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KINETICS OF HEROIN DEACETYLATION IN AQUEOUS ALKALINE SOLUTION AND IN HUMAN SERUM AND WHOLE BLOOD

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SUMMARY

A kinetic study of heroin hydrolysis in alkaline aqueous solution at room temperature was conducted by a gas chromatographic method to measure the consecutive reactions of diacetylmorphine to monoacetylmorphine and of monoacetylmorphine to morphine. A first-order reaction was observed in both instances, and the rate for the deacetylation of heroin was greater than that of monoacetylmorphine.

The rates of *in vitro* hydrolysis of diacetylmorphine in human whole blood and in serum were compared by the same method. Diacetylmorphine was hydrolyzed twice as rapidly in blood as in serum. While morphine was an end product of hydrolysis in the blood, it was absent in the serum.

INTRODUCTION

The hydrolysis of diacetylmorphine (heroin) to morphine occurs by a pathway of 6-monoacetylmorphine, as confirmed by manometric[1], colorimetric[2], and paper chromatographic[3] methods. However, the manometric and colorimetric procedures do not delineate the molecular entities produced in heroin degradation since the former measures the release of CO_2 during deacetylation and the latter quantitates the free phenolic group liberated from the cleavage of the acetyl groups from the heroin. The paper chromatographic approach does not provide a convenient measurement of the hydrolysis products.

Gas chromatographic (GC) techniques, on the other hand, are highly suited for kinetic studies, since consecutive reactions may be measured, the hydrolysis of intermediate products may be followed, and a precise quantitative analysis of reaction products is possible.

The lability of heroin in aqueous solution, particularly in concentrated solu-

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tions of hydroxyl and hydrogen ions, is of concern to the forensic chemist. Certain illicit drug preparations contain heroin in mixture with other drugs. Since the classical separation procedures described in The Bulletin on Narcotics[4] entail an alkaline treatment of samples prior to the extraction of basic drugs with organic solvents, it is possible that the heroin component of that sample would be subject to rapid degradation. The rate of that degradation would be of practical interest.

A comparative measurement of the rate of hydrolysis from diacetylmorphine to monoacetylmorphine and from monoacetylmorphine to morphine would be more meaningful than a graphic comparison by paper or thin-layer chromatography (TLC).

A kinetic study of the hydrolysis of heroin in an aqueous system can be used as a basis for further studies as a function of pH, of temperature, of prolonged storage, of relative amounts of humidity, and of enzyme catalysis in *in vitro* biological reactions. The present study was undertaken to elucidate the nature of heroin with respect to its structural stability in aqueous solution and in biological fluid as an initial step in establishing a method for its determination in blood.

Ellis[5] presented experimental evidence that the 6-acetyl and 3-acetyl groups of heroin were perhaps cleaved by separate enzymes in rabbit plasma.

Way *et al.*[6] noted the rapidity in which heroin was deacetylated in man and four other mammalian species when incubated in liver, kidney, blood, and brain; these investigators employed a countercurrent extraction system for separating 6-monoacetylmorphine from heroin. Nakamura and Ukita[7] demonstrated that heroin is deacetylated *in vivo* in dog within 10 min following an intravenous dose. Therefore, it seems futile to attempt detection of heroin in blood, and morphine would appear to be the metabolic product to be detected to imply the use of heroin. However, 6-monoacetylmorphine remained a possible entity for a systematic search in blood.

An *in vitro* study of heroin hydrolysis in human blood was undertaken to observe the rate of production and decay of heroin metabolites. A sample of fresh blood serum, obtained from a normal male subject who was not a drug user, and a sample of *post mortem* whole blood specimen freshly removed and heparinized were used. The specific objectives were to determine if complete enzymatic hydrolysis occurred in the serum or in the presence of blood cells, if postmortem blood was capable of degrading heroin and 6-monoacetylmorphine to morphine, if heroin was deacetylated by autohydrolysis in a buffer at physiological pH, and to study the rate of *in vitro* hydrolysis of heroin in blood.

EXPERIMENTAL

Reagents

Diacetylmorphine·HCl (heroin·HCl) was prepared according to a method described by Nakamura[8]. 6-Monoacetylmorphine was prepared according to the method of Wright[1]. It was found here that the crystallization of 6-monoacetylmorphine is hastened by dissolving heroin in a minimum amount of ethanol and then treating with an equivalent amount of hydroxylamine·HCl. Methadone·HCl USP was obtained from E. Lilly Labs. (Indianapolis, Ind., U.S.A.) and morphine sulfate USP from Mallinckrodt (St. Louis, Mo., U.S.A.).

Gas chromatography

A Microtek Model 220 dual-column gas chromatograph with a hydrogen flame ionization detector was used in this study. The 6 ft. × 4 mm I.D. column was treated with dimethyldichlorosilane. The liquid phase used was 3.8% silicone gum rubber UCW-98 (Hewlett-Packard), applied onto Chromosorb W HP, 80–100 mesh. The column was conditioned by a series of massive injections of free morphine (in methanol) to saturate its absorption sites. The temperature of the oven was maintained at 225°. The carrier gas (helium) flow-rate was 40 ml/min. The attenuation range was set at $10^2 \times 2$.

Mixed standard

Ten milligrams each of diacetylmorphine, 6-monoacetylmorphine, and morphine (each calculated as an anhydrous base) were dissolved in methanol containing 0.2 μg/ml methadone as internal standard. Two microliters were used for comparison purposes. A chromatogram of these standards is shown in Fig. 1.

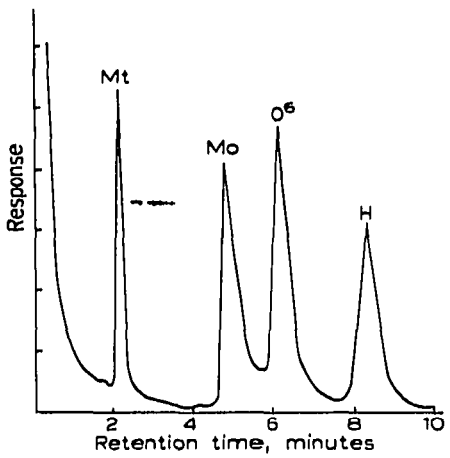


Fig. 1. A mixture of methadone (Mt), morphine (Mo), 6-monoacetylmorphine (O^6) and heroin (H) chromatographed under standard conditions. GC unit, Microtek 220 with flame ionization detector; column, 6 ft. × 4 mm glass, packed with 3.8% UCW-98 on Chromosorb W HP, 80–100 mesh. Injection temperature, 225°; detector temperature, 275°; carrier gas (helium) flow-rate, 40 ml/min; attenuation range, $10^2 \times 2$.

Aqueous solution

To 25 ml of water, 10 mg heroin hydrochloride and 25 ml of 1 *M* sodium carbonate were added. After various time intervals (1, 5, 10, 30, 60, 90, and 120 min), a 1-ml aliquot was removed and the pH was lowered to approximately 8.5 by adding 1 ml of saturated ammonium chloride to the aliquot. The mixture was quickly shaken with successive amounts of chloroform–isopropanol (8:2) in volumes of 25, 10, and 10 ml. The chloroform extracts were passed through filter paper to remove water and evaporated over a warm steam-bath under an air jet. Each extract was dissolved in 1 ml of methanol (GC-spectrograde, J. T. Baker, Phillipsburgh, N.J., U.S.A.) containing 0.1 mg methadone. One microliter was injected under standard conditions.

Hydrolysis in serum and whole blood, in vitro

To ten milliliters of fresh human serum and post-mortem whole blood, 1 ml of phosphate buffer, pH 7.4 and 20 and 30 mg, respectively, of diacetylmorphine hydrochloride were introduced. The mixture was incubated at 37.5° and a 1-ml aliquot was removed at intervals of 15, 30, 45, 90, and 120 min.

To each aliquot twelve drops of 40% dipotassium phosphate were added. The mixture was shaken with chloroform–isopropanol (9:1), first with 25 ml, then with 15 ml, and finally with 10 ml. Each succeeding extract was passed through a filter paper containing a pinch of powdered sodium sulfate and then evaporated to dryness on a warm steam-bath under an air jet. The residue was dissolved in 1 ml of methanol containing 0.1 mg of methadone. One microliter was injected under standard GC conditions.

Control

Ten milligrams of heroin hydrochloride were dissolved in 10 ml of 0.1 *M* phosphate buffer, pH 7.3. A 1-ml aliquot was treated with twelve drops of 40% K_2HPO_4 and then extracted and subjected to GC analysis in the same manner as that described for serum and blood hydrolysates.

Thin-layer chromatography

Each of the methanolic extracts from the above GC runs were evaporated to dryness and redissolved in 100 μl of methanol. The solution was quantitatively transferred to a TLC plate (Bakerflex silica gel 1B), each spot representing a particular hydrolysis time. The spots applied from the different hydrolysates were arranged on the plate according to a progressive sequence of time.

About 50 μg each of pure heroin, 6-monoacetylmorphine and morphine were applied as references on the same base line as the hydrolysates. These TLC plates were developed in a covered glass tank in an ascending mode in chloroform–methanol (8:2). The spots were revealed by spraying the plates with iodoplatinate spray reagent, as shown in Figs. 2 and 3. The TLC procedure was adapted from that used by Mary and Brochman-Hanssen[9].

RESULTS AND DISCUSSION

Gas chromatograms of a heroin hydrolysate (Fig. 4) of different time intervals from 1–120 min illustrate the deacetylation of heroin to O^6-monoacetylmorphine, as an intermediate product, and then to morphine, as the final product. The concentration values of reactants and products are shown in Table I. Fig. 5 shows the curves for the two-step series reactions for the degradation of heroin, resulting in a decrease in concentration, the appearance of 6-monoacetylmorphine, and its gradual degradation to morphine, and finally the increasing amount of the end product, morphine. Linear plots of reaction rates of heroin to monoacetylmorphine and of monoacetylmorphine to morphine, as a function of time, are shown in Fig. 6.

The plots of Fig. 5 make it quite evident that the acetyl group at the 3-carbon position, *i.e.*, on the phenolic hydroxyl group, is much more labile than that on the 6-carbon position of the alcoholic hydroxyl group. The reactants appear to be first-order, since a linear plot is obtained and the equation as shown below is obeyed, and

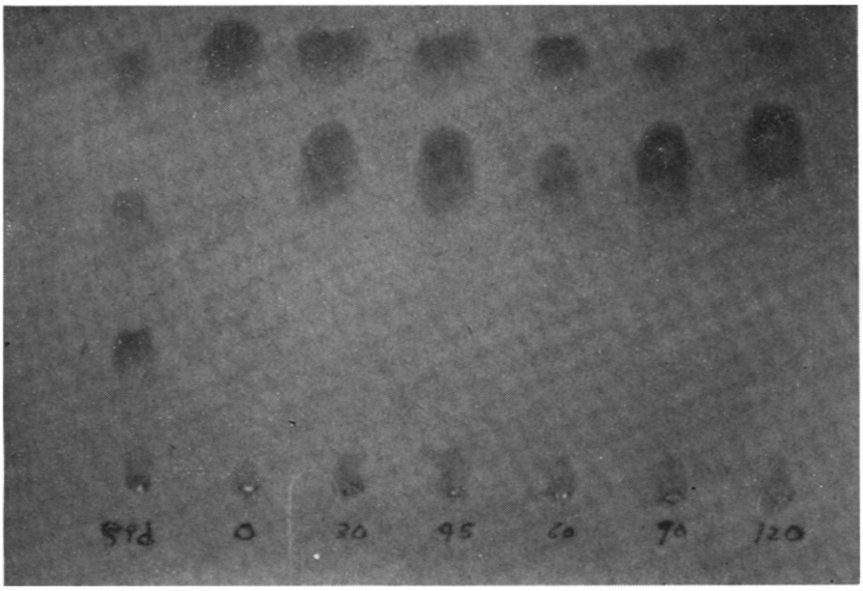


Fig. 2. Thin-layer chromatogram of series hydrolysis of heroin in serum, 0–120 min. From top to bottom: heroin, 6-monoacetylmorphine, and morphine. Std. = Reference standard.

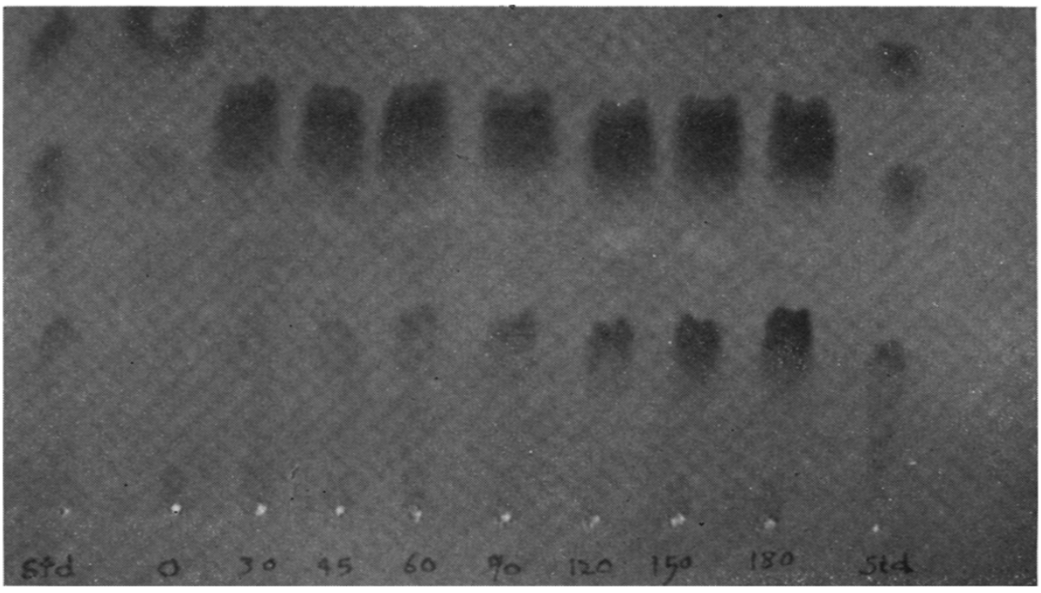


Fig. 3. Thin-layer chromatogram of series hydrolysis of heroin in whole blood, 0–180 min. From top to bottom: heroin, 6-monoacetylmorphine, and morphine. Std. = Reference standard.

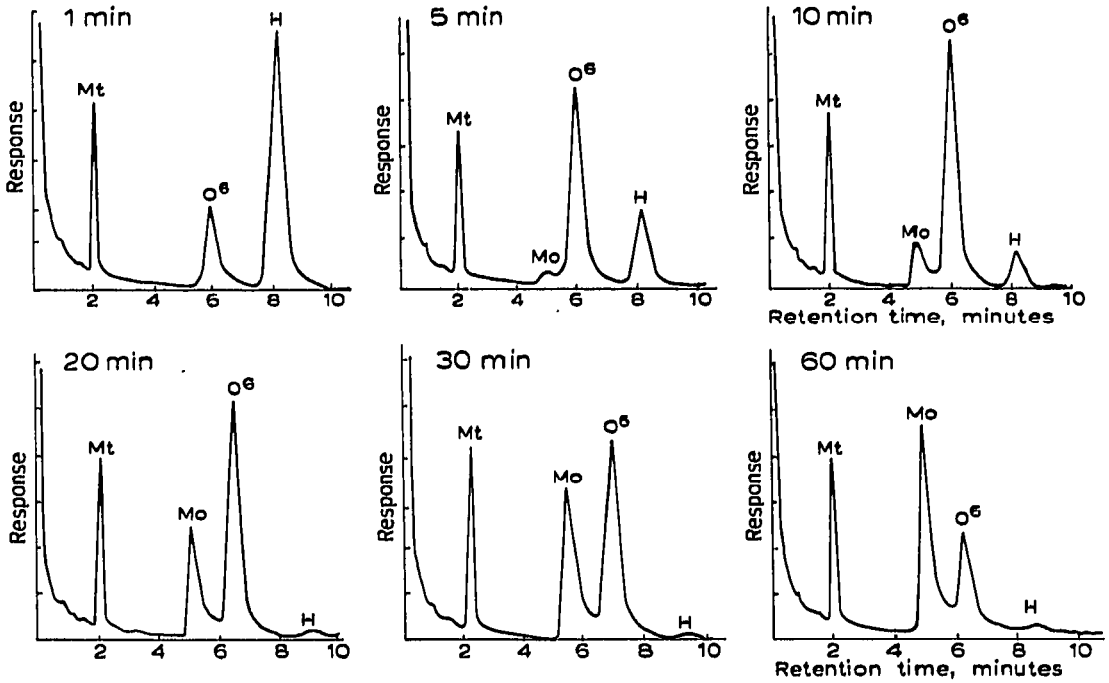


Fig. 4. GC profile of heroin and its hydrolysis products in 1 *M* Na_2CO_3 at different time intervals up to 60 min. The same analytical conditions, except for the length of hydrolysis, were observed in each case.

TABLE I

CONCENTRATION OF REACTANTS AND PRODUCTS IN HEROIN HYDROLYSIS MEASURED FROM GAS CHROMATOGRAMS

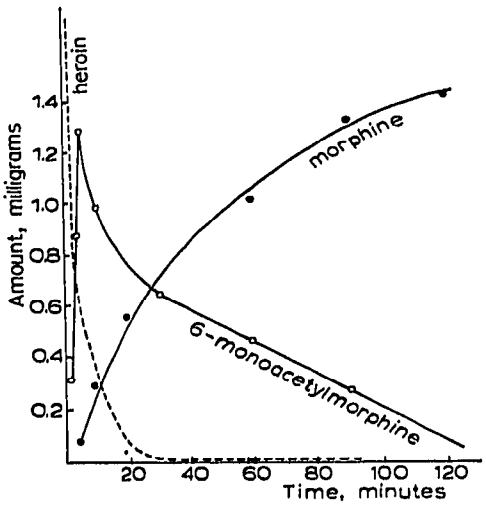
Mean values from two hydrolysis determinations are taken. Triangular method of area measurement made on GC peaks: ½ (height × width) = area.

Time (min)	*Concentration (mg)*		
	Morphine	*6-Monoacetylmorphine*	*Heroin*
1	0	0.33	1.67
5	0.08	1.28	0.56
10	0.28	1.00	0.32
20	0.55	0.77	0.07
30	0.72	0.64	0.04
60	1.06	0.48	0.02
90	1.32	0.29	0.02
120	1.52	0.18	0

since heroin, as shown in Fig. 5, decreases in an exponential manner as morphine increases. Also the entire amount of the reactant is exhausted under standard analytical conditions in which an excess amount of alkali is present in the reaction mixture.

The rate study as shown in Fig. 6 was based on the following eqn.[9]:

$$\log \frac{a}{a - x} = \frac{kt}{2.303} \qquad (1)$$



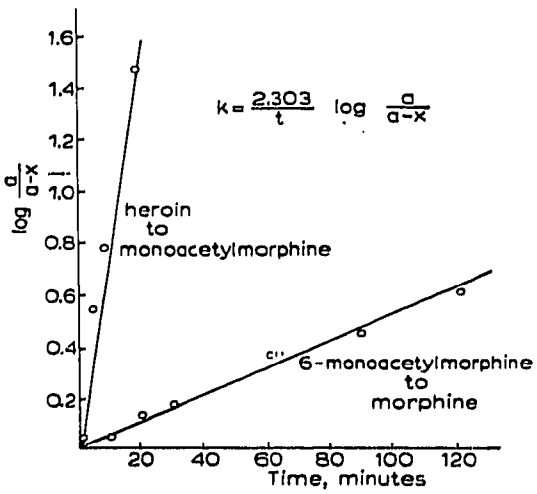


Fig. 5. Concentration *vs.* time plot for series degradation of heroin in 1 *M* Na_2CO_3.

Fig. 6. Linear plots of data in Fig. 4 as a first-order decomposition.

where a is the initial concentration of reactant and x is the product, k is the rate constant, and t is the time of reaction.

The rate constant k of heroin conversion to monoacetylmorphine was 16.6×10^{-2} min^{-1} and that of monoacetylmorphine to morphine was 1.25×10^{-2} min^{-1}. The relative stability of monoacetylmorphine in the aqueous media was inferred. In a separate experiment, it was found that 6-monoacetylmorphine did not completely degrade in hot water (98°) until after 114 h.

The differences in the rate of heroin hydrolysis in whole blood and in serum was studied by using GC and TLC methods. In the serum, as the thin-layer chromatogram of Fig. 2 shows, no cleavage of 6-monoacetylmorphine was observed, while in the whole blood both acetyl groups of heroin were cleaved, resulting in morphine being exhibited on the chromatogram (Fig. 3). The concentration *vs.* time plots of Figs. 7 and 8 demonstrate the rapid deacetylation of heroin as shown by the decrease in heroin concentration with a corresponding increase of monoacetylmorphine. Only for whole blood (Fig. 8) is morphine production apparent as soon as heroin is completely deacetylated. In both serum and whole blood, the amount of monoacetylmorphine remaining in the hydrolysate was relatively large. This finding suggests the advisability of examining *post mortem* blood specimens for the presence of monoacetylmorphine in acute heroin poisoning.

Hydrolysis proceeds at about twice the rate in the whole blood as in the serum; the biological half-life was observed to be 9 min in blood and 22 min in serum. The rate constant k for whole blood was 5.5×10^{-2} min^{-1} while that for the serum was 3.5×10^{-2} min^{-1} as calculated from the slopes shown in Fig. 9. Vincent[11] has characterized blood cells as having an esterase activity and Harthon and Hedstrom[12] demonstrated that whole blood was more vigorous than plasma in deacetylating acetylsalicylic acid.

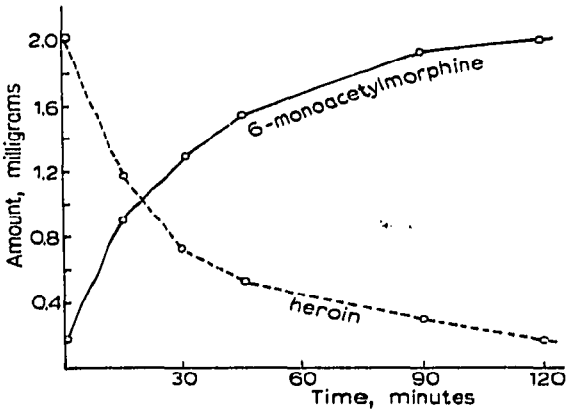


Fig. 7. Concentration *vs.* time plot for hydrolysis of heroin in human serum.

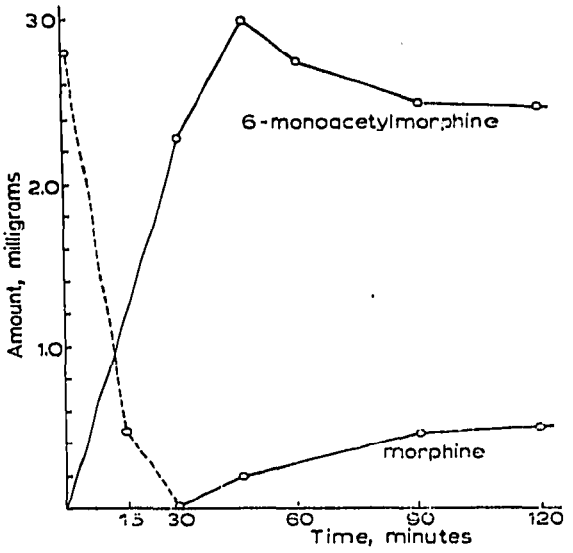


Fig. 8. Concentration *vs.* time plot for hydrolysis of heroin in human whole blood.

Whether 6-monoacetylmorphine can be detected in blood collected from man is left to conjecture, since the presence of this metabolite in human blood has not been reported. It is not infeasible that this could occur, however, since unchanged heroin itself has been detected in human urine samples by Elliott *et al.*[13] and Advenier *et al.*[14]. In an *in vivo* situation, the deacetylation of monoacetylmorphine may proceed at a much faster rate than in an *in vitro* system.

The spontaneous hydrolysis of heroin can be dismissed since a control test conducted in a non-enzymatic system at physiological pH showed that heroin was virtually left undegraded when incubated in phosphate buffer at pH 7.3. To prevent *post mortem* hydrolysis or bacterial action on heroin and 6-monoacetylmorphine in blood specimens, coroner's samples are usually collected in 1% sodium fluoride.

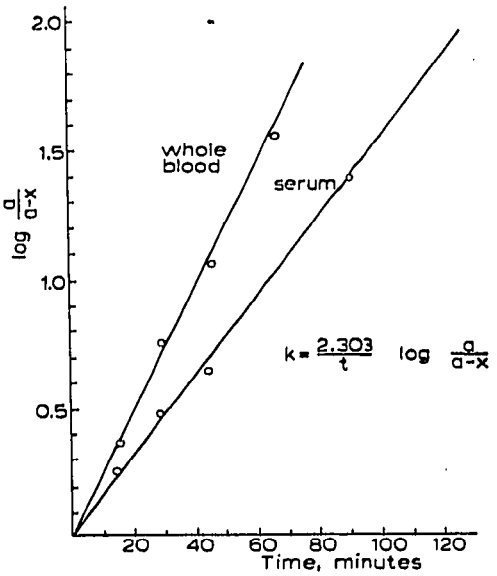


Fig. 9. Linear plots of data in Figs. 7 and 8 as a first-order decomposition.

Plueckhahn and Ballard[15] established that a concentration of more than 1.0% sodium fluoride was necessary to inhibit microbiological formation of ethanol from carbohydrates in stored blood samples.

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