were used to detect and identify individual amino-acids in all urines.

Experimental. The results summarized here were obtained on 3 rats, each studied in 3 metabolic periods of 3 or more days: I, in normal state during preliminary control periods; II, in the diabetic state following the injection of alloxan, treated with adequate doses of insulin, and III, after withholding insulin. Severely diabetic states were induced in every animal, as indicated by the rapidity of the development of acetonuria, acidosis and death within 3 to 5 days when insulin was withheld. The preliminary period I included three consecutive 24-hour collections of urine. After the administration of alloxan the ensuing diabetic state was controlled by daily injections of 1 to 3 units of protamine zinc insulin, the doses being adjusted to maintain the body weight constant and to keep the urine acetone-free. After the establishment of satisfactory control, 24-hour collections of urine were resumed and continued through periods II and III, until the animal died.

Data from 3 rats are presented in Table I. The individual amino-acids identified by the chromatographic method in the urines of the 3 animals in respective periods are listed collectively in Table II.

Discussion. During periods of severe and fatal acidosis in the alloxan-diabetic rat, deprived of insulin, the pattern of excretion of amino-acids in the urine remained essentially unchanged, compared with periods of control. The ratio of amino-acid nitrogen to total nitrogen excreted in the urines remained relatively constant, independent of the development of ketosis, acidosis, and increased excretion of total nitrogen. These facts suggest the conclusion that although protein catabolism is increased in alloxan-induced diabetes during periods of insulin insufficiency, normal metabolic pathways are utilized, and amino-acids are efficiently conserved by the kidney. This is in accord with the results of studies on human diabetics as reported by Hall.[10]

Summary. Nitrogen balance studies on 3 male rats during periods of normal control, of alloxan-induced diabetes with insulin therapy, and of fatal acidosis after withholding insulin, are reported.

The pattern of excretion of urinary amino-acids, and the ratio of total amino-acid nitrogen to total urinary nitrogen remained essentially unchanged throughout the 3 periods, including the terminal stage with greatly increased losses of total nitrogen.

[10] Hall, D. A., *Biochem. J. Proceedings*, 1948, **43**, lvii.

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17208. Lack of Identity of Hyaluronidase Inhibitor and Certain Mucoproteins in Blood Serum.*

DAVID GLICK, ROBERT A. GOOD, VINCENT C. KELLEY, RICHARD J. WINZLER, AND JOHN W. MEHL.

From the Departments of Physiological Chemistry and Pediatrics, University of Minnesota Medical School, Minneapolis, and Department of Biochemistry and Nutrition, University of Southern California, School of Medicine, Los Angeles.

Winzler and co-workers[1,2] observed that elevations of plasma mucoprotein levels occur in patients with cancer and pneumonia. Prinzmetal *et al.*[3] have noted a similar increase in patients with myocardial infarctions.

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[1] Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, 1948, **27**, 609.

[2] Winzler, R. J., and Smyth, I. M., *J. Clin. Invest.*, 1948, **27**, 617.

[3] Simpkin, B., Bergman, H. C., and Prinzmetal, M., *Am. J. Med.*, 1949, in press.

Seibert *et al.*[4,5] have found that the carbohydrate content of human serum proteins increases in cancer and tuberculosis, and Niazi and State[6] have confirmed this elevation in cancer and infectious diseases. These findings are in accord with similar results reported in the earlier literature by other investigators. Meanwhile, it was shown by Glick and coworkers that a similar elevation in the level of hyaluronidase inhibitor (not the inhibitor that is an antibody to hyaluronidase) occurs in cancer,[7] and in a wide variety of both virus and bacterial infections.[8-11] Increases of hyaluronidase inhibitor in infectious diseases were also reported by Thompson,[12] Friou and Wenner,[13] and others.

It could reasonably be supposed that the hyaluronidase inhibitor might be associated with mucoprotein or carbohydrate compounds that could compete with hyaluronic acid for the enzyme. This possibility appeared to be strengthened by the electrophoretic studies of Winzler *et al.*,[1] which indicated that the isolated mucoprotein migrated nearer to the albumin fraction than to the other plasma proteins at pH 8.3, and of Glick and Moore,[14] which showed that the hyaluronidase inhibitor in the serum migrated chiefly with the albumin at pH 8.6.

These considerations, along with the observed parallelism in the elevation of both the inhibitor and the mucoprotein in the same diseases, prompted the present investigation of the possibility of their association or identity.

Mucoprotein samples of human serum prepared from perchloric acid filtrates by the procedure of Winzler *et al.*[1] showed little inhibitory activity per gram of protein compared to serum. Plasma mucoprotein preparations isolated by an ammonium sulfate procedure[15] which were electrophoretically homogeneous also had very little hyaluronidase inhibitor activity. The very low isoelectric points of plasma mucoproteins permit their electrophoretic separation from other plasma proteins at pH 4 or 4.5.[16,17] We have isolated plasma mucoproteins electrophoretically and tested them for hyaluronidase inhibitor. One such sample which was isolated, dialyzed, and lyophilized had somewhat more inhibitory effect but was still much weaker than fresh serum on an equal total protein basis. Adding magnesium ions to these preparations had no influence.

In view of the instability of the hyaluronidase inhibitor, it was not felt that its lack of identity with plasma mucoproteins was established by the foregoing results. The experiment shown in Table I, however, shows more conclusively that hyaluronidase inhibitor is not associated with the acidic plasma mucoproteins.

A pooled sample of serum freshly obtained from several patients with lobar pneumonia was adjusted to pH 4.5 with approximately 2 N acetic acid. A sample was removed for the hyaluronidase inhibitor determination, and the remainder of the serum was centrifuged to remove the small amount of precipitate which had formed. This clarified serum was then immediately subjected to electrophoresis, without any preliminary dialysis, against an acetate buffer in sodium chloride. The total acetate concentration was 0.02 M,

4 Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W., *J. Clin. Invest.*, 1947, **26**, 90.

5 Seibert, F. B., Pfaff, M. L., and Seibert, M. V., *Arch. Biochem.*, 1948, **18**, 279.

6 Niazi, S. A., and State, D., *Cancer Res.*, in press.

7 Hakanson, E. Y., and Glick, D., *J. Nat. Cancer Inst.*, 1948, **9**, 129.

8 Glick, D., and Gollan, F., *J. Inf. Dis.*, 1948, **83**, 200.

9 Grais, M. L., and Glick, D., *J. Invest. Dermatol.*, 1948, **11**, 259.

10 Glick, D., and Campbell, B., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 29.

11 Grais, M. L., and Glick, D., *J. Inf. Dis.*, in press.

12 Thompson, R. T., *J. Lab. Clin. Med.*, 1948, **33**, 919.

13 Friou, G. J., and Wenner, H. A., *J. Inf. Dis.*, 1947, **80**, 185.

14 Glick, D., and Moore, D. H., *Arch. Biochem.*, 1948, **19**, 173.

15 Weimer, H., Mehl, J. W., and Winzler, R. J., submitted for publication.

16 Petermann, M. P., and Hogness, K. R., *Cancer*, 1948, **1**, 104.

17 Mehl, J. W., Golden, F., and Winzler, R. J., submitted for publication.

TABLE I.
Hyaluronidase Inhibitor in Mucoprotein Concentrates.

Sample	mg % mucoprotein-tyrosine	% hyaluronidase inhibition per 0.02 ml
Normal serum	2.2	21.2
Pooled pneumonia serum frozen immediately	14.0	59
Same adjusted to pH 4.5 and frozen	12.0	11
Same adjusted to pH 4.5 centrifuged and supernatant frozen	11.8	8
Same adjusted to p.H 4.5 centrifuged and supernatant held in electrophoresis bath during experiment	13.3	5
Compartment I (acidic mucoproteins)	11.4	8
Compartment II (normal globulin and mucoprotein with reduced globulin)	10.5	13
Compartment III (globulins, no mucoprotein)	0.8	8
Compartment IV. Normal albumins and globulins (reduced mucoprotein)	6.7	6

the ionic strength 0.1, and the pH was 4.5 at 22°C. A 4-compartment cell was employed, and compensation was used as needed to keep the upper edge of the albumin boundary at the middle junction of the cell on the side towards the anode. The electrophoresis experiment was terminated when the fastest mucoprotein component had reached a point about 2/3 of the distance up the upper half of the anodic limb (compartment I). At the same time, the most rapidly moving globulin had reached the top of the upper half of the cathodic limb (compartment III).

At least 3 positively-charged components migrated toward the negative electrode—the fastest with a mobility corresponding to the major mucoprotein component. Compartment I contained only these acidic components and when removed, the concentration of these components should have been 1/2 to 2/3 that in the serum. Compartment II was the lower half of this arm and contained mucoprotein and albumin in the original concentration with somewhat reduced amounts of globulin. Compartment III was the upper section of the arm ascending to the negative electrode and contained the globulins and very little albumin or mucoprotein. Compartment IV was the lower section of this arm and contained globulins and albumin at original concentration with a reduced mucoprotein concentration. These samples were frozen immediately after the electrophoretic separation and were kept frozen until analysis of hyaluronidase inhibitor in order to prevent deterioration. Appropriate controls were also carried as is indicated in Table I. The mucoprotein levels were carried out using the tyrosine and carbohydrate determinations previously described[1] and the hyaluronidase inhibitor was determined as described earlier.[8,18] The data of Table I show that there is no correlation between the hyaluronidase inhibitor activity and the mucoprotein content of the various samples—the sample from compartment III, containing little or no acidic mucoprotein, having as much inhibitor as other compartments. The partial inactivation of the inhibitor resulting from adjustment to pH 4.5 does not seriously affect our conclusion that the hyaluronidase inhibitor and acidic plasma mucoproteins are not associated.

During the course of a study on serum from

[18] Wattenberg, L., and Glick, D., *J. Biol. Chem.*, in press.

TABLE II.
Mucoprotein and Hyaluronidase Inhibitor Levels in "Lipoid Nephrosis."

Group of children	No.	Age range	Mean mucoprotein-tyrosine ± std. error of mean (mg %)	Mean % hyaluronidase inhibition ± std. error of mean per 0.02 ml fresh serum
Normal	40	1-15		21.2 ± 0.9
"	55	1-15	2.2 ± 0.1	
Nephrosis	9		1.5 ± 0.1	56.6 ± 3.7

TABLE III.
Hyaluronidase Inhibitor and Mucoprotein Levels in Miscellaneous Conditions.

Disease of child	Age	Mucoprotein-tyrosine (mg %)	% hyaluronidase inhibition per 0.02 ml fresh serum
Acute poliomyelitis	12	4.2	44
Acute lymphatic leukemia	8	9.4	29
Metastatic neuroblastema	1.3	9.8	56
Inactive rheumatic fever	16	5.1	11
Upper respiratory infection	11	2.6	31
Normal	5	3.8	23
"	12	2.6	33

children with nephrosis, which will be reported in detail elsewhere by Good, Kelley, and Glick, a further lack of correlation between the mucoprotein and inhibitor content of untreated sera was found (Table II). In this series the mucoprotein values measured by the method of Winzler *et al.*[1] were 32% less than the normal, while the inhibitor values were 167% greater than the normal. In other diseases, too, widely divergent serum values were found, as illustrated by a few examples given in Table III.

It is therefore clear that there is no identity of the hyaluronidase inhibitor and the acidic mucoproteins of blood serum as separated by electrophoresis at pH 4.5, by precipitation from perchloric acid filtrates, or by ammonium sulfate fractionation. This does not negate the possibility that the inhibitor may be found in other mucoprotein or glycoprotein components, nor is it at variance with the striking statistical correlation that has been observed between the elevation of serum mucoprotein and hyaluronidase inhibitor in cancer and infectious diseases.

Summary and conclusions. 1. Electrophoretically and chemically separated fractions of human serum containing high concentrations of mucoproteins showed no increase in non-specific hyaluronidase inhibitor when compared to native serum or other mucoprotein-poor fractions of serum.

2. In children suffering from "lipoid nephrosis" the hyaluronidase inhibitor levels of the serum were higher while the mucoprotein levels were significantly lower than normal.

3. In spite of the striking statistical correlation existing between the serum levels of mucoprotein and non-specific hyaluronidase inhibitor in a wide variety of human diseases, marked disparity in the serum levels of these substances occasionally occurs.

4. On the basis of these findings the lack of identity of the non-specific hyaluronidase inhibitor and these serum mucoproteins is pointed out.

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