	B_1	B_2	Niac.	Biot.	Folic	B_6	B_{12}
Normal 'h'	7	3·5	63·0	0·4	1·8	7·7	0·4
'High peak' from normal	—	45·1	—	—	—	—	—
'High peak' from 3'-Me DAB fed .	—	12·7	—	—	—	—	—

eine content of the 'high peak' (corresponding to 6–8th cm on the Figure was also determined (normal: 0·13%, dye containing: 0·11%).

Vitamin content. Only trace amount of vitamins have been found in the normal 'h' protein by microbiological assay. The riboflavin content of the 'high peak' was determined by fluorometry after BURCH *et al.*[9]. There is possibly a competition for binding in this segment between 3'-Me DAB and riboflavin, since only about one-fourth of the normal level of vitamin was found in this fraction, when it was isolated from dye containing supernatant fluid. The following Table gives the vitamins in μg/g of protein.

Nucleic Acids. The stepwise analysis of the perchloric extracts of the 'h' fraction has shown the complete absence of purine and pyrimidine bases. Thus, this fraction is devoid of nucleic acids and their acid soluble components, in good agreement with the results of SOROF *et al.*[10].

Molecular weight and bound dye. The molecular weight distribution of the 'h' fraction was studied in an analytical ultracentrifuge[11]. Two, nearly equal fractions have been observed, one rapidly moving (M.W. $\sim$ 45,000), an other slowly moving (M.W. $\sim$ 90,000).

The amount of bound 3'-Me DAB per unit weight of 'h' fraction was determined by colorimetry in 88% formic acid. 8 mg of this protein contained 2·15 γ of bound dye. Thus, assuming for the 'h' fraction an average molecular weight of 67,000 and an even distribution of the dye, only 27·2% of the protein molecules contain bound dye.

A full account of these and related investigations will be given elsewhere.

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McArdle Memorial Laboratory, University of Wisconsin, Medical School, Madison, Wis., December 24, 1957.

Résumé

Les auteurs ont étudié le profil électrophorétique du surnageant de foies de rats, nourris aux colorants azoïques cancérogènes. La fraction, dite protéine «h», a été isolée et on a déterminé son poids moléculaire, sa teneur en colorant lié, en vitamines et en acides nucléiques et sa composition en acides aminés.

[9] H. B. BURCH, O. A. BESSEY *et al.*, J. biol. Chem. *175*, 457 (1948); *180*, 755 (1949).

[10] S. SOROF, M. G. OTT *et al.*, Fed. Proc. *15*, 358 (1956).

[11] The molecular weight determinations were carried out by Dr. HAROLD F. DEUTSCH, to whom our thanks are due..

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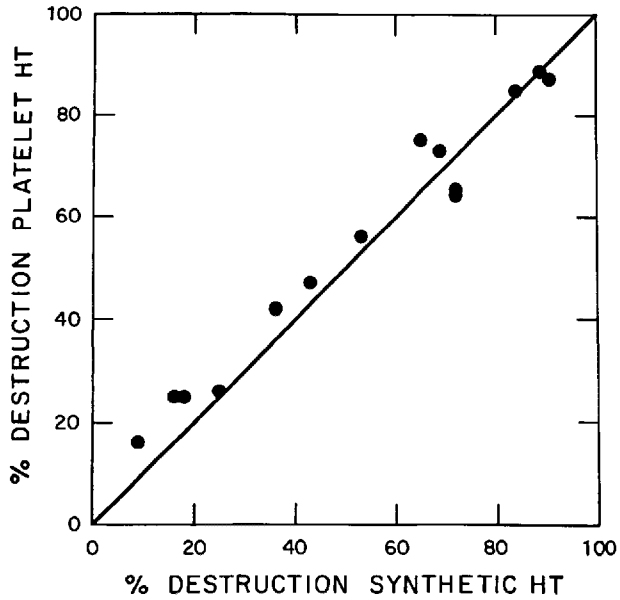
Serotonin Storage Mechanism and its Interaction with Reserpine

Serotonin (5-hydroxytryptamine, HT), a possible neurohumoral agent, is said to be 'bound' since tissue cells contain the amine in a form not immediately available to exert a biologic action. In studying HT storage, HUMPHREY and TOH[1] were the first to report that platelets can take up HT against a concentration gradient. The mechanism for HT uptake is not known; it could involve complexing of HT with an intracellular constituent or its passage across the cell boundary by active transfer. The present studies are concerned with the nature of the concentrating mechanism and how it is affected by reserpine.



Action of Monoamine Oxidase on Serotonin (HT) from Lysed Platelets. 0·1 to 2 ml of monoamine oxidase preparation was incubated with suspensions of disrupted platelets and with solutions of synthetic HT at pH 7·4 in air for 15 or 30 min at 37°C. Residual HT was measured as described previously[4]. Fraction of HT destroyed in lysed platelets is plotted against that destroyed in solutions of synthetic HT. The line represents the theoretical slope if the fraction of HT destroyed in lysed suspensions and in synthetic HT solutions were identical.

Evidence for a HT complex was sought in rabbit platelets which contain HT at a concentration thousands of times that in plasma. Platelets were isolated[2] at 4°C, washed twice with cold 0·1 *M* phosphate buffer, pH 7·4, suspended in 1 ml of buffer, lysed with 9 ml ice-cold water, and the suspension was subjected to ultrafiltration[3]. HT

[1] J. H. HUMPHREY and C. C. TOH, J. Physiol. *124*, 300 (1954).

[2] G. H. L. DILLARD, G. BRECHER, and E. P. CRONKITE, Proc. Soc. exp. Biol. Med., N. Y. *78*, 796 (1951).

[3] P. P. REHBERG, Acta physiol. scand. *5*, 305 (1943).

Serotonin (HT) and Bufotenine Uptake by Guinea Pig Platelets

Added Amine in µg/ml	Uptake of HT µg/mg protein	No. of Experiments	Uptake of Bufotenine µg/mg protein	No. of Experiments
0·66 (Normal Platelets)	0·42 ± 0·13	5	0·07 ± 0·02	8
(Reserpinized platelets)	0·05 ± 0·02	4	0·01 ± 0·01	6
6·6 (Normal Platelets)	0·81 ± 0·14	7	0·26 ± 0·05	8
(Reserpinized platelets)	0·17 ± 0·02	4	0·25 ± 0·06	6

4 ml of platelet-rich plasma, and 0·5 ml pH 7·4 buffer containing HT or bufotenine were incubated in siliconed beakers in air for 1 h at 37°C. Preparation of platelet-rich plasma, the isolation of platelets and the assay of HT and protein have been described previously[4]. Bufotenine was assayed by the same method as for HT. In experiments involving reserpine, the alkaloid was either added directly (1 µg/ml plasma) to normal platelets or platelets were taken from animals given drug (5 mg/kg) 16 h previously. Values represent net increase in HT or bufotenine concentration. Amine content is expressed as µg/mg platelet protein.

[4] B. B. Brodie, E. G. Tomich, R. Kuntzman, and P. A. Shore, J. Pharmacol. exper. Therap. *119*, 461 (1957).

levels[5] in ultrafiltrates were the same as that in unfiltered suspensions, indicating that binding to substances of large molecular size was negligible. HT may conceivably be localized in platelet granules, lysed by treatment with water; however, platelets homogenized in isotonic sucrose contained no bound HT. The possibility that HT was combined with diffusible substances of small molecular size was investigated by comparing the action of monoamine oxidase[6] on suspensions of lysed platelets and on solutions of the synthetic indole. HT in both preparations was metabolized at the same rates (Figure).

These results indicate that HT from disrupted platelets is indistinguishable from the synthetic amine. Accordingly, the high affinity of the amine for platelets cannot be explained on the basis of a stable HT complex. We were therefore led to consider active transport as an alternative mechanism for maintaining HT against a concentration gradient. This possibility was assessed by comparing the uptake of HT and its synthetic dimethyl analog bufotenine, by guinea pig platelets, which normally contain little HT. In general, cellular membranes are permeable to lipid-soluble compounds, but relatively impermeable to polar substances unless these are transported by a specialized carrier mechanism at the cell surface. The lipid solubilities of HT and bufotenine are extremely low; the partition coefficient of HT between chloroform and aqueous buffer pH 7·4 is 0·00035, and of bufotenine 0·00780. Consequently, these compounds should penetrate platelets slowly unless enforced by a special mechanism.

Comparisons were made of the uptakes of HT and bufotenine by guinea pig platelets. As reported previously, considerable amounts of HT were rapidly absorbed[7] from the surrounding fluid despite the adverse concentration gradient, whereas only small amounts of bufotenine were absorbed despite its 20 times greater partition coefficient (Table). Reserpine almost completely blocked the uptake of HT, but had no effect on the small uptake of bufotenine. These results are consistent with the view that HT is rapidly transported across the cellular boundary by a specialized transport mechanism with which reserpine interacts, whereas the more lipid-soluble bufotenine slowly enters the cells, perhaps by a passive diffusion[8].

Previous studies have shown that reserpine releases in 4 h about 50% of the HT normally present in platelets[9]. However, present studies showed that the addition of 1 µg/ml of reserpine to a platelet suspension almost immediately blocked the uptake of added serotonin. Consequently the *primary* effect of reserpine in depleting platelets of their *normal* HT content may be rapid inhibition of the transport mechanism which maintains the amine against a concentration gradient. With the blocking of the 'pump', HT leaves the cells by passive diffusion. The slow rate of HT release may be attributed to the difficulty with which this highly polar substance penetrates the platelet membrane in the absence of the postulated energetic mechanism. Additional evidence favoring transport mechanism is the observation that one reserpine molecule releases hundreds of HT molecules[9]. This finding is difficult to reconcile with the concept that reserpine detaches HT from a cellular constituent, but would be expected if reserpine inhibits a transport mechanism.

The question arises as to the possible source of energy for active transfer since HT is absorbed by platelets anerobically as well as aerobically. This energy could be derived from ATP present in platelets. Other workers[10] have implicated ATP in the localization of HT in platelets but postulate that the two substances form a chemical complex.

Further work is in progress to establish the validity of the concept that HT is held in cells by an active transport mechanism.

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Zusammenfassung

Es gelang nicht, mit physikalisch-chemischen Methoden einen HT-Komplex in hämolysierten oder homogenisier-

[5] H. Weissbach, T. P. Waalkes, and S. Udenfriend, J. biol. Chem. (in press).

[6] The enzyme is a preparation of soluble monoamine oxidase prepared according to H. Weissbach, B. S. Redfield, and S. Udenfriend, J. biol. Chem. (in press).

[7] J. H. Humphrey and C. C. Toh, J. Physiol. *124*, 300 (1954). – R. M. Hardisty and R. S. Stacey, J. Physiol. *130*, 711 (1955). – B. B. Brodie, E. G. Tomich, R. Kuntzman, and P. A. Shore, J. Pharmacol. exper. Therap. *119*, 461 (1957).

[8] Another kind of uptake which represents only a small fraction of added HT is evident at high concentrations of the amine. This uptake is not blocked by reserpine suggesting that it is simple binding onto platelet protein or the cell surface.

[9] A. Carlsson, P. A. Shore, and B. B. Brodie, J. Pharmacol. exper. Therap. *120*, 334 (1957).

[10] G. V. R. Born, G. I. C. Ingram, and R. S. Stacey, J. Physiol. *135*, 63P (1956).

* Riker Fellow.

ten Suspensionen von Kaninchen-Thrombozyten nachzuweisen. Versuchsergebnisse über die HT-Aufnahme durch Thrombozyten stimmen mit der Möglichkeit überein, dass das Amin in den Zellen durch einen aktiven Transportmechanismus konzentriert wird. Es wird angenommen, dass die Abgabe von normalem HT aus der Zelle unter Reserpineinwirkung, durch eine Hemmung des Aufnahmemechanismus und langsame, passive Diffusion aus den Zellen erfolgt.

Disparition de la 5-hydroxytryptamine dans les granulations des glandes de la peau de *Discoglossus pictus* et de *Rana temporaria* après injection de réserpine

L'injection de réserpine provoque la disparition de la 5-hydroxytryptamine (5-HT) de l'intestin grêle du lapin[1], tandis qu'elle augmente la teneur en acide 5-hydroxyindolacétique de l'urine de chien[2]. Comme ERSPAMER l'a montré chez le rat et le coq[3], cette diminution de la 5-HT s'observe non seulement dans le tractus gastro-intestinal, mais aussi dans le sérum, la rate et le cerveau. NAESS et SCHANCHE[4] l'ont confirmé pour le sérum de lapin. L'injection de réserpine fait disparaître la 5-HT des plaquettes sanguines chez l'homme[5]. Leur affinité pour la 5-HT est diminuée en présence de réserpine[6]. Aucun antagonisme n'a été constaté *in vitro* entre la réserpine et la 5-HT[4].

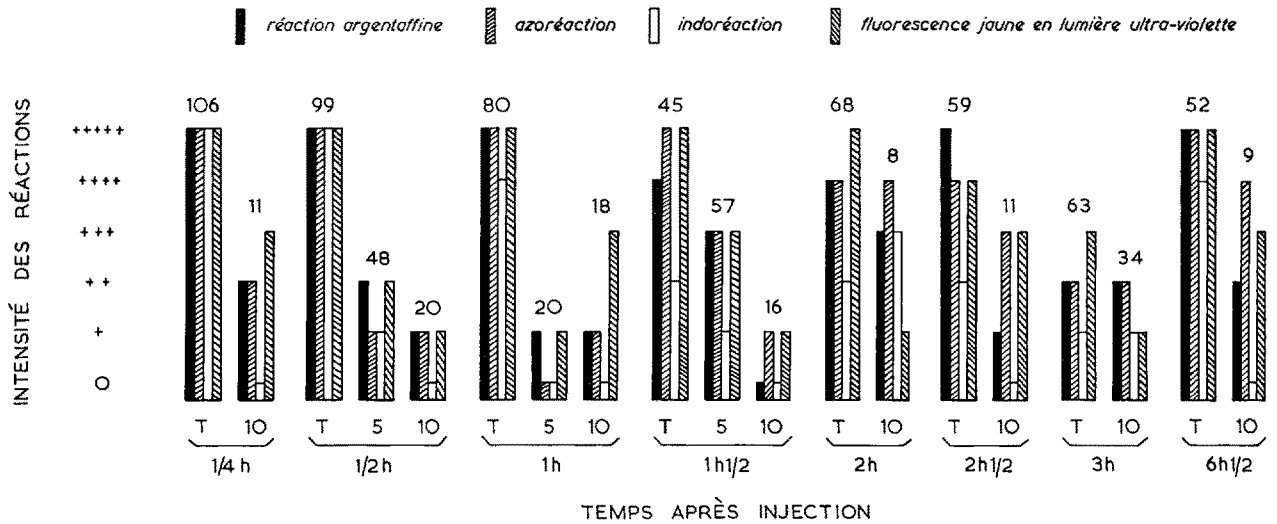
La peau de *Discoglossus pictus* contient d'après ERSPAMER[7] 410–450 μg de 5-HT par g de tissu (41–54 mg/kg de poids du corps). Celle-ci est localisée dans les granulations des glandes de la peau. Nous avons recherché la 5-HT par différentes réactions histochimiques sur des coupes de peau de grenouille et de discoglosse (*Rana temporaria* et *Discoglossus pictus*), les prélèvements étant faits à des temps différents après l'injection de réserpine (5 et 10 mg/kg Serpasil CIBA).

Comme il n'existe pas de réaction spécifique de la 5-HT, nous avons pratiqué les 3 réactions suivantes portant sur la mise en évidence des hydroxyles phénoliques libres en position *ortho* et *para*[8]: *a*) la *réaction argentaffine* selon la méthode de MASSON, *b*) l'*azo-réaction*, le sel de diazonium étant préparé à l'acide sulfanilique, *c*) l'*indoréaction*, la paradiamine aromatique utilisée étant la diméthyl*para*phénylènediamine. Ayant constaté qu'après la fixation au Bouin, la fluorescence attendue en présence de 5-HT était masquée, nous avons laissé séjourner ensuite les préparations 24 h dans du formol avant de les monter. Comme après la seule fixation au formol, une belle fluorescence jaune apparaît. Les résultats sont indiqués à la Figure pour les discoglosses.

Chez la grenouille, les résultats sont similaires. L'effet de 5 mg/kg de réserpine se marque surtout $^1/_2$ h et 1 h après l'injection. Même 7 h après les injections, les réactions ne sont pas encore aussi positives que chez les témoins.

En conclusion, la réserpine fait disparaître une substance qui montre toutes les réactions histochimiques de la 5-HT utilisées dans les granulations des glandes de la peau de *Rana temporaria* et de *Discoglossus pictus*. Cette perte est temporaire et est maximale $^1/_2$ h à 1 h après l'injection de 5 mg/kg chez la grenouille, 1 h après l'injection de la

[1] A. PLETSCHER, P. A. SHORE et B. B. BRODIE, Science *122*, 374 (1955).
[2] P. A. SHORE, S. L. SILVER et B. B. BRODIE, Science *122*, 284 (1955).
[3] V. ERSPAMER, Exper. *12*, 63 (1956); Naturwissenschaften *43*, 61 (1956).
[4] K. NAESS et S. SCHANCHE, Nature *177*, 1130 (1956).
[5] B. J. HAVERBACK, P. A. SHORE, E. G. TOMICH et B. B. BRODIE, Fed. Proc. *15*, 434 (1956).
[6] R. M. HARDISTY, G. I. C. INGRAM et R. S. STACEY, Exper. *12*, 424 (1956).
[7] V. ERSPAMER, R. C. scient. Farmitalia *1*, 1 (1954).
[8] L. LISON, *Histochimie et Cytochimie animales* (Gauthier-Villars, Paris 1953), p. 71.



Effet de la réserpine sur la teneur en 5-hydroxytryptamine des granulations de la peau de *Discoglossus pictus*.

+++++ Toutes granulations positives
++++ Granulations positives et faiblement positives
+++ Granulations positives, faiblement positives et négatives
++ Toutes granulations faiblement positives
\+ Granulations faiblement positives et négatives
O Toutes granulations négatives
T Témoin injecté de même volume d'eau distillée
5 Discoglosse injecté de 5 mg/kg de réserpine
10 Discoglosse injecté de 10 mg/kg de réserpine

Les chiffres correspondent au nombre des glandes portant des granulations compté dans 10 champs différents sur une longueur de 3,3 mm, chacun.