Tabelle

Pharmaka	Dosis mg/kg	Wirkungsdauer h	Histamingehalt µg/ml	% des Normalhistamingehaltes	P
Normalplasma			0,071 ± 0,0046	100	
Äther		1	0,054 ± 0,0026	75	< 0,001
Chloroform		1	0,068 ± 0,0048	95	0,6
Reserpin	1,0	3	0,081 ± 0,0052	114	0,001
Chlorpromazin	10,0	3	0,088 ± 0,0060	125	< 0,001
Numal	0,1	1	0,078 ± 0,0072	110	0,02
Iproniazid	100,0	24	0,102 ± 0,0044	144	< 0,001
Amphetamin	5,0	3	0,080 ± 0,0031	113	< 0,001
Mescalin	25,0	4	0,066 ± 0,0121	92	0,1-0,2
LSD	2,0	2	0,045 ± 0,0052	63	< 0,001

(+ 13%) zur Folge; eine Hemmwirkung auf die Aminoxydase ist unsicher. Die beiden bekanntesten Phantastika senken den Plasmahistamingehalt: *Mescalin* nur wenig und statistisch nicht sicher, aber *LSD* schon nach 2 h um 40%! Dieser Unterschied ist vor allem, verglichen mit der stark sedativen Wirkung der Antagonisten Reserpin, Chlorpromazin und Numal, interessant, da innerhalb dieser Gruppe eine Parallelität zwischen psychischer Wirkung und Histaminstoffwechsel besteht, was bei der Katecholamin- und Serotoninfreisetzung bekanntlich nicht der Fall ist.

Immerhin muss betont werden, dass die psychopharmakologischen Effekte aller untersuchten Stoffe keineswegs mit dem Plasmagehalt an Histamin vergleichbar sind. Doch wissen wir über die Beeinflussung der Bildung, der Freisetzung, des fermentativen Abbaus, der Ausscheidung des Histamins, wie auch über den zeitlichen Verlauf und den Ort dieser Wirkung noch sehr wenig.

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Pharmakologisches Institut der Universität Zürich, 9. Februar 1959.

Summary

The concentration of histamine in plasma of rats is changed by injection of psychopharmacons as follows: decreased by narcotics (ether, chloroform) and phantastics (LSD, mescaline), increased by tranquilizers (reserpine, chlorpromazine) a hypnotic (numal) and psychotomimetics (iproniacide, amphetamine).

PRO LABORATORIO

Apparatus for Spectrophotometric Microtitration Application to the Titration of Deuterohemin

In 1955, we built a small apparatus replacing the standard cell holder of a Beckman spectrophotometer Mod. DU, which allows concomitant measurement of the extinction and of the pH. A similiar arrangement has been recently described by BOAZ and FORBES[1] for the 'Cary' recording spectrophotometer. This encouraged us to publish the present note.

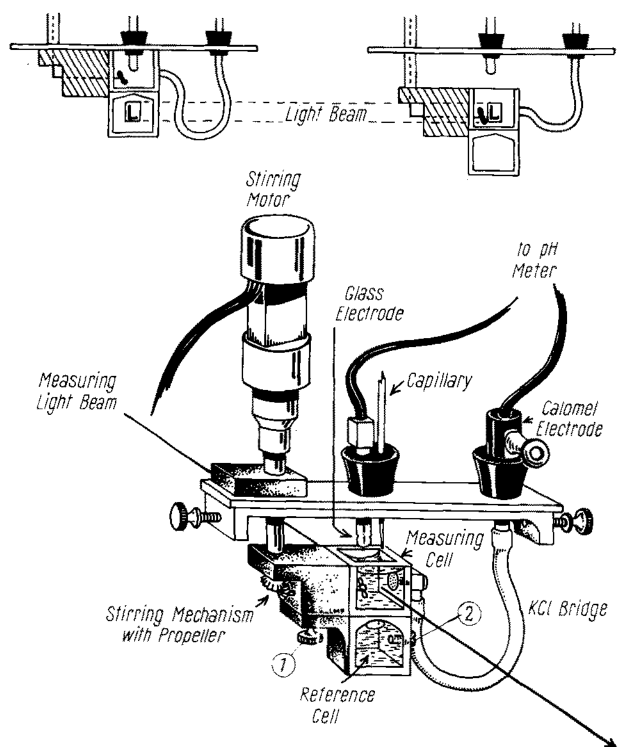

Fig. 1.—*Sketch of the apparatus*. The lock (made of brass) fits into the standard cell-holder compartment of a Beckman DU spectrophotometer. The lock carries: (1) the stirring motor, to which the cells (made of lucite) are attached; (2) a rubber stopper with the glass electrode and capillary from the micro-buret; and (3) a rubber stopper with a calomel electrode connected to KCl-bridge. The electrodes are by Beckman.—On top of the figure, the operation of the apparatus is schematically represented. For further explanations cf. the text.

The requirements of simplicity, light-tightness, and protection of delicate components (glass electrode and tip of microburet) demanded a minimum of moving parts. The sample and reference cells, made of lucite, are placed on top of each other and are movable in a vertical direction (Fig. 1). The glass electrode and the capillary tip of the

[1] H. E. BOAZ and J. W. FORBES, Anal. Chem. *30*, 456 (1958).

microburet (an 'Agla' syringe from Borroughs-Wellcome) remain fixed. The calomel electrode is connected to the sample cell by a piece of tygon tubing filled with KCl solution. Liquid connection between this salt bridge and the sample solution is made through small holes in the lucite cell wall plugged with cotton thread. Stirring is accomplished by a tiny propeller of pure nickel, driven by a small electric motor which also serves as a handle for raising and lowering the two cells.

Measurements are made as follows: the apparatus is removed from the spectrophotometer. The reference cell is disconnected [screw (1)] and filled at (2) by means of a syringe. After closing (2) with a threaded teflon plug, the cell is attached again. Gas bubbles collect in the roof of the cell. The KCl bridge is now freed from air bubbles, the parts reassembled, and checked for light leaks (black tape).

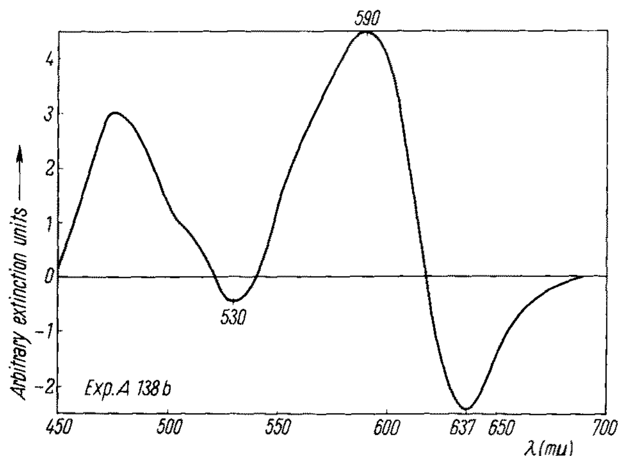

Fig. 2*a*. –The difference between the absorption spectra of deuterohemin at pH 10·3 (borate buffer) and at pH 5·7 (acetate buffer) respectively [Deuterohemin] ≈ 0·02 m*M*. The wavelengths 530 and 590 mμ were chosen for the titration experiment.

Abscissa: wavelength in mμ; ordinate: arbitrary extinction units (difference values).

Readings are made in 2 positions of the cell holder, as shown at the top of Figure 1. In *position A* (at left) an addition of the titrating solution to the sample solution is made, followed by 10–15 sec of stirring. The pH of the sample solution and the extinction of the reference solution are measured ('check' position of the spectrophotometer). In *position B* (at right) the extinction of the sample solution is read, whereupon a new 'cycle' is started.

The assembly was used for titrating deuterohemin (deallyl-protohemin IX). A difference spectrum (Fig. 2*A*) showed the two wavelengths at which the extinctions of deuterohemin at pH 5·7 and at pH 10·3 differ the most, namely 530 mμ and 590 mμ. The spectrophotometric titration was therefore carried out at these 2 wave lengths.

Pure, recrystallized deuterohemin was obtained by heating protohemin with three parts of resorcinol at 200° for 45 min and recrystallizing the crude product from warm acetic acid and NaCl. The crystals were dissolved in 0·1 *N*. NaOH. An appropriate volume of this solution was added to a buffer containing 40 m*M* of each of glycine, sodium acetate, disodium orthophosphate, sodium barbiturate, sodium citrate, sodium lactate, and boric acid. Preliminary tests had shown that this buffer has a good capacity over the pH range from 3 to 10. The microburette was filled with $N{\cdot}H_2SO_4$. The sample cell contained 1·60 ml deuteroheminbuffer solution, the reference cell was filled with freshly boiled distilled water.

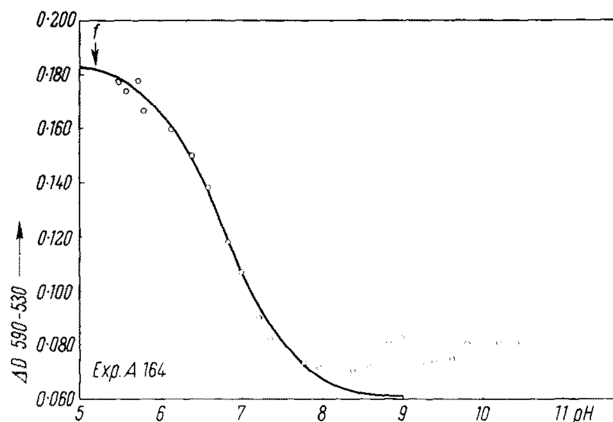

Fig. 2*b*.–The result of a typical spectrophotometric microtitration. Abscissa: pH. Ordinate: The difference of the extinction readings at a maximum (590 mμ) and a minimum (530 mμ) of the difference spectra of Fig. 2*A*. At *f*, the hemin begins to precipitate.

○ values of pH and ΔE actually read (corr. for dilution).,
—— theoretical dissociation curve for pK 6·80 and n = 1.

The points at pH values of >8 suggest a further pH dependent reaction.

Figure 2*B* shows the result of such a titration. The pK value was found to be 6·8 ± 0·05 at a high ionic strength.

pK values reported for the replacement of the hydroxyl group associated with the hemin iron by a water molecule

Hemin	pK	Wavelength	Authors references
Protohemin IX	7·6	?	SHACK and CLARK[2]
Mesohemin IX	7·0	607 mμ	COWGILL a. CLARK[3]
Deutero-hemin IX	6·8	590–530 mμ	this paper
Coprohemin I	7·44	600 mμ	CLARK a. PERKINS[4]

The Table lists the results of similar experiments with other hemins, published by CLARK *et al.* They added aliquots of alkaline hemin solutions to buffers of known pH and measured the extinction of the resulting solution at the wavelengths indicated.

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The Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia (Pa), December 12, 1958.

Zusammenfassung

Ein Apparat zur spektrophotometrischen Titration kleiner Substanzmengen wird beschrieben. Er erlaubt die Messung von pH und optischer Dichte in einem Arbeitsgang und ist so konstruiert, dass er in einen gewöhnlichen Beckmann-Spektrophotometer hineinpasst. Als Beispiel wird die Titration von Deuterohämin beschrieben. Der erhaltene p*K*-Wert ist 6,8 ± 0,05.

[2] J. SHACK and W. M. CLARK, J. biol. Chem. *171*, 143 (1947).
[3] R. W. COWGILL and W. M. CLARK, J. biol. Chem. *198*, 33 (1952).
[4] W. M. CLARK and M. E. PERKINS, J. biol. Chem. *135*, 643 (1940).
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