in the cystine-rich protein keratin when the latter was isolated as the thiosulfate, S-sulfokerateine, and in the cystine fraction isolated from hair. The source of selenium which is incorporated into hair protein is discussed.

The technical assistance of Benjamin R. Larke is gratefully acknowledged.

1. Anonymous, Vitamin E and Selenium, *Nut. Rev.*, 1958, v16, 149.

2. McConnell, K. P., Wabnitz, C. H., *J. Biol. Chem.*, 1957, v226, 765.

3. McConnell, K. P., Kreamer, A. E., Roth, D. M., *ibid.*, 1959, v234, 637.

4. Swan, J. M., *Nature*, 1947, v180, 643.

5. Gortner, R. A., Hoffman, W. F., *Organic Syntheses, Collective Vol. I*, John Wiley and Sons, Inc., New York, 1956, p. 194.

6. Rockland, L. B., Underwood, J. C., *Anal. Chem.*, 1954, v26, 1557.

7. Underwood, J. C., Rockland, L. B., *ibid.*, 1954, v26, 1553.

8. Rothman, S., *Physiology, and Biochemistry of the Skin*, Univ. Chicago Press, Chicago, 1954.

9. McConnell, K. P., Van Loon, E. J., *J. Biol. Chem.*, 1955, v212, 747.

10. McConnell, K. P., Wabnitz, C. H., Roth, D. M., *Fed. Proc.*, 1956, v15, 311.

11. Fleischer, S., Leitze, A., Walter, H., Haurowitz, F., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 860.

12. Moxon, A. L., Rhian, M., *Physiol. Revs.*, 1943, v23, 305.

13. Painter, E. P., *Chem. Revs.*, 1941, v28, 179.

14. Lemley, R. E., *Lancet*, 1940, v60, 528.

15. Lemley, R. E., Merryman, M. P., *ibid.*, 1941, v60, 435.

Received July 29, 1960. P.S.E.B.M., 1960, v105.

Effect of Bladder Distension on the Venous System of Man.* (26049)

GEORGE E. BURCH AND N. DEPASQUALE

Department of Medicine, Tulane University School of Medicine and Charity Hospital of Louisiana, New Orleans

Forty years ago Wernöe(1) observed that with certain painful visceral diseases "anemic zones" developed in the skin overlying the affected organ. He attributed the pallor to "ischemia" secondary to vasoconstriction. Stürup(2) described areas of hyperalgesic pallor in the skin of patients with acute appendicitis. Using a photometer Adams-Ray and Nórlen and Adams-Ray(3,4) observed that upon distension of the bladder, pallor developed in the cutaneous ventral segments of T_{11} and T_{12}. They believed the pallor to be due to a spinal reflex with the afferent fibers passing through sacral nerves S_2, S_3 and S_4 and efferent fibers traveling with the sympathetics producing vasoconstriction in the subcapillary venous plexus.

The present study was undertaken to learn the extent of this reflex by studying the effect of bladder distension on an intact isolated venous segment of the forearm of normal and diseased subjects.

Materials and methods. Twelve subjects ranging in age from 27 to 58 years (mean, 36 years) were studied; 5 were male and 7 were female. The cardiovascular system was normal in all but 2 subjects. One subject had luetic heart disease and chronic congestive heart failure and another had transection of the spinal cord at T_6 secondary to metastases from pulmonary carcinoma.

Venous pressure was measured in an isolated intact venous segment and in an adjacent vein open to the systemic venous system of the forearm as previously described(5,6). The pressures were recorded simultaneously and continuously by means of Statham strain gauge pressure transducers and a suitable multichannel direct writing recorder.

A straight French catheter was inserted into the bladder of the subject and was connected by means of rubber tubing, which led out of the subject's room, to a calibrated glass

* This investigation supported by grants from U. S. Public Health Service and Thibodeaux Research Foundation.

bottle containing 500 cc of isotonic saline solution warmed to body temperature. Vesical pressure was also recorded continuously. After insertion of the catheter into the urinary bladder all tubing was shielded from the subject's view so that he was unaware of the flow of fluid into his bladder.

The bladder was emptied and base line levels of the 3 pressures were recorded. Fluid was allowed to flow slowly into the bladder in 50 cc increments. After each 50 cc increment sufficient time was allowed for stabilization of any change in venous pressure. During these experiments care was taken not to disturb the subject. Conversation and activity in the observation room were restricted to a minimum.

Results. 1) *Normal subjects.* In all of the normal subjects the initial instillation of 50 cc of isotonic saline into the empty bladder produced an increase in segmental venous pressure. The magnitude of this rise varied considerably, the range being 20 to 445 mm H_2O with a mean of 120 mm H_2O. The rise in segmental venous pressure was transient, the pressure returning to the base line within 1 to 3 minutes after reaching the peak (Fig. 1). With each additional instillation of 50 cc of fluid, a similar increase in segmental venous pressure occurred; the increase in pressure was usually of essentially the same magnitude as the initial rise (Fig. 1). When the contents of the bladder reached between 300 and 350 cc, most of the patients experienced discomfort and the segmental venous pressure remained elevated until the bladder was emptied. In 4 subjects there was a slight increase in pressure in the systemic veins but the increase never exceeded 25 mm H_2O.

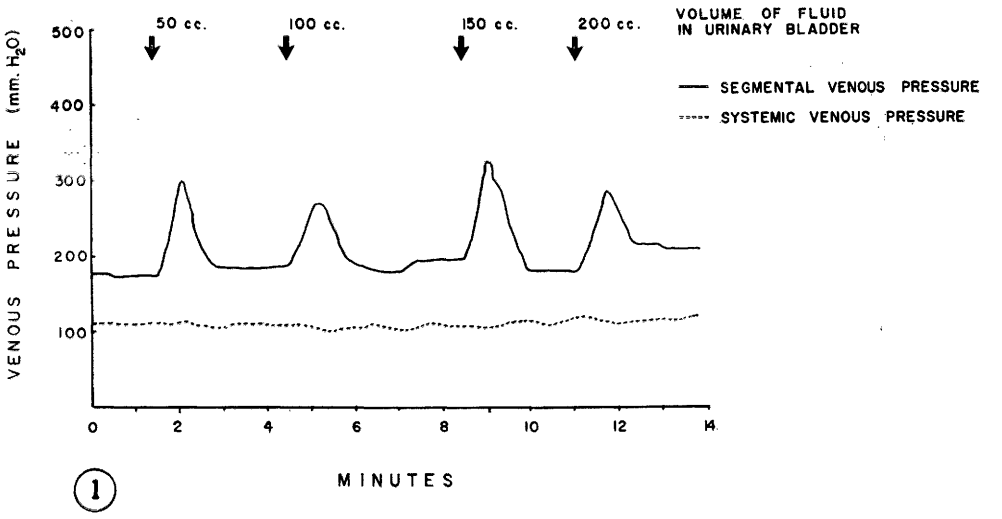
In 3 subjects in whom the nerve supply to the venous segment was interrupted by regional nerve blocking with procaine, there was no response of the isolated venous segment to instillation of the fluid into the urinary bladder (Fig. 2), whereas there was a slight increase in systemic venous pressure in 2 of the subjects.

2) *Patient with congestive heart failure.* The segmental and systemic venous pressures in this patient were elevated initially, basal levels of pressure being 776 mm H_2O and 380 mm H_2O respectively. When 50 cc of fluid was instilled into the bladder, there was a marked rise in segmental venous pressure (Fig. 3). When the pressure reached 1,000 mm H_2O the bladder was drained and the venous tone decreased. Instillation of 50 cc of fluid into the bladder a second time was again followed by a marked increase in segmental venous pressure. Systemic venous pressure increased sharply on both occasions. Coincident with the second increase in venous pressure, severe dyspnea and bubbling rales developed in both lungs so that it was necessary to treat the patient for acute pulmonary edema. Fig. 3 demonstrates the response of the segmental and peripheral veins to bladder distension as well as to various procedures employed in therapy.

3) *Patient with complete transection of spinal cord at the level of* T_6. There was no change in pressure in the isolated venous segment in this patient even when the urinary bladder was filled to capacity (Fig. 4).

Discussion. These observations indicate that slight filling of the urinary bladder can increase venous tone in the forearm veins of man. Since approximately 80% of total blood volume is contained in the venous system, changes in volume of the superficial veins should have a profound effect on the circulation.

The vesico-cutaneous reflex described is probably a spinal reflex mediated through receptors in the wall of the urinary bladder and carried by afferent nervous fibers through sacral nerves S_2, S_3 and S_4. Whether or not there are special higher venomotor centers in the spinal cord and cerebral cortex that control venous tone is not known. The afferent impulse may either pass through such centers or travel more directly to the veins by way of efferent sympathetic fibers from the spinal cord, or both. The reflex nature of the increase in peripheral venomotor tone is evident by the fact that the venomotor response to bladder distension was eliminated by regional nerve block. In addition, the failure of bladder distension to alter segmental venous pressure in the patient with transection of the spinal cord further supports a spinal reflex mechanism.



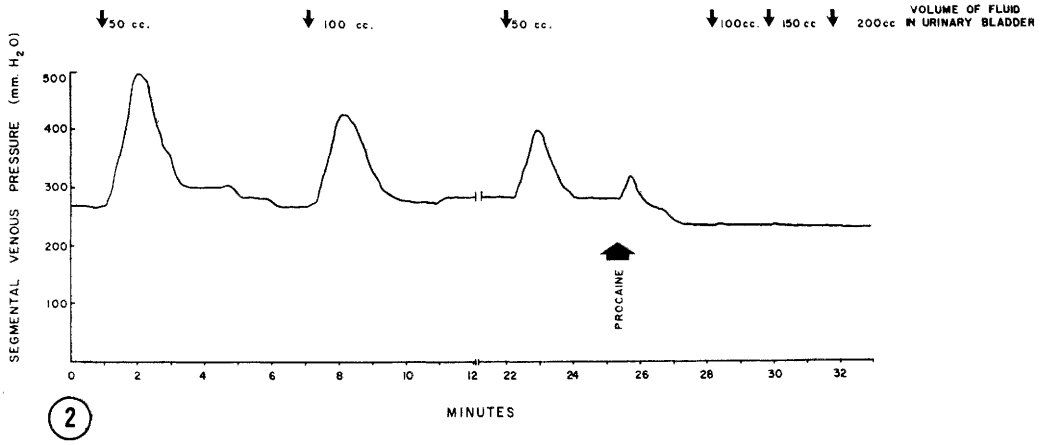


FIG. 1. Effect of instillation of fluid in 50 cc increments into the urinary bladder on segmental and systemic venous pressure.

FIG. 2. Effect of regional procaine block of nervous supply on response of the isolated venous segment to bladder distension. The sharp rise in pressure at time of procaine block was in response to pain and psychic stimulation associated with the performance of the block.

The increase in segmental venous pressure was not due to pain because the venomotor response occurred before the subjects developed discomfort or pain. However, it was intensified by pain, thus indicating again an increase in segmental venomotor tone in response to another type of pain(5,6), namely visceral pain.

It is characteristic of vesical pressure to remain low until the bladder contains a critical volume of fluid, usually about 300 cc in our subjects. However, an increase in segmental venous pressure developed well before such a critical volume of fluid was instilled into the bladder. Even though there was little or no increase in vesical pressure, an increase in segmental venous pressure occurred. Coincident with each instillation of fluid into the urinary bladder there was a slight rise in vesical pressure; adaptation to the new volume of fluid developed quickly, however, and vesical pressure returned to the original level within seconds. It is probable that stimulation of the stretch receptors in the wall of the urinary bladder initiated the venomotor reflex described.

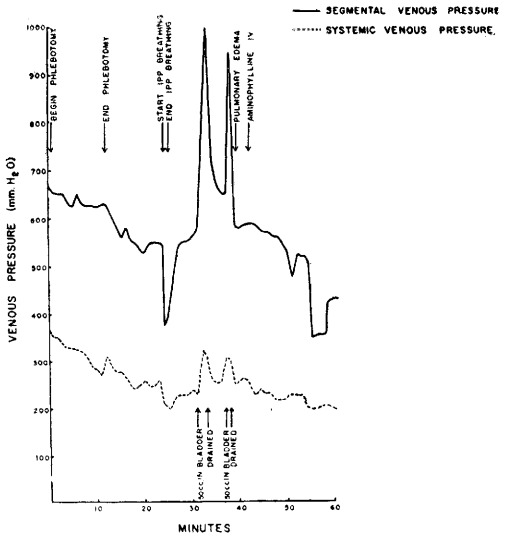


FIG. 3. Effect of bladder distension on venomotor tone in a patient with chronic congestive heart failure. (See text for details.)

Although there was a significant rise in segmental venous pressure with bladder filling in the normal subjects, there was only occasionally a slight rise in systemic venous pressure. The failure to obtain a rise in systemic venous pressure was probably due to the fact that the venous system was "loose" and could accommodate the blood squeezed centrally by constriction of the smaller veins, and/or other portions of the systemic venous system underwent a decrease in tone to compensate for an increase in tone in the superficial veins. However, in the patient with congestive heart failure who already had high venous and sympathetic nervous system tone, there was a considerable increase in peripheral systemic venous tone and pressure. The shifting of blood from the small veins into the pulmonary vessels and veins probably precipitated the pulmonary edema that developed. In this patient the central venous system was already "tight" so that it could not properly accommodate the blood "squeezed" centrally by peripheral venoconstriction. The vesico-venomotor reflex may have also involved the pulmonary veins, arteries and capillaries(7).

After each experiment close interrogation of the subjects indicated that 3 of them felt "something move" in the perineum as the fluid was instilled but felt nothing in the bladder. The remaining subjects felt nothing until the bladder was filled to the point of discomfort and pain.

Summary. Venomotor responses of an intact isolated superficial venous segment of the forearm of man to bladder distension showed that instillation of 50 cc of fluid into the empty bladder resulted in an increase in venous pressure and venomotor tone. The increase in segmental venous pressure could be inhibited by regional procaine block. The response was absent in a patient with complete transection of the spinal cord. Thus, distension of the urinary bladder initiates a spinal venomotor reflex which produces an increase in peripheral venous tone in the super-

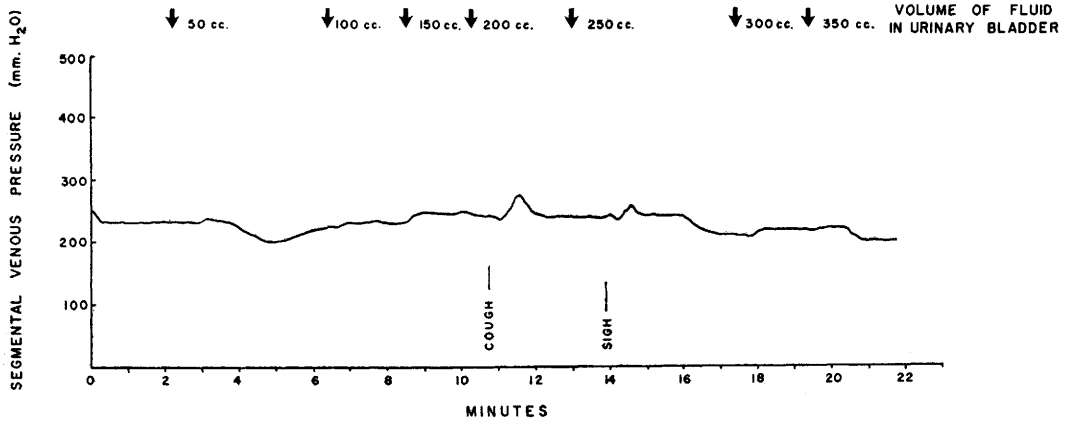


FIG. 4. Failure of bladder distension to influence venomotor tone in a patient with complete transection of spinal cord at level of T_6.

ficial veins of the forearm of man.

1. Wernoe, T. B., *Ugeskr. laegr,* 1920, v82, 1415.

2. Stürup, G., Visceral pain *Nyt Nordisk Forlag.,* Copenhagen, 1940.

3. Adams-Ray, J., Nórlen, G., *Acta physiol. scand.,* 1951, v23, 95.

4. Adams-Ray, J. *Acta chir. scandinav.,* 1952, v103, 100.

5. Burch, G. E., Murtadha, M., *Am. Heart J.,* 1956, v51, 807.

6. Malaret, G., Quiroz, A., Burch, G. E., *Human Biol.,* 1960, v32, 135.

7. Agrest, A., Roncoroni, A. J., *Circulation Research,* 1960, v8, 501.

Received August 30, 1960. P.S.E.B.M., 1960, v105.

Changes in Serum Complement Activity in Patients with Myasthenia Gravis.* (26050)

W. L. NASTUK, O. J. PLESCIA AND K. E. OSSERMAN (Introduced by Walter S. Root)

Dept. of Physiology, College of Physicians and Surgeons, Columbia University, New York City, Institute of Microbiology, Rutgers University, New Brunswick, N. J., and Myasthenia Gravis Clinic and Dept. of Medicine, Mt. Sinai Hospital, New York City

It has frequently been suggested that the deficit in neuromuscular transmission characteristic of patients with myasthenia gravis might be produced by a curare-like agent which can circulate in the blood. In a search for such an agent, previously described(1), serum samples obtained from a series of individuals afflicted with myasthenia gravis were tested for neuromuscular blocking action. The assay involved measurement of the changes in muscle tension output of an indirectly stimulated frog sartorius muscle-sciatic nerve preparation which was immersed in the diluted serum.

Serum samples obtained from a few myasthenic patients caused a reduction in muscle tension output, and this result appeared to parallel the cytolytic destruction of fibers lying on the surface of the sartorius muscle used in the assay(1). From these and more recent observations(2), it is clear that serum exhibiting the above type of cytolytic activity occurs in 44% of patients afflicted with myasthenia gravis and in 22% of normal controls. The relative strengths of the active serums found in each group are unknown.

The results described above led us to think about the nature of the cytolytic system involved and the possible connection between the activity of this system and etiologic factors which operate in myasthenia gravis. As to the nature of the cytolytic system, we speculated that serum complement (C′) might be concerned because, as is well known, C′ plays an essential part in immune hemolysis. For this and other reasons, it seemed worthwhile to carry out determinations of C′ activity on serum obtained from myasthenic patients. Encouragement was provided by the early results which showed that in many myasthenic patients, serum C′ activity was far outside the normal range.

In this paper we have reported results of serum C′ analyses performed on samples collected from a large series of patients with myasthenia gravis. Many of these individuals were studied for long periods, with particular attention to changes in serum C′ activity and clinical condition. A brief survey

* We wish to thank the following physicians whose generous help contributed in securing samples and data for this study. Drs. F. F. Foldes, I. M. Gerson, H. H. Goldberg, J. H. Jacobson, E. H. Lambert, R. R. Rascoe, L. P. Rowland, E. D. Weitzman and A. Wright.

We owe much to Mrs. L. V. Berk, Mr. J. T. Alexander and Mrs. Elaine Shapiro for their faithful, careful efforts in the assay work and the serum collection program.

Financial support was provided by grant from Dept. of H.E.W., P.H.S., Nat. Inst. Health, Bethesda, Md., and under Nat. Science Foundation Grant. Financial support for the earliest phase of the work was provided by Hoffman-La-Roche Inc., Nutley, N. J. to whom we express our gratitude.