leucocyte and differential counts were normal with the exception of a slight relative and absolute decrease in the monocytes.

The rabbit which was used as a control showed the differential normal throughout the immediate reaction period. On the next day, however, the total leucocyte count was increased with an increase in neutrophiles, but the characteristic basophilia did not occur. From this time the total leucocyte and differential counts were normal. This temporary rise in the leucocyte count was probably due to an infection, following thrombosis of the lateral vein of the left ear.

## 4313

## Duration of Anticoagulant Action of Heparin in Vivo in Relation to Dosage.

PAUL GROSS.* (Introduced by E. E. Ecker.)

*From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio.*

Reed[1] determined experimentally the "minimum dependable dose" of heparin as an anticoagulant. Takuji Shinoya,[2] upon injecting heparin intravenously into dogs, commented on the rapidity with which the coagulation time increased and then returned to normal. In the following experiments determinations were made of the rate of appearance and disappearance of the anticoagulant action of heparin *in vivo*.

Heparin was injected in varying amounts into the ear veins of rabbits, and the blood was collected from cut veins of the opposite ears. One method of determining clotting time which was used is as follows: Drops of blood were collected at intervals of 20 seconds or more upon glass slides cleaned with alcohol and ether. The time when each drop fell upon the slide was noted. Only blood which exuded and dropped readily was used. The blood was termed clotted when, upon being moved with a wire loop, clear or nearly clear serum was left behind. The slides were kept in a moist chamber at room temperature. A simpler method, used in the later series of experiments, is the capillary tube method. These 2 methods were

* Crile Research Fellow in Pathology.

[1] Reed, C. I., *Am. J. Physiol.*, 1925, lxxiv, 79.

[2] Shinoya, T., *J. Exp. Med.*, 1927, xlvi, 21.

used interchangeably without variations in results. The data of such an experiment are given in Table I.

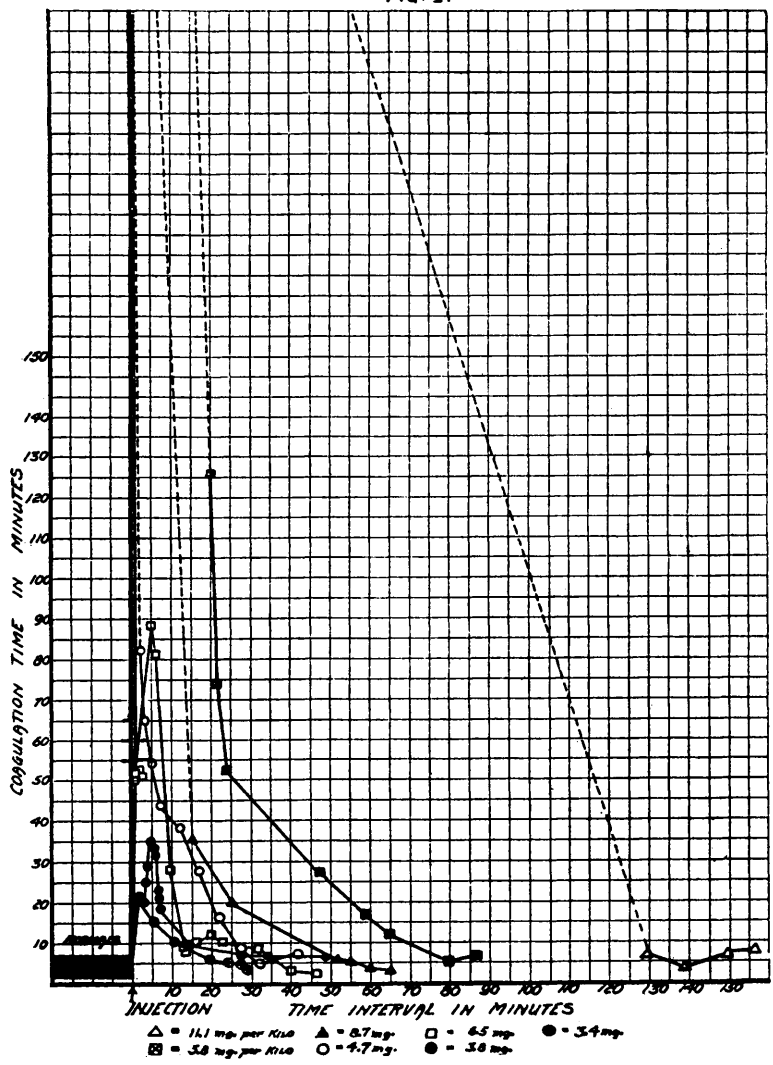
TABLE I.—*Anticoagulant Effect of Heparin In Vivo.*
Intravenous injection of 10 mg. of heparin into a rabbit weighing 1725 gm.

| Time | 11:54 | 11:55 | 12:09 | 12:10 | 12:22 | 12:22 | [illegible]:38 | 12:42 | 12:44 | 12:46 | 1:19 | 1:20 | 1:27 | 1:42 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Clotting Time in minutes | 5½ | 6 | 5½ | 6½ | Inject 10mg. hep'n | No clotting in 4 hours | | 126 | 74 | 52 | 27½ | 17 | 12 | 5 |

The clotting time was increased to more than 4 hours as soon as 30 seconds after the injection of heparin. Within 20 minutes after the injection, the clotting time began to return toward normal. The normal clotting time was reached within one and a half hours from the time of injection.

Upon plotting a number of curves from data similar to that in Table I, but for varying dosages of heparin, a similarity in the configuration of the different curves is observed (see Fig. 1).

FIG. I.



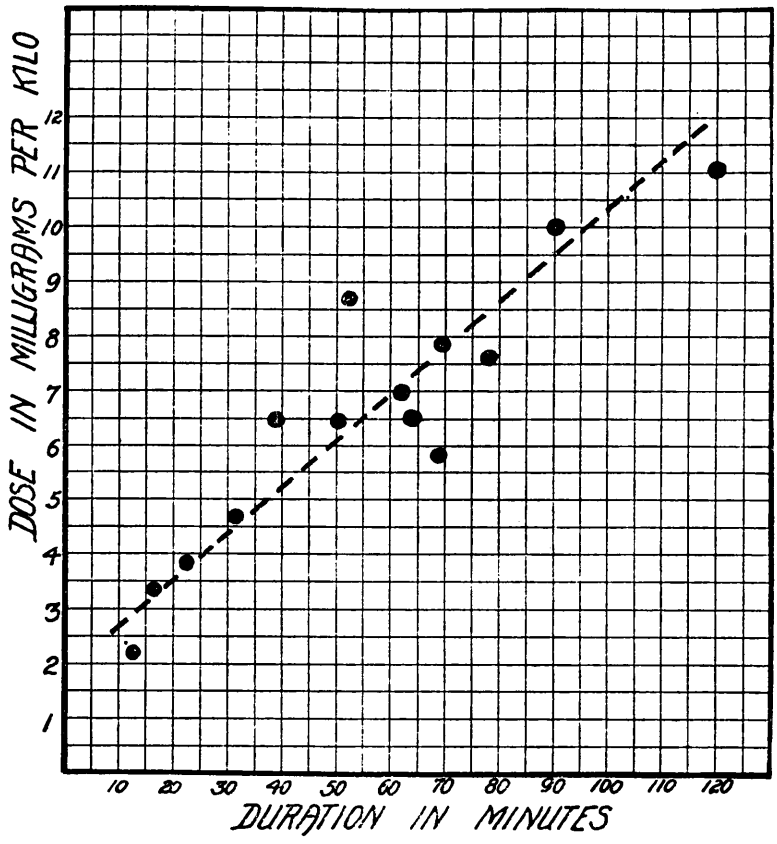

ANTICOAGULANT EFFECT OF HEPARIN IN VIVO

The interrupted lines connect points of definite coagulation time with points where the blood was last found to be incoagulable. The broad line labelled *normals* represents the range of variability in

the normal coagulation time of different animals taken for periods as long as 2 hours.

If the duration of the heparin action be plotted against the dosage employed, a representation such as in Fig. 2 is obtained. The moment at which the clotting time fell to within 2 minutes of the normal, was arbitrarily designated as the end of the heparin effect.

FIG. II




RELATION OF DOSE OF HEPARIN TO DURATION OF ITS EFFECT

*Summary:* 1. The intravenous injection of heparin is followed by an immediate increase in clotting time. Expressed graphically, this rise is practically perpendicular. The rate of return toward the normal clotting time is very rapid for one third of the duration of

the heparin action, and subsequently, more slowly. 2. Graphic expressions, for different dosages of heparin, of the changes in clotting time are similar. 3. In general, there is an approximate relationship, as expressed by the graph, between the dose of heparin per kilo of body weight and the duration of the heparin effect in rabbits.

## 4314

## Blood Phosphates in Milk Fever.

PIERRE A. FISH.

*From the Laboratory of Veterinary Physiology, Cornell University, Ithaca, N. Y.*

The work of Little and Wright,[1] confirmed in our laboratory,[2] and the work of Dryerre and Greig[3] have shown that there is a marked diminution in the amount of blood calcium in cows "down" with milk fever. It occurred to the writer that since calcium and the phosphates are intimately associated in many of the processes of body metabolism, the phosphates of the blood might also be affected.

Work along this line was begun in September, 1927, and, up to this time, 18 cases of milk fever have been tested. The results have been uniform in showing a subnormal amount of the inorganic phosphate and acid soluble phosphorus by the Fiske-Subbarow method prior to the inflation of the udder. In another investigation, we have found the average for the inorganic phosphates in normal milk cows to be 4.74 mg. per 100 cc. of serum, and for dry cows 6.45 mg.-100. The average of the acid soluble phosphorus for the milk cows was 7.95 mg.-100 and for the dry cows 9.23 mg.-100. The average of the inorganic phosphorus in the dry cows was 50% higher than that of the milk cows, and the acid soluble phosphorus was likewise higher, 16%, than in the milk cows. The calcium content was found to be the same in both groups of animals.

The accepted remedy for milk fever is inflation of the udder. The effects are usually rapid, the animal recovering from its collapse or coma, and standing on its feet again, often within a period of 6-8 hours. The first blood sample was always taken just prior to udder inflation and the second sample, and occasionally a third sample.

[1] Little and Wright, *Brit. J. Exp. Path.*, 1925, vi, 129.

[2] Fish, P. A., *J. Am. Vet. Med. Assn.*, 1928, lxxiii.

[3] Dryerre, H., and Greig, R., *Dumfries and Galloway Vet. Med. Assn.*, July 7, 1928.