Simplified Technic for Preparing Blood Group Specific Substance A. (17445)

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Since the blood group specific substances are carbohydrate like in nature it seemed logical that such a simple technic as preparing a protein free filtrate by the Folin-Wu method would be an easy way to prepare blood group substance A from Difco neopeptone. As will be shown the blood group substance was not destroyed by the reagents used in the Folin-Wu adaptation. The product which is obtained compares favorably in potency and purity with preparations made with more difficult procedures.

Materials and Methods. The starting material for this Folin-Wu adaptation was a 5% solution of Difco neopeptone in distilled water. For every 30 ml of neopeptone solution 15 ml of 10% sodium tungstate was added, followed by the slow addition of 15 ml of 2/3 N H_2SO_4. After all the sulfuric acid was added the container was shaken and then left to stand for 10-15 minutes. The filtrate which contains the blood group specific substance was dialyzed against cool running water until a negative test for tungstate, sodium and sulfate was obtained. The various chemical tests such as the picric acid, Molisch, etc. were run on this dialysate (recorded under results). Two procedures were used to prepare the final product: either, (1) the dialysate was precipitated with 10 volumes of acetone, centrifuged and dried in a vacuum desiccator, or 2) dialyzed against several changes of physiological saline and stored in the liquid form. The former product was tested in inhibition studies on a dry weight basis and compared with products prepared by the method of Goebel.[1] It should be recalled that this investigator prepared blood group specific substance A from neopeptone by multiple precipitations with 95% alcohol, using the deproteinizing method of Sevag.[2] In addition to comparing the latter product of our method with the product obtained by Goebel's method, it was compared with the blood group substance A content of a commercial product (Sharp-Dohme).

Anti-sera. Anti A sera for tests was prepared so as to contain as nearly as possible 8 units of isoagglutinin per unit volume.[3]

Inhibition tests. One drop of anti-A sera was added to one drop of the particular strength of the blood group specific substance. The tube was shaken and left to stand for 45 minutes at room temperature. One drop of a 2% suspension of A cells was added, the tube was shaken and left to stand for one hour. The presence or absence of agglutination was determined macroscopically. The technic is a modification of Wiener's[3] method for testing for secretors.

Results. The saline dialysate compared favorably with the commercial preparation in the inhibition tests for blood group specific substance A. The dialysate of our preparation inhibited agglutination through a titer of 256, while the commercial product inhibited agglutination through the titer of 128.

On a dry weight basis our product inhibited the agglutinating properties of 1 drop of anti-A sera when only 1 mg was present in 256 ml of physiological saline. The Goebel product which we prepared by his method gave inhibition when only 1 mg was present in 205 ml of physiological saline.

The Goebel method in our hands as well as the Folin-Wu technic gave a product free of amino acids according to the picric acid, Millons, Biuret and Hopkins-Cole tests. The commercial product gave a strongly positive Millons test. The Molisch reaction was strongly positive with all products.

1 Goebel, W. F., *J. Exp. Med.*, 1938, **68**, 221.

2 Sevag, M. C., *Biochem. Z.*, 1934, **273**, 419.

3 Wiener, A. S., 1945, Blood Groups and Transfusions, Charles C. Thomas, Springfield, Ill.

Discussion. The use of tungstate and sulfuric acid to prepare a protein free filtrate, while not removing the carbohydrate is a standard procedure in blood analysis work.[4] The fact that the blood group specific substances are carbohydrate like lends them admirably to this procedure. The method was probably never attempted before because of the nature of the reagents used since there was some doubt in our mind initially as to whether the blood group specific substance would withstand this treatment. It is not claimed that a superior product is obtained by the Folin-Wu technic in comparison to the method of Goebel, but the procedural simplicity of the former makes it a method of choice which should be easily used when animal tissues are the source of blood group specific substances.

Summary. A method for preparing blood group specific substance A from Difco neopeptone by the Folin-Wu technic is described and comparisons with other preparations are made. The method is simple and the product obtained compares favorably in purity and potency with products obtained by other methods.

[4] Andrews, J. C., and Kyker, G. C., 1947, A Laboratory Manual of Biological Chemistry, Edwards Brothers, Inc., Ann Arbor, Mich.

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Influence of Adrenal Cortical Steroids and Related Compounds on Sodium Metabolism.* (17446)

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In a previous paper from this laboratory[1] it was demonstrated that as little as one microgram of desoxycorticosterone produced a significant retention of sodium in the adrenalectomized male rat. This communication is concerned with the study of various adrenal cortical steroids and related compound on sodium metabolism by the same technic. These steroids include 11-dehydrocorticosterone, 17-hydroxycorticosterone, corticosterone, pregnanetrione-3, 11, 20, allopregnanetriol-3 (β), 17 (α), 21-one-20, alloprenanepentol-3 (β), 11(β), 17(α), 20, 21-3(β), 20, 21 triacetate, Δ^4-prenenol-21-trione-3,12,20 acetate, testosterone, estradiol, and pregnanetrione-3,12,20.

Animals, Materials, Methods. The rats were obtained from Carworth Farms. They were bilaterally adrenalectomized in one stage under ether anaesthesia. The animals subsisted exclusively on a chow diet both before and after adrenalectomy. When more than 24 hours elapsed between adrenalectomy and the day the experiment was run the rats were given normal saline in place of ordinary drinking water until the morning of the experiment.

The test material was injected subcutaneously in 0.25 cc of corn oil One hour later the rats received subcutaneously, 2 cc of a solution containing sodium chloride and the radiosodium.† The dose of sodium chloride was 35 μg per gram of body weight. The animals were placed in glass collecting cages and the urine collected for 6 hours. The urine was dried and the concentration of radiosodium in the residue determined as described previously.[1]

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[1] Dorfman, R. I., Potts, A. M., and Feil, M. L., *Endocrinology,* 1947, **41**, 464.

† The radiosodium was supplied by the Monsanto Chemical Company, through the U. S. Atomic Energy Commission.