certain fraction of the cellular protein of the kidney is more labile than other fractions under conditions of atrophy and hypertrophy.

*Summary.* 1. Distribution of kidney proteins in various cytoplasmic fractions has been determined in normal, hypertrophied, and atrophied rat kidney. The Soluble I fraction from normal and hypertrophied kidneys has been subjected to paper electrophoresis. 2. Despite a gain in total protein of approximately 35% in the hypertrophied kidney, and a loss of approximately 20% in total protein in the atrophied kidney, the distribution of proteins in the various cytoplasmic fractions shows only minor deviations from the normal. 3. No consistent differences could be seen in electrophoresis patterns of the Soluble I fraction between normal and hypertrophied kidney. 4. The significance of these findings is discussed.

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Received January 26, 1956. P.S.E.B.M., 1956, v91.

## Structures Responsible for the Characteristic Purple Color Development in Diphenylamine Reaction with Serum.* (22316)

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Numerous workers(1-4) have reported that the intensity of purple color which resulted when serum was heated with Dische reagent (5) was increased in a variety of diseases. The reaction has been associated with the serum proteins containing carbohydrate moieties: mucoproteins or glycoproteins found in the alpha globulin fraction of serum(2,6,7). Mucoproteins isolated from bovine tonsils and from human urine also gave the color reaction (8,9). The color reaction has been attributed to the presence of sialic acid(10) in the mucoprotein molecule(7). A number of polyhydroxylic acids, neuraminic(11,12), hemataminic(13), and lactaminic(14), have been isolated and appear to be related to sialic acid(15).

It is the purpose of this report to point out that the structure for sialic acid proposed by Gottschalk(16,20) is consistent with structures known to give the characteristic color development when heated with the Dische reagent.

*Absorption Studies.* Purple color which results when serum or mucoproteins are heated with the reagent is due to 2 absorption regions in the visible spectrum (Fig. 1). One absorption maximum occurs at about 520 mμ while the second occurs at 650 mμ. The absorption maximum at about 520 mμ is the significant one. The second absorbing region at 650 mμ can be attributed to conversion of common hexoses present in mucoproteins to 5-hydroxymethylfurfural when heated in an acid solution(17,18). Absorption maxima in the vicinity of 520 mμ, as will be shown below, can probably be attributed to a deoxyhexose struc-

* Supported in part by grant from U. S. Atomic Energy Commission Contract AT (11-1) 366 with The Rheumatic Fever Research Institute.

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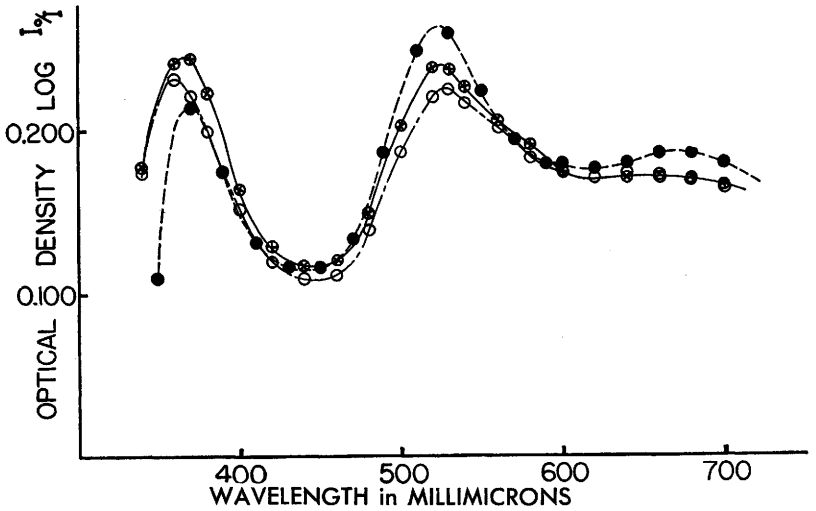


FIG. 1. Absorption curves from diphenylamine reaction obtained with human serum, sialic acid and bovine tonsil mucoprotein. × (circled) Human serum 0.12 ml serum/ml solution according to published procedure(2) using Dische reagent. ○ Bovine tonsil mucoprotein 590 μg protein per ml solution. ● Sialic acid 130 μg/ml solution. To 1 ml solution was added 2 ml of reagent. The mixture heated for 30 min. at 100°, cooled to room temperature and read in a Beckman Model DU Spectrophotometer.

ture which has been postulated(16,20) to be present in sialic and neuraminic acids.

The absorption curves obtained when 40 μg of 2-deoxygalactose§, 40 μg of D-galactal, and 10 μg of fructose, were heated with Dische's diphenylamine reagent may be seen in Fig. 2. In the case of 2-deoxygalactose, and D-galactal, the 40 μg samples were dissolved in 1 ml of water. Two ml of the reagent were added to the aqueous solution and the mixture was heated for 30 minutes in a boiling water bath. Fructose treated in this manner did not develop the characteristic color. When the Dische reagent was added directly to as little as 10 μg of fructose, however, in the absence of water, and the mixture was heated, a pink color developed within a few minutes.‖ The absorption curve obtained with dry fructose heated with the Dische reagent closely resembled the curves obtained from D-galactal and 2-Deoxygalactose as can be seen in Fig. 2. Many substances have been tested with the Dische reagent both in our laboratory and elsewhere(18). Of all the simple substances tested, only the deoxyhexoses and hexals gave the characteristic color development and absorption behavior, when heated in aqueous solution with the unmodified Dische reagent. The ketoses in aqueous solution, when heated with a modified reagent containing a larger fraction of sulfuric acid, also give the characteristic color development. The absorption curves are similar in this case to those obtained when serum, mucoproteins, the hexals and the deoxyhexoses are heated with the Dische reagent.

The absorption curves obtained when sialic acid and a purified mucoprotein were heated with Dische reagent are shown in Fig. 1. Comparison of curves in Fig. 1 and 2 indicate a striking similarity in chromogenic structures present in all substances reacting with the reagent. Acid-catalyzed dehydration of hexoses is well known(19). The conclusion that water is removed from 2-deoxyhexoses and from ketoses under the conditions discussed above, seems justified. The ethylenic ring seen in Fig. 3 thereby becomes a feature common to a dehydrated deoxyhexose, dehydrated fructopyranose, and to hexals. This structure or a common derivative of this

§ 2-deoxyglucose also shows this characteristic behavior.

‖ Sorbose, galaheptulose, mannoheptulose and sucrose also behave in similar manner.

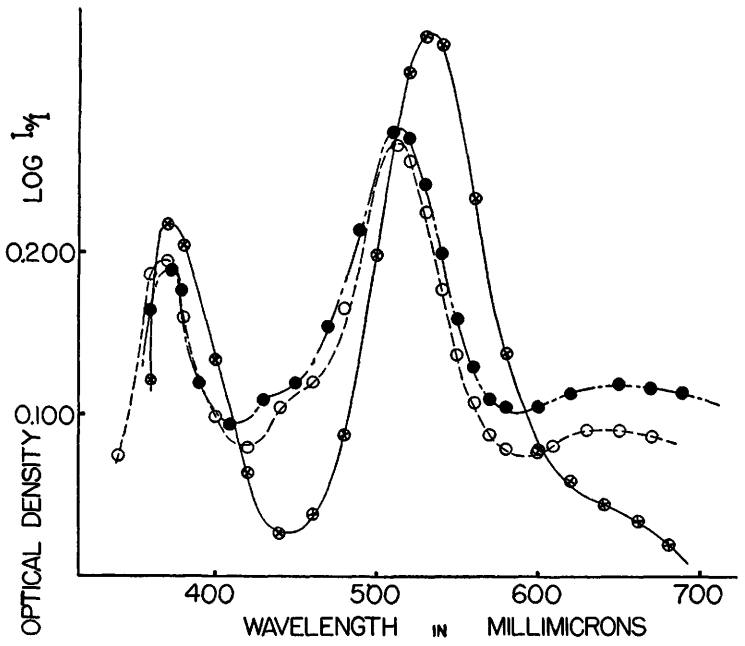


FIG. 2. Absorption curves from diphenylamine reaction obtained with 2-deoxygalactose, D-galactal and fructose. ○ 2-deoxygalactose 40 μg/ml solution. ● D-galactal 40 μg/ml solution. × (circled) Fructose 10 μg. To 1 ml solution was added 2 ml of reagent. The mixture was heated 30 min. at 100°, cooled to room temperature and read in a Beckman Model DU Spectrophotometer. In the case of fructose 3 ml of reagent was added to 10 μg of dry fructose and the mixture heated 30 min. at 100°.

structure accounts for the absorption maxima at approximately 520 mμ. A deoxyketose structure in sialic acid(16,20) would therefore account for the color reaction of this substance with the Dische reagent.

The chemistry of the deoxyhexoses(21) and the hexals(22,23) is also consistent with the viewpoint that a deoxyhexose is present in the sialic acid molecule. A characteristic of the deoxy sugars is their great reactivity and their instability in solutions containing mineral acids. 2-deoxygalactose is almost completely destroyed, with marked discoloration, when heated with 1 N HCl for one minute at 100°. The glycals are stable in alkaline solution, form humins in acid solution and are extremely reactive substances(22,24). The pronounced tendency of sialic acid(25) and of the gangliosides(26) to form humin when heated with acids has been described.

*Summary.* It has been shown that deoxyhexoses and hexals react with the Dische diphenylamine reagent in a characteristic manner. No other structures except sialic acid and related compounds have been found to react in the same fashion. A deoxyhexose structure for sialic acid has been proposed by Gottschalk. The color given by sialic acid when heated with the reagent is therefore accounted for by the presence of the deoxyhexose. Since sialic acid is considered to be a common constituent of the glycoproteins (mucoproteins), the color given by these substances and serum when heated with the Dische reagent appears to be due to the deoxyhexose present in sialic acid.

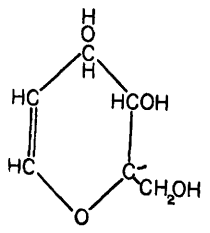


FIG. 3. Ethylenic ring structure common to glycals, and after elimination of a molecule of water, to deoxyhexoses and pyranose form of ketohexoses.

The assistance of Miss Saima Lagg is gratefully acknowledged. Discussions with Dr. G. Blix have been helpful. We are grateful to Dr. A. Gottschalk for calling attention, prior to our seeing the published reports, to the deoxyhexose character of his proposed structures. A number of substances used in this study were obtained through the kindness of several individuals. Dr. T. Reichstein sent generous samples of 2-deoxygalactose, 2-deoxyglucose and D-galactal. The late Dr. G. S. Hudson provided galaheptulose and mannoheptulose. The sample of sialic acid was obtained from Dr. L. Odin.

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Received January 30, 1956. P.S.E.B.M., 1956, v91.

## Hormonal Requirements of Implantation in the Rabbit.* (22317)

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The phenomenon of implantation in mammals is accomplished by a definite sequence of events which in part are dependent on the endocrine system. It has been adequately demonstrated(1,2,3) that blastocyst growth in the rabbit is controlled by the secretion of the corpus luteum. Partial progestational proliferation in the ovariectomized rabbit could be produced within 5 days by 0.2 mg progesterone daily(4). The injection into rabbits, ovariectomized 20 hours after mating, of progesterone in dosage up to 1.5 mg a day maintained implantation until the 11th day of pregnancy(5). Crude corpus luteum extracts of similar unitage maintained embryos to full term(6). In ovariectomized rabbits, implantation is maintained for 11 days if at least 0.5 mg of progesterone is given twice daily; 1.0 mg given twice daily after the 11th day of pregnancy maintains the embryo to term(7). In the intact non-ovulated rabbit, implantation of transplanted ova requires an initial high dose of progesterone; subsequent

* Thanks are due to Miss Anne P. Merrill and Miss Pauline S. Longo for technical assistance and to Chemical Specialties Co., (Syntex) for progesterone preparations. This investigation was aided-in-part by research grants from the Josiah Macy, Jr. Foundation and the Planned Parenthood Federation of America.

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