Serum Glycoprotein Changes in Pregnancy.* (28200)

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A number of studies have reported serum protein changes during pregnancy, but comparatively few investigations have been made of serum glycoprotein changes. Total serum glycoprotein levels have been reported to increase in late pregnancy(1,2,3); and by salt fractionation methods, hexose content of the albumin was shown to be significantly elevated(2). This elevation was not due to an increase in seromucoid as the seromucoid for pregnant subjects was essentially normal. Total serum protein decreases until near the 28th week of pregnancy, then increases slightly until term. Serum albumin falls progressively as pregnancy advances while the serum globulins show slight gain(4).

The following study was designed to make parallel protein and glycoprotein studies during pregnancy utilizing newer paper electrophoretic methods.

Methods. Fifty patients were studied during their period of pregnancy. Thirty-one patients were primigravidas and 19 were multigravidas. Twenty-four of the patients were Caucasian; 24, Negroid; one, Indian; and one, part Japanese, part Caucasian. All subjects had normal pregnancies; no cases with hypertension, toxemia, or other diseases were included.

Sera from these patients were subjected to paper electrophoretic studies using a Spinco Model R Paper Electrophoresis Cell. A current of 5 milliamperes per cell of 8 paper strips was applied for 16 hours. Eight microliters of serum were applied to each strip used for protein studies, and 30 μl to each strip was used for glycoprotein studies. Following the 16-hour run, the strips were dried immediately in an oven at 110°C for 30 minutes. The protein strips were stained with bromphenol blue by the Spinco Method B and quantitated in the Spinco Model RB analytrol. The glycoprotein strips were stained by the Periodic-Acid-Schiff (P.A.S.) method and quantitated as described by Shetlar *et al.*(5). Total serum glycoprotein as bound hexose was determined by the tryptophane method(6); total serum protein by a biuret method.

Results. Table I summarizes the average total serum protein and serum glycoprotein changes during pregnancy and early post partum. There was a gradual decrease in total serum protein until the 35th week of gestation, when the value increased slightly to the end of pregnancy. During the first 24-48 hours post partum, serum protein dropped to a low level and then rose abruptly. In several patients, with apparently normal nutrition and pregnancy, total serum protein concentration tended to continue to fall until near term. There was an increase in total serum glycoprotein, whether expressed in mg/100 ml of bound hexose or as a percentage of the serum protein, throughout pregnancy. The increases are more striking between 15 and 25 weeks with values leveling off at 35 weeks. After delivery, there is a further sharp increase in total serum glycoprotein.

Fig. 1 summarizes changes of the serum protein fractions expressed as percentage of total serum protein. The most striking change was the decrease in the albumin fraction during the entire period of gestation. During the first 4 days post partum, albumin values increased. The α_1-globulin showed an increase throughout pregnancy. The increase illustrated post partum was quite variable for different patients, some exhibiting a decrease rather than an increase during this period. The α_2- and β-globulin protein fractions also steadily increased during pregnancy. The α_2-globulin values rose in the early post partum, while the β-globulin fraction values decreased rather sharply. The protein γ-globulins remained rather constant throughout pregnancy and the early post partum.

Table II summarizes average changes in the glycoprotein of various fractions, expressed as milligrams hexose/100 ml serum during pregnancy and early post partum.

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TABLE I. Total Serum Protein and Glycoprotein in Pregnancy.

Constituent	Before pregnancy	During pregnancy (expressed in wk) 6	10	15	20	25	30	35	40	Post partum 2 days	4 days
Protein	7.25 g%	7.15	7.10	6.95	6.85	6.65	6.50	6.50	6.70	6.15	6.70 g%
Glycoprotein	110 mg%	116	119	124	134	145	161	170	173	180	200 mg%
Glycoprotein ÷ protein	1.52	1.62	1.68	1.78	1.96	2.18	2.48	2.62	2.58	2.93	2.98

TABLE II. Serum Glycoprotein* Fractions in Pregnancy.

Fraction	Before pregnancy	During pregnancy (expressed in wks) 6	10	15	20	25	30	35	40	Post partum 2 days	4 days
Albumin	14	20	26	31	31	33	35	37	38	32	30
α_1-Globulin	20	19	17	16	17	22	25	25	24	32	42
α_2-Globulin	32	34	34	36	42	36	35	34	42	52	64
β-Globulin	28	28	28	27	28	30	32	32	38	37	38
γ-Globulin	16	17	17	16	19	26	34	39	33	29	24

* Expressed in milligrams of bound hexose per 100 ml of serum.

The albumin fraction glycoprotein increased during pregnancy until term. A steep drop in the albumin glycoprotein fraction was noted in the early post partum. The α_1-globulin glycoprotein, expressed as mg per 100 ml, decreased until the 24th week, from which time the values were above the non-pregnant value through the remainder of gestation. The α_2-globulin glycoprotein, expressed as mg per 100 ml, was increased above the non-pregnant value throughout pregnancy. The α_1- and α_2-glycoprotein fractions showed a rather sharp increase in the early post partum period. The β-globulin glycoprotein fraction, expressed as mg per 100 ml, was above the non-pregnant value after the 25th week. The β-globulin glycoprotein changed only slightly in the early post partum. From the 20th week, the γ-globulin glycoprotein expressed as mg per 100 ml, increased until the 35th week when a gradual drop was noted. This fraction decreased sharply in the early post partum.

Fig. 2 graphically depicts data derived by expressing the bound hexose (glycoprotein) figures as a percentage of the corresponding protein electrophoretic fraction. This treatment will indicate changes of bound carbohydrate relative to protein in each fraction. From Fig. 2 it is readily seen that the carbohydrate content of albumin increased consistently during pregnancy and decreased post partum. The α_1-globulin and β-globulin glycoproteins decreased during pregnancy, while α_2-glycoprotein, relative to protein, increased until the twentieth week and then decreased. The percentage of bound hexose to protein of α_1-, α_2- and β-globulin, when expressed in this way, all increase post partum. The γ-globulin glycoprotein increased after the 15th week until the 35th week and then dropped post partum.

Discussion. Total serum protein. The decrease in total serum protein can be explained as dilution by expanding blood volume. Several investigators have found that the total absolute serum protein is increased in pregnancy when the greatly expanded plasma and blood volumes are taken into account(4). Thus, it would appear that in pregnancy more serum protein is made than in the non-pregnant state, but the concentration (g/100 ml) falls due to the greatly expanded fluid volume. The sharp decrease in concentration in the first 0-48 hours post partum can be explained by the further increase in plasma volume caused by release into the plasma of excess tissue fluids held during gestation. The rapid increase noted after 2 days post partum, may be explained by diuresis occurring following delivery.

Serum protein fractions. The increase of total serum glycoprotein in the face of decreasing total protein concentration can be explained largely on the basis of the decreased albumin fraction. This difference between the albumin and other serum fractions is of considerable interest. There may be increased production of serum fractions other

than albumin, or albumin may be preferentially used by the tissues. There may be an increased need for the alpha and beta protein fractions as carriers for specific substances whose needs are increased in pregnancy.

Serum glycoprotein changes. The increase of total serum glycoprotein in the face of decreasing total serum protein is an indication of increase of high carbohydrate protein fractions relative to low carbohydrate fractions. These alterations are readily verified by examination of the individual electrophoretic fractions. Earlier work using salt fractionation had indicated that the albumin fraction in pregnancy increases in bound hexose(2). These results, however, could be explained on the basis of contamination of the salt fractionated albumin fractions with some α-globulins. Similar findings in the above electrophoretic studies strengthen the concept that a qualitative change occurs within the albumin fraction during pregnancy. Examination of Fig. 2 indicates that this is a progressive process during pregnancy. Further correlation with pregnancy is indicated by the decrease occurring post partum. Similarly the decreases of bound carbohydrate relative to protein of α_1- and β-globulin and increases of γ-globulin glycoprotein during pregnancy indicate changes within these fractions. The α_2-globulin changes are more complicated, and perhaps indicate the influence of 2 types of processes related to biosynthesis of α_2-globulins. The increases of bound carbohydrate relative to protein in the α_1-, α_2- and β-globulin fractions after delivery are similar to those occurring in inflammatory states(7), and may therefore be due to injury. The post partum decrease of γ-globulin bound carbohydrate may indicate that this fraction reverts quickly to the normal state after pregnancy.

The changes of glycoproteins found in pregnancy may be contrasted with those found in inflammatory states. In active rheumatoid arthritis, elevations of bound hexose, expressed as a percentage of the protein moiety, occur in the α_1- and α_2-globulin; β- and γ-globulin glycoprotein levels are not significantly changed and albumin is only slightly affected(7). In contrast, decreases in α- and β-globulin glycoprotein levels and increases in albumin and γ-globulin glycoproteins were found in pregnancy in the current study. In addition, earlier work(2) had revealed normal seromucoid levels in pregnancy in contrast to elevated levels of this constituent in inflammatory states. Thus, it appears that the elevated serum glycoprotein levels of pregnancy are quite different from those found in inflammatory states.

The changes described above indicate that

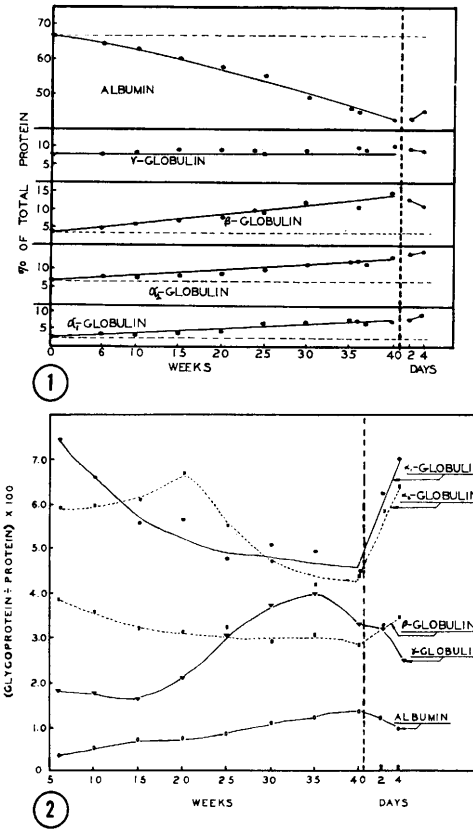

FIG. 1. Changes in serum protein fractions during pregnancy. Each paper electrophoretic fraction is expressed as a percentage of total serum protein. In all cases except γ-globulin, a dotted line indicates avg non-pregnant level. In the case of γ-globulin, dotted line coincides with experimental line. Vertical dotted line indicates time of delivery. Post partum results are plotted on a different time scale at right of this line.

FIG. 2. Changes in serum glycoprotein fractions during pregnancy. All results are expressed as grams of glycoprotein hexose in 100 g of serum protein. Vertical dotted line indicates time of delivery. Post partum results are plotted on a different time scale at right of this line.

further studies of serum proteins in pregnancy utilizing more refined zone electrophoretic methods would produce useful information. The use of immunoelectrophoretic techniques is particularly indicated.

Summary. During pregnancy, total serum protein and serum albumin concentration decreased, while the α- and β-globulin protein fractions increased and the γ-globulin protein fraction remained at a rather constant level throughout pregnancy. Total serum glycoprotein, expressed as protein bound hexose, increased throughout the gestational period. This was accompanied by a rise in the levels of all glycoprotein fractions expressed as mg per 100 ml. Relative to the protein content of each fraction, the glycoprotein content of the albumin and γ-globulin fractions increased during pregnancy, while the α_1-, α_2- and β-fractions decreased in relative percentage of bound carbohydrate. During the early post partum period, albumin and γ-globulin glycoprotein decreased, while α_1-, α_2- and β-globulin glycoprotein increased. These results indicate that striking alterations of plasma protein metabolism occur in pregnancy.

1. Lustig, B., Novak, J., *Exp. Med. Surg.*, 1946, v4, 252.

2. Shetlar, M. R., Kelly, K. H., Foster, J. V., Shetlar, C. L., Everett, M. R., *Am. J. Obst. and Gynec.*, 1950, v59, 1140.

3. Stary, Z., Bursa, F., Teezok, O., Cindi, R., *Hoppe-Seylers Z. Physiol. Chem.*, 1955, v288, 55.

4. Mack, H. C., *The Plasma Proteins in Pregnancy*, Charles C Thomas, Publisher, Springfield, Ill., 1955.

5. Shetlar, M. R., Cahill, C., Stidworthy, G., Shetlar, C. L., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 44.

6. Shetlar, M. R., Foster, J. V., Everett, M. R., *ibid.*, 1948, v67, 125.

7. Stidworthy, G., Payne, R. W., Shetlar, C. L., Shetlar, M. R., *J. Clin. Invest.*, 1957, v36, 309.

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Effect of pH on Thermal Stabilization of Oral Poliovirus Vaccine by Magnesium Chloride. (28201)

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Polioviruses(1) and other enteroviruses(2) are stabilized at elevated temperatures in high ionic concentrations of $MgCl_2$. In molar $MgCl_2$ at 50°C, poliovirus is stabilized for at least 2 hours; at 37°C for 3 days; and at room temperature (25°-28°C) for 21 days. This report is concerned with the role of pH in the stabilization of live poliovirus vaccines with $MgCl_2$, and the effect of long term (18 months) storage at 4°C.

Materials and methods. Oral poliovirus vaccines (type 1, LSc; type 2, P712; and type 3, Leon) were kindly provided by Wyeth Laboratories.

For virus assay, kidneys from immature rhesus monkeys were trypsinized and grown in lactalbumin hydrolysate medium containing 0.2 mM $AlCl_3$ to suppress adventitious agents(3). Cultures were maintained in the same M-E medium but free of Al ions. Titrations were carried out by determination of plaque-forming units (PFU), using bottle cultures, with 25 mM $MgCl_2$ in the agar-nutrient medium to expedite plaque counting(4).

Results. Effect of pH on stabilization by $MgCl_2$. On occasion, samples of poliovirus in molar $MgCl_2$ have failed to stabilize the virus at 28°C to the extent expected. In these instances the caps on vaccine vials were found to be loose, and an increase in pH was evident in the samples in question. To determine the effects of long term exposure to the atmosphere, types 1, 2, and 3 poliovirus vaccines were diluted to contain 10^7 PFU/ml in M $MgCl_2$ and in M-E medium, respectively. The pH of the vaccines in M $MgCl_2$ was 6.4 at time of mixing, but after 24 hours the pH equilibrated to 6.8-7.0 and remained constant for 30 days at 28°C