(heated to 60°C for 20 minutes) has also been pointed out by Quick and his associates(8). Whether the platelet factor and the thromboplastic factor of brain tissue are chemically identical has still to be determined.

Summary. 1. On the basis of heat stability studies, the clotting properties of brain tissue extracts are due to 2 components: a) a thrombokinase, which has the ability to convert prothrombin directly to thrombin, and b) a thromboplastin, which is an accessory factor and must react first with another plasma factor, such as proconvertin, before it is capable of converting prothrombin to thrombin. 2. The indirect effect of thromboplastin must be distinguished from the direct action of thrombokinase on prothrombin if the mode of action of so-called "thromboplastic" brain tissue extracts is to be understood.

1. Milstone, J. H., *Medicine,* 1952, v31, 411.
2. Morawitz, P., *Deutsch. Arch. klin. Med.,* 1904, v79, 1.
3. Nolf, P., *Arch. internat. physiol.,* 1908, v6, 1.
4. Owren, P. A., *Acta med. Scandinav.,* suppl., 1947, v194, 1.
5. Jensen, H., Gray, E. J., and Schaefer, Jr., E. H., *Army Med. Research Laboratory Rep.* No. 131, 15 Dec. 1953.
6. Quick, A. J., *The physiology and pathology of hemostasis,* Lea and Febiger, Philadelphia, 1951.
7. Soulier, J. P., and Larrieu, M. J., *J. Lab. and Clin. Med.,* 1953, v41, 849.
8. Quick, A. J., Stapp, W. F., and Hussey, C. V., *ibid.,* 1952, v39, 142.
9. Biggs, R., Douglas, A. S., and MacFarlane, R. G., *J. Physiol.,* 1953, v122, 554.
10. Koller, F., Loeliger, A., and Duckert, F., *Acta Haematol.,* 1951, v6, 1.

Received April 30, 1954. P.S.E.B.M., 1954, v86.

Levels of Serum Protein Fractions in Diabetic Patients with *Retinitis proliferans.** (21109)

NILS RUD. KEIDING.† (Introduced by Alexander Marble.)

From the Baker Clinic Research Laboratory New England Deaconess Hospital, Boston, Mass.

There has been much speculation as to the etiology of the degenerative vascular lesions seen in long-term diabetes. The question has been raised as to what role, if any, shifts in serum protein and, in particular, serum mucoproteins and lipoproteins, play in the development of such abnormalities(1-3). Changes in the major serum protein fractions in diabetes have been reported. Schneider, Lewis, and McCullagh(4) described changes in the albumin and β-globulin fractions of the serum from 31 diabetic patients with retinitis. The reduction in albumin and the elevation of the β-globulin fraction were more marked in the retinitis cases than similar changes observed in uncontrolled diabetics. That these changes may not represent the same thing was indicated by a difference in response to treatment. Rifkin and Petermann(5) have reported the results of an electrophoretic study of the serum proteins of patients with clinically established diabetic glomerulosclerosis. A decrease in the albumin and a significant elevation of the α_2-globulin were found in all 10 cases. The β-globulin was normal or only slightly elevated. Changes in the serum lipoproteins in diabetics with vascular degenerative changes have been reported by Keiding *et al.*(6) and by Engelberg *et al.*(7). The elevation of the S_f 12-20, S_f 21-35, and S_f 35-100 lipoproteins was found to correlate with the presence of both diabetic retinopathy and nephropathy. Changes in the serum mucopolysaccharides and mucoproteins in patients with vascular degenerative disease have been investigated systematically both by Berkman *et al.*(8) and by Keiding and Tuller(9). However, during the work of the latter authors it was evident that it would be of interest to follow changes in the major serum protein fractions along

* Aided by Diabetic Fund of Boston and by Nordisk Insulin Fond.

† Present address: Steno Memorial Hospital, Gentofte, Denmark.

TABLE I. Clinical Characteristics of 14 Patients Studied.

Sex	Age, yr	Duration of diabetes, yr	*Retinitis proliferans*	Proteinuria	Hypertension	Renal insufficiency
♂	38	23	+	+	+	+
♂	34	18	+	+	+	0
♀	34	23	+	+	0	0
♂	37	26	+	+	+	0
♂	24	13	+	+	0	0
♂	24	2+	+	+	0	0
♂	33	20	+	+	+	0
♂	28	25	+	+	+	+
♀	28	19	+	0	0	0
♂	38	21	+	0	0	0
♂	58	21	+	0	0	0
♀	33	28	+	0	0	0
♀	70	14	+	0	+	0
♂	50	10	+	0	(+)	0

with the determination of the protein-bound carbohydrates in patients showing vascular disease in various stages. Two groups of diabetic patients were selected for study, one with marked retinal changes (*retinitis proliferans*) and no clinical evidence of kidney damage, and the other with both *retinitis proliferans* and renal damage.

The results of the determination of serum protein changes through the use of paper electrophoresis and of the analysis of protein-bound carbohydrates on the patients are reported below.

Methods. Blood was taken from patients before or 3 hours after breakfast. Analyses for protein-bound carbohydrates were carried out at once by 2 methods. The non-hexosamine carbohydrate content of an alcohol precipitate of the proteins in serum was determined by the anthrone method(10). Hexosamine content was determined by a modified Elson-Morgan technic(11). The concentrations of these protein-bound carbohydrates are reported as mg/100 ml of serum. *Paper electrophoresis* of the serum proteins was performed by the basic technic of Kunkel and Tiselius(12). Whatman No. 3MM filter paper was placed between siliconized glass plates after the application of 0.01 ml of serum to the paper. Separation was carried out for approximately 4 hours in a barbital buffer, 0.05 ionic strength, pH 8.6, and for applied voltage of 15 V/cm of paper. The albumin fraction moved approximately 12-13 cm. Bromophenol blue was used to stain the protein. The strips were evaluated by eluting 0.5 cm segments in 0.01 N NaOH and the color intensity read with a Klett-Summerson colorimeter. The readings were plotted and the areas under the curves determined with a planimeter. The concentration of each fraction is expressed in g/100 ml of serum. A strictly standardized technic was used, and normal serum was always run simultaneously with pathological serum to maintain maximum reproducibility and comparability. The average variation of separate runs of the same serum was: albumin plus α_1-globulin $\pm 1\%$, α_2-globulin $\pm 2.9\%$, β-globulin $\pm 2.4\%$, and γ-globulin $\pm 1.5\%$.‡ Proportionality was found between protein concentration and stain uptake for the albumin fraction within the concentration limits used. Although the globulins did not take up the dye quite in proportion to protein concentration at high levels, the differences were small. Since comparison was made between normal and pathological sera run by the same technic, no correction factor was introduced. Determinations of the *total protein* were made using the biuret reaction as modified by Mehl(13) in 1945. Purified human albumin was used as the standard reference.

Material. Non-diabetic subjects used were doctors and laboratory personnel in the hospital. They were healthy, showed no signs of infection and no albumin in their urine. The group consisted of 4 females (ages 21, 38, 41,

‡ These determinations were checked in the laboratory of the Steno Memorial Hospital, Gentofte, Denmark.

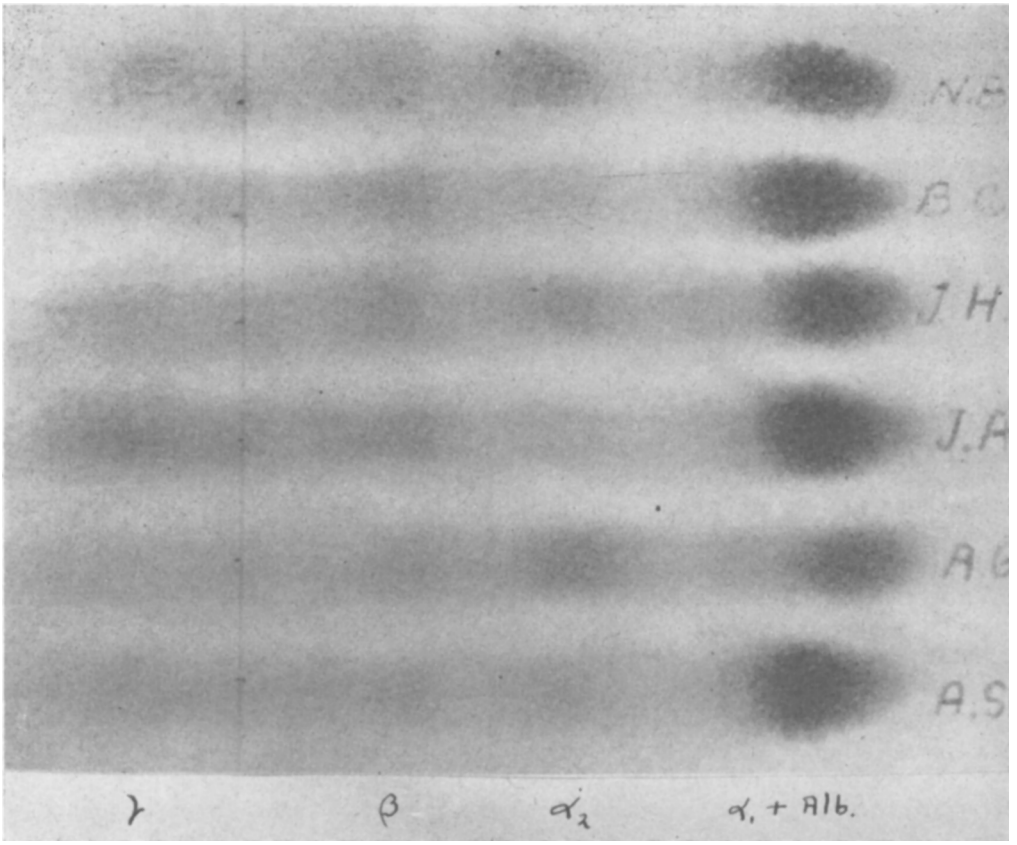

FIG. 1. Paper electrophoresis patterns of the proteins in representative sera. (B.C.), (J.A.) normal subjects; (N.B.), (J.H.), (A.G.) diabetics with *retinitis proliferans* and proteinuria; (A.S.) diabetic with early *retinitis proliferans* without proteinuria.

and 50 years) and 6 males (ages 22, 28, 32, 34, 37, and 37 years). The diabetics were patients in the New England Deaconess Hospital. The clinical data are presented in Table I. The diagnosis of *retinitis proliferans* was made in all cases by an ophthalmologist. Proteinuria was based on repeated findings of more than 20 mg % albumin in the urine. Azotemia was said to be present if the non-protein nitrogen concentration was elevated consistently above 40 mg %.

Results. The separation of serum proteins obtained in the paper electrophoresis is shown in Fig. 1. Six different sera are represented; 2 from normal subjects, 3 from diabetics with *retinitis proliferans* with proteinuria, and one from a diabetic with early *retinitis proliferans* without proteinuria. It is evident that there is a distinct increase in the α_2-globulin fraction in the diabetics with both *retinitis proliferans* and proteinuria, while there is only a slight increase in that of the diabetic without proteinuria. In the 3 diabetics with proteinuria, the serum albumin spot is less dense than in the non-diabetics.

Fig. 2 and 3 present graphically the quantitative changes in protein-bound carbohydrates and in the serum proteins respectively for the non-diabetics and the 2 groups of diabetics. As may be seen readily from Fig. 2, both groups of diabetics showed an increase in the protein-bound carbohydrate of both the non-hexosamine and hexosamine type. However, only in the group with proteinuria was there a change in total protein (Fig. 3).

From Fig. 3 it is evident that both groups of diabetics have a low combined albumin + α_1-globulin value in contrast to the shift in total protein. Likewise, both groups of diabetics show an elevated α_2-globulin value. The

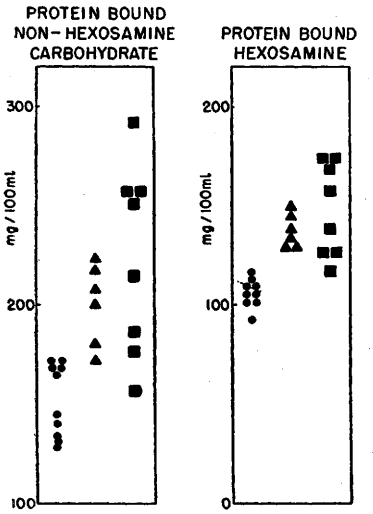

FIG. 2. Levels of protein-bound carbohydrate. ● Non-diabetics. ▲ Diabetics with *retinitis proliferans* without kidney disease. ■ Diabetics with *retinitis proliferans* and kidney disease.

β-globulin and γ-globulin values are not markedly different in the diabetics and non-diabetics. However, there does appear to be a trend toward higher levels of γ-globulin in the serum of diabetics with *retinitis proliferans* without proteinuria compared to the diabetics with both conditions.

Discussion. These findings of a disturbed pattern of serum proteins in diabetic patients with *retinitis proliferans* are comparable to other reports of protein disturbances in this disease within the limitations of differences in the quantitative methods. Thus, the discrepancy in the high β-globulin values reported in diabetic retinitis by Schneider *et al.* (4) and the almost normal values given here might be accounted for in this manner. The measurement in the Tiselius electrophoresis depends on the refractive index which changes with the quality and quantity of the lipoproteins while the paper electrophoresis measurements depend only on the amount of protein present and do not change with lipid content. That an increased concentration of lipoprotein in the β-globulin fraction is present in diabetes has been demonstrated by Russ *et al.* (14).

The decrease in the concentration of the combined albumin + α_1-globulin fraction and the increase in the α_2-globulin fraction is parallel to that reported by Rifkin and Petermann (5) in a study of 10 diabetics between 60-70 years showing "diabetic glomerulosclerosis," *i.e.*, all having marked proteinuria, hypertension, and edema.

In the data presented here, only 2 patients had renal disease advanced enough to show an elevated non-protein nitrogen. These and 3 others with proteinuria also had hypertension, and 3 had proteinuria as the only evidence of renal disease. However, a close rela-

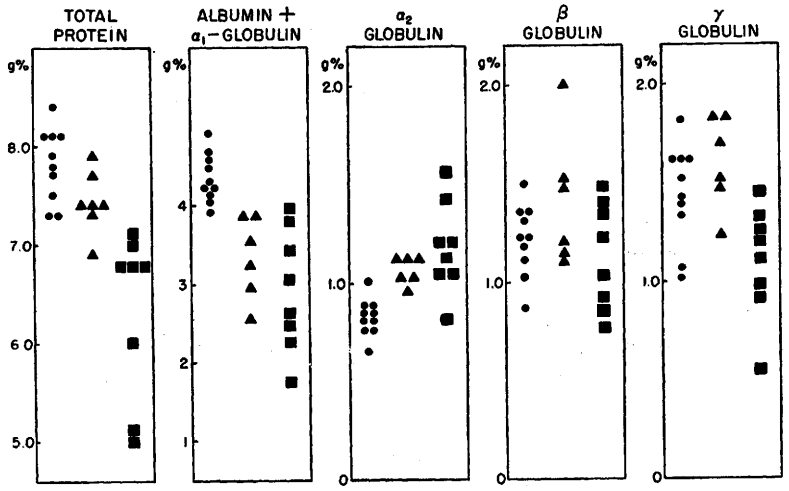

FIG. 3. Levels of total protein and of protein fractions in sera. ● Non-diabetics. ▲ Diabetics with *retinitis proliferans* without kidney disease. ■ Diabetics with *retinitis proliferans* and kidney disease.

tionship exists between *retinitis proliferans* and the Kimmelstiel-Wilson lesions as has been discussed by Root(2). Thus, it might be concluded from the data reported herein that the alterations in the serum protein pattern involving the albumin and a_2-globulin fractions are present in diabetic patients having *retinitis proliferans* and at a stage at which the protein changes cannot be accounted for by clinically detectable kidney damage. The protein changes become more marked with the development of clinical signs of kidney involvement.

The increase in concentration of total protein-bound carbohydrates found in this study roughly paralleled the increase in the a_2-globulin fraction. Similar parallelism was found by Seibert *et al.*(15) in their work on patients with tuberculosis. These authors on the basis of data obtained on Cohn fractions estimate the amount of carbohydrate bound to the a_2-globulin fraction to be 5.8%, whereas the amounts bound to albumin, a_1, β, and γ fractions are 0.1, 1.6, 2.7, and 1.6, respectively. Therefore, the variations of total protein-bound carbohydrates in the data presented here are probably principally influenced by the increase in concentration of the a_2-globulin fraction. Considerable influence on the total protein-bound carbohydrate value can be caused by variation in concentration of the β and γ fractions, but normally the content of bound carbohydrate is not so great in these fractions.

Summary. 1. The serum protein-bound carbohydrates and the serum protein fractions were studied in diabetic patients with *retinitis proliferans*. The serum protein-bound carbohydrates were found to be present in a higher concentration than in non-diabetes. By the method of paper electrophoresis, a definite decrease in the values for the albumin + a_1-globulin fraction and an increase in the a_2-globulin fraction was also found in all of the diabetics, although only those with both *retinitis proliferans* and proteinuria showed a decrease in the total protein. In the β-globulins no essential change was seen. In the group showing both retinitis proliferans and nephropathy, the γ-globulin concentration tended to be lower than in the group with *retinitis proliferans* alone, but none of the groups had values distinctly different from those of non-diabetics. 2. It is concluded that disturbances in the protein pattern exist which particularly involve the albumin and a_2-globulin fractions at a stage in the development of the diabetic vascular lesions where the changes cannot be accounted for by loss of protein in the urine.

The author is indebted to Miss Anna Gotsis for technical assistance and to Dr. Elizabeth F. Tuller for her help and cooperation in all aspects of the work and in the preparation of the manuscript.

1. McManus, J. F. A., *Proc. Am. Diabetes Assn.*, 1949, v9, 303.

2. Root, H. F., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 458.

3. Friedenwald, J. S., *J. Am. Med. Assn.*, 1952, v150, 969.

4. Schneider, R., Lewis, L. A., and McCullagh, E. P., *Am. J. Med. Sci.*, 1946, v212, 462.

5. Rifkin, H., and Petermann, M. L., *Diabetes*, 1952, v1, 28.

6. Keiding, N. R., Mann, G. V., Root, H. F., Lawry, E. Y., and Marble, A., *ibid.*, 1952, v1, 434.

7. Engelberg, H., Gofman, J., and Jones, H., *ibid.*, 1952, v1, 425.

8. Berkman, J., Rifkin, H., and Ross, G., *J. Clin. Invest.*, 1953, v32, 415.

9. Keiding, N. R., and Tuller, E. F., in prep.

10. Tuller, E. F., and Keiding, N. R., *Anal. Chem.*, 1954, v26, 875.

11. Shetlar, M. R., Foster, J. W., Kelly, K. H., and Everett, M. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 509.

12. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 89.

13. Mehl, J. W., *J. Biol. Chem.*, 1945, v157, 173.

14. Russ, E. M., Edler, A., and Barr, D. P., *Am. J. Med.*, 1951, v11, 468.

15. Seibert, F. B., Pfaff, M. L., and Seibert, M. V., *Arch. Biochem.*, 1948, v18, 278.

Received May 20, 1954. P.S.E.B.M., 1954, v86.