This study was conducted with avian plasma only. There is no reason to suspect, however, that the procedure would not be applicable to mammalian plasma. Whether it could be used for extraction and analyses of Evans Blue in other tissues or in plasma-dextran mixtures requires further investigation.

Summary. A simple procedure is described for determination of Evans Blue in plasma based on precipitation of the dye-protein complex with trichloroacetic acid and extraction of the acidified precipitate with n-butanol. The standard curve is linear to at least 40 μg/ml of dye.

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A Method for the Isolation of Pyroglutaminyl Peptides from Glycoproteins.* (30645)

T. IKENAKA AND K. SCHMID

Department of Biochemistry, Boston University School of Medicine, Boston, Mass.

A considerable number of human plasma proteins including α_1-acid glycoprotein(1), Zn-α_2-glycoprotein(2), 3S γ_1-globulins(3) and fibrinogen(4) are known to possess N-terminal amino acids that do not react with dinitrofluorobenzene. Other blood proteins such as the 7S γ-globulins(5,6) when subjected to the same analysis yield only small amounts of N-terminal aspartic and glutamic acid. In view of the importance of the terminal amino acids of proteins for elucidation of the chemical structure of these macroglobulins, a procedure was developed that permits determination of the N-terminal amino acids of the mentioned glycoproteins. As model compound α_1-acid glycoprotein(7) was selected.

Extensive studies of proteolytic digests of the native and denatured form and of several derivatives of α_1-acid glycoprotein revealed the following: (a) Initial removal of the sialic acid is a prerequisite for separation of the peptides resulting from the subsequent hydrolysis. If sialic acid was not cleaved off, any N-terminal peptides with substituted α-amino groups and the sialic acid-containing glycopeptides were found together in the effluent of the Dowex-50 column mentioned below. Removal of sialic acid was achieved with a neuraminidase or by mild acid hydrolysis (0.025 N H_2SO_4, 80°, 1 hour). (b) Reduction of the disulfide bonds and alkylation of the formed sulfhydryl residues appear necessary for the complete and permanent unfolding of the polypeptide chain. (c) Succinylation of the free ϵ-amino groups(8) and benzylation of the guanidinium groups (9) were carried out to make possible the isolation of any N-terminal peptides that may contain lysine and/or arginine.

The hydrolysis of the glycoprotein modified as described above was carried out with pronase. The obtained hydrolysate was fractionated on a Dowex-50 column (X2, H-cycle) in the cold(10). The acidic peptides together with a small amount of glycopeptides present in the effluent were next separated from each other by high voltage electrophoresis at pH 3.5 in pyridine acetate buffer.

The investigation of α_1-acid glycoprotein by this procedure showed that high voltage electrophoresis of the acidic peptides yielded a major and 4 minor fractions. The main

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component proved to be a tripeptide that did not react with dinitrofluorobenzene and accounted for approximately 0.7 mole per mole of protein. This peptide was composed of N-terminal pyroglutamic acid, proline and C-terminal i-leucine. The presence of N-terminal pyroglutamic acid was demonstrated by alkaline hydrolysis of the tripeptide (1.0 N NaOH, 22°, 3 days) to open the pyrrolidone ring followed by dinitrophenylation, acid hydrolysis and identification of the isolated DNP-compound as DNP-glutamic acid. The absence of acetyl groups was established by the technique of Ludowieg and Dorfman (11) and by hydrazinolysis. It should be added that certain proteins and peptides(4, 12,13) have already been shown to possess pyroglutamyl as N-terminus.

Summary. A method was developed for the isolation of the N-terminal peptides of glycoproteins with N-substituted N-terminal amino acid. The N-terminal tripeptide derived from α_1-acid glycoprotein, a constituent of normal human plasma, was composed of N-terminal pyroglutamic acid, proline and C-terminal isoleucine.

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Renal Handling of 5-Hydroxyindoleacetic Acid in the Dog.* (30646)

M. E. KAHIL, E. L. SIMONS, J. CASHAW AND H. BROWN (Introduced by M. Lane)
Department of Medicine, Baylor University College of Medicine, and Metabolic Research Laboratory, Veterans Administration Hospital, Houston, Texas.

The present knowledge of the renal handling of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, is incomplete. Despopoulos and Weissbach(1) have shown that rabbit kidney cortex slices, incubated in a medium containing 5-HIAA, concentrate 5-HIAA by an active aerobic process. In one patient with the carcinoid syndrome, the estimated clearance of 5-HIAA varied from 343 to 372 ml/minute, values well in excess of glomerular filtration rate, suggesting that 5-HIAA might be actively secreted into the urine by the renal tubular cells.

The purpose of this communication is to report the results of studies in which the clearance of 5-HIAA was measured in the dog.

Materials and methods. Renal clearance studies were carried out on healthy mongrel dogs of both sexes using the methods outlined by Smith(2). The dogs were anesthetized with Na Pentobarbital, 30 mg/kg administered intravenously. Blood samples were drawn from an indwelling catheter in the jugular vein. Blood was collected in chilled siliconized tubes containing heparin.

5-HIAA (Sigma) and creatinine were dis-

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