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The Different Effects of Soluble and Crystalline Hydrocortisone on Bone* **

Howard Duncan, Charles A. Hanson, and A. Curtiss
Department of Medicine and Orthopaedics, Henry Ford Hospital, Detroit, Michigan

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The effects of hydrocortisone on growing bone administered in two different physical forms (soluble and crystalline) in an identical dosage of 5 mg/kg/day for six weeks were studied in 52 growing rabbits. The animals were divided into (1) controls, (2) soluble hydrocortisone treated and (3) crystalline hydrocortisone treated. The rabbits treated with soluble hydrocortisone showed changes different from those given crystalline hydrocortisone. When compared with the control group, the soluble hydrocortisone treated group gained less weight, had less longitudinal bone growth, had some suppression of bone formation at all surfaces and had some increased bone resorption at the cortical-endosteal surface. However, the crystalline hydrocortisone treated group lost weight, ceased longitudinal bone growth, ceased bone formation at all surfaces and had marked bone resorption at the cortical-endosteal surface resulting in dramatic cortical thinning. It is believed that the determining factor in this differential effect is the prolonged elevation of plasma cortisol following injection of the crystalline hydrocortisone. While other investigators have shown a strong drug-dose relationship, this study indicates that the duration of action of the drug or the frequency of its administration per day may be equally important.

Key words: Bone — Corticosteroids — Hydrocortisone — Osteoporosis.

Les effets de l'hydrocortisone, administrée sous deux formes physiques différentes (solubles et cristallines), à une dose identique de 5 mg/kg/jour ont été étudiés sur l'os en croissance chez 52 lapins pendant 6 semaines. Les animaux sont divisés en 1) un groupe témoin, 2) un groupe traité à l'hydrocortisone soluble et 3) un groupe traité à l'hydrocortisone cristalline. Les lapins traités à l'hydrocortisone soluble présentent quelques différences avec ceux traités à l'hydrocortisone cristalline. Par rapport aux témoins, le groupe traité à l'hydrocortisone soluble a augmenté plus faiblement de poids, présente moins de croissance osseuse longitudinale, montre un arrêt de l'ostéogenèse des surfaces et présente une augmentation de la résorption osseuse au niveau des surfaces corticales et de l'endoste. Cependant, le groupe traité à l'hydrocortisone cristalline a perdu du poids, ne présente pas de croissance osseuse longitudinale, ni d'apposition osseuse au niveau de toutes les surfaces, avec cependant une résorption osseuse marquée au niveau des surfaces corticales et de l'endoste, provoquant un amincissement net des corticales. Il semble que le facteur déterminant de cette action différente est l'élévation prolongée du cortisol plasmatique après injection d'hydrocortisone cristalline. Alors que d'autres aufeurs ont montré un rapport étroit médicament-dose, cette étude indique que la durée de l'action du médicament ou la fréquence de son administration par jour peut également être importante.

Die Wirkung von Hydrocortison auf den wachsenden Knochen wurde bei 52 Kaninchen untersucht. Das Hydrocortison wurde in zwei Formen (löslich und kristallin), bei gleicher Dosierung von 5 mg/kg/Tag, während 6 Wochen verabreicht. Die Tiere wurden in folgende

For reprints: H. Duncan, M.B., M.R.C.P., Division of Rheumatology, Henry Ford Hospital, Detroit, Michigan 48202, U.S.A.

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Gruppen unterteilt: 1. Kontrollen; 2. mit löslichem Hydrocortison behandelte Tiere; 3. mit kristallinem Hydrocortison behandelte Tiere. Die mit löslichem Hydrocortison behandelten Kaninchen wiesen nicht dieselben Veränderungen auf wie die mit kristallinem Hydrocortison behandelten. Im Vergleich mit der Kontrollgruppe zeigte die mit löslichem Hydrocortison behandelte Gruppe folgende Veränderungen: weniger Gewichtszunahme, weniger Wachstum der longitudinalen Knochen, leichte Unterdrückung der Knochenbildung auf allen Oberflächen und leicht erhöhte Knochenresorption an der corticalen Endostoberfläche. Die mit kristallinem Hydrocortison behandelten Tiere jedoch zeigten: Gewichtsverlust, Stillstand des longitudinalen Knochenwachstums, Stillstand der Knochenbildung auf allen Oberflächen und deutliche Knochenresorption an der corticalen Endostoberfläche, welche zu dramatischer corticaler Verdünnung führte. Es wird vermutet, daß der ausschlaggebende Faktor in dieser unterschiedlichen Wirkung die anhaltende Erhöhung der Cortisolkonzentration im Plasma ist, welche auf die Injektion von kristallinem Hydrocortisol folgt. Während andere Forscher eine starke Dosisabhängigkeit gezeigt haben, deutet diese Untersuchung an, daß die Wirkungsdauer der Substanz oder die Häufigkeit ihrer Verabreichung pro Tag ebenso wichtig sein können.

In clinical medicine, few commonly used drugs can equal the diverse and profound effects of the adrenal corticosteroids. The changes they can cause in bone have been recognized since the description of vertebral collapse in Cushing's Syndrome in 1932 (Cushing, 1932). Following the development of synthetic corticosteroids in the early 1950's, considerable clinical and experimental study has been directed toward elucidating the nature and mechanisms of the bone changes produced by these drugs and their analogues. The overall effect of the corticosteroids on bone is to produce an osteoporosis by altering the balance between bone formation and bone resorption (Villaneuva, 1967; Jett *et al.*, 1970; Duncan, 1967; Jee *et al.*, 1972). The degree of this change depends upon such factors as the *species* of animals studied, the *derivative* of cortisone and naturally the *dose* and *duration* of treatment (Jee *et al.*, 1966, 1972).

In this paper we show that two physical forms of the same corticosteroid (soluble hydrocortisone and crystalline hydrocortisone) given in identical doses to growing rabbits produce different effects upon the animals as a whole and upon the bone in particular.

Materials and Methods

New Zealand white rabbits, 90 days old, were used in this study. They were acquired from the same rabbit agency and were treated in three groups. The groups were: (1) controls, (2) those which received soluble hydrocortisone succinate[1], (3) those which received crystalline hydrocortisone[2], an aqueous suspension. Groups of at least five or six male littermates were studied. Each group was treated simultaneously, with one or two animals serving as controls and the remainder given hydrocortisone. A total of 35 animals served as controls and received daily injections of saline (0.1 ml/kg). A total of 17 animals received soluble hydrocortisone (5 mg/kg/day) and 35 received soluble hydrocortisone (5 mg/kg/day). All injections were given intramuscularly six days per week. Each animal was weighed on Day 0 and twice weekly thereafter to ensure that the dosage of drug would remain directly proportional to the body weight. The rabbits were kept in individual cages and fed Purina Lab Chow Checkers with water ad libitum. Each animal had its entire right femur X-rayed on Day 0 and again on the day of sacrifice. Also, a tetracycline[3] "bone marker" (25 mg/kg)

1 Hydrocortisone succinate (Solu-Cortef).

2 Hydrocortisone (Cortef), an aqueous crystalline suspension.

3 Tetracycline (Panmycin). All drugs kindly provided by the Upjohn Company, Kalamazoo, Michigan.

was given six days prior to the first day of treatment and again six and three days prior to sacrifice. Litters were sacrificed after two, four or six weeks.

Histology

The right femur and the right fifth and sixth ribs were removed from each animal, and six fresh fully mineralized sections were cut from the midpoint and prepared (Frost, 1958) for bright field and fluorescent microscopy. The total cross sectional area of each section, cortical bone area and marrow cavity area were measured (Frost, 1969). Newly-formed bone was recognized by the tetracycline labelling technique and quantitation was conducted according to methods previously described (Frost, 1969).

X-Rays

Uniformly oriented lateral X-rays of the right femur were obtained on Day 0 and again on the day of sacrifice by flexing the knee of the rabbit to 90 degrees, taping the thigh and foreleg to the underlying cassette to secure position, and abducting the other leg out of the range of the X-ray beam. By immobilizing the leg with the knee in this flexed position, rotational variations of the femur were avoided. The tube distance from the cassette was 40 in. (102 cm) and the exposure was $^1/_{20}$ second at 50 kVp and 200 mA. The quality and reproducibility of X-rays permitted accurate measurement of the periosteal diameter, and marrow cavity diameter at the midpoint of the femur. X-ray measurements were correlated with histological measurements of the cross section of the same bone sampled at the same site. In addition, femoral length was determined.

Plasma Cortisol Level

In eight animals, plasma cortisol levels were determined at intervals following the routine injection of soluble hydrocortisone and crystalline hydrocortisone. The method of estimating plasma cortisol levels has been described by Murphy (1967).

Results

Although all animals in this study were sacrificed either after 2, 4, or 6 weeks of therapy, it has been our observation in other studies (Hanson *et al.*, 1970) that no animal which received crystalline hydrocortisone in a dose of 5 mg/kg/day would survive beyond the 7th week of therapy, whereas those which received the same dose of soluble hydrocortisone continued to grow and gain weight well beyond 16 weeks as in the study of Epker (1970).

Weight

The mean weight at zero time was 2.57 ± 0.41 kg[4] being determined from 87 animals. The weight gain of the control animals in six weeks averaged 0.89 ± 0.07 kg. Those animals which received soluble hydrocortisone gained an average of 0.4 ± 0.06 kg in six weeks, whilst those receiving crystalline hydrocortisone gained weight during the first week and then actually *lost* weight during the subsequent five weeks for a net loss of 0.64 ± 0.18 kg (Fig. 1). Animals treated with crystalline hydrocortisone at times developed bloating, muscle wasting and weakness during the fifth and sixth weeks of treatment.

Femoral Dimensions

1. Length. X-rays showed that the femur length of control animals increased 8.1 ± 0.5 mm in six weeks. In the soluble hydrocortisone-treated group, the length

4 Standard Error.

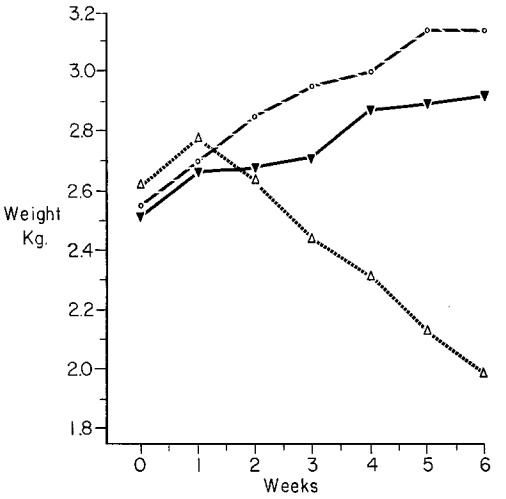


Fig. 1. Weight changes in rabbits due to cortisol (soluble and crystalline). ○ Control saline (35–5); ▾ soluble hydrocortisone 5 mg/kg (17–8); ▵ crystalline hydrocortisone 5 mg/kg (35–5)

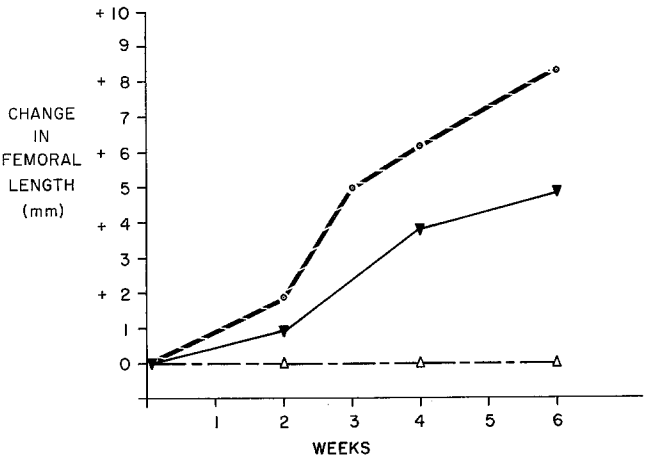


Fig. 2. Femoral length changes in rabbits due to hydrocortisone (soluble and crystalline). ⊙ Control saline; ▾ soluble hydrocortisone 5 mg/kg; ▵ crystalline hydrocortisone 5 mg/kg

increased 5.6 ± 0.8 mm in six weeks whilst *no* growth occurred in those animals which had received crystalline hydrocortisone (Fig. 2).

2. Total Diameter. Measurements of X-rays revealed the control animals increased the periosteal diameter of the femur at the midpoint of 0.32 ± 0.1 mm in six weeks, whilst those on the soluble hydrocortisone increased their femoral periosteal diameter 0.31 ± 0.1 mm in six weeks but no increase was seen in this dimension in any animal receiving crystalline hydrocortisone.

Measurements of X-rays obtained at the time of sacrifice correlated well with direct histological measurements.

Histological Measurements

The total cross sectional area and the cross sectional area of the cortex were measured at the midpoint of each femur. The ratio of the cortical bone area (C) to the total cross sectional area (T) of the femur was determined and recorded as the C/T ratio (Frost, 1969). Since there is normally a constant relationship between area of bone cortex and total cross-sectional area, the C/T ratio is a valid expression of induced changes in bone and permits comparison between animals. The C/T ratio can be utilized as an index of cortical thickness, an increasing value indicating cortical thickening and a decreasing value indicating cortical thinning. In the control group, the C/T ratio increased gradually and

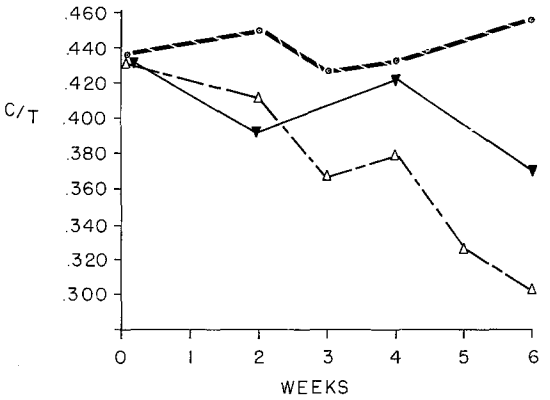
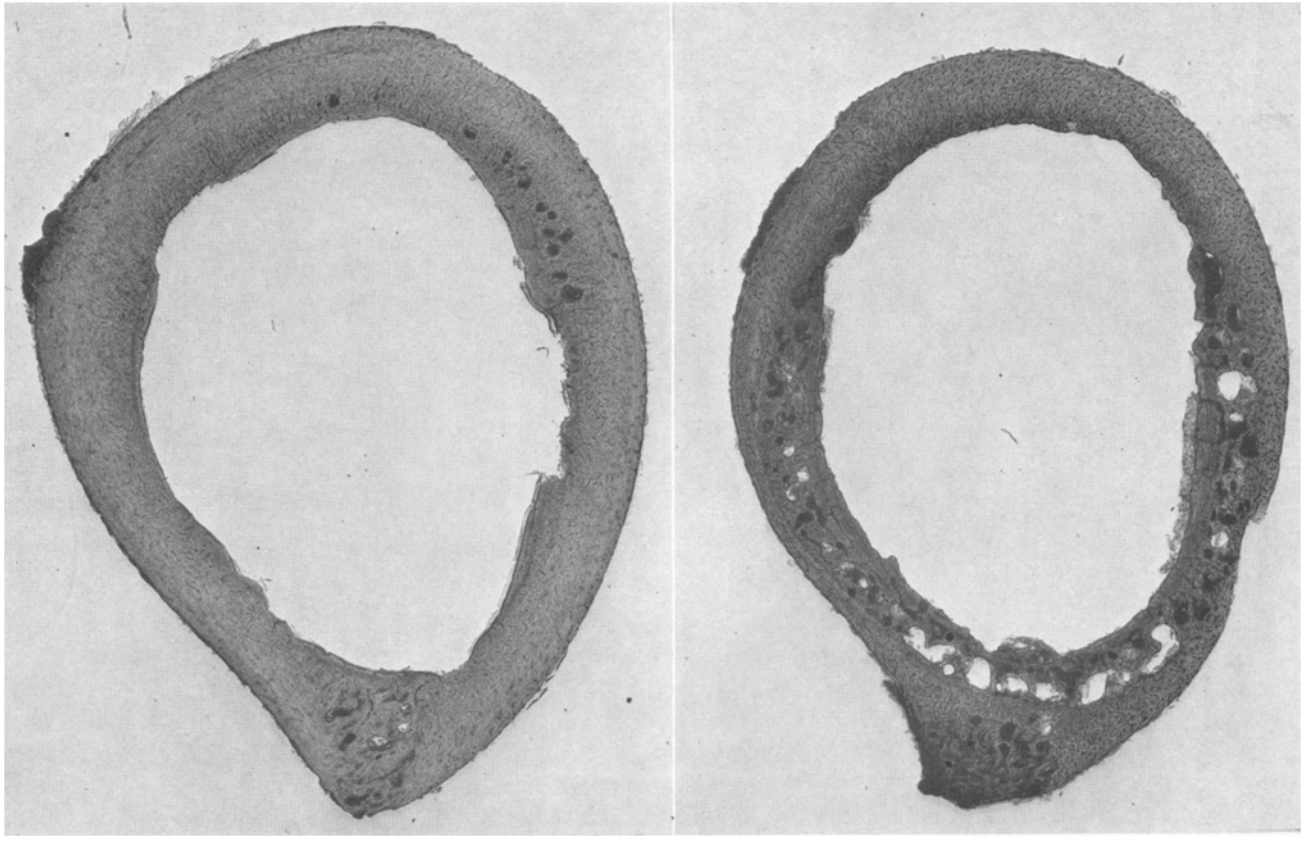


Fig. 3. Mean values of cortical and total area ratios (C/T ratio) determined from a total of 87 rabbits sacrificed at various intervals. ⊙ Control saline; • soluble hydrocortisone 5 mg/kg; ▵ crystalline hydrocortisone 5 mg/kg

slightly over six weeks from 0.435 to 0.458, as might be expected in growing animals. In the soluble hydrocortisone treated group, the C/T ratio decreased from 0.435 to 0.370, while in the crystalline hydrocortisone treated group, the C/T ratio decreased dramatically from 0.435 to 0.308 over the six week treatment period (Fig. 3).

The reason for the marked decrease in C/T ratio or cortical thinning was rapid resorption in the endosteal one third of the cortex due to a sequence of events that has been described by the authors (Hanson *et al.*, 1970). The sequence begins during the second week of treatment with the development of resorption spaces in the sub-endosteal cortex. The number and size of the resorption spaces in this area gradually increases and extends around the majority of the cortical circumference. By the end of the fourth week of treatment, the large and numerous resorption spaces begin to coalesce and undermine most of the endosteum (Fig. 4). The confluence of resorption spaces as the junction of the inner and middle one third of the cortex could be consistently appreciated on radiographs of the femur as a linear radiolucent line (Fig. 5). During the fifth and sixth weeks of treatment, the endosteal "slough" or resorption was completed, resulting in dramatic and sudden cortical thinning.



A B

Fig. 4. Cross sections of right femur of littermates after four weeks of treatment. Section A is a control animal. Section B is a crystalline hydrocortisone-treated animal, 5 mg/kg per day

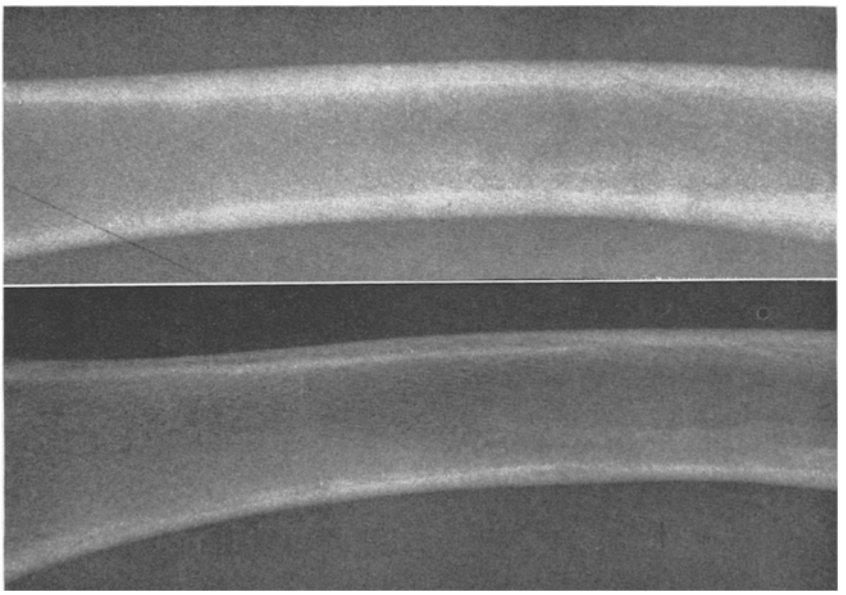


Fig. 5. Lateral X-rays of the right femur midshafts of littermates (control *above* and crystalline hydrocortisone-treated *below*) after four weeks of treatment. Note the linear radiolucent lines within the cortices. Its location corresponds to the confluence of resorption spaces seen at 4 weeks on cross-section preparation (see Fig. 4)

Tetracycline Labelling—Periosteal and Endosteal Bone Formation

Tetracycline, when given orally or parenterally, is incorporated in new bone as it calcifies. It fluoresces under ultraviolet light and can be utilized to identify sites and rates of bone formation. The initial tetracycline label was given six days prior to Day 0 and in all three groups of animals, 90–100% of the periosteal surface was labelled. In the control group and soluble hydrocortisone treated group, the label was "buried" under new bone. In the soluble hydrocortisone treated group the label was also buried, but under a lesser amount of new periosteal bone than the controls indicating that some bone formation had continued. In the crystalline hydrocortisone treated group, the original 6-week-old label was still at the periosteal surface, indicating that bone formation had been halted (or severely impaired) at the time therapy was begun.

The terminal tetracycline label, given at six and three days prior to sacrifice, was accepted by 90–100% of the periosteal surface in both the control group and soluble hydrocortisone treated group, but none was accepted in the crystalline hydrocortisone group indicating a complete absence of any bone formation at this surface.

The initial and terminal tetracycline labels at the endosteal surface were also evaluated at 4 weeks—prior to the loss of endosteal slough. The *initial* label in the controls occupied some 43.2 ± 9.0 [5] percentage of the endosteal surface

5 Standard Error.

whereas endosteal resorption had occurred in both the soluble hydrocortisone-treated group and crystalline hydrocortisone-treated group and there was remaining only $12.6 \pm 7.6\%$ and $8.4 \pm 4.5\%$ respectively at four weeks. There was almost complete failure to accept the *terminal* endosteal label in the crystalline hydrocortisone treated group. However, in the soluble hydrocortisone treated group and control group, the terminal endosteal label was present on $5.0 \pm 0.6\%$ of the surface and $9.2 \pm 2.7\%$ of the surface respectively at *six* weeks.

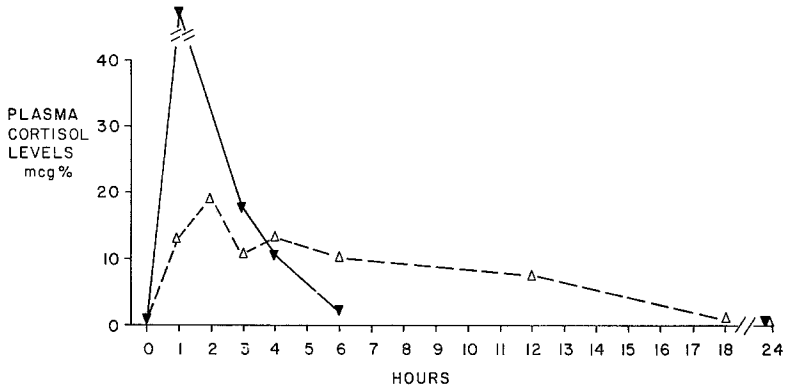


Fig. 6. Plasma cortisol levels following i.m. hydrocortisone in rabbits. ▾ Soluble hydrocortisone 5 mg/kg; ▵ crystalline hydrocortisone 5 mg/kg

Plasma Cortisol Measurements

The plasma levels of the cortisol after injection of soluble hydrocortisone (5 mg/kg) rose rapidly reached a peak[6] within 1–2 h, declined rapidly during the next 4 h and remained below measurable levels for the remaining 18 h. Following injection of crystalline hydrocortisone (5 mg/kg), the plasma cortisol level rose less dramatically but remained in a clearly elevated range for 12 h, was still present in a measurable amount at 18 h, and was below a measurable level by 24 h (Fig. 6).

Discussion

The differential effect of hydrocortisone given in the same dosage but in different physical forms (soluble and crystalline) was demonstrated and supported by a number of parameters including weight change, longitudinal bone growth, bone formation and bone resorption. Whereas both drugs qualitatively have similar effects, the effect of soluble hydrocortisone is less profound than that of crystalline hydrocortisone. As compared to the control group, the soluble hydrocortisone treated group gained less weight, had less longitudinal bone growth, had some suppression of bone formation at all surfaces and some increased bone resorption at the cortical-endosteal surface. However, the crystalline hydrocortisone treated group lost weight, ceased longitudinal bone growth, ceased bone

6 The peak values of plasma cortisol were found to be in excess of 80 μg/100 ml, but the absolute values were not determined.

formation at all surfaces and had marked bone resorption at the cortical-endosteal surface resulting in dramatic cortical thinning.

The reason for this differential effect may rest with the fact that although a single dose of soluble hydrocortisone produces a very high level of plasma cortisol rapidly, it remains circulating in measurable amounts for only 4–6 h before falling back below the measurable range. By contrast, the same dose of crystalline hydrocortisone results in sustained plasma cortisol levels for at least 12–18 h, the peak level being less than $^1/_4$ of that seen with the soluble form. The more prolonged exposure of the various systems to the elevated levels may be the reason for the more profound effect of crystalline hydrocortisone. The late stage debilitation of the animals may well have contributed to some alteration of the bone dynamics due to loss of muscle mass and consequently bone stresses, but however there was a suppression of bone formation with transient increased resorption which was determined at the second and third week before the physical changes had progressed sufficiently to alter the animals' activity. Even though the *degree* of change may have been affected by the weight loss, the *direction* was not altered. In support of this concept we have shown (in studies to be reported) that doses of 2.5 mg/kg and 1.25 mg/kg of the crystalline hydrocortisone show changes which are in excess of those which followed the use of soluble hydrocortisone in the dosage 5 mg/kg. In these studies there was no severe inanition, although initial weight loss did occur and growth was suppressed, and animals survived well beyond 16 weeks on the lower dose.

The pattern of bone resorption due to crystalline hydrocortisone suggests that the stimulus to the progenitor cells was such as to promote an abnormal response with increased number of sites of osteoclast activity developing from the subendosteal osteons. The result was that these simultaneously enlarging resorption spaces coalesced and ultimately "undermined" the old endosteum. By the fourth week there was progressive resorption of this "slough" which finally left the new endosteum much closer to the periosteal surface. Consequently the cross sectional cortical area was rapidly reduced, and this is reflected in the C/T ratio. We have also noted that in animals allowed to recover from this six week therapy, there is *no* renewal of lost cortex, although some transient "rebound" remodelling occurred at all bone surfaces.

Jee *et al.* (1972) have demonstrated that some of the corticosteroid effects (in rabbits and rats) are dependent upon the *magnitude* of the dose as well as the *derivative* of corticosteroid used. These parameters were both controlled in our studies where the only variable was the physical state of the drug. This study indicates that the *duration of the action* of the drug may be equally important. Our data suggests that whenever possible, it might be beneficial (or less detrimental) if corticosteroids were administered in single doses at intervals as great as possible, rather than multiple small doses, i.e., twice or three times per day.

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