same as for the total circulation(6). Further, the BVR_{cells} for control and eviscerated dogs are the same(7).

Previously, after an overtransfusion, the ratio of the two hematocrits remained the same. In the present experiments when the liver was bypassed and then an overtransfusion was given, the circulatory hematocrit increased more than the venous hematocrit resulting in a BVR_{cells} ratio of 1.0. Under these circumstances the involvement of the spleen in the BVR_{cells} ratio was probably negligible because of the anesthetics used(8). Also, observation of the size of the spleen at the end of the experiment indicated that it was contracted. This suggests that at this time the circulating red cells were evenly distributed throughout the vascular system. There are two possible explanations for this phenomenon. Either a portion of the capillary bed in which cells and plasma were differentially distributed has been eliminated from the circulation, or, while a small area of the circulation was shut down containing the red cells trapped, the majority of the capillaries had the same concentration of red cells as the large vessels. In view of the increased portal venous pressure after the transfusion and the fact that on gross inspection the vessels of the splanchnic area were found to be dilated and well-filled, the latter alternative seems to be the likely one. Whether the rise in portal venous pressure plays a role in the trapping of red cells and loss of plasma cannot be decided on the basis of these experiments.

Summary. In anesthetized dogs, the measured cell and plasma volumes, protein concentration, venous hematocrit and BVR_{cells} were the same before and after bypassing the liver with a portal vein to femoral vein shunt. An overtransfusion of blood after the bypass produced significant changes in all measured circulatory parameters. At the end of the experiment the volume of trapped cells was 33% of the cells infused, and the escaped plasma was 93% of the infused. Protein lost represented 78% of the infused. Circulatory and venous hematocrits were the same after the bypass and transfusion, suggesting that under these circumstances cells and plasma were evenly distributed throughout the circulation. Mean arterial pressure was reduced slightly after insertion of the bypass and also after the overtransfusion. Portal venous pressure increased when the liver was bypassed and further increased after the transfusion following the bypass.

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Quantitative Determination of the Group Specific Protein in Normal Human Serum.* (29827)

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Three phenotypes of the group specific α globulin (Gc) can be recognized in human serum by starch gel and immunoelectrophoresis; a fast component Gc 1-1, a slow component Gc 2-2 and an intermediate com-

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ponent Gc 2-1(1). The synthesis of this serum protein is controlled by two co-dominant autosomal alleles designated Gc^1 and Gc^2(2). Two mutations have been described affecting the Gc locus; one was found in a population of Chippewa Indians (Gc Chippewa) and the second in a population of Australian Aborigines (Gc Aborigine)(3). Thus far no serum has been encountered which lacks the Gc protein. Although preliminary observations in this laboratory suggested that the group specific protein existed in the serum in only small amounts(4), more recent evidence suggested the possibility that quite large quantities of the Gc protein are normally present in human serum. To obtain more precise information on this point, a method was developed to determine the concentration of the Gc protein in normal human serum by a quantitative precipitin reaction.

Materials and methods. Specific antisera against Gc 1-1 protein were obtained by immunizing Chinchilla rabbits with a purified Gc 1-1 preparation(4). 0.20 mg of Gc protein in complete Freund's adjuvant was injected into each footpad and an additional 0.20 mg injected into the hind footpads 9 days later. Three weeks after the first injection the rabbits were bled and the serum from 2 animals pooled. Examination of the antiserum disclosed 2 contaminating antibodies in addition to the Gc antibody. These were identified as the anti α_1-antitrypsin and antialbumin. Preparations of these 2 antigens were made by starch block electrophoresis of human serum (Gc 2-2) and, after titration, the contaminating antibodies were absorbed selectively. Phenyl mercuric nitrate in a final concentration of 1:30,000 was added to the serum as a preservative and this specific anti-Gc serum was used throughout.

Quantitative precipitin curves were constructed using a purified preparation of Gc 1-1 and whole serum of types Gc 1-1, Gc 2-2 and Gc 2-1. The reaction was conducted in a total volume of 0.2 ml and allowed to proceed for 24 hours at 4°C; the precipitate was washed 3 times, dissolved in 0.1 ml N/10 NaOH and the protein estimated using the Folin-Ciocalteau method. A calibration curve for the protein method was constructed

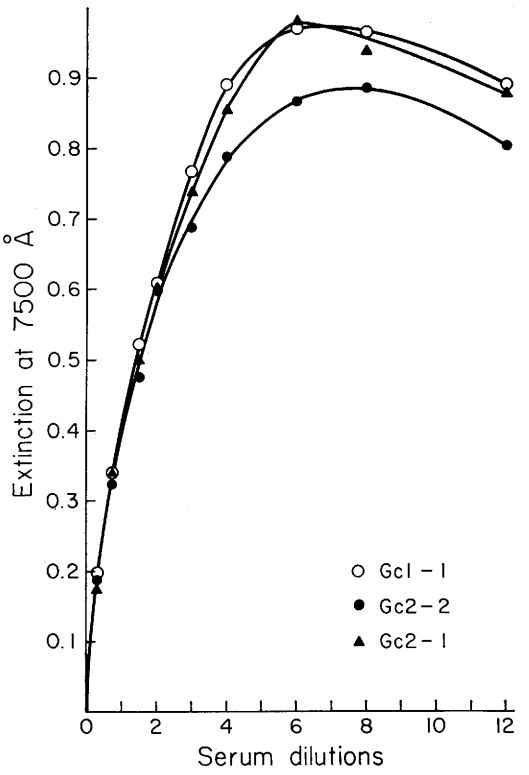

FIG. 1. Quantitative precipitin curves for 3 common phenotypes, Gc 1-1, Gc 2-2 and Gc 2-1. Serum dilutions range from undiluted to 1:32.

using 3 times recrystallized human albumin and the final color read on a Beckman DU spectrophotometer at 7500 Å. Serum was obtained from 21 normal human volunteers, drawn under sterile conditions in the fasting state, and stored at —4°C. The serum samples were analyzed in duplicate at various dilutions. Immunoelectrophoresis was carried out according to the method of Scheidegger (5) as modified by Hirschfeld(6).

Results. The precipitation curves of the 3 phenotypes Gc 1-1, Gc 2-2 and Gc 2-1 are shown in Fig. 1. The serum samples were serially diluted with normal saline in ratios of 1:1.5, 1:2, 1:3, 1:4, 1:6, 1:8, 1:16, 1:32. A linear relationship of protein concentration and optical density was found between the serum dilutions 1:32 and 1:6. This corresponds to an extinction-coefficient E = 0.20 to 0.625 and a range of 2.0 to 10.5 μg of Gc protein. Quantitative determination of the Gc protein was performed in the region of the curve where no significant differences be-

TABLE I. Comparison of the Serum Group Specific Protein Concentration in the 3 Common Phenotypes.

Gc phenotype	Sex	Age	mg/100 ml	Total	Mean	S.D.	S.E.
1–1	♀	31	78.4	8	75.8	9.1	3.2
	♂	40	72.7				
	♀	64	76.6				
	♀	28	62.4				
	♂	78	88.4				
	♀	33	88.0				
	♂	75	67.5				
	♀	30	72.5				
2–1	♂	30	80.6	10	74.4	5.2	1.6
	♂	48	72.0				
	♀	30	76.0				
	♂	31	75.2				
	♂	59	68.0				
	♂	68	80.0				
	♀	73	76.5				
	♂	32	80.0				
	♀	39	68.0				
	♀	51	67.5				
2–2	♂	26	67.2	3	72.8	5.2	3.0
	♀	46	73.6				
	♀	49	77.5				
				21	74.8	6.5	1.4

S.D., standard deviation; S.E., standard error.

tween the Gc phenotypes were observed. In each group of sera analyzed a control sample of type Gc 1-1 was included. The mean level of Gc protein in 5 analyses of this sample, performed on separate occasions, was 72.7 mg/100 ml S.D. ± 2.6 mg. The results obtained on all sera analyzed are illustrated in Table I. The mean concentration of serum Gc protein in 21 normal individuals was 74.8 mg per 100 ml ± 6.5 mg.

No significant difference in the levels of the Gc protein in the 3 common phenotypes examined was detected, and, in this small sample, the values appeared independent of age and sex.

Discussion. The development of a quantitative precipitin method has enabled the amount of serum group specific protein to be determined. Insufficient quantities of purified Gc were available to construct a standard protein curve for the group specific component and thus the results are expressed in albumin equivalents. A comparison of the amino acid composition of the group specific components(4) with that of serum albumin (7) would suggest that any error introduced on this account would be extremely small.

Summary. The normal level of the serum group specific protein (Gc) has been found to be 74.8 mg/100 ml ± 6.5 as estimated by an immunochemical method. No significant differences were found in the mean level of the protein in the 3 phenotypes Gc 1-1, Gc 2-2 and Gc 2-1.

ADDENDUM: Since this report was submitted, low serum levels of Gc protein have been found in portal cirrhosis (58.4 and 59.4 mg/100 ml), in acute virus hepatitis (59.4 mg/100 ml) and in terminal hepatic coma (27.9 mg/100 ml). These data reinforce the previous evidence(8) that the liver is the principal site of Gc protein synthesis.

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Some Antigenic Characteristics and Immunologic Reactions of Horse Spleen Ferritin.* (29828)

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The usefulness of ferritin, the electron dense iron-storage protein, in various studies, particularly in the field of electron microscopy, suggested the need for additional information about some of its immunologic characteristics. Ferritin has a molecular weight of about 750,000 and exists as a protein shell covering an iron containing core(1). Anti-ferritin antibody had been prepared in rabbits(2) and goats(3). It may be stained with a suitable iron stain. The use of ferritin in labeling of antibodies recently has been reviewed(4).

The present studies describe the preparation of a solution of ferritin with a single antigenic component and its labeling with I^{131}. Studies of certain characteristics of rabbit and chicken anti-ferritin sera are illustrated, particularly utilizing the technique of precipitation of isotope labeled ferritin as a measure of the chicken and rabbit antibodies. A technique of localization of ferritin-anti-ferritin precipitin arcs by an iron staining procedure applied to immunoelectrophoretic preparations provides a means of study of types of antibodies against ferritin. The technique, similar to that of autoradiographic immunoelectrophoresis, may be used for study of immunoglobulins where ferritin can be used as the antigen without the necessity of an isotope label.

Methods and materials. Ferritin was obtained commercially (horse spleen ferritin, Pentex Laboratories, Kankakee, Ill.). The commercial ferritin was recrystallized 8 times by cadmium sulfate and reprecipitated 4 times by ammonium sulfate precipitation(5). The ferritin was finally purified by passage through Sephadex G-200 (Pharmacia, Uppsala, Sweden) in 0.1 M NaCl and 0.1 M TRIS pH 8.0 buffer. This purified ferritin was utilized in subsequent experiments and compared with the commercial and recrystallized preparations. Ferritin concentration in the gel filtration effluent was measured in a Beckman D.U. Spectrophotometer at 255 mμ, at which wave length maximal absorption by ferritin appeared to occur.

Three preparations of ferritin were studied. These were preparations A, B and C shown in Table I. All 3 were labeled with I^{131} ($I^{131}F$) using the method of labeling proteins described by Talmage *et al*(6).

Adult albino rabbits were immunized with commercial ferritin in incomplete Freund's adjuvant(7). Each rabbit received 50 mg ferritin subcutaneously weekly for 6 weeks. Rabbits were bled 8 days after the last injection. Adult White Leghorn roosters received commercial ferritin in 0.15 M NaCl. Because of toxicity of cadmium salts, immunization was carried out over a period of 2 days using divided (30 mg) doses intravenously to a total dose of 40 mg per kg body weight. Primarily immunized roosters were bled 7 days after initiation of immunization. Some roosters received a second immunization with ferritin 4 weeks after primary immunization and were bled one week later.

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