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.

W. L. PACHA

Pharmaceutical Chemical Laboratories, Sandoz Ltd., 4000 Basel 13 (Switzerland), 19 September 1968.

[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.

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[3] A. KOHN and D. DANON, Experientia *21*, 296 (1965).
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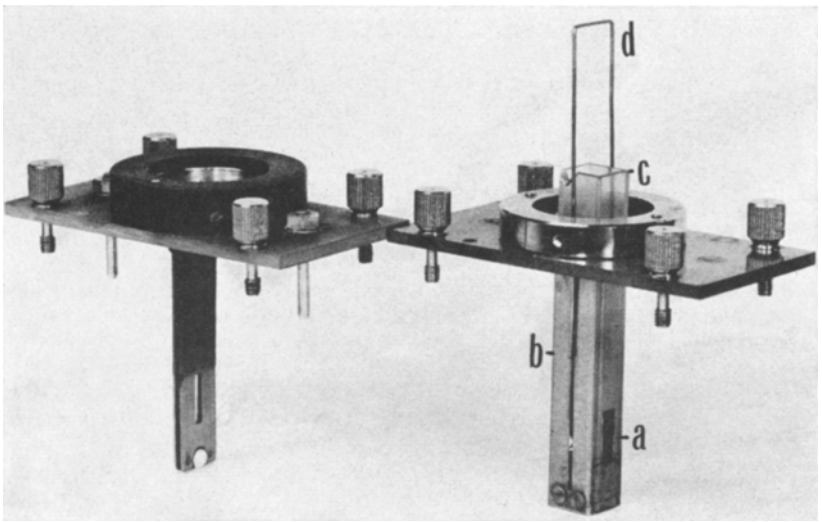

Fig. 1. The accessory for introducing the glass or plastic cuvette into the optical pathway of the fragiligraph for measuring the rate of agglutination of cells (on the right) replacing the original part of the instrument (on the left) used when automatic recording of osmotic hemolysis is required. (a) Optical aperture, (b) non-magnetic stainless steel square holder for the cuvette, (c) cuvette, (d) a wire to facilitate the removal of the cuvette after the test.

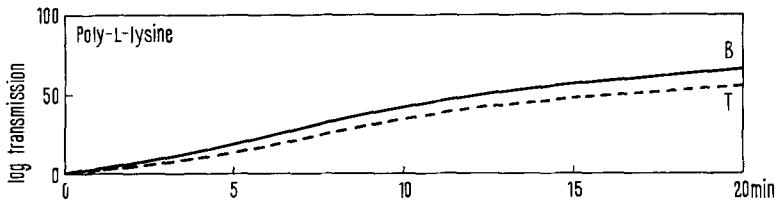

Fig. 2. The rate of agglutination by poly-L-lysine. Agglutination curves of young red cells (T, top fraction) and old red cells (B, bottom fraction).

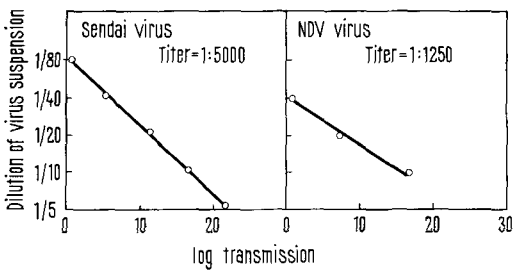

Fig. 3. Hemogglutination by Myxovirus parainfluenza Sendai and NDV virus. A graph representing the correlation between the rate of agglutination and the dilution of Sendai virus (left) and NDV virus suspension (right) (see text).

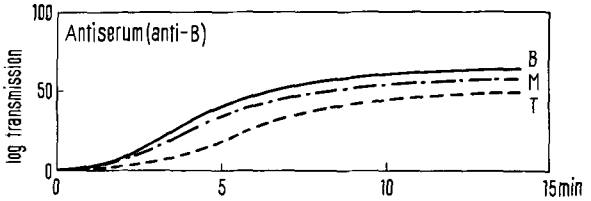

Fig. 4. Agglutination by antibodies. Agglutination curves of: old red cells (group B) (B); young red cells (T) and mixed red cell population (M) using undiluted antiserum (anti B).

The accessory (Figure 1) consists of a plastic plate, similar to that in the instrument which holds the electrodes for measuring the conductivity of the dialysing medium and the space for introducing the container cell. This space is square now rather than round, and a non-magnetic stainless steel square tube is attached to it with an aperture in the optical path and a wire to facilitate removal of the cuvette. Distilled water (7 ml) in a glass container is necessary for maintaining the optical properties of the instrument and for good conductivity between the thermostatically controlled circulating water and the optical cuvette that will be introduced into the accessory.

Agglutination by poly-L-lysine: Heparinized or citrated human blood was used. Separation of old and young red cell fractions was carried out as previously described[7], using a battery of non-water miscible phthalate ester mixtures of predetermined specific gravity. The separated fractions were washed twice before use. The suspension for the test was prepared by resuspending 1 vol. of packed red cells in 500 vol. of 0.9 NaCl solution buffered to pH 7.3 with veronal acetate.

1.8 ml of the test suspension is introduced into the siliconized square optical cuvette. The siliconization is necessary to avoid adhesion of aggregates to the internal wall of the container. The baseline is adjusted to the first line of the recording paper. A teflon coated magnetic stirrer (size $^3/_8$ inch) is then introduced into the optical cuvette. The magnetic bar is automatically activated as the recorder is switched to the 'on' position. After a few seconds of stirring and recording at baseline level, 0.4 ml of poly-L-lysine hydrobromide solution, degree of polymerization n = 100 (Yeda Research and Development Co., Rehovot, Israel) (100 μg/ml in 0.9% NaCl) is added rapidly. The baseline should be slightly readjusted because of the dilution by the added transparent polymer solution.

[7] D. Danon and Y. Marikovsky, J. Lab. Med. *64*, 668 (1964).

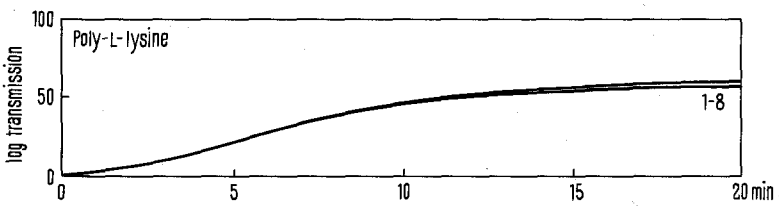

Fig. 5. The range of 8 successive agglutination curves by poly-L-lysine showing the reproducibility of the method.

Two recorded curves, representing the difference in the rate of agglutination by poly-L-lysine of a top (young red cells) and bottom (old red cells) fraction of the same blood sample (Figure 2) are presented.

Viral hemagglutination: Preparation of chicken red cell suspension and working procedures were identical as for the measurement of the rate of agglutination with poly-L-lysine. However, since at room temperature or higher, myxoviruses elute from the red cell, the recording was carried out in the cold room (4 °C).

Higher temperatures could be obtained by adjusting the temperature to the thermostatically controlled circulating water. Hemagglutination was initiated by adding 0.2 ml of virus suspension to 1.8 ml of red cell suspension in the cuvette. The viruses used were: NDV virus, strain HP, and Myxovirus parainfluenza Sendai as stock suspensions of HA titer 1:1250 and 1:5000, respectively. The recorded hemagglutination curves were measured after a given recording time for the degree of displacement, i.e. % transmissivity. The correlation between the deviation of the curve and the virus dilution of a representative experiment is depicted in Figure 3.

Agglutination by antibodies: A volume of washed packed human red blood cells was suspended in 500 vol. of isotonic NaCl solution. 1.8 ml of this suspension was introduced into the cuvette containing the magnetic bar mixer. 1 drop of concentrated antiserum was added. Commercial blood grouping sera were used (Hyland, Los Angeles, California). The rate of agglutination of old and young cell fractions by antiserum of the same titers is illustrated in Figure 4.

Discussion. The steady continuous stirring in the cuvette prevents sedimentation. With the progress of agglutination the cross section of the cells that stopped or scattered the light before agglutination is markedly reduced, resulting in increased light transmission. The bigger the aggregation of cells, the higher the loss of cross section of cells available for stopping or scattering the light.

The character of the curve indicates that the function is relatively simple. A practically straight line is obtained when the original curve is plotted on semilogarithmic paper so that the curve, and hence the rate of agglutination, can be characterized by a rather simple numerical expression. The reproducibility of the method is fairly satisfactory (see Figure 5). However, if the blood suspension is left for more than 30 min at room temperature, changes in the rate of agglutination may occur.

It is concluded that rate of agglutinability, whether with polylysine, virus or antibody, can be automatically recorded with the fragiligraph. In addition, the distance between the level of the plateau and the 100% transmission indicates the proportion of cells that remained non-agglutinated, a factor the knowledge of which is important for the estimation of both virus and antibody titers.

Photometric quantitation of hemagglutination has recently been reported[8]. However, the technique implied separate measurements of different times after induction of agglutination. The advantage of the automatic recording is obvious.

Résumé. Une méthode est décrite qui permet de mesurer et d'enregistrer automatiquement la vitesse d'agglutination des globules rouges par les hémagglutinines virales, la poly-L-lysine et les antisérums. Le fragiligraphe modèle D2 muni d'un nouvel accessoire a été utilisé. La différence de vitesse d'agglutination entre cellules jeunes et agées a été enregistrée.

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PRO NATURA INTEGRA

Editor's note. Under the title of 'Pro Natura Integra', papers on fundamental research in the field of bio-protection will appear. Over-population, under-nutrition and changes in environment have led to ecological disturbances in the balance of Nature which threaten the existance of mankind. Man is faced with uncertainty through the changes in his environment. This most critical crisis can only be over-come by a society which has the will to carry out a biophylaxis which is scientifically founded, ecologico-economically co-ordinated and biopolitically responsible. H. M.