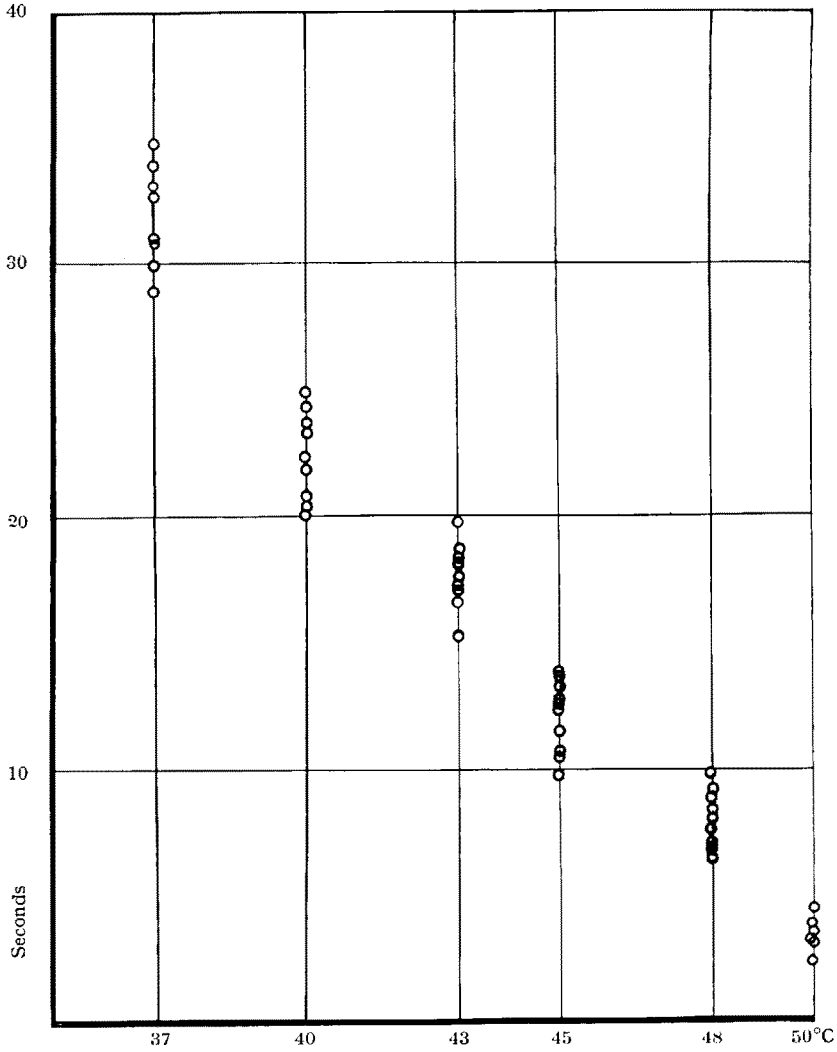

Fig. 3

fixative itself extracts nucleotide material[4], this method seems largely unsuitable. Consequently, the preparation of microtome sections of fresh-frozen tissue[5] in recent modifications[6], or mounting of the sections on slides without using water, has to be adopted.

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Department of Histology, University of Lund (Sweden), November 5, 1957.

Zusammenfassung

Aus fixiertem Gewebe bzw. Paraffinschnitten, die mit normaler Technik auf Wasser ausgebreitet werden, gelingt es, Nukleotide und Eiweissderivate zu extrahieren. Quantitative Daten werden mitgeteilt, und es wird deren Bedeutung für die Histochemie diskutiert.

[5] K. Linderstrøm-Lang and K. R. Mogensen, C. R. trav. lab. Carlsberg Sér. Chim. *23*, 27 (1938).

[6] B. W. Grunbaum, J. R. Geary, Jr., and D. Glick, J. Histochem. Cytochem. *4*, 555 (1956). – W. Thornburg and P. E. Mengers, J. Histochem. Cytochem. *5*, 47 (1957).

PRO EXPERIMENTIS

Adaptation of the Methyl Orange Method for the Determination of Reserpine*

The method proposed in the present paper represents an adaptation of a general reaction for organic bases developed by Brodie and Udenfriend[1], and has the advantages over previous methods[2] of being simple, rapid, and applicable to both pharmaceutical preparations and biological materials. Reserpine is extracted from slightly acidic solutions (pH adjusted to between 4–6 with 0·1 N HCl) with five times as much ethylene dichloride (the solvent phase is washed with an equal volume of 0·1 N sodium hydroxide), and then reacted

* A grant for this study, and the reserpine used in these experiments, were generously supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[1] B. B. Brodie and S. Udenfriend, J. biol. Chem. *158*, 705 (1945).

[2] S. M. Hess, P. A. Shore, and B. B. Brodie, J. Pharmacol. *118*, 84 (1956). – A. J. Glazko, W. A. Dill, L. M. Wolf, and A. J. Kazenko, Pharmacol. *118*, 377 (1957). – R. B. Poet and J. M. Kelley, 126th National Meeting of the A.C.S., Division of Biological Chemistry, New York (September 1954).

with methyl orange to form a soluble complex in the organic solvent. Color is then developed by the addition of acid alcohol. The details of the technique are otherwise essentially as described in the original article[1].

Results and Discussion.—Reserpine-methyl orange colored complex has a maximum absorption peak ranging from 521 to 535 millimicrons. Selection of the midpoint of this plateau gives the most reproducible results in analysis.

Table I
Effect of time on the optical density of the reserpine-methyl orange complex.

Elapsed time in minutes	Optical density
5	0·310
10	0·312
20	0·309
40	0·308
60	0·310
120	0·310
240	0·310
480	0·309
640	0·310

Table I shows the effect of time on the optical density of the final colored reserpine-methyl orange complex. Color development is apparently complete within 5 min after the addition of the acid alcohol and is stable at room temperatures for at least 8 h. Optical density of the color is proportional to concentrations of reserpine in ethylene dichloride ranging from 0·3 to 15 μg/ml; the maximum values corresponding to an optical density at the upper limit of sensitivity of the colorimeter.

Although varying losses occur with changes in the pH of the medium from which reserpine is extracted, over 92% recoveries are possible between a pH of 4 and 8. Table II shows the effect of pH on the recovery of reserpine from plasma. When the pH of the medium was set at 4–6, recoveries generally ranged from 92–96%. Representative figures for tissue homogenates, plasma, urine, aqueous solutions, and tablets are presented in Table III. In all cases, blanks were low, corresponding to less than 0·05 μg of reserpine per ml of ethylene dichloride.

Table II
Effect of pH on recovery of reserpine from plasma[1]

pH	μg reserpine added	μg reserpine per ml	Percentage[2] reserpine recovered
1·0	25·0	2·5	89·0 ± 2·4
4·0	25·0	2·5	95·5 ± 1·9
6·0	25·0	2·5	95·8 ± 2·6
8·0	25·0	2·5	94·0 ± 3·3
11·0	25·0	2·5	90·0 ± 3·0

Of several sulfonic acids, methyl orange proved to be the one of choice because it is a water soluble dye which is apparently insoluble in ethylene dichloride and has a high color index. It forms a reserpine-methyl orange complex which is very soluble in ethylene dichloride and enters the organic phase in amounts proportional to a wide range of concentrations of reserpine. Different lots of methyl orange produce slight but constant, variations in optical density for a given amount of reserpine although the absorption spectrum retains the small relative shape. Therefore, it is necessary to make a new standard curve for each new batch of methyl orange used. In this study, a standard was always run along with unknowns thereby eliminating this variable.

Table III
Recovery of reserpine with the pH of the medium set at 4–6.

Substance analysed	μg reserpine added	μg reserpine per ml	Percentage[3] reserpine recovered
Aqueous solutions	12·5	1·25	96·8 ± 2·6
	25·0	2·50	97·6 ± 2·1
Tablets	50·0	5·00	96·5 ± 1·8
	100·0	10·00	97·2 ± 1·5
Plasma	12·5	1·25	92·9 ± 2·9
	25·0	2·50	93·2 ± 3·3
Urine	12·5	1·25	93·5 ± 3·0
	25·0	2·50	94·8 ± 2·8
5% homogenate[4] .	12·5	1·25	92·8 ± 3·1
	25·0	2·50	93·6 ± 2·6

The proposed method has already been used to estimate the plasma disappearance of reserpine in the unanesthetized dog[5]. GLAZKO *et al.*[6] subsequently found also that the methyl orange method was suitable for determinations of reserpine and methyl reserpate; however, no data on the method appears in their article. Further possibilities remain that the methyl orange method for reserpine could also be used for studies concerning tissue metabolism of reserpine and, in combination with prior paper or column chromatography, to assay reserpine in various Rauwolfia species.

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Résumé

Une méthode microcolorimétrique est proposée pour la détermination de la réserpine dans les préparations pharmaceutiques et le matériel biologique. La réserpine est extraite des solutions légèrement acides avec du dichlorure d'éthyle, puis est mélangé au méthyl orange pour former un complexe dans le solvent organique. La couleur se forme, par l'addition d'alcool acide, et elle est proportionnelle aux concentrations de réserpine allant de 0·3 à 15 μg/ml de dichlorure d'éthyle. En général on obtient des récupérations dépassant 92%. La méthode a été considérée comme applicable à des études concernant la disparition de la réserpine du protoplasme.

[1] Aqueous solutions, tablets, urine, and tissue homogenates give essentially the same results.

[2] Figures represent means ± standard deviations of ten consecutive determinations.

[3] Figures represent means ± standard deviations of ten consecutive determinations.

[4] 5% homogenates of dog liver, kidney, and spleen give essentially the same results.

[5] E. A. DE FELICE, Exper. *13*, 373 (1957).

[6] A. J. GLAZKO, W. A. DILL, L. M. WOLF, and A. J. KAZENKO, J. Pharmacol. *118*, 377 (1957).

[7] Preliminary aspects of this study were completed while the author was Ciba Fellow in Pharmacology at Boston University School of Medicine. Currently the author is Professor of Biological Science at the New England College of Pharmacy.