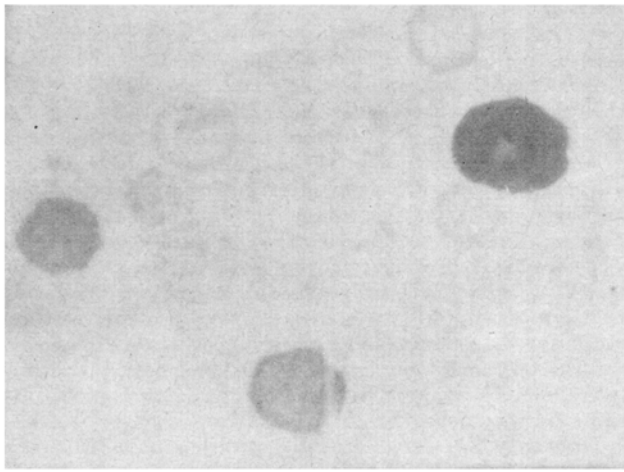

Blood smear from a 15 day old BDF_1 fetus stained by the ferric ferricyanide reduction stain for free sulphydril groups following acid extraction. Negative (extracted) red blood cells are presumably of maternal origin (C57BL/6); the unextracted (dark) cells are 'fetal' red cells staining similarly as the red cells of the father (DBA/2). × 600

acid solubility or resistance for the respective strain. Whereas human and mouse red blood cells give approximately similar intense Prussian blue stains, considerable less positive reaction is obtained in mice (tan) than man (brown) by the iodination-coupled tetrazonium stain. There were no readily perceptible differences in staining intensity among different strains of mice, or adult compared to fetal red blood cells.

Hybrid fetuses resulting from crosses of mothers having acid-extractable hemoglobin (C57BL/6 and BALB/c) and fathers with acid-resistant hemoglobin (A/J and DBA/2) contain the two types of red blood cells (Fig.). Since adult hybrids (CAF_1, BDF_1, BAF_1) have circulating red cells of only one kind, identical to their fathers in that they are not or incompletely eluted by acid, transplacental passage of maternal blood may be inferred. The presence of two kinds of hemoglobin in e.g. BDF_1 fetuses (C57BL/6 females × DBA/2 males) has also been verified by agar gel electrophoresis, showing a single spot and diffuse pattern superimposed.

Circular paper chromatography was employed using Whatman filter paper No. 1; solvents applied were 10–50% aqueous pyridine and combinations of *N*-butanol, pyridine and water. The chromatographic data reveal that the hemoglobin variants lie within a narrow range of mobility; Rf values do not differ by more than ± 0.04 units independent of the eluent used, strain of mouse, and adult or fetal origin of the hemoglobin.

Zusammenfassung. Hämoglobin und Erythrozyten von prä- und postnatal untersuchten Inzuchtmäusen verhalten sich, was verschiedene physikalisch-chemische Analysen betrifft, ähnlich. Die Existenz eines fötalen Hämoglobins konnte nicht bewiesen werden.

H. MEIER[7]

Roscoe B. Jackson Memorial Laboratory, Bar Harbor (Maine), November 22, 1961.

[7] This investigation was supported by a grant from the National Institutes of Health, P.H.S. C-4691.

Estimation of Decamethonium in Biological Fluids

In a previous work, it has been demonstrated that aqueous solutions of methonium salts, if mixed with a small amount of Nessler's reagent, develop a turbidity proportional to salts concentration. Through this very sensitive reaction, it has been possible to estimate very small amounts of methonium salts. Since this reaction is enhanced by the length of the carbon chain, it may be of a particular interest for detection of decamethonium[1] at low concentrations. On account of its strong curarizing action, C_{10} is widely employed in clinical and experimental pharmacology, and therefore its accurate estimation is very much needed in biological fluids, for which we previously postulated the use of the isotopic methods[2].

The direct application of the turbidimetric reaction to biological fluids, and specially to urine and serum, is impossible, owing to the presence of various interfering substances. It is necessary to discard these substances, without loss of C_{10}.

In this paper the results will be reported which relate to the application of this new reaction for quantitative estimation of C_{10} in biological fluids, such as blood serum and urine.

Urine procedure. To 10 ml of freshly collected normal human urine were added 100 mg of solid $HgCl_2$ and 1 ml of 2 *N* NaOH. The massive black precipitate was discarded by filtration, obtaining a slightly turbid filtrate. By addition of 2 ml of 10% HCl in glacial acetic acid, the solution becomes clear on mixing. Samples of 5 ml of the clear filtrate are collected and 0.2 ml of Nessler's reagent added. After 30 min, the turbidity was read in a Beckman spectrophotometer mod. D.U. at 580 mμ wavelength against a blank containing urine without C_{10}, similarly treated. In Figure 1, the data are given of the detection of different amounts of C_{10} added to urine.

As is shown, the urine method allows accurate determination of C_{10} concentrations between 20–200 γ/ml. It must be pointed that in the acid medium the reaction is

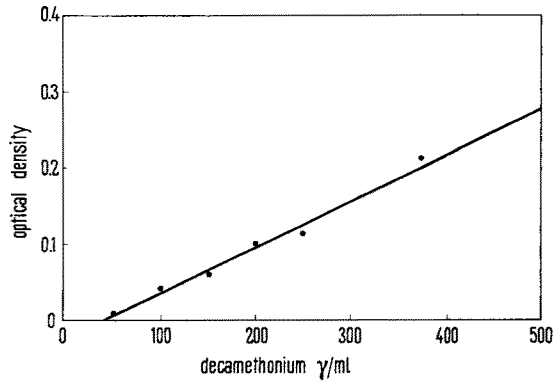

Fig. 1. Estimation by turbidity reaction of decamethonium in urine.

[1] further called C_{10}.

[2] C. MANNI, G. MORICCA, B. GIOVANELLA, and P. MAZZONI, Simposio Internazionale su Curaro, Curarosimili e Curarizzanti (I.T.E. 1958), p. 240.

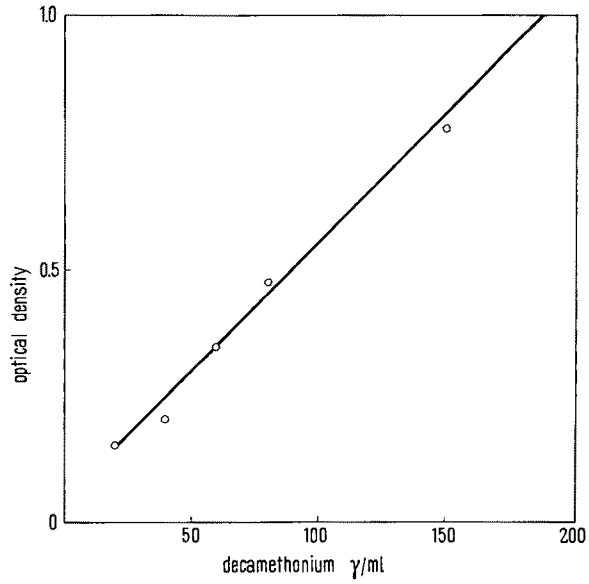

Fig. 2. Estimation by turbidity reaction of decamethonium in human blood serum.

not so specific as in alkaline. Two substances (promethazine (Fargan) and Lobeline), employed in clinical surgery, also give some turbidity.

Serum procedure. Since Nessler's reagent gives a partial protein precipitation, the main problem is represented by deproteinization without loss of C_{10} with a clear supernatant. The $HgCl_2$ treatment leaves the supernatant very turbid. The commonly used deproteinizing methods were employed unsuccessfully, and therefore the following technique was adopted: 5 ml of serum were dialized for 24 h with constant stirring, against 10 ml 0.5 *M* NaCl in order to improve the linearity of the reaction[3]. To a sample of 5 ml, 0.2 ml of Nessler's reagent were added and the turbidity was read as described for the urine, against a blank of serum without C_{10} dialysed against 0.5 *M* NaCl. As shown in Figure 2, there is a perfect linearity of the reaction; the sensitivity of the method falls between 100–500 γ/ml. The reaction occurs in alkaline medium and is absolutely specific.

Résumé. Les auteurs ont modifié la méthode de dosage de sels de méthonium, proposée dans leur travail précédent, pour rendre possible son application à la recherche du C_{10} dans les sérums et les urines.

Cette méthode est rigoureusement spécifique pour la détermination du décaméthonium dans le sérum entre 100 e 500 γ/ml mais moins spécifique pour les urines.

G. Moricca, B. Giovanella, C. Manni, and R. Cavaliere

Istituto Regina Elena for Cancer Research and Clinica Chirurgica della Università di Roma (Italy), December 23, 1960.

[3] B. Giovanella, C. Manni, and G. Moricca, Exper. *15*, 393 (1959).

N-Bromosuccinimide Action on Creatine Phosphokinase

Creatine phosphokinase was treated with *N*-bromosuccinimide (NBS) according to the method of Patchornik et al.[1]. Changes in the ultraviolet spectrum as well as appearance of new *N*-terminal residues were studied. Figure 1 gives the absorption spectra of the enzyme after addition of varying amounts of NBS in 8 *M* urea, – 0.1 *M* acetate–pH 4.0. (In each case readings were taken until they remained constant with time.) With approximately 10 *M* of NBS per mole of protein appreciable alterations in the spectrum begin to appear. Above an NBS concentration of 240 *M* no further changes occur. In Figure 2, where differences in the absorbancy at 280 mμ as a function of NBS concentration are plotted, the maximum amplitude of the drop in molar absorbancy for the phosphokinase is $38.2 \times 10^3/M$. If a decrease of $2.8 \times 10^3/M$ of free tryptophan is accepted[2], presence of 13–14 tryptophan residues is indicated. This value compares fairly with that of 11–12 residues obtained on chemical analysis[3].

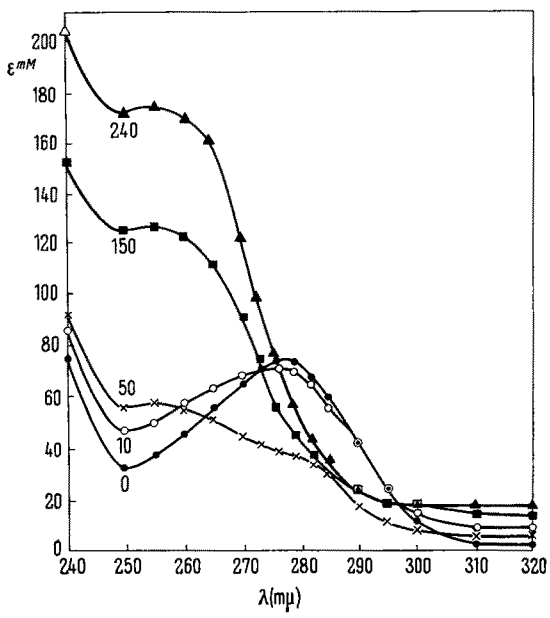

Fig. 1. Absorption spectra of creatine phosphokinase in 8 *M* urea – 0.1 *M* acetate pH 4.0 after treatment with different amounts of NBS. Numerals indicate moles of NBS/*M* of protein. Samples were read against a urea-bufferblank containing identical concentrations of NBS.

The phosphokinase used in these studies had been crystallized once according to the method of Kuby et al.[4] and appeared as one peak on examination in the ultracentrifuge. When it was not treated with NBS (control) but was dinitrophenylated (after exposure to urea-acetate buffer) for $2^1/_2$ h at room temperature in alcoholic solution made alkaline with $NaHCO_3$, dialysed against water, lyophilized, hydrolyzed for 16 h at 110°C with 6 *N* HCl and chromatographed[5], there appeared 5 spots identified as glutamic-aspartic, serine, threonine, glycine,

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[2] T. Peters, Jr., C. R. trav. lab. Carlsberg *31*, 227 (1959).
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[5] H. Fraenkel-Conrat, J. I. Harris, and A. L. Levy, in D. Glick, *Methods of Biochemical Analyses* (Interscience Publishers Inc., New York 1955), Vol. 2, p. 359.