Rheumatoid arthritis. Osteoporosis and fractures related to steroid therapy. *Acta Med. Scand. suppl.* 341, 211-224, 1958.
 56. *Simonsen, D. G., Wertman, M., Westover, L. & Mehl, J. W.*: The determination of serum phosphate by the molybdivanadate method. *J. Biol. Chem.* 166, 747-755, 1946.
 57. *Sissons, H. A.*: The osteoporosis of Cushing's syndrome. *J. Bone Jt. Surg.* 38B, 418-433, 1956.
 58. *Sissons, H. A.*: Osteoporosis of Cushing's syndrome. In: *Bone as a Tissue*. Eds.: K. Rodahl, J. T. Nicholson and E. M. Brown. McGraw-Hill Book Company Inc., 3-17, 1960.
 59. *Sjöberg, H. E., Reizenstein, P. & Arman, E.*: Retention of orally administered ⁴⁷Ca in man as measured in a whole-body counter. *Scand. J. Clin. Lab. Invest.* 26, 67-71, 1970.
 60. *Storey, E.*: The influence of adrenal cortical hormones on bone formation and resorption. *Clin. Orthop.* 30, 197-217, 1963.
 61. *Teicher, R. & Nelson, C. T.*: Osteoporosis and pathological fractures following treatment with ACTH and cortisone. *J. Invest. Dermatol.* 19, 205-210, 1952.
 62. *Thomas, W. C. & Howard, J. E.*: Disturbances of calcium metabolism. In: *Mineral Metabolism. An Advanced Treatise*. Eds.: C. L. Comar and F. Bronner. Volume II, part A, Academic Press New York and London, 445-482, 1964.
 63. *Urist, M. R.*: Observations bearing on the problem of osteoporosis. In: *Bone as a Tissue*. Eds.: K. Rodahl, J. T. Nicholson and E. M. Brown. McGraw-Hill Book Company Inc., 18-45, 1960.
 64. *Wajchenberg, B. L., Pereira, V. C., Kieffer, J. & Ursic, S.*: Effect of dexamethasone on calcium metabolism and ⁴⁷Ca kinetics in normal subjects. *Acta Endocrin.* 61, 173-192, 1969.
 65. *Werne, S.*: Osteoporosen vid reumatoida arthritiser och cortisonterapi. *Svenska Läkartidningen* 58, 2722-2731, 1961.
 66. *Whedon, C. D.*: The combined use of balance and isotopic studies in the study of calcium metabolism. In: *Proceedings of the 6th International Congress of Nutrition, Edinburgh 1963*. E. & S. Livingstone Ltd., Edinburgh and London, 425-438, 1964.
 67. *Virkkunen, M. & Lethinen, L.*: Side effects of corticosteroids. Observations after five years' continuous use. *Acta Med. Scand. suppl.* 341, 205-210, 1958.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.