changed beyond its normal state. Such an interpretation would be consistent with the recent findings by Seifter *et al.*(14), that the alarm reaction in rabbits makes the synovial membrane less susceptible to the action of hyaluronidase. It might then be suggested that cortisone changes the character of connective tissue in such a manner that it no longer reacts to the stimuli which bring about its breakdown in the various diseases of connective tissue. An altered reactivity after ACTH therapy in response to experimental wounds has been shown to occur in rabbits by Ragan *et al.*(15).

Summary. ACTH brings out a marked decrease in the level of the "nonspecific" hyaluronidase inhibitor in patients with rheumatic fever. This decrease parallels the drop in sedimentation rate and clinical course. It is suggested that this may be a reflection of the effect of adrenal hormones on changing the state of connective tissue so that it no longer reacts to the precipitating stimuli in the diseases of connective tissue.

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Renal Glycosuria Induced by Adrenocorticotrophic Hormone.* (17782)

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That anterior pituitary hormones will, under suitable conditions, produce temporary or permanent diabetes in the experimental animal is well recognized(1,2). Conn and his co-workers(3) have studied the temporary diabetic state which was induced in normal adult human beings by the continued administration of moderately high doses of pituitary adrenocorticotrophic hormone (ACTH) and have demonstrated that the diminished glucose tolerance and glycosuria which may follow multiple injections of ACTH are associated with diminished blood glutathione levels. Indeed, it was shown that the administration of glutathione will bring about temporary diminution of the glycosuria and hyperglycemia induced by ACTH(4). This finding as Conn *et al.* have pointed out, is of considerable interest in relation to the studies of Lazarow(5) in which it was demonstrated that the effectiveness of alloxan as a diabetogenic agent could likewise be reduced by glutathione and other sulfhydryl-containing compounds.

In the course of studies dealing with the effect of ACTH on acute infectious diseases we have observed, in agreement with Conn *et al.*, that glycosuria could usually be ininduced if sufficiently large doses of ACTH were administered, and that hyperglycemia and impaired glucose tolerance generally accompany the glycosuria. Significant decreases in glutathione levels in the blood have always accompanied these evidences of altered carbohydrate metabolism. It has been suggested that one of the effects of ACTH is to bring about lowering of the "renal threshold" for glucose(3). However, the patients whose

* Aided by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service.

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responses to ACTH suggested such an effect have demonstrated abnormal glucose tolerances and usually hyperglycemia as well. The possibility exists therefore that the glycosuria was due to transitory peaks of alimentary hyperglycemia which, because of the diminished tolerance to glucose induced by ACTH, reached higher levels than usual and thus permitted the blood sugar levels to exceed otherwise normal renal thresholds.

Increased excretion of sulfur-containing compounds in the urine and feces is induced by ACTH(6). This finding, and the decrease in blood glutathione which accompanies ACTH-induced glycosuria, suggested the possibility that some of the apparent glycosuria was due to increased excretion of sulfhydryl compounds. In the case to be described, apparent glycosuria occurred in the absence of evidence of impaired glucose tolerance in a patient with pneumococcal pneumonia who was being treated with ACTH. The glycosuria was accompanied by diminished blood glutathione levels, and both returned to their normal values after ACTH was withdrawn. Blood reduced glutathione was determined by the method of Woodward and Fry(7).

The patient, a 16 year old schoolboy, was admitted to the Boston City Hospital with left lower lobe type 8 pneumococcal pneumonia with bacteremia. At entry he was acutely ill with a temperature of 104°F, pulse 120 and respirations 36. The white blood count was 16,500 with 88% polymorphonuclear leucocytes and the urine was entirely negative. After an 8-hour period of observation, the patient was given 25 mg (Armour Standard) ACTH‡ per dose for 2 doses, 50 mg for 2 more, 200 mg per day for the next 2½ days, and then 175, 150, 100, 50 and 12.5 mg per day, in 4 doses, for each of 5 successive days. All injections were intramuscular. During the 12 hours after the onset of therapy the patient rapidly became afebrile, and within 18 hours he was entirely free of symptoms. By the fourth hospital day, 3 days after the onset of therapy, the leucocyte count had dropped to 8000, the chest was clear by physical examination and virtually clear by X-ray, and the patient continued to be entirely free of symptoms. Sputum was no longer available but type 8 pneumococci were present in the throat culture.

Glycosuria developed after 24 hours of therapy with ACTH and continued until this therapy was stopped. Seven fasting blood sugar determinations made at regular intervals during the period of ACTH therapy were between 76 and 103 mg%. The urine on the third day of therapy, reduced Benedict's reagent to yellow and continued to do so as long as 200 mg per day of ACTH were administered. As the dose of ACTH was diminished the glycosuria slowly disappeared. During the second day of ACTH therapy, when the patient appeared clinically well, 6.3 g of glucose were recovered from his urine. On the third day after the onset of treatment the fasting blood sugar was 103 mg% and a glucose tolerance test after the intravenous injection of 50 g of glucose showed blood glucose levels of 160 and 87 mg% in ½ and 2 hours, respectively. The blood glutathione was 15.3 mg% on the fasting specimen and was 14.7 on the following day.

In order to determine the nature of the reducing substance, the urine was treated with washed yeast cells for 30 minutes; no reducing substances were demonstrable by Benedict's reagent after such treatment whereas prior to fermentation the reagent was reduced to a yellow color. In addition, an osazone was isolated from the urine and after recrystallization had the characteristic crystalline structure of glucosazone. The patient was seen 2 weeks after his discharge from the hospital and at this time had no glycosuria and his blood glutathione was 24.0 mg%.

Discussion. Despite the lowered blood glutathione content and evidence that the urinary excretion of sulfur-containing compounds is increased during the administration of ACTH(6), the reducing substance in the urine of a patient who received the hormone and exhibited normal glucose tolerance was primarily glucose. The methods used for

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7. Woodward, G. E., and Fry, E. G., *J. Biol. Chem.*, 1932, v97, 465.

‡ The ACTH was supplied by Dr. John R. Mote, Medical Director of Armour Laboratories.

urinary sugars do not detect minute increases in the urinary sulfhydryl content. It appears then that glycosuria may occur during the administration of ACTH with no apparent evidence of disturbed carbohydrate metabolism, but in the presence of diminished blood glutathione levels. Such a finding offers evidence that ACTH acts to depress the renal threshold, and that a temporary renal glycosuria may be induced by the hormone. It is obviously of interest to study the sulfhydryl levels in patients with true renal glycosuria. The renal threshold has been shown to be dependent upon at least 2 variables, namely, the rate of glomerular filtration of glucose, and the rate of tubular reabsorption from the glomerular filtrate(8-10). The first of these factors has been investigated and there is evidence that ACTH and cortisone act to increase glomerular filtration(1). The amount by which glomerular filtration is so increased is small, as measured by inulin clearance, and it appears unlikely that the glomerular filtration factor alone could account for the rather large amount of glycosuria in this patient. That tubular function of seemingly normal kidneys is markedly altered in the presence of large doses of ACTH or cortisone has been observed(11), and while no studies of the tubular maximal rate of absorption of glucose have been conducted as yet, it appears likely that the renal glycosuria induced in this case is due more to tubular functional changes than to alterations in the rate of glomerular filtration.

The findings in this patient also suggest that the general metabolic effect of ACTH on carbohydrate metabolism as evidenced by hyperglycemia and impaired glucose tolerance is separable, perhaps merely as a matter of degree, from the more localized effect of ACTH on renal tubular function, the latter effect occurring at lower levels of ACTH activity before the more generalized effect appears. The kidney would appear to be peculiarly susceptible to the action of adrenocortical hormones.

It has been suggested that the effect of ACTH on carbohydrate metabolism and perhaps also on the renal threshold for glucose varies with the preparation of ACTH, and may therefore represent a factor separable from other effects of ACTH(12). Thus, Conn *et al.* have demonstrated that whereas the effect of ACTH on carbohydrate metabolism may be reversed by glutathione, the sulfhydryl compound did not affect the rate of corticoid excretion(4). Individual variations in response to the drug are, of course, to be anticipated, but there are not as yet sufficient data to decide whether the effects which have been observed are due to a contaminant or are related to the fundamental action of cortical hormones in tissue metabolism. The reproducibility of the effect and the agreement with experimental observations of the effect of adrenal cortical hormones on carbohydrate metabolism would favor the latter concept (13,14).

The possibility has been considered that the changes in glucose threshold herein described are related to the patient's infection. The patient was clinically asymptomatic at the time the observations were made. Furthermore, the disturbed glucose tolerance characteristic of severe infection was not present(15). It is of interest that disturbance of renal function observed in some patients with lobar and primary atypical pneumonias who were receiving ACTH could be reproduced in noninfected patients previously known to have normal kidney function(16), indicating that the renal functional changes were referable to the

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ACTH and not to the infections. To what degree the alteration of glucose resorption and other tubular functions as well as altered carbohydrate metabolism are related to sulfhydryl activity is a problem that deserves further attention.

Summary and conclusions. Glycosuria in the presence of normal blood sugar levels and normal glucose tolerance was demonstrated in a patient recovering from pneumococcal pneumonia during treatment with ACTH. The reducing substance in the urine was shown to be glucose and the glycosuria was accompanied by lowered blood glutathione levels. These observations indicate that renal tubular function may be affected by ACTH and that sulfhydryl activity may be of importance in tubular function.

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Hormone Replacement Therapy in the Aged Female—Estrogen Bioassay.* (17783)

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The practical importance of a "human unit" as opposed to a comparative animal unit in the clinical investigation and utilization of estrogen preparations is well recognized. A useful means by which such a unit may be derived lies in the comparative abilities of various forms of estrogen to produce withdrawal bleeding from the uteri of castrate women. The study presented here applies this method, described by Allen(1) to the determination of the relative effectiveness of pure alpha estradiol,† beta estradiol,‡ pure estrone,† and estradiol benzoate§ in causing withdrawal bleeding from the uteri of postmenopausal women.

Method of study and results. The group used for these determinations consisted of women between the ages of 60 and 86. Initially each member was given one milligram of estradiol benzoate three times per week intramuscularly and caused to bleed periodically by the withdrawal of this hormone. This manner of reactivation of the senile endometrium has been described in detail in previous publications(2,3). When a well stimulated and responsive endometrium had been achieved the bioassays were carried out as in Table I.

TABLE I.
Typical Estrogen Bioassay.
(9 patients in each series for 3 wk).

Drug	Dosage, mg/wk	Total dosage, mg	No. showing withdrawal bleeding
Estradiol benzoate	1 × 3	9	6
Alpha estradiol	1 × 3	9	2
Estradiol benzoate	1 × 3	9	5
Estrone	1 × 3	9	2
Estradiol benzoate	1 × 3	9	2
" "	1 × 3	9	6
Beta estradiol	5 × 3	45	0
Estradiol benzoate	1 × 3	9	1

It had become evident that in order to obtain uniform results, it was necessary to interpose between each 2 of the drugs being assayed "priming cycles" of an estrogen preparation known to be a good stimulator. By previous investigation it had been found that alpha estradiol benzoate when given in dosage of 1 mg 3 times per week was capable consistently of causing withdrawal bleeding in over half of the trials. Since this comes close to a theoretical human unit or bleeding dosage of the medication, estradiol benzoate was used as a control preparation to which the estrone, alpha estradiol, and beta estradiol

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* The medication used in this study was generously supplied by † The Food and Drug Administration, ‡ Organon, Inc., § Schering Corp.

2. Masters, W. H. and Allen, W. M., *J. Gerontol.*, 1948, v3, 183.

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