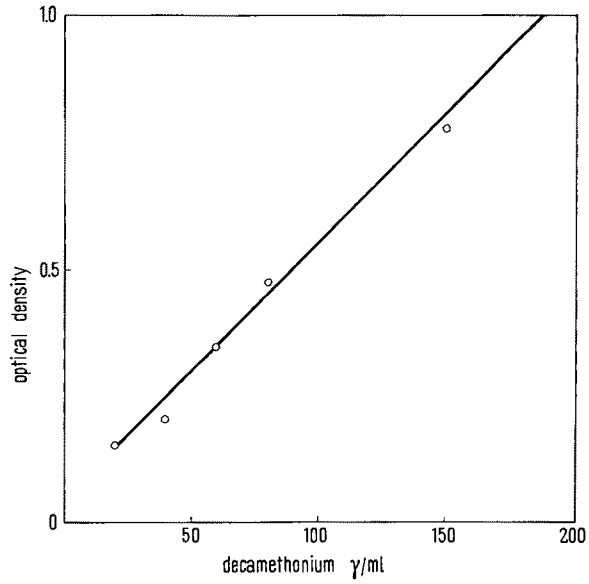

Fig. 2. Estimation by turbidity reaction of decamethonium in human blood serum.

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.

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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].

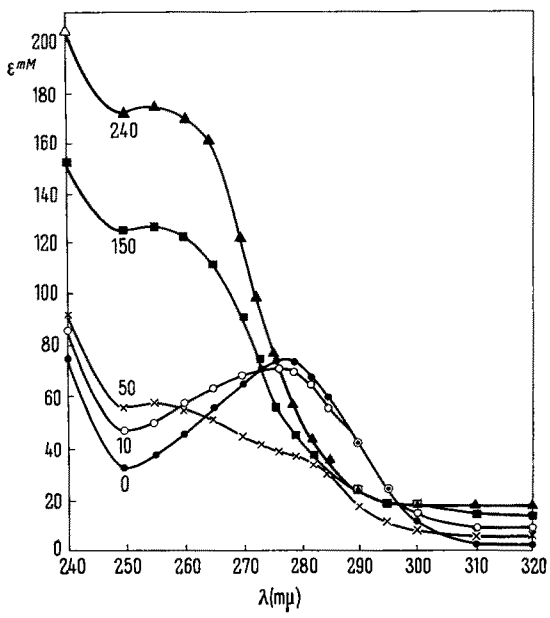

Fig. 1. Absorption spectra of creatine phosphokinase in 8 *M* urea – 0.1 *M* acetate pH 4.0 after treatment with different amounts of NBS. Numerals indicate moles of NBS/*M* of protein. Samples were read against a urea-bufferblank containing identical concentrations of NBS.

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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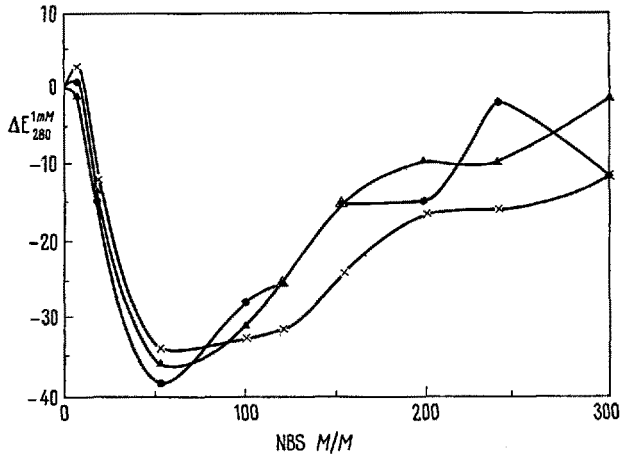

Fig. 2. Change in *mM* absorbancy of creatine phosphokinase treated with increasing amounts of NBS. After 15 min incubation (×). After 160 min incubation (●). After 290 min incubation (▲).

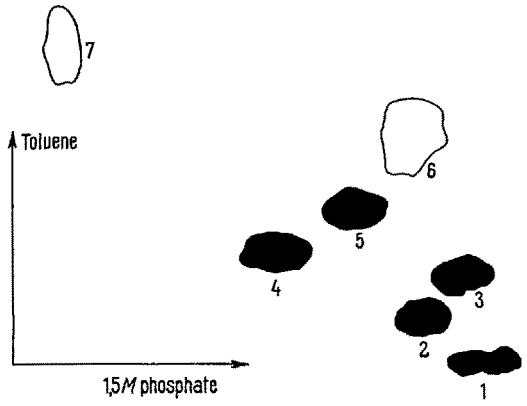

Fig. 3. Paper chromatography of DNP-amino acids from the hydrolysate of intact, dinitrophenylated creatine phosphokinase without NBS treatment (full spots). The additional compounds obtained after NBS reaction are indicated by hollow spots.

	M/*M* of protein		*M*/*M* of protein
(1) glutamic-aspartic	0.044	(5) alanine	0.024
(2) serine	0.022	(6) valine	0.152
(3) threonine	0.096	(7) unknown	0.078
(4) glycine	0.018		

Solvents: Toluene–pyridine–chloroethane and 1.5 phosphate buffer (pH 6.0)[5]. The unknown spot was neither Di-DNP lysine nor Di-DNP tyrosine.

and alanine in quantities, however, so small as to make interpretation of the results difficult (Fig. 3). It might be that 1) protein impurities or absorbed amino acids in the preparation are responsible for these *N*-terminal traces; that 2) certain bonds are somewhat hydrolyzed during dinitrophenylation in alkaline medium or that 3) structure hindrances prevent proper activity by the fluoro-dinitrobenzene onto the end terminals. Two additional spots, valine and an unidentified substance are observed when the protein is exposed to 100 moles of NBS per mole of protein in the 0.1 *M* urea-acetate buffer, pH 4.0 for 1 h (followed by dialysis against 0.5% $NaHCO_3$) before dinitrophenylation, suggesting the existence of a try-val bond in the molecule. (Analysis of the aqueous layer remaining from the ether extraction yielded no additional *N*-residues.) Considered on the mole per mole basis, the quantity of the new *N*-terminal group formed by the action of the NBS is very small. Such bond cleavages, however, have been reported only in yields averaging 20–40% and sometimes even as low as 5–10% or less[6]. Why the number of new *N*-terminals formed does not correspond to the number of tryptophans in the molecule cannot be explained at present. A similar observation has been made with regard to lysozyme where seven residues had been expected[6]. It should also be mentioned that paper electrophoresis at pH 8.6 in barbiturate buffer before and after sulfite exposure[7] of the NBS treated protein did not reveal the presence of any split products.

Zusammenfassung. Behandlung von Kreatinphosphokinase mit überschüssigem *N*-Bromsuccinimid bewirkt eine Umwandlung der vorhandenen peptidartig gebundenen Tryptophanreste, welche sich durch die für diesen Vorgang charakteristische Veränderung des Spektrums anzeigt. Am unbehandelten Enzym liessen sich auch mit einem grossen Überschuss von NBS keine freien endständigen NH_2-Gruppen eindeutig nachweisen.

F. A. QUIOCHO, M. O'DELL, and F. FRIEDBERG[8]

Department of Biochemistry, Howard University Medical School, Washington (D.C.), December 30, 1960.

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[8] This work was aided by a grant from the Muscular Dystrophy Associations of America, Inc.

The Inhibitory Effect of Duodenal Souring on Shay Ulceration in Rat

SOKOLOV was the first to observe in 1904 that the gastric juice introduced into the dog duodenum intensively reduced the gastric secretion in the Pavlov pouch[1]. The presence of this duodenal inhibitory mechanism was consequently supported by several authors[2–6]. Various investigators produced from the duodenal mucosa the hormonal substances responsible for this effect[7,8]. Enterogastron decreases the gastric secretion also of cats, thus establishing the fact that the substance is not race-specific[9]. The degree of inhibition of experimental ulcers, a method renewed by SHAY[10], and originally described by TALMA[11], is extensively used in the evaluation of duodenal extracts, that is, enterogastron preparates[12]. We were interested in the question of whether duodenal

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