The absence of spectrally detectable hemoglobin after the 80% saturated $(NH_4)_2SO_4$ dialysis was established in the following way. In the region 530 mμ to 585 mμ, carbonyl hemoglobin (COHb) and COMb have different absorption spectra so that a change in the percentage of COHb in the solution results in changes in the absorption spectrum of the mixture. If the log optical density (OD) is plotted against wavelength in the 530–585 mμ region, the shape of the curve will depend only on the percentages of each absorbing component present. From the Figure it can be seen that after the 80% saturated $(NH_4)_2SO_4$ dialysis the percentages of COHb and COMb remained unchanged upon dialysis against more saturated $(NH_4)_2SO_4$ solutions. Since hemoglobin starts to precipitate at a lower ionic strength than Mb, it is probable that the curves obtained from the supernatant after the 80% and 100% saturated dialysis were identical because all spectroscopically detectable hemoglobin was removed.

Although the above procedure yielded purified Mb extracts which proved suitable for kinetic experiments, several additional attempts were made to carry the purification one step further by crystallizing Mb. The purified Mb solution was fractionated against more concentrated $(NH_4)_2SO_4$. After several days the resulting precipitate contained particles which resembled the globuliform crystals described by ROSSI-FANELLI *et al.*[10]; however, no macroscopic crystals were obtained on continuing the dialysis for six weeks.

Following the failure to obtain crystalline Mb by $(NH_4)_2SO_4$ fractionation, the samples obtained after step 3 in the above procedure were chromatographed on Amberlite IRC 50. Some non-chromoprotein contaminants were separated by this ion exchange resin. The results of this exploratory investigation are supported by similar findings for Mb from other animal species[11] and suggest that ion exchange chromatography may be used for further purification. An additional method of Mb purification which merits consideration is continuous paper electrophoresis, which was recently used in purifying fish Mb[12]. However, it is our belief that the dialysis technique described in the present report is simpler and more likely to yield larger amounts of Mb which can be used in spectroscopic measurements of kinetic reactions.

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Biophysics Laboratories, Pennsylvania State University, University Park (Pennsylvania), February 26, 1959.

Résumé

Une technique est décrite pour préparer de la myoglobine pure par fractionnement avec sulfate d'ammonium.

[10] A. ROSSI-FANELLI, D. CAVALLINI, and C. DEMARCO, Arch. Biochem. Biophys. *50*, 496 (1954).

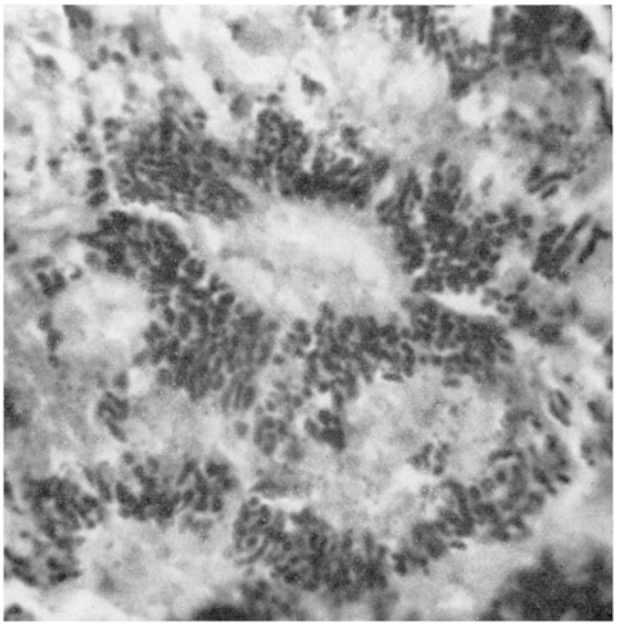
[11] N. K. BOARDMAN and G. S. ADAIR, Nature *177*, 1078 (1956). – R. TRIMMER, H. J. VAN DER HELM, and T. H. J. HUISMAN, Nature *180*, 239 (1957).

[12] E. ANTONINI and B. MONDOVI, Boll. Soc. ital. Biol. sper. *31*, 1388 (1955).

PRO EXPERIMENTIS

Rapid Staining of Mitochondria

Several methods have, up till now, been suggested for the demonstration of mitochondria in permanent mount tissues; but most of them, requiring special fixatives, mordants and lenght of time, are not suitable for routine laboratory work.



Mitochondria in hepatic cells of Guinea pig (× 1500).

I have obtained excellent results with the following rapid and simple method, and I advice it not only for routine but also for special histological study (pathological changes as cloude swelling, and so on).

Fixation: fix exclusively in 10% formalin. Embed in paraffin.

Method of staining

(1) After removal of the paraffin in the usual way, rinse in water and place the sections into a warm normal solution of hydrochloric acid (about 60°C) for 3 min.
(2) Rinse in water.
(3) Place the sections in fuchsin acid aqueous solution 1% for 30 sec.
(4) Rinse in water.
(5) Place the sections in light green aqueous solution 1% for 1 to 3 min.
(6) Rinse in water.
(7) Dehydrate in 95% and absolute alcohol.
(8) Clear in xylol and mount in balsam.

Mitochondria stand out sharply, stained purple-red with peripheral wall green, chromatin and collagen green, muscle tissue purplish, erythocites brilliant red.

A. NOVELLI

Department of General Pathology of the University of Genova (Italy), March 6, 1959.

Riassunto

Viene descritta una nuova semplice tecnica per la colorazione rapida dei mitocondri nelle sezioni di tessuti: i pezzi devono essere fissati in formalina, le fettine, dopo sparaffinamento, vengono immerse in una soluzione calda (60°C) di acido cloridrico normale per 3 min e poi colorate con fucsina acida (1%) per 30 sec e con verde luce (1%) per 1–3 min. I mitocondri risaltano intensamente colorati in rosso con una sottile parete colorata in verde.