The author is grateful to Dr. H. Zaman, W.H.O. Fellow from the Medical College in Dacca, East Pakistan, who participated in initial phase of this work. Thanks are also due to the following persons who kindly supplied human kidney specimens: Drs. A. A. Liebow, W. B. McAllister, and R. R. Berneike, Yale School of Med. and Grace-New Haven Community Hospital, Dr. R. N. Barnett of Norwalk Hospital, Dr. R. E. Kendall, of Hartford Hospital, Dr. H. C. Loh of St. Mary's Hospital, Waterbury, Dr. C. E., McLeod of Middlesex Memorial Hospital, Middletown, and Dr. W. L. Chow of Bridgeport Hospital.

1. Weller, T. H., Enders, J. F., Robbins, F. C., Stoddard, M. B., *J. Immunol.*, 1952, v69, 645.

2. Committee on Enteroviruses, Nat. Found. for Infantile Paralysis, *Am. J. Pub. Hlth.*, 1957, v47, 1556.

3. Hsiung, G. D., Melnick, J. L., *J. Immunol.*, 1957, v78, 137.

4. Hsiung, G. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 387.

5. Melnick, J. L., *Diagnostic Procedures for Virus and Rickettsial Diseases*, 2nd Ed., 1956, APHA, N.Y. City, 115.

6. Henderson, J. R., Taylor, R. M., *Am. J. Trop. Med. & Hyg.*, 1959, in press.

7. Dalldorf, G., Sickles, G. M., Plager, H., Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

8. McLaren, A., Sanders, F. K., *J. Hyg.*, 1959, v57, 106.

Received August 10, 1959. P.S.E.B.M., 1959, v102.

Zone Electrophoretic Studies of Proteins and Glycoproteins of Bovine Serum and Synovial Fluid. (25336)

BARRY DECKER,* BERNARD F. MCKENZIE,† WARREN F. MCGUCKIN†
(Introduced by G. W. James III)
Dept. of Medicine, Medical College of Va., and Mayo Clinic, Rochester, Minn.

The electrophoretic patterns of bovine serum and synovial fluid were determined as part of a broader study in the human. The scarcity of information concerning bovine synovial fluid glycoproteins prompted this report.

Methods. Undialyzed samples of serum or synovial fluid were subjected to electrophoresis at room temperature, on Whatman 3 mm paper, 3 cm. wide, in barbital buffer of ionic strength 0.075, at pH 8.6, for 16 hours at a constant current of 0.6 milliampere/strip. The Spinco model R series C, paper electrophoresis apparatus was used. After oven drying at 110°C, for 15 minutes, glycoproteins were additionally fixed to the papers and cleared of buffer salts by a wash in absolute alcohol. Paper strips were dyed for proteins with amidoschwarz 10 B dye and for glycoproteins by a modification of the periodic acid-Schiff technic(1). The dyed paper strips were analyzed in a Spinco model RA densitometer, using Wratten filters No. 58 and 22 for proteins and an interference filter with maximal transmission at 561 mu for glycoproteins. Synovial fluids were digested by 140 turbidity reducing units of bull testicular hyaluronidase at 37°C for 1½ hours prior to electrophoresis to remove the viscous anomaly. Weight of protein applied to each paper strip was equivalent to 10 microliters of a 4.5 g/100 ml solution of protein. For glycoprotein determinations 30 microliters of serum and 50 microliters of synovial fluid were applied to the paper. Protein concentration was determined by biuret method and protein-bound hexose by modification of the anthrone tryptophane reaction(2).

Material. Serum from 13 cows was obtained immediately after slaughter. Synovial fluid was aspirated from third joint of foreleg at the same time. In eight animals bilateral joints were aspirated making a total of 21 specimens of synovial fluid. This material was obtained through the courtesy of Hormel Co., Austin, Minn.

Results. Table I describes the mean protein and glycoprotein patterns of 13 bovine sera. Mean values from 13 human control sera are included for comparison. There was

* Director Connective Tissue Study Group, Medical College of Va.

† Section of Biochemistry, Mayo Clinic.

TABLE I.

	Protein, g/100 ml	% of total protein				Protein, g/100 ml				Hexose, mg/100 ml	% of total protein-bound polysaccharide				Hexose/protein (PR) ratio
		Alb.	$(\alpha_1+\alpha_2)$	β	γ	Alb.	$(\alpha_1+\alpha_2)$	β	γ		Alb.	$(\alpha_1+\alpha_2)$	β	γ	
Normal human serum															
Mean	7.09	53	7 11	14	17	3.72	.47 .75	.96	1.18	100	13	16 25	25	20	1.42
S.D.	.27	3	1 2	1	2	.37	.05 .06	.07	.20	8	3	4 5	4	3	.12
Normal bovine serum															
Mean	8.08	42	15	15	28	3.37	1.19	1.23	2.28	124	7	51	23	20	1.53
S.D.	.64	3	2	2	4	.29	.10	.15	.49	12	4	6	4	5	.12
Normal bovine synovial fluid															
Mean	1.80	50	14	16	21	.90	.24	.29	.37	36	10	49	28	14	1.98
S.D.	.66	5	2	2	3	.27	.08	.10	.11	11	5	8	5	4	.46

8.08 ± .64 g of protein and 124 ± 12 mg of protein-bound hexose/100 ml of bovine serum. Alpha 1 and alpha 2 globulins were only infrequently separated by the conditions of electrophoresis used. There was 3.37 ± .29 g of albumin, 1.19 ± .10 g of alpha globulin, 1.23 ± .15 g of beta globulin and 2.28 ± .49 g of gamma globulin/100 ml of bovine serum. In comparison to human serum, bovine serum contained significantly more beta and gamma globulin, significantly less albumin and essentially the same concentration of alpha globulins. The marked increase in gamma globulin accounted for the higher bovine serum protein.

The protein-bound hexose of bovine serum (124) was significantly higher than human serum (100) but the mean bovine hexose/protein (PR) ratio of 1.53 ± .12 did not differ significantly from the mean human value of 1.42 ± .12. The distribution of protein-bound polysaccharide in bovine serum was similar to human serum with slightly less albumin polysaccharide and slightly more alpha globulin polysaccharide.

Table I also describes the mean protein and glycoprotein patterns of 21 bovine synovial fluids. There was 1.80 ± .66 g of protein and 36 ± 11 mg of protein-bound hexose/100 ml of bovine synovial fluid. The protein concentration of synovial fluid was only 22% of the serum protein, but the synovial fluid had a higher relative concentration of albumin (50%) and a lower relative concentration of gamma globulin (21%) than the comparable fractions in serum (albumin—42%, gamma globulin 28%). The distribution of bovine synovial glycoproteins was essentially comparable to serum glycoproteins but the failure to distinguish the alpha 1 and alpha 2 zones may have masked significant differences between fluid and serum.

The mean hexose/protein (PR) ratio of bovine synovial fluid (1.98 ± .46) was significantly higher than bovine serum (1.53 ± .12) indicating preferential permeability of one or more glycoproteins.

Comment. The electrophoretic pattern of bovine serum (or plasma) has been studied in a few animals by moving boundary electrophoresis using a variety of buffers(3-5). Con-

siderable variation in these reports resulted from differences in technic, with albumin ranging from 38-43% and gamma globulin from 11 to 28%. In a large series of 51 Devon steers, Brandish *et al.*(6), using moving boundary electrophoresis in a phosphate buffer of pH 7.5 and an ionic strength 0.18, noted albumin to comprise 46.6%, alpha globulins 14%, beta globulins 8.9% and gamma globulins 30.5% of a mean serum protein of 7.0 ± .5/100 ml. They noted that albumin remained relatively stable in their normal bovine population with a mean concentration of 3.2 g/100 ml while total serum protein varied with changes in gamma globulin. The thirteen bovine sera studied, in the present report, had an albumin concentration of 3.37 g per 100 ml with a higher total protein (8.08) which related to an increase in gamma globulin.

Hesselvick(7), using moving boundary electrophoresis, demonstrated hyaluronic acid, albumin, beta and gamma globulins in equine synovial fluid. Subsequent investigators(8) have shown alpha globulins to be present in bovine synovial fluid and have measured higher relative concentrations of albumin and lower relative concentrations of globulin in synovial fluid in comparison to serum. The results of the present report are in accord with these findings.

Neuhaus *et al.*(9) eluted the alpha, beta and gamma globulins of bovine synovial fluid after electrophoresis on starch and measured the protein and hexosamine concentration of each fraction, noting an increase in the hexosamine/protein ratio of the fluid in comparison to serum. The increase in the hexosamine/protein ratio was predominantly in the alpha globulin zone and was considered to result from the preferential permeability of seromucoid. The present report supports the finding of a relative increase in the glycoproteins of synovial fluid as compared to serum but does not offer evidence as to the identity of this glycoprotein(s). Neuhaus(9), other(10), and the present authors were not able to readily separate the alpha 1 and alpha 2 globulins of bovine serum and synovial fluid.

Summary. 1) Zone electrophoresis of 13 bovine sera yielded a mean concentration of 3.37 ± .29 g (42%) albumin, 1.19 ± .10 g (15%) alpha globulin, 1.23 ± .15 g (15%) beta globulin and 2.28 ± .49 g (28%) gamma globulin/100 ml. Serum protein-bound hexose was 124 mg/100 ml or 1.53 ± .12% of the mean serum protein concentration of 8.08 g/100 ml. Zone electrophoretic separation showed albumin to contain 7 ± 4%, alpha globulins 51 ± 6%, beta globulins 23 ± 4% and gamma globulins 20 ± 5% of the protein-bound polysaccharide. 2) Twenty-one synovial fluids from the same animals had a mean concentration of .90 ± .27 g (50%) albumin, .24 ± .08 g (14%) alpha globulins, .29 ± .10 g (16%) beta globulins and .37 ± .11 g (21%) gamma globulin/100 ml. Synovial fluid protein-bound hexose was 36 ± 11 mg/100 ml or 1.98 ± .46% of the mean synovial fluid protein of 1.80 g/100 ml. Zone electrophoretic separation showed albumin to contain 10 ± 5%, alpha globulins 49 ± 8%, beta globulins 28 ± 5%, and gamma globulins 14 ± 4% of the protein-bound polysaccharide. 3) The synovial fluid had relatively more albumin (50%) and relatively less gamma globulin (21%) than serum (albumin 42%, gamma globulin 28%) and a higher hexose/protein (PR) ratio indicating preferential permeability of albumin and one or more glycoproteins. Alpha globulins were not separated into alpha 1 and alpha 2 zones.

1. McGuckin, W. F., McKenzie, B. F., *Clin. Chem.*, 1958, v4, 476.

2. Decker, B., McKenzie, B. F., McGuckin, W. F., Slocumb, B. F., *Arthritis and Rheumatism,* 1959, v2, 162.

3. Deutch, H. F., Goodloe, M. B., *J.B.C.,* 1945, v161, 1.

4. Hogness, K. R., Giffee, J. W., Koenig, V. L., *Archives of Biochemistry,* 1946, v10, 281.

5. Cann, J. R., Brown, R. A., Kirkwood, J. G., *J. Am. Chem. Soc.,* 1949, v71, 1609.

6. Brandish, C. J., Henderson, W. M., Brooksby, J. B., *Biochem. J.,* 1954, v56, 329.

7. Hesselvick, L., *Acta Medica Scand.,* 1940, v105, 153.

8. Meriel, P., Ruffie, R., Sardou, M., Fournie, A., *Presse Med.,* 1954, v62, 1207.

9. Neuhaus, O. W., Letzring, M., *J.B.C.,* 1958, v232, 177.

10. Platt, D., Pigman, W., Holley, H. L., Patton, F. M., *Archives of Biochem. and Biophysics,* 1956, v64, 152.

Received August 10, 1959. P.S.E.B.M., 1959, v102.