

PHOSPHATE CONTAMINATION OF COMMERCIAL HEPARIN

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(RECEIVED FOR PUBLICATION OCTOBER 8, 1954)

Heparin is widely used as an anticoagulant in biochemical laboratories. Hawk, Oser, and Summerson (1947) state that heparin is an excellent anticoagulant, as it does not alter the distribution of diffusible substances between cells and plasma. Jorpes (1946) states that heparin neither influences the volume of the blood corpuscles nor interferes with the chemical analysis. In the course of routine estimations of plasma inorganic phosphorus (P), it was noted that the heparin used was heavily contaminated with phosphate and was responsible for erroneously high values. We have not been able to find any report suggesting that heparinized blood is not suitable for the estimation of phosphate.

Materials and Methods

Phosphate Estimations.—These were carried out, using the method of Fiske and Subbarow (1925).

A crude preparation of heparin is normally used in this laboratory for the preparation of heparinized blood tubes. This preparation consists of short-chain molecules which are a by-product of the penultimate stage in the manufacture of therapeutic heparin. The preparation is equivalent to 30 units heparin per mg. For routine use 0.1 ml. of a solution of 5 g./165 ml. of this preparation is added to each 10 ml. blood tube and the tubes are heated in an oven at 37° C. to dryness. The pure preparation of therapeutic heparin made by the same manufacturer, which contains 99.8 units per mg., was also used. For purposes of comparison the five other commercial preparations of heparin available in the British Isles were obtained, using the solution of 1,000 units per ml.

Results

TABLE

Heparinized Plasma (mg. P/100 ml.)	Serum (mg. P 100 ml.)
7.7	4.0
6.3	3.8
8.1	3.4
7.7	3.8
5.4	3.7
7.3	4.2
5.9	3.7

It was consistently found that heparinized plasma had a higher P content than serum. The table shows a number of typical values.

The heparin solution was shown to be heavily contaminated with phosphate.

Discussion

An attempt was made to introduce a correction factor for the amount of phosphate contamination, but this was abandoned because (1) it would necessitate the accurate measurement of 0.1 ml. heparin solution by the unskilled women who prepare the tubes for blood; (2) the amount of blood added from the syringe in the ward could not be accurately measured without a great deal of difficulty, as it would entail the cooperation of the large number of doctors, students, and nurses who withdraw blood samples; (3) it appeared likely that the amount of the contamination might vary from batch to batch of heparin. It was considered that more reliable results would be obtained if estimations of P were carried out on serum rather than on heparinized plasma.

The therapeutic heparin preparation of the firm which supplied our heparin, and all the other preparations of heparin available, were shown to contain traces of P. These are not ordinarily used in this laboratory because of their high cost.

Four of the firms which manufacture heparin have informed us that they use phosphoric acid in the process, but in most cases steps to remove it are carried out. Nevertheless it seems very likely that at least traces of P may remain in the refined product. One firm which does not use phosphoric acid has informed us that during the autolysis of the starting tissue ammonia is added, and that ammonium magnesium phosphate is formed from magnesium and phosphorus present in the tissue. This tends to adhere to their stainless steel containers. They have also noticed that some batches of charcoal used in their purification process are contaminated with phosphate. Traces of P have

been found in their preparation. In either of these manufacturing processes P is likely to be present as a contaminant, and the actual amount may vary from batch to batch.

In these circumstances it seems highly undesirable to add heparin as an anticoagulant to blood on which an estimation of inorganic phosphorus is to be carried out.

Summary

Phosphorus has been found as a contaminant in heparin which was being used as an anticoagulant in a routine biochemical laboratory.

The processes employed in the commercial preparation of heparin are likely to lead to the contamination of the finished product with phosphate.

One of us (M. G. McG.) wishes to acknowledge the receipt of a personal grant from the Medical Research Council, and one (E. M.) a grant from the Research Fund of the Queen's University of Belfast.

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