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Antihemophilic Factor (AHF): Plasma Levels After Administration of AHF Preparations to Hemophilic Dogs.* (23417)

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Intravenous administration of plasma fractions rich in antihemophilic factor (AHF) has been shown to be followed by a rapid rise in the plasma level of AHF. In normal and hemophilic dogs, half of the plasma AHF was lost in 2-6 hours(1,2). A similar rise and fall in AHF was observed in human hemophiliacs following injections of large volumes of plasma(2) or bovine AHF fractions(3). Recently it was shown that high plasma AHF levels occurred following intravenous injection of a human plasma fraction(4). Similar quantitative studies on other routes of administration of AHF are not available, although many years ago it was observed(5,6) that the clotting time in hemophilia was shortened after intramuscular injections of a plasma globulin substance. Other workers (7,8) indicated subsequently that intramuscular injections were both ineffective and dangerous. Data presented in this paper demonstrate the relative effectiveness of various routes of administration of a plasma AHF fraction to hemophilic dogs. After each injection, plasma tides of AHF were determined. A preliminary report of some of these data was given earlier(9).

Materials and methods. Lyophilized bovine AHF was prepared by a modification of Bidwell's procedure(10). On the basis of protein and AHF determinations the prepara-

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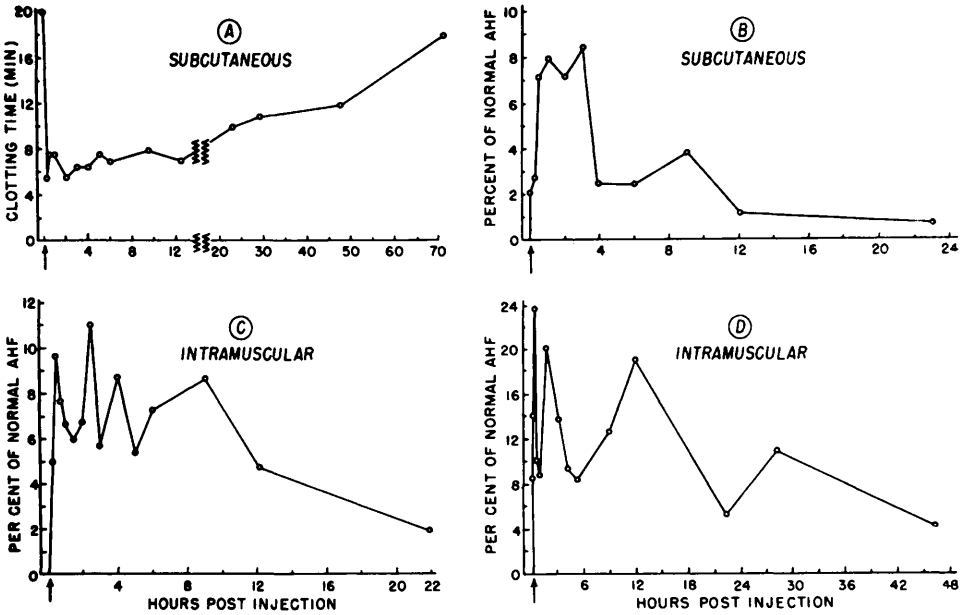

FIG. 1. AHF levels following injections of AHF preparations. *(A) Subcut.:* 3.5 kg female hemophilic dog was given 19 ml AHF preparation dissolved in citrate-saline solution, 27 times the activity of dog plasma, in 4 equal doses, each thigh. Inj. time 10 min. *(B) Subcut.:* 11.9 kg female hemophilic dog was given 25 ml AHF preparation dissolved in 0.11 M citrate, 19 times the activity of dog plasma, in 4 equal doses, each thigh. Inj. time 4 min. Pre-inj. clotting time 38 min. *(C) Intramusc.:* 3.5 kg female hemophilic dog given 19 ml AHF preparation dissolved in citrate-saline solution, 25 times the activity of dog plasma, in 4 equal doses, each thigh. Inj. time 12 min. Pre-inj. clotting time 26 min. *(D) Intramusc.:* 13.2 kg female hemophilic dog given 62 ml AHF preparation, dissolved in 0.11 M citrate, 15 times the activity of dog plasma, intramusc. Inj. time 11 min.

tions were found to be about 50 times as concentrated as the initial plasma. The dry powder was reconstituted either in *citrate-saline* solution (0.02 M sodium citrate, 0.127 M NaCl) or in *sodium citrate* (0.11 M). AHF activity of the solutions used for injection was 4-27 times greater than normal canine plasma. AHF was injected into hemophilic dogs(11) by subcutaneous, intramuscular, or intravenous routes, and the plasma AHF was assayed periodically(12). Pooled plasma from 3 normal dogs served as the control (100% AHF) in the assays. Whole blood clotting times were done by a modified Lee-White procedure. Normal values were 5-7 min.

Results. Subcutaneous injection. Results of 2 experiments are given. In the first, AHF was injected as a solution in citrate-saline, in the second, as a solution in strong citrate. The details of the first experiment are indicated in Fig. 1A. The dose injected was calculated to be sufficient to raise the plasma AHF to 200% of the normal dog level had it been given intravenously. Actually, the AHF did not reach assayable levels (about 1-3%) at any time during the experiment. Sufficient circulating AHF was present, however, to maintain clotting time at a normal level for many hours. The injection sites became firm but not discolored within an hour after the injections and remained so for 24 hours. In the second experiment (Fig. 1B), the dose, if given intravenously, should have raised the plasma AHF to 80%. Five min after injection AHF was detected in the plasma. The values rose to 7-8% and remained there for 2½ hours. Clotting times, not shown on the figure, were reduced to the normal range for 29 hours. Injection sites remained pliable.

Intramuscular injection. Results of 2 experiments, similar in design to the subcutaneous injections presented above, are shown in Fig. 1. In the first experiment (Fig. 1C),

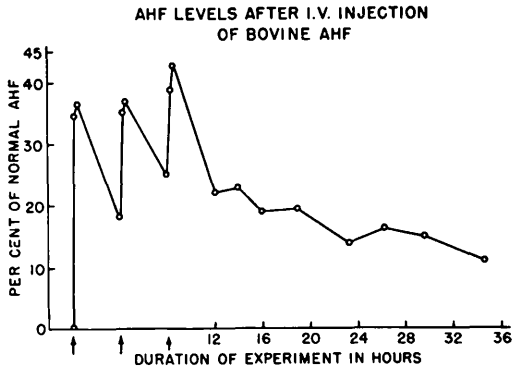

FIG. 2. AHF levels following repeated, closely-spaced intrav. injections of AHF fractions. 12.6 kg female hemophilic dog was given a priming dose of 58 ml AHF in citrate solution, 4 times as active as dog plasma at 0 time. 29 ml doses inj. at 4 and 8 hr respectively. Pre-inj. clotting time 31 min.

the AHF was injected as a solution in citrate-saline; the same dose, if injected intravenously, should have raised the AHF to 200%. Promptly after intramuscular injection, AHF appeared in the plasma; the highest level, 11%, was reached 2½ hours after injection. Clotting times were reduced and remained within the normal range for 2 days. The injection sites became firm. In the second experiment of this group (Fig. 1D), AHF was injected as a solution in strong citrate; the dose if injected intravenously should have raised the AHF to 80%. As before, the AHF appeared promptly in the plasma, but it reached higher levels than in any of the previous experiments. Measurable amounts were still present 46 hours after injection. Clotting times remained normal for the same period. The injection sites in this experiment remained pliable.

Closely-spaced intravenous injections. A series of experiments was performed in which the fall-off in plasma AHF was followed after moderate levels had been maintained for several hours. The results of one experiment are given in Fig. 2. There was good correlation between expected and observed plasma AHF values after each injection. The doses of AHF administered were expected to raise the plasma levels to 37, 36, and 44% respectively; observed peak levels after each injection were 37, 37, and 43%. It will be noted that plasma AHF was maintained at or above the 18% level for 12 hours. After each injection, the decline in AHF was rapid with the level dropping to about one-half the peak value in 4 hours. In contrast to the rapid fall observed in the first part of the experiment, the decline was relatively slow in the last part. Approximately 23 hours elapsed while the AHF fell from 22% to 11%.

Discussion. In comparing the relative efficacy of subcutaneous and intramuscular injection of AHF, it was observed that in either case the factor appeared promptly in the plasma. However, plasma AHF was maintained at higher levels and for longer times after intramuscular injection. It appears likely that the firmness of the injection sites, noted when weak citrate solutions of AHF were used, was due to local clotting of fibrinogen in the bovine AHF fractions. Impaired absorption as well as local destruction of AHF by thrombin(13) may well have occurred in these experiments. Better results were obtained when strong citrate solutions of AHF were used, despite the fact that the doses employed were only 40% as large as those in weak citrate. It has been suggested(1) that in hemophilia "an AHF level consistently maintained somewhat above 5% will allow sufficient functioning of the hemostatic apparatus to control hemorrhage in many instances." It will be noted that AHF levels in this range were reached and maintained for varying periods of time in 3 of the 4 experiments shown in Fig. 1. With potent preparations of AHF, these modes of administration appear feasible and perhaps desirable.

AHF may appear in relatively large amounts in the plasma after extravascular injection. The reverse situation is to be expected: in time, some intravenously injected AHF should be redistributed to the extravascular spaces. This process may have been responsible, in part, for the different rates of disappearance of plasma AHF observed in the course of experiments of the type illustrated by Fig. 2. The rapid disappearance of AHF from the peak levels attained immediately after intravenous injection accords with previous observations in this laboratory(1,2) and could be mainly a manifestation of redistribution. Contrasted with this is the slow disap-

pearance of AHF after time had been available for equilibration—22% to 11% in 23 hours. Depending on the extent to which equilibration between plasma and extravascular spaces had actually taken place, the AHF half-life would appear to be at least 23 hours.

Summary. 1. The plasma tide of AHF was measured after subcutaneous and intramuscular injections of bovine AHF fractions into hemophilic dogs. Better results were obtained with intramuscular than with subcutaneous administration; higher plasma AHF levels were maintained for longer times. Citrate in adequate concentration in the AHF solutions appeared to have an adjuvant action. 2. The rate of fall off of plasma AHF was followed after moderate levels of this clotting factor had been maintained for several hours. It is suggested that the biologic half-life of AHF in the dog may be at least 23 hours.

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Delayed Nidation in the Rat Induced by Progesterone.* (23418)

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Delayed implantation of blastocysts occurs in many diverse forms of mammals such as the roe deer, armadillo, bear, and many of the Mustelidae(4), and in the laboratory rat and mouse if the female is nursing a sufficient number of young while pregnant(3,4). Numerous reports have been published describing experiments done in an attempt to prevent or shorten delay under these conditions, but relatively little investigation has been done to artificially delay nidation in non-lactating females. The object of these studies was to determine whether nidation could be delayed by altering the normal hormonal relationship during early pregnancy in the laboratory rat.

Materials and methods. Mature female rats of the Sprague-Dawley strain were caged with mature males and the day of insemination determined by the presence of sperm in vaginal smears taken between 8:00 and 10:30 a.m. daily. The day that sperm were found was designated as Day 1 of pregnancy. The rats were maintained on Rockland rat diet and tap water. Surgical procedures were done under ether anesthesia and semi-sterile conditions. Laparotomies and ovariectomies were performed through dorso-lateral in-

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