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CHRONIC TOXICITY OF DEXTRAN SULPHATE IN RABBITS

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The chronic toxicities of two samples of low molecular dextran sulphate from different sources were studied in rabbits. Both samples caused retardation in weight gain, cachexia and general osteoporosis with spontaneous fractures and epiphyseolyses. Heparin-treated controls were used to exclude the possibility that the toxic effects could be connected with prolonged anticoagulant treatment. It is concluded that prolonged administration of dextran sulphate causes systemic toxic effects which are not related to its anticoagulant activity.

Grönwall, Ingelman, and Mosiman (1945) showed that the toxicity of dextran sulphate declined with decreasing molecular weight. Later Walton (1954) investigated the toxicity of dextran sulphates with still lower molecular weights and found that " compounds with an average molecular weight of about 7,500 show qualitatively similar anticoagulant action to that of heparin and comparable low toxicity."

However, the chronic toxicity of such compounds has not been definitely established. In 1952 we began with investigating the effects of prolonged administration of low molecular dextran sulphate to rabbits. We regularly obtained a typical syndrome consisting of increasing weakness, spontaneous fractures of the bones and/or paresis of the legs. The doses used were 10 to 50 mg./kg. administered intravenously three to six times weekly. The syndrome described was seen in practically all animals if the treatment was extended over a sufficient period of time which varied between 4 and 12 weeks and depended on the compound and dose used. With heparin, no such symptoms could be produced with doses up to 50 mg./kg. under similar conditions.

The purpose of the present work was to describe the results of a typical experiment with low molecular dextran sulphate prepared in our laboratories and to ascertain if a sample of commercially available dextran sulphate produced for clinical use showed qualitatively similar effects.

In the two experiments recorded in this paper we used heparin-treated animals as controls to eliminate the possibility that the anticoagulant effect *per se* might influence the results. The doses used were chosen on the basis of our earlier experiments. No attempt was made to correlate the dosage with the doses used clinically for anticoagulant treatment.

MATERIALS AND METHODS

Dextran Sulphates.—In the first experiment a compound synthesized in our laboratories by Ingelman and Mårtensson was used. This preparation had an intrinsic viscosity of 0.042 at 25°. Using the light scattering method, the average molecular weight was determined by K. Granath and found to be about 3,000. The sulphur and nitrogen contents were 17.5% and less than 0.001% respectively. The substance will be referred to as dextran sulphate I. It was employed as a 1% sterile solution of the sodium salt in physiological saline, prepared immediately before use. No buffering agents were used.

In the second experiment, a British commercial dextran sulphate (Dexulate, Glaxo, Batch No. 2) was used. This preparation contained 1,000 dextran sulphate units/ml. and 1% buffering agents. It will be referred to as dextran sulphate II. [One international dextran sulphate unit is approximately equivalent in anticoagulant activity to one international unit of heparin (Ricketts, Walton, van Leuven, Birbeck, Brown, Kennedy, and Burt, 1953).]

Heparin.—In both experiments a Swedish heparin (Vitrum Lot A-54), sodium salt, containing 100 i.u./mg. was used. It was freshly prepared as a 1% sterile solution in physiological saline before use.

Experimental Animals.—Albino rabbits of the same breed and of both sexes and about five to six months old were used. The animals were housed in individual cages at room temperature. The food consisted of grain mixture, clover hay, beets and carrots. Food and water were unrestricted.

Plan of Experiments.—The drugs were administered intravenously. The animals were weighed at least once a week and examined regularly. The doses were not adjusted to animal weight changes during the course of treatment.

Experiment I.—Three groups of rabbits were injected once daily except on Saturdays and Sundays with dextran sulphate I, heparin or saline. According to earlier experience, the dose of dextran sulphate producing definite changes within four to eight weeks was found to be about 20 mg./kg. As we intended to estimate the lowest toxic dose, we started with a dose of 10 mg./kg. and later raised the doses of both dextran sulphate and heparin to 20 mg./kg.

Experiment II.—A group of five rabbits was injected once daily with dextran sulphate II. Another group of five rabbits served as controls and were injected with doses of heparin equipotent in anticoagulant activity. As we had no previous experience with dextran sulphate II and wished to produce definite changes within a reasonable time of treatment, the doses used were approximately 500 dextran sulphate units/kg. and 500 i.u. of heparin respectively. These doses corresponded to about 45 mg./kg. of dextran sulphate II and 5 mg./kg. of heparin.

Results

Experiment I.—As can be seen from Fig. 1, the dextran sulphate I group shows only slight retardation in weight gain during the first seven weeks of treatment during which 10 mg./kg. daily of dextran sulphate I was administered. The average weight gain was 0.47 kg. in the dextran sulphate group I as compared to 0.67 kg. and 0.71 kg. in the heparin and saline groups respectively.

After increasing the doses of both drugs to 20 mg./kg. daily, signs of toxicity became apparent within two weeks in the dextran sulphate group. The animals lost weight and showed gradually increasing weakness and cachexia while the control groups remained in good condition. The growth rate in the heparin group seemed to be slightly depressed as compared to the saline group.

The rabbits in the dextran sulphate I group developed paresis of the hind legs and/or spontaneous fractures. According to earlier experience, animals with such symptoms die within a few days if the treatment is continued. Therefore, these animals were killed with pentobarbitone sodium given intravenously at times indicated in Fig. 1.

At autopsy, the only gross lesions found were those in the skeletal system. Signs of increased fragility of the bones were observed in all animals of the dextran sulphate I group. Further characteristic lesions were seen near the large joints in form of epiphyseolyses and deformation of articular surfaces. Two of the

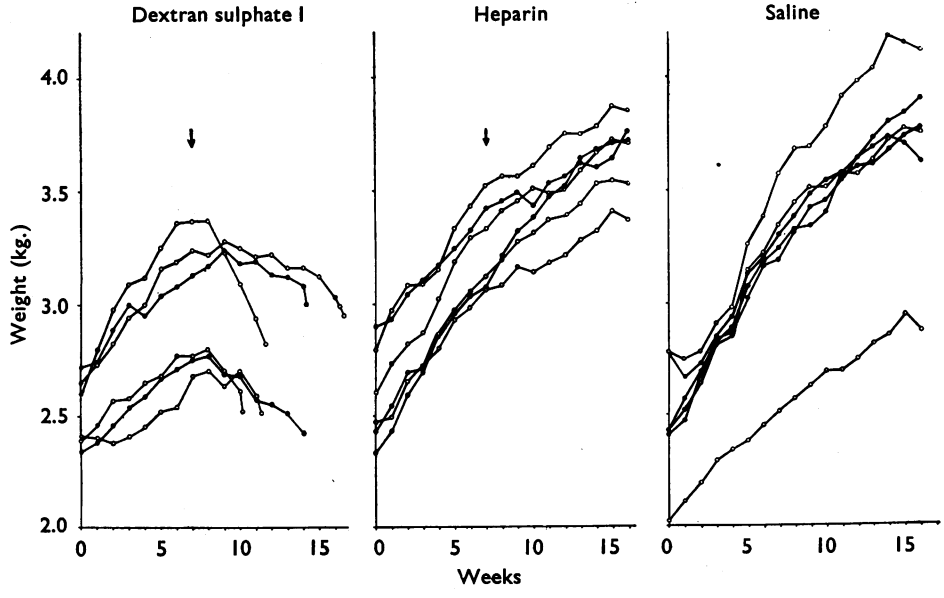

FIG. 1.—Growth curves for rabbits treated with dextran sulphate I, heparin and saline respectively. The first values represent the weight at the start of treatment. The arrows indicate the day when dosage was raised from 10 mg./kg. to 20 mg./kg. for both drugs. The injections were given i.v. daily except on Saturdays and Sundays. The last values represent the weight on the day on which the animals were killed.

rabbits had fractures of the humeral diaphysis. Five out of six rabbits in this group showed epiphyseolyses of the femur, tibio-fibula or humerus. In two animals fractures of the lumbar vertebrae were found.

Haematomas were seen only in connexion with fractures. Small haematomas were found typically around loosened parts of the compacta, the latter remaining attached to muscular insertions. Apparently, the pareses observed may be explained by the occurrence of fractures of the vertebrae and/or loosened muscular insertions as well as by epiphyseolyses.

Post mortem, *x*-ray examination of the skeleton revealed considerable thinning of the compacta, rarefaction of the spongiosa and numerous fractures in all dextran sulphate I treated animals. The rarefaction of the spongiosa was pronounced in the epiphyses of the long bones, in the pelvis and the lumbar vertebrae.

No gross lesions were found in the heparin and saline groups. The histological findings of experiments I and II will be described together.

Experiment II.—As can be seen from Fig. 2 all rabbits receiving dextran sulphate II showed retardation in weight gain. During the first three weeks of treatment this group had an average weight gain of 0.21 as compared to 0.53 kg. in the heparin group. After the third week, the dextran sulphate II group exhibited marked weight loss, while the heparin group showed a further average weight gain of 0.27 kg. during the second three weeks.

On physical examination, no signs for external or internal bleedings which primarily could be explained by decreased coagulability of the blood could be observed. From the fourth week of treatment, all animals in the dextran sulphate II group showed increasing weakness, spontaneous fractures of the bones and/or paresis of the legs. One of the animals died after the sixtieth injection. The others were killed with pentobarbitone sodium at times indicated in Fig. 2 because of pareses and spontaneous fractures.

At autopsy gross lesions identical to those described in experiment I were found. Three out of five rabbits in this group showed multiple epiphyseolyses of the humerus or femur. Two of the rabbits had fractures of the femoral diaphysis.

As in experiment I, post mortem *x*-ray examination of the skeleton disclosed considerable thinning of the compacta and rarefaction of the spongiosa. To illustrate the degree of osteoporosis, the thickness of the compacta in the femoral diaphyses was measured from roentgenograms magnified twenty times. The average thickness of the femoral compacta measured at corresponding sites was found to be 0.9 mm. in the dextran sulphate II group and 1.3 mm. in the heparin group.

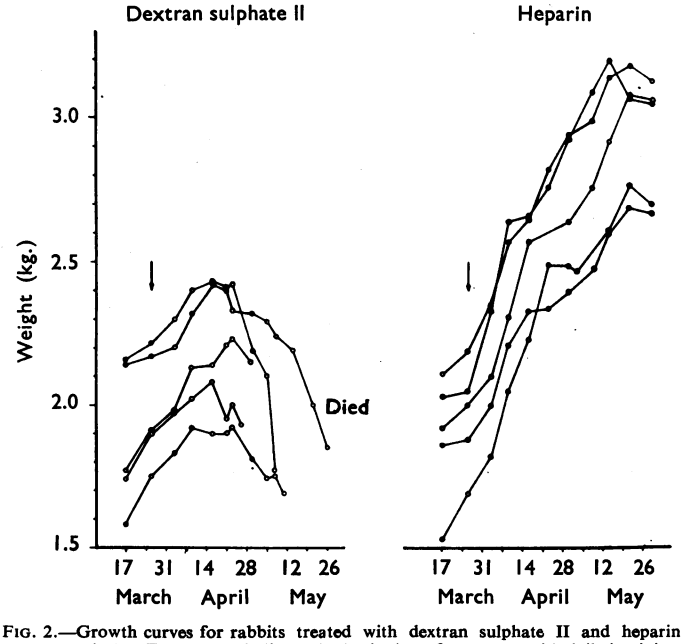

FIG. 2.—Growth curves for rabbits treated with dextran sulphate II and heparin respectively. The arrows indicate the beginning of treatment with daily i.v. injections of 500 i.u. of dextran sulphate and with 500 i.u. of heparin respectively. The last values represent the weight on the day on which the animals were killed.

Histopathological Findings.—No constant histopathological findings were found in the liver, kidneys, spleen, and adrenals of the animals treated with dextran sulphates when compared with the controls. The histopathological study of decalcified slices from costochondral junctions showed generalized bone absorption. The number of osteoblasts was diminished while osteoclasts were present in great numbers. The bone trabeculae in the growth zone were thin and diminished in number. They often appeared devoid of the osteoblastic layer, the latter being replaced by an almost continuous seam of osteoclasts.

In the arteries of the lungs, another typical lesion was seen fairly constantly. The lumina of

the arteries were often wholly or partially occluded with fat and/or haemopoietic tissue and occasionally contained small fragments of bone or cartilage. These lesions are probably embolic in nature and secondary to osteoporosis and fractures.

Discussion

Walton (1954) concluded that the toxicity of large molecular dextran sulphates depends on the interaction of these compounds with plasma proteins and the formed elements of the blood. The substances used by us are of such a low molecular size that, according to the results of Walton (1954), no interaction with blood components can be expected. Therefore the findings described in this paper appear to have a different pathogenesis.

The location of the principal lesions in the skeleton and especially in the epiphyseal growth zones suggests that dextran sulphate may be deposited at these sites. However, such deposition has as yet not been demonstrated.

The interpretation of the pathogenesis of the osteoporosis described lies beyond the scope of this work, but as a working hypothesis we assume that dextran sulphate might act by competition with naturally occurring sulphated polysaccharides in bone and cartilage and in this way might interfere with normal bone metabolism.

It is evident from the growth curves that the growth rate was depressed long before spontaneous fractures occurred. Therefore, the cachexia observed is not necessarily the result of osteoporosis or of trauma caused by fractures. Possibly, the cachexia may indicate more general systemic effects not demonstrable anatomically.

In spite of the fact that, in experiment I, 1,000 to 2,000 i.u./kg. of heparin was used, no comparable chronic toxic effects were noticed. It is therefore concluded that dextran sulphate exhibits systemic toxic effects which are not related to its anticoagulant activity.

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