LVI. A METHOD FOR THE PREPARATION AND RECRYSTALLISATION OF OXYHAEMOGLOBIN.

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Many methods for the preparation of oxyhaemoglobin in crystalline form have been described. In most of these the corpuscles, after having been separated from the serum, are laked by the addition of ether or of varying amounts of ether and alcohol, and from the solutions so obtained oxyhaemoglobin crystals are more or less readily deposited. These crystals, when blood yielding easily crystallisable oxyhaemoglobin is employed, are well formed and have the appearance of a pure substance. They are recrystallised by dissolving in water and adding alcohol or ammonium sulphate in appropriate quantities. But, although the crystals appear to be homogeneous, the analyses of oxyhaemoglobin prepared by these methods, even from the same species, vary very considerably, and in this connection it is interesting to note that, according to Abderhalden, oxyhaemoglobin which has been once recrystallised can still contain as much as 15 % of foreign protein.

One of us, wanting some pure oxyhaemoglobin, prepared a specimen from horses' blood by Abderhalden's method using alcohol as the reagent for bringing about crystallisation. Excellent crystals were easily obtained, but it was noted that it was never possible to bring the entire crystalline mass into solution in water. There was always an insoluble residue, indistinguishable from the soluble portion as far as crystalline character was concerned. After each recrystallisation a similar insoluble residue was encountered.

We suspected that this difficulty was due to the denaturating effect of alcohol on the protein, and we sought means to avoid completely the use of denaturating agents of every description, finally devising the following method, which up to the present we have used only in connection with horse corpuscles. It depends on the facts that corpuscles are laked by water and that oxyhaemoglobin is less soluble than haemoglobin in water.

Defibrinated horse blood is centrifuged and the corpuscles washed by aid of the centrifuge with isotonic saline solution until the supernatant liquid gives no turbidity when heated to boiling. The thick corpuscle paste is then transferred to collodion tubes and dialysed under the pressure of a column of mercury, first against running tap water for about three days, and finally against frequent changes of distilled water for about two days. The corpuscles

are completely laked and the haemoglobin becomes partly reduced during this operation, a deep purple solution being obtained. This is centrifuged and can then be poured off from the deposit of stromata etc. Oxygen is next bubbled through the clear solution until crystallisation of the oxyhaemoglobin occurs. As a rule this is somewhat delayed, crystallisation suddenly taking place after about 20 minutes' oxygenation. The pasty mass obtained is then centrifuged, the crystalline oxyhaemoglobin settling to the bottom of the tube as a thick scarlet paste. Under the microscope the crystals appear as very fine hair-like needles. The supernatant liquid is poured off. The crystalline oxyhaemoglobin so produced is *completely* soluble in water.

In order to recrystallise the material it is suspended in about two to three times its volume of water. The aqueous suspension is then warmed in a water bath to 37° and the containing flask is attached to a vacuum pump. Oxygen is pumped off and the oxyhaemoglobin reduced to haemoglobin, which, being much more soluble in water than oxyhaemoglobin, gives a clear, deep purple solution. This solution is then cooled and reoxygenated, when oxyhaemoglobin again crystallises out. The crystals are centrifuged down and the supernatant liquid discarded. This operation of recrystallisation can be repeated as often as may be desirable.

We propose to study the properties of oxyhaemoglobin so prepared and to apply the method to other bloods which give less easily crystallisable haemoglobins than that of the horse.