A Method for the Fluorimetric Determination of Thioridazine (Mellaril®) or Mesoridazine (Lidanil®) in Plasma

We needed a method for determining thioridazine which, in addition to being sensitive enough for estimating blood levels, would also be suitable for assaying a large number of samples in a clinical laboratory.

Phenothiazines have a relatively low intrinsic fluorescence, which can be only slightly increased by cooling the samples to a low temperature or by using an alkaline rather than an acid medium. Most authors have tried, therefore, to increase the fluorescence by oxidation reactions.

RAGLAND et al.[1] have described a method for the determination of phenothiazines where a fluorophor is formed by oxidation with hydrogen peroxide. As the intensity of the fluorescence with this procedure decreased continuously with time, it was difficult to obtain reproducible results.

In the procedure of MARTIN[2] fluorescent derivatives are produced by reacting the phenothiazines with concentrated sulfuric acid. According to the author the fluorophor formed with thioridazine is, however, relatively unstable and the reading has to be taken within 15 min following the reaction.

MELLINGER and KEELER[3] have developed a method for fluorescence titration of phenothiazines using potassium permanganate. In their procedure a solution of potassium permanganate is added successively until maximum fluorescence is reached; with further addition of the reagent the intensity again decreases.

The method we have developed is a modification of the potassium permanganate titration procedure of MELLINGER and KEELER[3]. An excess of permanganate is allowed to react with the drug for a short period and then oxidation is stopped by addition of a dilute solution of hydrogen peroxide. In this way very reproducible readings are obtained.

In order to test the stability of the fluorophor formed from thioridazine, the fluorescence reading in the spectrofluorimeter was recorded for 3-min periods with continuous illumination immediately after adding hydrogen peroxide and then 10 and 30 min later. The reading decreased by 2% after 13 min and by 6% after 33 min.

Procedure. The details of our procedure for measuring thioridazine (Mellaril®) or its side-chain sulfoxide, mesoridazine (Lidanil®), in plasma are as follows:

Plasma or urine, 1–5 ml samples in a 25 ml glass-stoppered centrifuge tube, are alkalinized with 0.5 ml of 10 *N* sodium hydroxide and extracted with 10 ml of heptane containing 1.5% isoamyl alcohol by shaking on a machine for 15 min. Then the tubes are centrifuged for 5 min at 3000 rpm. A 9-ml aliquot of the heptane is transferred to another centrifuge tube, and the thioridazine (mesoridazine) returned to an aqueous phase by shaking with 2 ml of a 2 *M* acetate buffer, pH 3. After centrifugation the organic layer is removed by aspiration and 1.5 ml of the buffer solution is used for the assay.

The samples, acidified with 0.5 ml of 0.2 *N* sulfuric acid, are oxidized by addition of 0.1 ml of a 0.1% solution of potassium permanganate. After 5 min at room temperature the reaction is stopped by adding 0.1 ml of a 0.6% solution of hydrogen peroxide. The fluorescence is determined in a Zeiss spectrofluorimeter at 355 nm excitation, and 440 nm fluorescence.

Internal standards are obtained by adding known amounts of the drug to plasma or urine. A stock solution of 100 µg/ml in 0.1 *N* hydrochloric acid, from which the standards are prepared daily by dilution, can be stored in a refrigerator for at least 2 weeks. Blanks are prepared by omitting the drug from the samples, which are otherwise treated identically.

Discussion. Sensitivity: The intensity of fluorescence shows a linear relationship in the concentration range studied up to 10 µg. Approximately 80% of the thioridazine and 90% of the mesoridazine standard added to blood samples can be recovered by this extraction procedure. From dog urine, recoveries of approximately 95% are obtained with both drugs.

The sensitivity – defined as the smallest difference between the contents of the substance in 2 different samples that can be distinguished by the method[4] – allows the detection of approximately 0.05 µg of thioridazine or mesoridazine.

Specificity: The metabolism of thioridazine in the rat has been studied using the ^{35}S-labelled compound[5]. The

Table I. Blood levels of thioridazine in the rat

Min.	20 mg/kg, i.v. µg/ml ± s.d.	N	50 mg/kg, i.p. µg/ml ± s.d.	N	10 mg/kg, p.o. µg/ml ± s.d.	N
5	3.25 ± 0.19	2	1.95 ± 0.22	2	–	
10	3.52 ± 0.38	4	5.19 ± 1.87	9	0.05 ± 0.03	5
30	2.55 ± 0.48	4	5.11 ± 0.85	13	0.17 ± 0.08	6
60	2.27 ± 0.62	4	4.89 ± 1.26	8	0.16 ± 0.04	6
120	2.26 ± 0.20	4	4.25 ± 1.27	9	0.26 ± 0.14	6
180	1.48 ± 0.17	2	3.38 ± 1.25	6	0.24 ± 0.02	6
240	1.01 ± 0.02	2	2.67 ± 0.89	7	0.26 ± 0.10	6
360	–		–		0.11 ± 0.05	3
480	–		–		0.04 ± 0.02	3
$T\frac{1}{2}$[a]	2.3		3.3		1.5	

[a] Biological half-life, h. Mean ± s.d. = 2.4 ± 0.9.

Table II. Human plasma levels of thioridazine[a]

h	3	8	24	48	72	96	$T\frac{1}{2}$[b]
Patient							
1	2.24	1.87	0.75	0.16	0.00	–	11.6
2	2.13	1.85	0.89	0.29	0.06	0.00	15.1
3	1.20	1.81	0.51	0.07	0.00	–	8.4
4	0.93	1.51	0.99	0.41	0.15	0.03	19.4
5	2.43	1.97	0.73	0.16	0.00	–	11.2
Mean ± s.d.	1.79 ± 0.67	1.80 ± 0.17	0.77 ± 0.18	0.22 ± 0.13			13.1 ± 4.2

[a] µg/ml. [b] Biological half-life, h.

[1] J. B. RAGLAND, V. J. KINROSS-WRIGHT and R. S. RAGLAND, Analyt. Biochem. *12*, 60 (1965).

[2] E. A. MARTIN, Can. J. Chem. *44*, 1783 (1966).

[3] J. MELLINGER and C. E. KEELER, Analyt. Chem. *36*, 1840 (1964).

[4] P. A. ANASTASSIADIS and R. H. COMMON, Analyt. Biochem. *22*, 409 (1968).

[5] K. ZEHNDER, F. KALBERER, N. KREIS and J. RUTSCHMANN, Biochem. Pharmac. *11*, 535 (1962).

metabolic reactions observed were *N*-demethylation, oxidation at both sulphur atoms and the formation of glucuronides of hydroxylated derivatives. The majority of metabolites in the bile (over 80%) consists of conjugated hydroxylated derivatives. These conjugated, acidic derivatives are not taken up by heptane from alkaline medium and should therefore not interfere with the determination of the parent compound.

The predominant derivative of the free metabolites is the thioridazine disulphoxide. The possibility of separation of potential metabolites from the parent compound by extraction has been tested by studying the distribution of these compounds between various buffer solutions and heptane-isoamyl alcohol. Only a minute quantity of the thioridazine disulphoxide is extracted into the organic phase at an alkaline pH. Nor-thioridazine and its side chain sulphoxide are transferred almost entirely from a pH 10 buffer into heptane. At pH 3, however, only about 50% of these latter 2 derivatives are returned into the aqueous phase. Therefore at best half of these 2 metabolites would be included in the determination.

The side-chain sulphoxide of thioridazine, mesoridazine, is extracted to the same extent as the parent compound and the intensity of fluorescence is also the same. Thus, this method can also be used for the determination of mesoridazine.

Another phenothiazine, chlorpromazine, and the dibenzazepine, imipramine, were studied for possible interference in the determination of thioridazine. Both drugs should not interfere, since under the conditions used for fluorophor formation the fluorescence of 10 μg of chlorpromazine corresponds to the blank reading and 5 μg of imipramine also produces no significant fluorescence.

Results. The method has been applied to the determination of blood levels of thioridazine in laboratory animals and man[6].

The data obtained with rats after i.v. injection of 20 mg/kg, i.p. administration of 50 mg/kg, and peroral administration of 10 mg/kg of thioridazine are contained in Table I.

From these data a biological half-life of about 2.5 h has been calculated for the disappearance of thioridazine from rat blood. The biological half-lifes were obtained by selecting the best fitting line for the exponential part of a semilogarithmic plot by means of the 'least squares' method.

In the same way a half-life of approximately 13 h was found for the disappearance of thioridazine from the plasma of patients treated with a single oral dose of 200 mg of the drug (Table II).

These results demonstrate that the rate of disappearance of thioridazine from human plasma is much slower than from rat blood.

Zusammenfassung. Es wird eine Methode zur fluorimetrischen Bestimmung von Thioridazin (Melleril®) und dessen Seitenkettensulfoxid Mesoridazin (Lidanil®) beschrieben. Die Erfassungsgrenze der Methode in Plasma und Urin liegt für beide Wirkstoffe bei 0.05 μg pro Probe.

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[6] The human blood was kindly provided by the courtesy of Drs. PÖLDINGER and RÜMMELE of the Psychiatric University Hospital, Basel.

Red Cell Agglutination Kinetics: A Method for Automatic Recording with the Fragiligraph

The fragiligraph is an instrument designed primarily for the automatic recording of the osmotic fragility of red blood cells[1]. Measurement of the degree of hemolysis as a function of decreasing salt concentration is based on recording the increasing transparency of red cell suspensions as hemolysis progresses.

Other applications of the instrument for hematological tests have recently been reviewed[2]. Among the tests which can be performed exploiting the advantage of automatic recording in the fragiligraph, are the quantitative determination of viral hemagglutinins and hemagglutinins-inhibiting antibodies[3,4], the recording of the rate of agglutination and sedimentation by positively charged polyelectrolytes[5] and the agglutination of red cells by antibodies[6]. In the latter test, the fact that the curve obtained in fragiligraph measurement was a composite one, representing the rate of agglutination by the agglutinating agent and the rate of sedimentation of the agglutinated cells, made difficult, if not impossible, analysis of the curve with regard to the phenomena taking place during the agglutination process. In the present communication a method is described in which the rate of agglutination alone can be automatically recorded using the Fragiligraph Model D2 (Elron, Electronic Industries, Haifa, Israel) equipped with a 'linearizer', a unit which enables recording the log transmission on a linear scale, and a simple accessory specifically made for the purpose. Advantage is taken of the sensitive optical system of the instrument and of the steadiness and accuracy of the magnetic stirrer which is independent of line current variations. The dialysing membrane cell is replaced by a siliconized square optical cuvette or a plastic one containing a magnetic bar. When the agglutination curve reaches a plateau, the sedimentation rate curve of the aggregates and single cells can be recorded by removing the magnetic bar stirrer. Thus the agglutination and sedimentation rate curves are recorded separately.

[1] D. DANON, J. clin. Path. *16*, 377 (1963).
[2] D. DANON, Br. J. Haemat. *13*, (Suppl.) 61 (1967).
[3] A. KOHN and D. DANON, Experientia *21*, 296 (1965).
[4] A. KOHN and D. DANON, Int. Symp. Immun. Methods of Biol. Stand. in Series immunobiol. Standard, Basel *4*, 319 (1967).
[5] Y. MARIKOVSKY, D. DANON and A. KATCHALSKY, Biochim. biophys. Acta *124*, 154 (1966).
[6] I. COHN, Ph. D. Thesis, The Hebrew University, Jerusalem (1965).