mouse serum is the source of the non-erythropoietin stimulator of erythroid colony formation. The essential difference of this system is ability to form clones without erythropoietin, whereas in all other cases described above its presence is essential.

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INCORPORATION OF LABELED CORTISOL INTO DIFFERENT TYPES OF CONNECTIVE TISSUE

V. P. Torbenko, B. S. Kasavina, and T. V. Ukhina

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Adrenocortical hormones and, in particular, hydrocortisone have a marked effect on the state of the skeletal system and the organ of vision, in which they give rise to metabolic changes [1, 8, 12, 14]. Corticosteroids change the activity of lysosomal enzymes of bone tissue and of the tissues of the eye; activity of glycosidases in different types of connective tissue is variously changed [10]. Investigations [3, 4, 6] have shown that corticosteroids can affect lysosomal enzyme activity in the tissues of the eye, and on that basis it has been postulated that enzyme systems of connective tissue are especially sensitive to the action of hormones. It has also been shown that these tissues are target tissues for the action of cortisol [7]. No investigations into incorporation of steroid hormones into bone and cartilage could be found in the accessible literature. Meanwhile, the possibility of biochemical parallels in the tissue metabolism of the eyes and of bone and cartilage tissues can be deduced from observations showing that lesions of the skeletal system (osteogenesis imperfecta, Paget's disease, osteolathyrism, mucopolysaccharidoses, etc.) are accompanied by disturbances in the organ of vision [2, 5, 13, 15].

The object of this investigation was to study the distribution, dynamics of accumulation, and excretion of cortisol-^{3}H in the cortical and cancellous bones, costal cartilage, and also in the sclera and cornea, in order to examine the specificity of the link between these tissues and the hormone.

N. N. Priorov Central Research Institute of Traumatology and Orthopedics, Ministry of Health of the USSR, Moscow. Helmholtz Moscow Research Institute for Eye Diseases.

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TABLE 1. Incorporation of Cortisol-^{3}H (in cpm/g) into Different Types of Connective Tissue (M ± m)

Tissue	Maximal incorporation of cortisol-^{3}H	Competitive binding of hormone
Compact bone	0,85±0,12	0,31±0,07
Cancellous bone	1,41±0,01	1,30±0,09
Cartilage	1,50±0,20	1,33±0,20
Sclera	5,42±0,09	2,40±0,07
Cornea	3,62±0,10	2,10±0,05

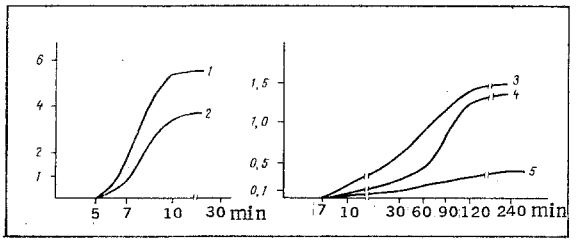

Fig. 1. Incorporation of cortisol-^{3}H into different types of connective tissue (experiments in vitro). Abscissa, incubation time; ordinate, activity (in cpm/g). 1) Sclera, 2) cornea, 3) cancellous bone, 4) cartilage, 5) cortical bone.

EXPERIMENTAL METHOD

Experiments were carried out on 45 Chinchilla rabbits weighing 1-1.5 kg and also on samples of human bone and cartilage [5]. The tests were done in vitro; the isolated tissues were incubated for 5, 7, 10, and 30 min and 1, 2, 4, 6, 20, and 24 h in medium containing 2.5×10^{-6} M cortisol-^{3}H and 0.001 M EDTA (pH 7.4) at 37°C. After incubation the tissues were homogenized in 85% formic acid solution during heating. Samples of 0.2 ml were taken from the homogenates and added to special flasks containing 5 ml of toluene-alcohol scintillator. Radioactivity was counted by means of a "Multim-212" automatic liquid scintillation counter. Accumulation of the hormone in the tissues was expressed in cpm/g wet weight of tissue.

EXPERIMENTAL RESULTS

Data on incorporation of cortisol-^{3}H into different types of connective tissue are given in Fig. 1. Cortisol-^{3}H was incorporated into cartilage and bone tissues as early as 10 min after the beginning of incubation. Incorporation of the hormone reached a maximum after incubation for 2 h. Cortisol-^{3}H was incorporated into sclera and cornea 5-7 min after the beginning of incubation, and reached a maximum for the tissues of the eye after 10 min. The intensity of incorporation was greater into the sclera than into the cornea. Cancellous bone and cartilage were similar to the cornea in the intensity of incorporation of labeled hormone. The lowest level of incorporation of cortisol was found in cortical bone.

The rate of excretion of the hormone, reflecting the degree of its binding with the tissues, was higher for the sclera and cornea than for bone and cartilage tissues.

The comparative study of incorporation of cortisol-^{3}H showed that the time of beginning of incorporation of the hormone into human cortical bone and cartilage, as in the corresponding tissues of rabbits, was 10 min. With respect to absolute incorporation of cortisol-^{3}H into these tissues, the values are a little higher in man than in the rabbit; the total time of elimination of the hormone from human tissues was longer than in rabbits. The small differences discovered do not amount to differences of principle, and it can be concluded that the

dynamics of incorporation of cortisol-^{3}H into bone and cartilage tissue is identical in man and animals.

To study the question of the presence of specific receptors for hormone binding by the tissues experiments were carried out to determine competitive binding of cortisol by bone and cartilage tissues and also by sclera and cornea. For this purpose, the isolated tissues were placed in incubation medium containing nonradioactive cortisol. Incubation lasted long enough to produce maximal incorporation of the hormone into the tissue: 60 min for bone, 120 min for cartilage, and 30 min for sclera and cornea. The tissues were then washed and incubated with cortisol-^{3}H. The results of these experiments showed (Table 1) that incorporation of labeled cortisol into the sclera and cornea was reduced, whereas into bone and cartilage tissue it was unchanged. This suggests that the sclera and cornea contain specific receptors for binding with cortisol, whereas the hormone accumulates in bone and cartilage by diffusion.

Accepting the well-known views that target tissues are characterized by rapid incorporation of the hormone, the presence of specific receptors, and changes in enzyme activity [9-11], it can be postulated that bone and cartilage tissues are not target tissues for cortisol. The sclera and cornea, which may perhaps contain specific receptors, are target tissues for this hormone. This may be one reason for the difference in the optimal therapeutic doses of hydrocortisone during the treatment of eye and bone diseases.

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