Specific Activity of ^{14}C-Labelled Serotonin Released from Human Blood Platelets[1]

Blood platelets which are induced to aggregate by, for instance, ADP[2], release a variety of substances, among which are adenine nucleotides and biogenic amines. This so-called 'release reaction' is often assessed by measuring the release of ^{14}C by platelets which have been incubated previously with serotonin labelled with ^{14}C[3-6]. Platelets take up serotonin rapidly and efficiently in vitro, and release much of it during the release reaction. Also the assay of ^{14}C is both simpler and more sensitive than the assay of serotonin. The content of ^{14}C in the extracellular fluid is, however, a true measure of the serotonin released only if the serotonin-^{14}C taken up is mixed homogenously with the serotonin already present in the cell. This is not trivial since the content of the platelets is compartmentalized, and certain substances are distributed between separate pools. Serotonin is localized predominantly in the so-called very dense bodies or osmiophilic granules[7,8]. Some, however, has been found dispersed throughout the platelets[7,9]. DAVIS and WHITE[9] studied platelets by autoradiography after labelling with serotonin-^{3}H; they presented evidence that the nongranular serotonin is released preferentially upon addition of ADP or upon clotting. STACEY[10] found that human citrated PRP[2] releases serotonin upon treatment with ADP only if the platelets have been loaded previously with serotonin, and has concluded that only excess serotonin is released together with part of the adenine nucleotides.

Hence it is possible that serotonin is stored in two or more separate pools; that these pools are labelled at different rates and that they are released to different degrees. Under these conditions, the amount of ^{14}C released would not represent the release of the endogenous serotonin.

In view of this possibility I have measured the specific activity of the serotonin released by ADP from platelets which had been loaded with serotonin-^{14}C, of the serotonin remaining in the platelets after this treatment, and of the serotonin of a sample of labelled platelets not treated with ADP.

Human citrated PRP (pH 6.8) was shaken for 45 min at 18 °C with serotonin-^{14}C at a final concentration of 1 μM and 0.04 μc/ml. 2.5 ml of the labelled PRP, supplemented with KCl, $CaCl_2$ and *Tris*-HCl, was stirred in a turbidimeter[11,12] in a total volume of 5 ml at 37 °C and at a pH of 7.3. The final concentrations were: K^+, 13.5 mM; total Ca, 3 mM; free C^{++}, 0.22 mM[13]; *Tris*, 51 mM; platelets, ca. 2×10^9/ml. After 2 min, ADP was added to a final concentration of 0.1 mM and the stirring continued for 5 min during which strong aggregation occurred. EDTA was then added to a concentration of 7 mM, the mixture placed in ice and centrifuged for 10 min at about 3000 g. The sediment was homogenized in 5 ml of water. 2 ml of the homogenate and of the supernatant were deproteinized by the addition of 0.8 ml 7% $ZnSO_4$ and 0.4 ml 1 N NaOH, followed by centrifugation. The extracts were assayed for serotonin fluorometrically according to CROSTI and LUCCHELLI[14] with the aid of an Aminco-Bowman spectrofluorometer, and for ^{14}C in a Tricarb liquid scintillation counter, after mixing 0.2 ml of extract with 5 ml of methanol and 10 ml of a 1% solution of Butyl-PBD in toluene. A control sample of the labelled PRP was diluted with saline to the same concentration of platelets and treated further like the aggregated suspension of platelets.

PRP was prepared from the pooled buffy coats of ACD-blood collected for the Central Laboratory of the Swiss Red Cross Transfusion Service in Berne as described before[15]. Serotonin-^{14}C was purchased from the Radiochemical Centre, Amersham (U.K.) (5-hydroxytryptamine-3-^{14}C creatinine sulphate, 56 mc/mmol). The scintillator Butyl-PBD (2-(4′-t-butylphenyl)-5-(4″-biphenylyl)-1, 3, 4-oxadiazole) was obtained from CIBA Ltd., Basle (Switzerland).

The radioactivity and serotonin content of the supernatant and the sediment, and the specific activity of the serotonin are presented in the Table for the aggregated and the control sample. It is evident that the specific

[1] Supported by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung.

[2] Abbreviations: ADP, adenosine-5-diphosphate; PRP, platelet-rich plasma; *TRIS*, *tris* (hydroxymethyl) methane; EDTA, ethylene diamino tetra-acetate.

[3] T. H. SPAET and J. CINTRON, Br. J. Haemat. *11*, 269 (1965).

[4] Z. JERUSHALMY and M. B. ZUCKER, Thromb. Diath. haemorrh. *15*, 413 (1966).

[5] M. A. PACKHAM, E. S. WARRIOR, M. F. GLYNN, A. S. SENYI and J. F. MUSTARD, J. exp. Med. *126*, 171 (1967).

[6] D. C. B. MILLS, I. A. ROBB and G. C. K. ROBERTS, J. Physiol., Lond. *15*, 715 (1968).

[7] M. DA PRADA, A. PLETSCHER, J. P. TRANZER and H. KNUCHEL, Nature *216*, 1315 (1967).

[8] J. P. TRANZER, M. DA PRADA and A. PLETSCHER, Adv. Pharmac. *6*, part A, 125 (1968).

[9] R. B. DAVIS and J. G. WHITE, Br. J. Haemat. *15*, 93 (1968).

[10] R. S. STACEY, Experientia *24*, 908 (1968).

[11] J. R. O'BRIEN, J. clin. Pathol. *15*, 452 (1962).

[12] G. V. R. BORN and M. J. CROSS, J. Physiol. Lond. *168*, 178 (1963).

[13] Calculated from the concentration of citrate and the stability constant of the calcium citrate chelate, $K = 1400\,M^{-1}$. J. SCHUBERT and A. LINDENBAUM, J. Am. chem. Soc. *74*, 3529 (1952).

[14] P. F. CROSTI and P. E. LUCCHELLI, J. clin. Pathol. *15*, 191 (1962).

[15] M. BETTEX-GALLAND and E. F. LÜSCHER, Thromb. Diath. haemorrh. *4*, 178 (1960).

^{14}C and serotonin content of the $Zn(OH)_2$-extracts of the supernatant and the sediment of aggregated and control platelets labelled previously with ^{14}C-serotonin

Sample	Sediment			Supernatant			
	^{14}C-content (cpm/ml)	Serotonin (μM)	Specific activity (cpm/pmole)	^{14}C-content (cpm/ml)	% ^{14}C of total	Serotonin (μM)	Specific activity (cpm/pmole)
Aggregated	8,600	3.3	2.6	6,200	42	2.3	2.7
Control	13,800	6.6	2.1	420	3	0	–

Mean of 2 samples.

activity of the serotonin released by ADP, of that remaining in the platelets and that of the total serotonin of the untreated platelets are almost identical. 3 similar experiments gave the same result.

It can be concluded that the serotonin taken up by the platelets is mixed homogeneously with the serotonin already present in the platelets, and that the percentage of release, obtained from counting the radioactivity of the supernatant of an aggregated platelets suspension, is a true figure for the proportion of serotonin released during the treatment.

In this respect serotonin differs from the adenine nucleotides. IRELAND[16] and HOLMSEN[17] have shown that platelets labelled with adenosine-^{14}C or phosphate-^{32}P release very little radioactivity in the release reaction and concluded that the nucleotides to be released are stored in a pool not readily labelled. This pool is possibly localized in the granules which also store serotonin[18,19].

Zusammenfassung. Menschliche Blutplättchen in Zitratplasma wurden mit Serotonin-^{14}C inkubiert und danach mit ADP zur Aggregation gebracht. Die spezifische Aktivität des freigesetzten Serotonins war gleich der des in den Plättchen verbliebenen Serotonins.

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[16] D. M. IRELAND, Biochem. J. *105*, 857 (1967).
[17] H. HOLMSEN, Scand. J. Lab. Invest. *17*, 239 (1965).
[18] Professor H. AEBI kindly made available his facilities for spectrofluorometry and for liquid scintillation counting; Miss M. SCHNEIDER gave competent technical assistance.
[19] L. M. ALEDORT, H. GILBERT and E. PUSZKIN, Blood *34*, 535 (1969) recently confirmed STACEY's[10] earlier findings (see text); they furthermore concluded that serotonin taken up in vitro does not equilibrate with endogenous serotonin. Whereas the possibility can not be excluded that preincubation with serotonin sensitizes the platelets for the release reaction by small amounts of ADP, the results reported by ALEDORT et al. do not necessarily imply that serotonin taken up in vitro does not equilibrate with its endogenous pool.

Application of the Electroprecipitin Test to the Detection of the Virus of Canine Distemper

In research facilities utilizing random source dogs, canine distemper or a distemper-like syndrome is frequently seen. Despite signs suggestive of canine distemper (i.e. fever, malaise, anorexia, catarrhal nasal and conjunctival exudate) it is impossible to accurately diagnose the disease without clinical laboratory evidence or seeing the clinical signs of paralysis, chorea, or convulsions.

Since available laboratory tests for canine distemper are unreliable or impractical in most research facilities utilizing large numbers of random source dogs, a rapid diagnostic test for canine distemper was sought. This is to report the results achieved by the production of an antibody to the virus in rabbits, and its subsequent use to detect the virus of canine distemper via the electroprecipitin test.

Materials and methods. Antigen and antiserum. A commercially available modified live virus canine distemper vaccine[1] was mixed with complete Freunds adjuvant[2] (1:1) and injected s.c. into 3 kg New Zealand rabbits[3]. After 3 weeks, blood was collected via saphenous venapuncture, the serum separated and stored frozen until tested. Dilutions of antigen were made in saline to be tested.

Electroprecipitin test. Canine distemper viral dilutions were prepared in saline and tested against the rabbit antiserum by the electroprecipitin test[4–6]. A veronal barbital buffer[7], pH 8.6, ionic strength 0.05, was used in all electroprecipitin tests. Cellulose acetate electrophoretic support strips[8] were pre-soaked for 30 min prior to using, then blotted and placed in the electrophoretic chamber. A 5 λ drop of antiserum was placed 1 inch from the cathode, and a current of 250 V, 5–7 ma, was passed for 30 min. The current was shut off, and the antigen(s) (approximately 5 λ) were applied with a fire-polished capillary tube (0.5–0.9 mm i.d.) as a straight line immediately behind (on the cathode side) the point of antiserum application (Figure 1). Current was restored for 20 min. At the end of that time, the strip was transferred to 0.85% saline solution and rinsed for 5–10 min, with occasional movement of the saline solution. The strip was then dried and placed in Ponceau S stain[9] for 5 min. The strip was then rinsed twice in 5% acetic acid and once in tap water. After drying, the strips were examined for precipitin bands by means of illumination from behind (Figure 2). Control tests consisted of pooled canine serum from dogs immune to canine distemper, and saline alone tested against the antiserum.

Gel diffusion tests. Double diffusion in one dimension[10] in tubes was used in all instances to confirm electroprecipitin test results. A 0.6% agar solution in 0.85% NaCl was employed as the interphase between antiserum and antigen.

Serum neutralization test. The rabbit antiserum prepared against the distemper virus was tested for serum neutralizing antibody, and the titer calculated by the Reed and Muench system[11]. Serum from 3 pre-immune rabbits, randomly selected, were also tested for neutralizing antibody.

[1] D-Vac, Bio-Centic Laboratories, St. Joseph (Mo., USA).
[2] Difco Laboratories, Detroit (Michigan, USA).
[3] Shankin Rabbitry, Warren (Michigan, USA).
[4] F. A. ZYDECK, E. E. MUIRHEAD and H. SCHNEIDER, Nature *205*, 189 (1965).
[5] F. A. ZYDECK, E. E. MUIRHEAD and H. SCHNEIDER, Am. J. clin. Path. *44*, 596 (1965).
[6] F. A. ZYDECK, E. E. MUIRHEAD and H. SCHNEIDER, Am. J. clin. Path. *45*, 323 (1966).
[7] Merck and Co., Rahway (New Jersey, USA).
[8] Gelman Instrument Co., Ann Arbor (Michigan, USA).
[9] Harleco, Haritman Leddan Co.
[10] J. R. PREER, J. Immun. *77*, 52 (1956).
[11] C. M. SHELDON, Eli Lilly and Co.