the result of an increase in the rate of production and not to be due to any alteration in the life span of the red blood cell.

Summary. Six individuals taken from sea level to 14,900 feet showed a normal red cell life span during the period of acclimatization; therefore, the polycythemia secondary to altitude is due entirely to an increase in the rate of red cell formation.

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Distribution of Glycoproteins in Normal Human Plasma.* (21300)

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(Introduced by Charles M. Carpenter.)

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The glycoproteins† of plasma are a heterogeneous group of conjugated proteins consisting of peptides in firm union with polysaccharides. Their occurrence, chemistry, significance, and methods for determination have been reviewed by Winzler(1). Due to the complex fractionation procedures required for the isolation of individual glycoproteins (2-4), the most generally employed method for the estimation of the protein-bound carbohydrate of plasma is the determination of total plasma glycoprotein. Although such determinations have yielded valuable data on the levels of plasma glycoproteins in many physiologic and pathologic conditions, they provide no information on the distribution of the protein-bound carbohydrate among the protein fractions present in blood plasma.

In the present investigation data obtained from the analyses of fractions isolated by a low temperature-low salt-ethanol procedure (5) from normal human plasma are correlated with the results of the more widely employed total plasma glycoprotein and seromucoid determinations.

Materials and methods. Plasma. Seventeen ml of blood were obtained by venipuncture from 10 male and 10 female donors in apparent good health and varying in age from 21 to 45 years. The blood was immediately added to 3 ml of A.C.D. solution(5). Following separation by centrifugation from the blood cells, the A.C.D. plasma was stored at −20°C until the fractionations and the chemical determinations could be conducted. *Fractionation.* Five ml aliquots of A.C.D. plasma were fractionated by the low temperature-low salt-ethanol procedure of Lever and associates (5). The separations were carried out in a −5°C bath containing 50% by volume of ethanol and equipped with an effective stirrer. Precipitates were recovered by centrifugation at −5°C. *Chemical analyses.* Fractions I + III, II, and VI were dissolved and made up to 10 ml and Fraction IV + V was dissolved and made up to 25 ml with the same reagents employed by Lever *et al.*(5). The protein-bound carbohydrate (hexose) content of the plasma fractions was determined as follows. One-ml

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†Terminology employed is in accord with the recent suggestions of Winzler(1). The term "total plasma glycoprotein" has replaced "total plasma polysaccharide" and "seromucoid" has been substituted for "mucoprotein."

TABLE I. Distribution of Protein-Bound Carbohydrate and Protein in Normal Human Plasma. Twenty samples in each determination.

Component	Protein-bound carbohydrate			Protein			Protein-bound carbohydrate ÷ protein × 100	
	Mean* (mg %)	CV†	% of total	Mean* (g %)	CV†	% of total	Mean* (%)	CV†
Total	109 ± 2.1	.086	100	7.4 ± .12	.072	100	1.5 ± .03	.093
Fraction I + III	38 ± 1.2	.141	34.9	1.3 ± .04	.138	17.6	2.9 ± .07	.108
" II	13 ± .7	.241	11.9	.9 ± .04	.199	12.2	1.4 ± .04	.128
" IV + V	41 ± 1.2	.131	37.6	4.9 ± .06	.055	66.2	.8 ± .03	.225
" VI	13 ± .5	.172	11.9	.2 ± .02	.447	2.7	6.5 ± .82	.538
Sum of primary fractions	105 ± 2.0		96.3	7.3 ± .10		98.7		
Albumin	12 ± .2‡	.075	11.0	4.0 ± .07	.078	54.0	.3§	
Fraction IV‖	29 ± .4	.062	26.6	.9 ± .06	.298	12.2	3.2 ± .22	.307
Seromucoid	10 ± .4	.179	9.2					

* Including stand. error of mean. † Coefficient of variation. ‡ Value calculated from albumin concentration and % of carbohydrate in human serum albumin (Cutter). § Value obtained by analysis of human serum albumin (salt-poor), Cutter Laboratories, Berkeley, Calif. ‖ Fraction IV values represent difference between values for Fraction IV + V and albumin.

aliquots of the protein solutions were added dropwise to 10 ml absolute ethanol at room temperature. After standing 10 minutes, the precipitate was centrifuged at 1800 rpm for 10 minutes. The ethanol was decanted and the precipitate drained by inverting the tube. One ml of distilled water was added and the hexose content determined by an orcinol reaction(6). The protein content of the isolated fractions was estimated in one ml aliquots by the biuret method of Weichselbaum(7). Albumin was determined in Fraction IV + V by the hematin-binding procedure of Rosenfeld and Surgenor(8). Total plasma glycoprotein, seromucoid, and total plasma protein were determined by methods previously reported(6). The mean, standard error of the mean, and coefficient of variation were determined by standard statistical methods(9).

Results. The summary of the results of the chemical analyses is presented in Table I.

Distribution of protein-bound carbohydrate. The greatest concentration of protein-bound carbohydrate occurred in Fraction IV + V (α-globulin + albumin). Seventy-one percent of the value for the combined fraction was attributable to Fraction IV. Albumin with the lowest carbohydrate content of any of the determined fractions, nevertheless, accounted for a substantial percentage of the total plasma glycoprotein due to its high concentration in plasma.

Although Fraction II (γ-globulin) has been studied extensively in relation to its antibody content and physicochemical properties, little attention has been directed to the polysaccharide moiety of the fraction. The results of the current study indicate that carbohydrate forms an integral part of the γ-globulin molecule. The hexose value for the fraction, 13 mg %, represented 11.9% of the total plasma glycoprotein.

Protein-bound carbohydrate values for Fractions II, V, and VI were similar, notwithstanding the large differences in the protein content of the fractions.

The polysaccharide level of the perchloric acid-soluble, phosphotungstic acid-insoluble seromucoid fraction was lower than the corresponding values of the fractions isolated by ethanol precipitation. The major component of Fraction VI and seromucoid is orosomucoid, a glycoprotein with a very low isoelectric point, pH 1.8, and characterized by a high degree of solubility(10).

The 4 primary fractions accounted for 96.3% of the total plasma glycoprotein. The difference is attributable to losses common to all fractionation procedures. Variability as measured by the coefficient of variation was most pronounced in Fraction II.

Distribution of protein. Fraction IV + V accounted for 66.2% of the total protein content of plasma. The concentration of albumin represented 54% of the total plasma protein. The ratio was in good agreement with plasma

albumin values obtained by electrophoretic analyses(11,12). The calculated ratio for Fraction IV (α-globulin) in plasma was lower than corresponding values based on electrophoretic measurements(11,12). It is to be noted, however, that α-globulin is also a constituent of Fractions I + III and VI(5). The percentage of γ-globulin (Fraction II) was intermediate between 2 reported values obtained by electrophoresis(11,12). The sum of the protein concentrations of the 4 isolated fractions represented 98.7% of the total plasma protein. Individual values for Fraction VI were subject to the greatest degree of variability. The magnitude of the variation is due in part to the technical difficulties encountered in the determination of very low concentrations of protein by a biuret method.

Protein-bound carbohydrate as per cent of protein. Two of the 4 primary fractions (I + III and VI) were observed to have carbohydrate/protein ratios greater than the value obtained for whole plasma. Fraction IV + V with the lowest percentage of glycoprotein, however, accounted for two-thirds of the protein content and three-eighths of the protein-bound carbohydrate content of plasma. The protein-bound carbohydrate of Fraction VI represented 6.5% of the fraction, presumably due to the presence of orosomucoid, with a hexose content of 17%(4,10). The high hexose content of Fraction I + III, 2.9% was somewhat surprising in view of the low values obtained by similar determinations on β-globulin and fibrinogen from pooled, normal plasma(13). The possibility exists, however, that the relatively high value of the fraction is due to the presence of α-globulin, which represents approximately 17% of the protein content(5).

The fractionation procedure of Lever *et al.* (5) has certain limitations with respect to its employment in the study of plasma glycoproteins. The most important are the marked heterogeneity of Fraction I + III (α-globulin + β-globulin + fibrinogen) and the separation of α-globulin and albumin into the same Fraction IV + V. Albumin and α-globulin have been shown to decrease and increase, respectively, in a large number of pathologic and experimental conditions(14). The latter disadvantage has been obviated to a certain extent, as demonstrated in the current study, by the development of improved methods (8,15) for the determination of albumin in Fraction IV + V.

Advantages of the separation procedure include the isolation of fractions of known composition in an undenatured state, permitting the determination of their chemical, physical, and physiologic properties.

Summary. Five-ml samples of normal, human plasma have been separated by a low temperature-low salt-ethanol procedure into 4 primary fractions, I + III, II, IV + V, and VI. The concentrations of protein-bound carbohydrate (hexose) and of protein in whole plasma and the 4 primary fractions and the protein-bound carbohydrate content of seromucoid were determined. Fraction IV + V, of the primary fractions, contained the greatest amount of protein-bound carbohydrate and of protein. The percentage of protein-bound carbohydrate with respect to protein was highest in Fraction VI.

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Effect of Caloric Restriction on Cholesterol Atherogenesis in the Rabbit. (21301)

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The effect of caloric restriction on the genesis of atherosclerosis has received very limited attention. Studies of human obesity(1-3) and surveys of the nutritional and health status in various countries(4,a,b) have resulted in a general impression that low caloric intake exerts a protective action in any atherogenic situation. Scanty information is available concerning the influence of caloric restriction on the development of experimental atherosclerosis(5,6). The present study was undertaken to furnish more detailed findings regarding the effects of severe caloric restriction in rabbits fed cholesterol.*

Our experimental results are significant because they 1) illustrate the fallacy of regarding severe caloric restriction as the opposite of obesity and 2) point out the necessity for paying careful attention to the diet and weight of rabbits subjected to experimental procedures or unusual diets designed to elucidate factors involved in atherogenesis.

Material and methods. Male, adult Swiss rabbits weighing between 3.5 and 5.5 kg were kept in the laboratory for several weeks prior to the experiments and the average daily food intake necessary to maintain weight equilibrium was estimated. A standard food mixture† was used which has the following content of nutrients, minerals and vitamins: fiber 15%, fat 3%, protein 17.50%, calcium 1.66%, P .37%, I .0033%, salt 1.00%; pantothenic acid 13-14 mg/lb., niacin 17.5 mg/lb., thiamin 1.28 mg/lb., riboflavin 3.40 mg/lb., biotin 0.16 mg/lb., vit. C .81 mg/lb., vit. D 450 USP units/lb., vit. E 30 mg/lb., choline 640 mg/lb. This diet was supplemented with 10-20 g/day raw carrots. Two series of experiments were carried out differing in 1) duration, 2) degree of caloric restriction, 3) dose of cholesterol, and 4) mode of cholesterol administration. The control groups in both experiments were fed *ad libitum;* the average consumption approximated 130 g/day of the standard ration. There were 18 animals in Exp. A and 22 in B. Half of this number were on restricted food intake, the rest served as control. *Duration.* Exp. A lasted 12 weeks, Exp. B 8 weeks. *Degree of caloric restriction.* The underfed group in Exp. A received 50 g/day, Exp. B 30 g/day of rabbit pellets plus 20 g raw carrot. This constituted approximately ½ and ⅓ of average control ration. *Dose of cholesterol.* 1 g/day in Exp. A and 2 g/day in Exp. B. Thus the absolute cholesterol content in the groups on adequate and on restricted calories was the same, but the percentile content varied, being considerably higher in the restricted diets. *Mode of Administration.* Measured amounts of crystalline cholesterol were mixed thoroughly with shredded raw carrots without adding any oil. In Exp. A the rabbits consumed the carrot-cholesterol mixture at the same time as their pellets. In Exp. B regular feeding was delayed until 4 to 5 hours after completion of cholesterol ingestion. The uneaten

* While the manuscript was being prepared for publication, an article appeared by McMillan, Whiteside, and Duff (*J. Exp. Med.*, 1954, v99, 261) dealing with the same experimental problem. It is of interest that the findings and conclusions are virtually in agreement with ours.

† B-B Laboratory Rabbit Diet (Maritime Milling Co., Inc., Buffalo, N. Y.)