EFFECT OF THE ORGANOPHOSPHORUS COMPOUND 307 ON REPARATIVE OSTEOGENESIS IN DOGS WITH EXPERIMENTAL FRACTURES

U. Ya. Bogdanovich, D. A. Veselovskii, I. M. Spektor, and I. L. Shulutko

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The theoretical basis for the clinical application of anticholinesterase drugs is continually being widened [5]. Reports have been published indicating that acetylcholine and certain anticholinesterase preparations stimulate regeneration and reparation osteogenesis after experimental fractures [2, 4, 14].

The object of the present investigation was to study the healing of fractures in experimental animals receiving the organophosphorus compound 307.

Compound 307—the dimethyl ester of α-acetoxy-β, β, β-trichloroethylphosphinic acid—was synthesized by K. V. Nikonorov [11]. Investigations by I. D. Neklesova and co-workers [9, 10] demonstrated its low toxicity for warm-blooded animals and, at the same time, its high anticholinesterase activity.

EXPERIMENTAL METHOD

Experiments were carried out on 42 adult dogs. In aseptic conditions and under general anesthesia a transverse osteotomy of the femur was performed in the middle third of the diaphysis; the fragments were fixed by a Bogdanov intramedullary metal rod. The wound was closed in layers with catgut and a spirit dressing applied. No additional immobilization was used.

In the experiments of control series I (18 dogs) the animals received no preparations. In series II (24 dogs), after the operation the animals received subcutaneous injections, at intervals of 1-2 days, of a 10% oily solution of preparation 307 in doses of between 3 and 100 mg/kg (in the course of the experiment the optimal dose of the compound was chosen). Altogether from 7 to 20 injections were given.

The process of healing of the fracture was studied roentgenologically and histologically. In the animals of series II, in addition, the serum cholinesterase activity was determined by Hestrin's method [17] before and after injection of the preparation, the serum sialic acid concentration was determined before and 1 h after its injection by the method described by Hess and co-workers [16], changes in the absolute serum protein content were investigated, changes in the ratio between the protein fractions were studied by electrophoresis, and a general analysis of the blood was made periodically.

The blood acetylcholine concentration was determined in the animals before and 1 h after injection of compound 307 by the following method (I. M. Spektor).* Into each of two centrifuge tubes 0.03 ml heparin was poured and to one of them 1 ml of 0.15% neostigmine solution in physiological saline was added. Blood from a vein was collected in each tube in a volume of 3 ml, with shaking. After 15-20 min, 1 ml of 0.15% neostigmine solution was added to the tube not previously containing it. Next, 4 ml of 10% trichloroacetic acid was added to each tube and the contents were filtered. To 3 ml of filtrate, 6 ml of alkaline hydroxylamine was added, followed 2-3 min later by 3 ml of 1:2 hydrochloric acid, and then by 3 ml of 0.37 M ferric chloride. Colorimetry was then performed on the FÉK-M apparatus with a working layer 30 mm in thickness (green filter). Extinction of the second sample was subtracted from that of the first sample. The difference obtained was converted into gravimetric units of acetylcholine by means of a calibration graph.

*This method is published for the first time.

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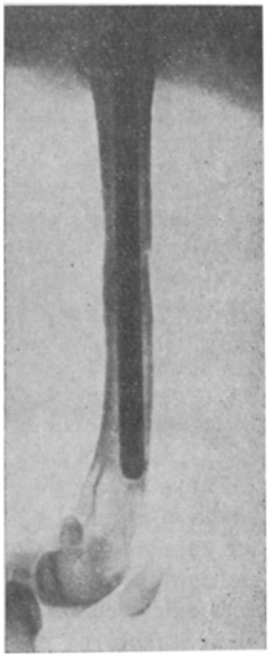

Fig. 1. Roentgenogram of dog No. 3 (group 1) one month after operation. Fracture uniting by "first intention."

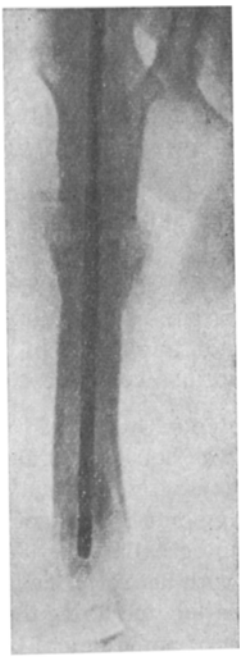

Fig. 2. Roentgenogram of dog No. 2 (group 3) four months after operation. Pseudarthrosis.

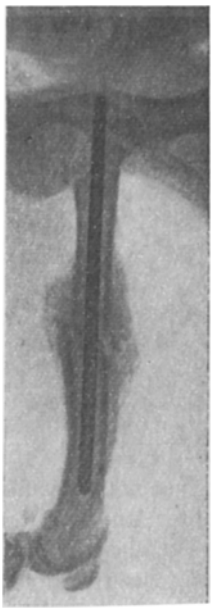

Fig. 3. Roentgenogram of dog No. 15 (control) two months after operation. Well marked periosteal reaction, no consolidation present.

EXPERIMENTAL RESULTS

A single subcutaneous injection of compound 307 in the doses used lowered the serum cholinesterase activity on the average by 44.4% ($P < 0.001$). The activity returned to its initial level after 1-2 days, and an interval of 1-2 days was therefore selected for injection of the preparation. When repeated injections of the compound were given, the serum cholinesterase activity remained permanently lowered.

The acetylcholine concentration in the blood of the animals was increased 1 h after injection of the preparation on the average by 43% in all the experiments except four: its value rose from 4 μg/ml before injection of the preparation to 7 μg/ml 1 h after injection ($P < 0.02$).

During repeated injection of compound 307 the serum sialic acid concentration rose simultaneously with the decrease in cholinesterase activity, and this served as an indirect index of mucoprotein and mucopolysaccharide metabolism, regarded as playing an exceptionally important role in osteogenesis [13].

In the postoperative period a significant decrease in the relative weight of the albumin fraction of the blood proteins and an increase in the fraction of α_1- with α_2-globulins, consisting partly of mucoproteins [12], took place in agreement with the results of investigation of the sialic acid level.

Many authors have reported a decrease in the absolute content of blood proteins after a fracture [1, 8], and this was confirmed by our experiments.

General analysis of the blood, repeated at intervals, showed that the changes in the blood observed after operation were of the usual type observed after operations of the character of osteosynthesis using metal rods [3].

Analysis of the roentgenograms and histologic preparations showed that the patterns of fracture healing obtained depended on the magnitude of the single and total dose of the compound.

Depending on the results of the experiments, the animals in series II could be subdivided into three groups.

Group 1 (8 Dogs). The compound was injected in a dose of 3-60 mg/kg per injection 7-10 times in the course of 17-30 days. In 7 dogs of this group the fracture united by endosteal callus within 60-90 days (Fig. 1). Well marked osteogenesis was observed histologically in the fracture region, resulting in primary union of the bone [7]. One dog (No. 36) was lost on the 31st day. The serum cholinesterase activity in the animals of this group was lowered on the average by 75%.

Group 2 (12 Dogs). The compound was injected also in a dose of 3-60 mg/kg per injection, but the number of injections was increased to 20, and the period of injection of the compound to 31-46 days. In 11 dogs of this group the fracture healed within 90-133 days. The fracture in one dog (No. 31) did not unite because the metal rod was removed prematurely. Morphologically the process of reparative osteogenesis developed more slowly in the animals of this group, with a more marked periosteal reaction than in the animals of group 1. As a result of prolonged administration of compound 307, a considerable fall was observed in the serum cholinesterase activity (on the average by 67%).

Group 3 (4 Dogs). Compound 307 was injected in a dose of 60-100 mg/kg per injection over a period of 30-45 days; the total number of injections was between 10 and 12. In three dogs of this group the fracture had not united after 138 days. Roentgenologically, absorption of bone tissue was observed along the fracture line, and the diameter of the bone fragments in contact was increased in the shape of a funnel because of the periosteal reaction (Fig. 2). Histologically the wide lumen of the Haversian canals could be seen at the fracture site, many of them filled with necrotic masses; many areas of "melting" [6] bone trabeculae and with numerous osteoclasts were located along the fracture line. The histologic picture described above is suggestive of the development of pathological bone formation due to general factors rather than to local conditions [15]. The serum cholinesterase activity in the dogs with ununited fractures was lowered on the average by 68%.

Roentgenograms of the animals of the control series of experiments showed considerable outgrowth from the periosteum, and by the 3rd month the intensity of their shadows was close to that of bone tissue (Fig. 3). Histologically the bone in the region of the fracture was indistinguishable in structure 6 months after operation from the rest of the bone.

Hence, when compound 307 was injected subcutaneously in doses of between 3 and 60 mg/kg for a period of 3-4 weeks from the day of fracture (7-10 injections), primary healing of a fraction of the femur in dogs was observed 60-90 days after the operation of internal fixation with a metal rod. Injection of the preparation in the same doses but for a longer period—up to 46 days (11-20 injections), and also its administration in doses of 60-100 mg/kg, led as a rule to delay of repair, and to the formation of the typical roentgenological and morphological picture of a pseudarthrosis at the fracture site. In these circumstances the serum cholinesterase activity was considerably lowered.

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