known concentration in the series of unknowns analyzed.

Application of the method to a series of blood samples and comparison with Gentzkow's(7) and Karr's(9) methods using urease hydrolysis (Table IV) resulted in satisfactory agreement of values.

*Summary.* The acid hydrolysis method of measuring urea has been revised so that it will furnish accurate and reliable results when applied to either whole or cell-free blood.

The computation of standard errors was carried out by Mr. Myron J. Willis of the Statistics Section, Epidemiologic Services, Communicable Disease Center.

Received October 9, 1951. P.S.E.B.M., 1951, v78.

## Abrupt Improvement of Serum Electrophoretic Pattern in Nephrosis After ACTH-Induced Diuresis. (19074)

SIDNEY E. LENKE AND HENRY M. BERGER. (Introduced by Albert E. Sobel.)
*From the Mattie Billings Lowe Laboratory, Jewish Hospital of Brooklyn, N. Y.*

This paper deals with the partial correction of the abnormal electrophoretic pattern, which is characteristic of the serum in nephrosis(1-4), in patients who responded to ACTH with intense diuresis. In the nephrotic syndrome there occurs a progressive accumulation of fluid in the interstitial spaces and the serous cavities, the edema increasing until, quite unpredictably, it is relieved by massive diuresis. Until recently the onset of diuresis has been fortuitous, and it has therefore been difficult to follow the chemical and physical changes which occur in blood just before and after diuresis. It is now possible to produce diuresis in many nephrotic patients with a variety of drugs such as ACTH, cortisone, urea, cation exchange resins, and occasionally by nitrogen mustard. The effect of the diuresis thus produced upon some chemical constituents of the blood has been described(5-7).

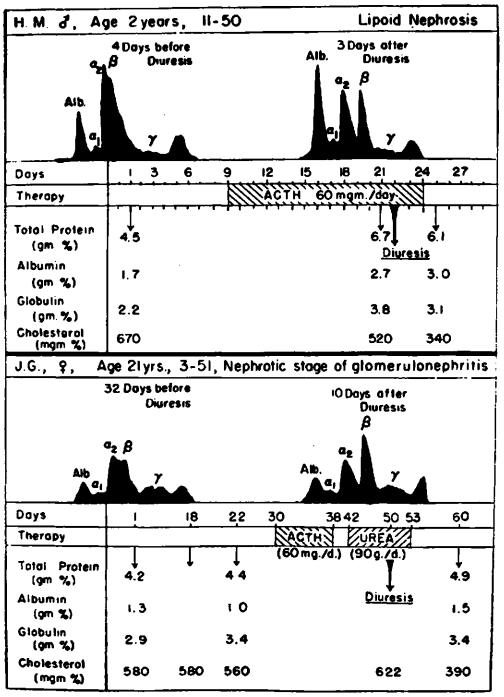


FIG. 1. Total protein, albumin, globulin results listed were determined by chemical analyses.

This paper describes the marked changes observed in the electrophoretic pattern of the serum proteins.

*Methods and materials.* Observations are presented from 4 cases, 3 infants suffering from lipoid nephrosis and one adult female, 21 years of age, suffering from chronic glomerulonephritis. Her nephrotic syndrome

1. Zeldis, L. J., and Alling, E. L., *J. Exp. Med.*, 1945, v81, 515.
2. Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exp. Med.*, 1939, v70, 399.
3. Longsworth, L. G., and MacInnes, D. A., *J. Exp. Med.*, 1940, v77, 71.
4. Routh, J. I., Knapp, E. L., and Kobayshi, E. K., *J. Ped.*, 1948, v33, 688.
5. Block, W. M., Jackson, R. L., Stears, M. P., *Ped.*, 1948, v7, 33.
6. Schwartz, H., Kohn, J. L., *Am. J. Dis. Child.*, 1938, v49, 579.
7. Gotfried, S. P., Steimen, J. T., and Kramer, P., *Am. J. Dis. Child.*, 1946, v74, 283.

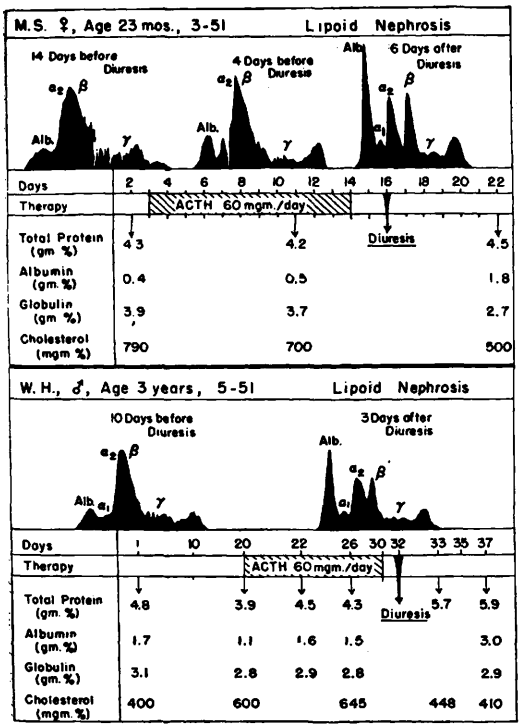


FIG. 2. Total protein, albumin, globulin results listed for W.H. were determined chemically. Those listed for M.S. were determined by electrophoretic calculations.

began late in pregnancy, 6 months before the present observations. ACTH alone was used to produce diuresis in the 3 cases of lipoid nephrosis (W.H., H.M., M.S.). Sixty mg of ACTH was given intramuscularly in divided doses each day for 8 to 15 days (as indicated in Fig. 1 and 2).

In the adult case (J.G.) when ACTH failed to produce diuresis in 8 days, 4 additional days were allowed to elapse and then urea was administered orally (90 g daily). Diuresis occurred during the administration of urea.

The following analyses were done before therapy and after diuresis: 1. Electrophoretic analysis of the serum proteins; 2. Total proteins (by the micro-Kjeldahl method); 3. A/G ration (electrophoretically calculated); 4. Total serum cholesterols. The technic used in the electrophoretic analysis has been described(8,9). All analyses were made using a barbiturate buffer of pH 8.6, ionic strength 0.1. The apparatus used was the Tiselius electrophoresis apparatus.

*Results.* It is evident from Fig. 1 and 2 that within a few days after diuresis a remarkable change occurred in the serum proteins of these 4 cases. This change as determined electrophoretically was toward the normal.

The most notable change occurred in the region of the $\alpha_2$ and $\beta$ globulins. This is the region which characteristically fails to show resolution in the nephrotic serum before diuresis, appearing as an amorphous, roughly outlined area. After diuresis this area showed excellent resolution of these $\alpha_2$ and $\beta$ globulins. The total of $\alpha_2$ and $\beta$ globulins decreased after diuresis, but still remained above normal limits (Table I).

Concurrently there was a rise in the serum albumin, varying from 16.7% to 350%. The concentration of the protein components is presented in Table I. Calculations of the $\alpha_2$ and $\beta$ globulins has not been attempted in the patterns before diuresis because of the characteristic lack of resolution of these peaks. The $\alpha_1$ and $\gamma$ globulins seem to show no significant change after diuresis.

The total protein showed a moderate increase after diuresis, an increase reflecting the additional albumin, as well as in one case a rise in the total globulin. In 3 cases the total globulin decreased after diuresis.

The serum cholesterol levels showed a marked decrease in all 4 cases following diuresis (Fig. 1 and 2), and the gross lipemia which is typical of nephrotic serum became visibly clearer after diuresis. However, even after diuresis some lipemia is evident and the serum cholesterol values, even though markedly decreased, remain considerably above normal limits.

*Discussion.* The marked change in the electrophoretic serum protein pattern which occurred in these nephrotic patients after diuresis, the rise in the albumin, the decrease in the $\alpha_2$ and $\beta$ globulins, with clearer resolution of these globulins, is surprisingly abrupt. In this laboratory's experience with over 700

8. Weiner, A. S., Berger, H. M., and Lenke, S. E., *Lab. Digest,* 1951, v14, 11.

9. Lenke, S. E., and Berger, H. M., *Lab. Digest,* 1951, v15, 4.

TABLE I. Concentration of Protein Components in Serum Before and After Diuresis as Determined Electrophoretically.

| Case | Date | T.P. | Alb. | Glob. | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ |
|---|---|---|---|---|---|---|---|---|
| S. | 3/21 | 4.3 | .4 | 3.9 | | | | |
| S. | 3/30 | 4.2 | .5 | 3.7 | | | | |
| Diuresis | | | | | | | | |
| S. | 4/11 | 4.5 | 1.8 | 2.7 | .3 | 1.1 | 1.1 | .2 |
| H. | 4/28 | 4.5 | .5 | 4 | .2 | ——3.5—— | | .3 |
| Diuresis | | | | | | | | |
| H. | 5/10 | 5.9 | 2.1 | 3.8 | .3 | 1.6 | 1.3 | .6 |
| G. | 4/12 | 4.1 | .6 | 3.5 | .3 | ——2.4—— | | .9 |
| Diuresis | | | | | | | | |
| G. | 5/18 | 4.4 | .7 | 3.7 | .3 | 1.2 | 1.6 | .6 |
| M. | 11/3 | 5.5 | 1.1 | 4.4 | .2 | ————4.2———— | | |
| Diuresis | | | | | | | | |
| M. | 11/28 | 5.5 | 1.8 | 3.7 | .3 | 1.7 | 1.3 | .5 |

electrophoretic patterns in a variety of clinical investigations, we have been impressed with the essential stability of an individual's serum protein pattern. The hypoalbuminemia of nephrosis has been singularly refractory to improvement, even after the daily injection of large amounts of albumin. The improvement after ACTH-induced diuresis is therefore notable, since no such change occurs even with diuresis induced by giving 50 g of salt-free human albumin intravenously for 15-30 days* (4,10). Work is in progress to ascertain whether the increased serum albumin is due partly or wholly to a decreased proteinuria.

One possible source of the sudden increase in the serum albumin is the interstitial fluid. Though relatively protein-poor, a diuresis of 5 liters should yield at least 25 g of albumin to the body. Another possibility, that some of the increased albumin which was noted after diuresis was actually present in the serum before diuresis, but migrated in the region of the $\alpha_2$ or $\beta$ globulins due to an abnormal electrostatic surface charge, is now under investigation in this laboratory.

The second impressive change after diuresis is the decrease of the $\alpha_2$ and $\beta$ globulins and the marked improvement in the resolution of these peaks. This may be considered as additional evidence of the importance of lipids and protein-lipid complexes in the nephrotic syndrome. These globulins have an affinity for lipids and seem to serve as a transport system for lipids and fat soluble pigments (11) much as hemoglobin serves to transport oxygen.

The improvement in the $\alpha_2$ and $\beta$ globulin region of the electrophoretic pattern occurs as the serum cholesterol and gross lipemia improve. It may be assumed that the physiological mechanism which produces the salutory diuresis, produces as well, an improvement in the basic lipid disturbance of nephrosis. That this occurs without ACTH or cortisone has not been observed by us, nor reported by those who used nitrogen mustard or urea alone.

A notable difference is evident between the response of infants suffering from lipoid nephrosis and the adult case of glomerulonephritis. Although the resolution of the globulin peaks improved in both, the adult case showed only a slight rise in the serum albumin. Experiments are now being pursued to ascertain whether this distinction is repeatable and whether it is due to the nature of the two diseases or to a specific effect of ACTH on the serum albumin. (The diuresis in the adult case occurred some time after ACTH, during the administration of urea).

*Conclusion.* 1. A striking change is noted in

* Albumin excretion was followed in a later group of patients and will be presented in another paper.

10. Thorn, G. W., Armstrong, S. H., Jr., Davenport, V. D., and Tyler, F. H., *J. Clin. Invest.*, 1945, v24, 802.

11. Blix, G., *J. Biol. Chem.*, 1942, v137, 485.

the electrophoretic pattern of the serum in nephrotic patients soon after diuresis induced by ACTH, or ACTH plus urea. The pattern which is characteristic of nephrotic serum (low albumin and large, unresolved $\alpha_2$ and $\beta$ globulin peaks) reverts toward the normal after diuresis. The serum albumin rises sharply and the $\alpha_2$ and $\beta$ globulin region shows clear resolution. 2. Possible explanations of these abrupt changes in the electrophoretic patterns and concentrations of serum proteins are discussed.

We are grateful to Dr. Dan Moore of the College of Physicians and Surgeons, Columbia University, Dr. William Dock, Professor of Medicine, State University Medical Center at New York, Dr. Albert E. Sobel, Head of the Department of Biochemistry and Dr. Benjamin Kramer, Chief of Pediatrics, at the Jewish Hospital of Brooklyn, for their help in preparing this report.

Research funds were donated in part by the Perkin Elmer Corporation.

Received September 4, 1951. P.S.E.B.M., 1951, v78.

## Acetoacetate-Induced Changes in Blood Lactic and Blood Ascorbic Acids; Prevention by Insulin and Amellin. (19075)

MADHAB CHANDRA NATH AND CHITTA HARAN CHAKRABARTI.

*From the University Department of Biochemistry, Nagpur, India.*

Diabetes is frequently associated with acidosis, a symptom characterized by a lowering of the blood alkali reserve. It has recently been shown by the authors(1) that the injection of $\beta$-hydroxybutyrate into normal rats caused a depletion in liver and muscle glycogen and an increase in the tissue lactic acid. Green and Brosteaux(2) reported that pyruvic acid almost completely inhibits the enzymatic oxidation of lactic acid *in vitro,* and Holmes and Holmes(3) have observed a direct relationship between the blood sugar value and the brain lactic acid content in both hypo and hyperglycemic conditions. The increase of lactic acid in diabetes has been observed by Maranon and his associates(4) and Genes(5). Marenzi(6) has observed that the injection of anterior pituitary extract into dogs causes an increase in blood sugar and blood lactate values. Vit. C has been found by Sigal and King(7) and Banerjee and Ghosh(8,9) to play a role in carbohydrate metabolism. Sigal and King(7) have shown that the successive stages of Vit. C depletion are associated with a corresponding rise in the fasting blood sugar level and a distinctly lowered glucose tolerance; the administration of Vit. C restores the carbohydrate metabolism to normal. The injection of acetoacetate in a dose of 18 mg per 100 g body weight has recently been reported by Nath and his associates(10) to cause a marked depression in the blood Vit. C level of rabbits. An attempt was therefore made to study the cumulative effects of acetoacetate on the lactic acid and the Vit. C contents of the blood of rabbits.

*Experimental.* In the first set of experiments (Group A), 6 healthy male rabbits

1. Nath, M. C., and Chakrabarti, C. H., *Ind. J. Physiol. and Allied Sci.,* 1951, v5, 43.

2. Green, D. E., and Brosteaux, J., *Biochem. J.,* 1936, v30, 1489.

3. Holmes, B. E., and Holmes, E. G., *Biochem. J.,* 1927, v21, 412.

4. Maranon, G., Collazo, J. A., and Almela, J., *Vol. Jubilaire honneur Parhon,* 1934, 331; *Ber. ges. Physiol. Exp. Pharmakol,* 1934, v85, 94.

5. Genes, S. G., *Bull. biol. med. Exp. U.R.S.S.,* 1939, v8, 461.

6. Marenzi, A. D., *Compt. rend. Soc. Biol.,* 1934, v117, 464.

7. Sigal, A., and King, C. G., *J. Biol. Chem.,* 1936, v116, 489.

8. Banerjee, S., and Ghosh, N. C., *J. Biol. Chem.,* 1947, v168, 207.

9. Banerjee, S., *Nature,* 1944, v153, 344.

10. Nath, M. C., Chakrabarti, C. H., Hatwalne, V. G., and Laul, K. S., *Nature,* 1948, v162, 660.