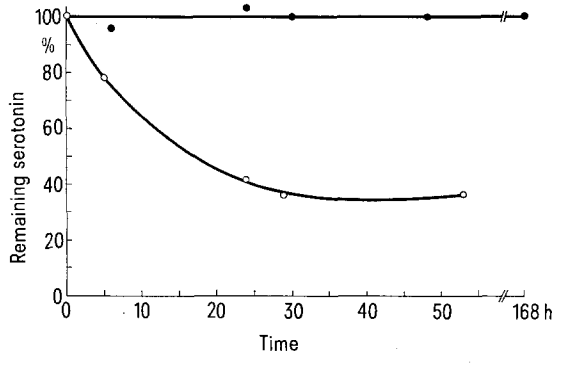
A Simple Routine-Method to Preserve and Determine Blood Serotonin

Several methods for the quantitative fluorometric determination of serotonin in whole blood or platelets are described in the literature[1-8]. These methods are time-consuming (extractions with organic solvents or condensation reactions) and need too much blood, so there is still a need for a method that can be used in a routine laboratory without any difficulty[9]. Moreover, the instability of serotonin in stored blood complicates its assay. We describe here a rapid, sensitive and reproducible method for the determination of serotonin, as well as a medium in which the serotonin content of blood can be kept stable for at least 1 week. The small quantity of blood necessary for the determination (1 ml) allows its application to the blood of babies.

Materials and methods. In plastic tubes, 1 ml blood is added to 0.1 ml of a 3% solution of ascorbic acid in saturated EDTA Na_2 (15 g/100 ml)[1] and frozen in a mixture of dry ice and acetone. When not immediately used, the blood can be stored in this form for at least 7 days at −18 °C. Just before determination of serotonin, the blood is unfrozen and 2.5 ml water is added.

From that time and during the whole procedure, the tubes are kept in ice. After 10 min, in order to ensure total hemolysis, 1 ml 10% $ZnSO_4$ and 0.5 ml 1 *N* NaOH are added to precipitate the proteins. After addition of each reagent, mixing is done either manually or with a Whirlimixer. The suspension is centrifuged in a refrigerated Sorvall RC2B centrifuge at 3000 *g* for 5 min. To 1 ml of the clear supernatant, 0.3 ml 12 *N* HCl (containing 5 mg ascorbic acid per 10 ml HCl)[9,10] is added. This is done just before measuring the fluorescence of the sample with an Aminco-Bowman spectrofotofluorometer at 540 nm after excitation at 295 nm (both uncorrected instrument values)[11].

A stock solution of serotonin-creatinine sulfate in 0.1 *N* HCl (corresponding to 100 µg serotonin/ml) is diluted with 0.1 *N* HCl so as to obtain standard solutions containing resp. 500, 250 and 125 ng/ml, which are handled in the same way as the samples; 1 ml 0.1 *N* HCl is used as a blank. This should be done for every set of determinations. All reagents are stored at 4 °C.



Stability test: ●, blood samples added to ascorbic-acid EDTA and cooled in dry ice-aceton. ○, blood samples added to Heparine (1,000 IU/ml) cooled in ice.

Results and discussion. The serotonin determinations made in our laboratory according to methods described in the literature gave very low values. Experiments showed that serotonin disappears by oxidation as a function of time, the oxidating agent being oxyhemoglobin[12]. The addition of ascorbic acid together with fast cooling in dry ice-aceton prevents this destruction.

The sensivity and linearity of the method described here were tested with standards of serotonin-creatinine sulfate in the range 20–5000 ng, expressed as serotonin. When a given amount of serotonin is added to a blood sample, the recovery lies between 80 and 85%.

By this method we determined the blood serotonin concentration of 50 normal children aged between 6 months and 16 years. The blood serotonin levels were calculated from the fluorescence of the samples compared with the fluoresence of the standards. The mean total serotonin blood level was 189 ng/ml, with a range from 76 to 317 ng/ml (These values are not corrected for the 80–85% recovery of serotonin added to blood.) whole blood. These results are in agreement with those found by Tu and Partington[13] and others.

Stability tests show (see Figure) that, for at least 7 days, there is no variation in the serotonin content if the sample is frozen immediately and kept at −18 °C until determination.

Résumé. Une nouvelle méthode de routine pour le dosage par spectrophotofluorométrie de la sérotonine dans 1 ml de sang est décrite. Grâce à l'emploi d'un antioxydant et à une congélation immédiate, le taux de sérotonine reste constant pendant au moins une semaine.

F. Geeraerts, L. Schimpfessel and R. Crokaert

Vrije Universiteit Brussel, Laboratorium voor Biochemie, Waterloolaan 115, B-1000 Brussels (Belgium), 29 November 1973.

[1] G. W. Ashcroft, T. B. B. Crawford, J. K. Binns and E. J. MacDougall, Clin. chim. Acta *9*, 364 (1964).
[2] M. L. Das, Biochem. Med. *6*, 299 (1972).
[3] T. P. Waalkes, J. Lab. clin. Med. *53*, 824 (1959).
[4] A. Yuwiler, S. Plotkin, E. Geuer and E. R. Ritvo, Biochem. Med. *3*, 426 (1970).
[5] F. Rosner, B. H. Ong, R. S. Paine and D. Mahanand, Lancet *1*, 1191 (1965).
[6] Y. Chauvel, Y. Ollivier, P. Jouan and S. Samperez, Annls Biol. clin. *31*, 231 (1973).
[7] M. Da Prada, J. P. Tranzer and A. Pletscher, Experientia *28*, 1328 (1972).
[8] H. Weissbach, T. P. Waalkes and S. Udenfriend, J. biol. Chem. *230*, 865 (1958).
[9] C. Dreux, Annls Biol. clin. *31*, 283 (1973).
[10] J. H. Thompson, C. A. Spezia and M. Angulo, Analyt. Biochem. *31*, 321 (1969).
[11] S. Underfriend, D. F. Bogdanski and H. Weissbach, Science *122*, 972 (1955).
[12] J. J. Blum and N. S. Ling, Biochem. J. *73*, 530 (1959).
[13] J. B. Tu and M. W. Partington, Cerebr. Palsy Bull. *14*, 457 (1972).

Isolement de fragments nucléés et anucléés d'œufs de l'oursin *Sphaerechinus granularis*

De nombreuses expériences biologiques et biochimiques ont été faites sur des fragments nucléés et anucléés d'œufs d'oursin. Elles ont toutes été effectuées sur le même genre d'oursin, *Arbacia;* en effet, il est facile de couper en deux moitiés les œufs vierges de cette espèce par centrifugation, comme l'a montré Harvey dès 1936[1]. Il serait, évidemment, souhaitable d'étendre ces expériences à d'autres espèces, afin de savoir si on est en droit de généraliser les