Uptake of Radiosulphur in Growing Bones of Cockerels Treated with Cortisone and 17-Ethyl-19-Nortestosterone.* (23765)

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It is apparent that cortisone exhibits catabolic effects both in birds and in mammals. Recently Hublé has shown that chondrogenesis in proliferation zones of proximal and distal ends of the 3 long bones of the cockerel's leg was inhibited by cortisone treatment (1). This author was using as criteria of hormonal effect, the histological changes induced in cartilage and bone. We were able to demonstrate that catabolic and anabolic changes in cartilage and bone of rat, may be studied satisfactorily with S^{35} labelled sulphate uptake procedure(2-6). It is well established that the selective deposition of radiosulphur in certain tissues is due to utilization of sulphate ions in the synthesis of chondroitin sulphate of connective tissue ground substance and in collagen(7,8). We showed that S^{35} uptake by the healing fractured bone in rats may be significantly inhibited by cortisone(4,5) and stimulated by certain anabolic steroids(2,3). This was interpreted that cortisone inhibited and anabolic steroids stimulated production of certain mucopolysaccharides which are essential in collagen fibrillogenesis and which specifically incorporate labelled sulphate. The same procedure was also used to investigate the catabolic effect of AT-10 (dihydrotachysterol in rats) (6). It was shown that an anabolic steroid 17-ethyl-19-nortestosterone (Nilevar)(9) counteracted the catabolic effects of both cortisone (4,5) and AT-10(6). It was apparent from these results that the hormonal effect on bone repair may be studied with radiosulphur, and that this procedure may be also practical for the study of chondrogenesis and osteogenesis.

The purpose of the present experiment was to compare effects of cortisone and 17-ethyl-19-nortestosterone on the S^{35} uptake by the 3 long bones of the young cockerel's leg. An attempt was also made to offset the catabolic effect of cortisone by simultaneous treatment of birds with 17-ethyl-19-nortestosterone.

Materials and methods. Cockerels of White Rock-Broiler strain, were obtained from a commercial hatchery when 3 days old and immediately confined to thermostatic brooders (Start-Gro) 24 x 36 inches capacity. The birds were kept in groups of 15 per brooder, and received routine chicken food with tap water *ad libitum*. Hormones were injected, beginning the sixth day of bird's life, and administered during the 3 following weeks, 4 doses the first week and 3 doses weekly for the next 2 weeks. Following 4 groups were studied: 1. Control group; no treatment. 2. Given cortisone acetate (Merck, Co.); 5 mg in 0.5 cc saline, subcutaneously. Total dosage 50 mg/bird. 3. Given 17-ethyl-19-nortestosterone (Nilevar, Searle & Co.); 5 mg in 0.2 cc of sesame oil, intramuscularly. Total dosage bird 50 mg. 4. Given both cortisone and 17-ethyl-19-nortestosterone, as Groups 2 and 3. Isotope was administered 24 hours after last dosage of hormone. Radiosulphur (S^{35} in H_2SO_4, Atomic Energy, Canada) was injected subcutaneously, one mc/kg of bird's body weight. The dose was dissolved in 5 ml of distilled water together with 40 mg of sodium sulphate(10). Immediately after sacrifice the femur, tibiotarsus and tarso-metatarsus were separated, cleaned from adhering tissues and dried at room temperature 24 hours, following which their weights were recorded. The bones were then dissolved by nitric-perchloric acid wet-digestion procedure described previously(10). The final precipitate of radioactive barium sulphate was transferred to planchettes and counting carried out using a thin mica window Tracerlab G-M tube. A Tracerlab Superscaler S.C. 18A was used for recording. Results were calculated in counts/minute (c.p.m.) per 100 mg of tissue. The changes

* Supported in part by research grant from G. D. Searle & Co., Chicago.

in body weight were also recorded and presented as percentages of gain in body weight, during the experimental period of 3 weeks.

Results. Two tables summarize our results. The gain of body weight observed in 4 groups of birds during the 3-week experimental period is recorded in Table I. It may be noted that normal control birds gain about 265% of body weight as compared with about half of this value found in cortisone-treated cockerels. Anabolic steroid Nilevar not only promotes body growth but also partially alleviates the inhibitory effect of cortisone.

Table II records the data on measurement of radioactivity of bones. Because dosage of isotope corresponded to body weights of cockerels (1 mc/kg B.W.), the recording of results in c.p.m./100 g of bone may be considered as most appropriate. It is evident that cortisone significantly reduced radiosulphur uptake in bones, as compared with normal control. The effect of cortisone is more pronounced here than the effect of this catabolic hormone on body growth.

Anabolic steroid 17-ethyl-19-nortestosterone significantly promoted the binding of labelled sulphate by the studied bones. Simultaneous treatment with both steroids offset the effect of cortisone.

Discussion. The inhibitory effect of cortisone on chondrogenesis and osteogenesis in birds, demonstrated by histological technics (1), seems also to be proved in our experimental conditions. Radiosulphur uptake procedure is apparently an adequate method for study of hormonal effect on production of mucopolysaccharides in connective tissue(11). This method shows that cortisone inhibits and

TABLE I. Effect of Various Steroids on Gain in Body Weight in Cockerels. Expressed as % of gain after 3 weeks of treatment.

Treatment	No. of birds	% increase in body wt
Control (no treatment)	15	265 ± 10*
Cortisone, 50 mg	15	140 ± 9
17-ethyl-19-nortestosterone, 50 mg	12	300 ± 12
Cortisone, 50 mg, and 17-ethyl-19-nortestosterone, 50 mg	12	188 ± 11

* Mean ± S.D.

TABLE II. Radiosulphur Uptake by 3 Long Bones (Femur, Tibiotarsus and Tarso-metatarsus) of Growing Cockerels. Expressed in cpm (count/min.) per 100 mg of bone.

Treatment	No. of birds	cpm × 100
Control	15	6.20 ± .87 * (4.70-7.93) †
Cortisone, 50 mg	15	1.98 ± .26 (1.56-2.50)
17-ethyl-19-nortestosterone, 50 mg	12	15.87 ± 3.2 (12.8-20.6)
Cortisone, 50 mg, and 17-ethyl-19-nortestosterone, 50 mg	12	10.75 ± 1.2 (8.9-12.0)

* Mean ± S.D. † Range.

17-ethyl-19-nortestosterone (Nilevar) promotes synthesis of collagen during the process of growing of long bones in young cockerels. Combined therapy protects connective tissue of growing bones against the catabolic effect of cortisone. It is apparent that Nilevar has highly anabolic property not only during the process of healing of connective tissue(2,3) but may also accelerate its growth in young animals.

Summary. 1. The effects of cortisone and 17-ethyl-19-nortestosterone (Nilevar) on radiosulphur uptake by the three long bones of the young cockerel's leg, were investigated. Cortisone diminished and Nilevar increased the binding capacity of labelled sulphate by the growing bones of birds. This was interpreted as the catabolic and anabolic effect of these hormones respectively, on synthesis of certain mucopolysaccharides of growing connective tissue, which specifically incorporate radiosulphur. 2. The anabolic steroid 17-ethyl-19-nortestosterone counteracted the catabolic effect of cortisone on growing bone, when both hormones were administered simultaneously.

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Received December 16, 1957. P.S.E.B.M., 1958, v97.

Nutrition Studies in the Cold. II. Curative Effect of Cold on Fat Fatty Livers.* (23766)

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The comparative response of rats maintained at 25° and at 1° to variations in protein and fat content of the diet has been described recently(1). Diets of a low protein or high fat content produced elevated levels of liver fat at 25° while at 1° the liver fat was within the normal range at all protein and fat levels which were employed. It has been demonstrated that in addition to the preventive effects of supplementary choline and methionine in hypolipotropic diets, these lipotropic factors have a curative effect on fat fatty livers, *i.e.*, will decrease an elevated level of liver fat due to previous ingestion of a hypolipotropic diet(3).

It became of interest to determine if cold also had a curative effect on fat fatty livers† as well as the preventive effect described recently(1). The data presented below clearly demonstrate that cold exhibits both of these characteristic effects of the other lipotropic factors.

Methods. Young male rats (50-80 g) of the Carworth strain were used. The care of the animals and the general experimental conditions were the same as described earlier(1). Three diets (Table I) which in our earlier experiments produced fatty livers at 25° were used. Choline and inositol were omitted from the vitamin mixture so that the lipotropic factors of the diets were supplied entirely by the methionine of the dietary casein. At the end of the appropriate periods, as described below, the animals were sacrificed and the livers were removed, weighed, and analyzed for total lipids essentially as described by Dury and Treadwell(4). Twenty-two or 23 rats were placed on each of the 3 diets for 21 days at 25°. At the end of this first period 7 rats on each diet were sacrificed. Those remaining on each diet were divided into 2 groups and maintained on the same diet for an additional 28 days; during this second period, one group was continued at 25°, the other group was kept at 1°. At the end of this 28-day period all rats were sacrificed and the livers analyzed for total lipids. This experimental design provided an evaluation of the curative effect of cold (level of liver lipids after 21 days at 25° compared with that after additional 28 days at 1°) and confirmation of our earlier results on the preventive

TABLE I. Experimental Diets.

Constituents	Diets, g/100 g No. 1	No. 2	No. 8
Casein*	5	10	20
Salt mixture†	5	5	5
Cellu flour	2	2	2
Starch	33	31	16
Sucrose	33	30	15
Vitamin mix‡	2	2	2
Lard	18	18	38
Cod liver oil	2	2	2

* Vitamin-free casein.
† Hubbell, Mendel, and Wakeman(5).
‡ For composition see Treadwell *et al.*(1).

* This research was supported in part by U. S. Air Force under Contract, monitored by Alaskan Air Command, Arctic Aeromedical Laboratory, Seattle, Wash.

† Several types of fatty livers have been distinguished, principally on mode of dietary production. Fat fatty liver is produced by ingestion of a diet consisting of purified protein, carbohydrate, salts, vitamins, and some easily available form of neutral fat. This type of diet supplemented with sufficient cholesterol produces the cholesterol fatty liver(2).