alkaline phosphatase activity of the kidneys. The larger dose of the parathyroid extract produced a greater excretion of phosphorus(1). The method of autolysis of the homogenate, the type of substrate and the pH of the enzyme determination did not reveal any differences between the two groups of animals. Similar results were obtained in rats studied at 2, 4, 6, and 8 hours (Bonelli and Sala) and 6 hours (Kochakian and Reed) after an injection of 1 ml of parathyroid extract.

The divergent findings previously reported (1) were very likely due to observations based on an insufficient number of animals.

It should be noted that greater enzyme activities were obtained at pH 9.7, by the King-Armstrong (K-A) method, and by autolysis at 5°.

Conclusion. Parathyroid extract produces an increased excretion of phosphorus, but no change in the alkaline phosphatase activity of the kidney of male rats.

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Influence of Administration of ACTH on Urinary Amino Acids.* (20158)

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The present paper describes some changes produced by the administration of ACTH‡ on the urinary output of nitrogenous substances with particular emphasis on amino acids. Urine samples were obtained during 3 periods of study in 2 female psychotic patients. Since the results for all 3 collection periods were similar, the results for only one will be discussed in detail. Both of these patients responded in a similar manner to each other and as reported for normal subjects in the literature and other psychotic patients tested in this laboratory(1) with regard to drop in eosinophils, sodium retention, potassium diuresis, and negative nitrogen balance (see Fig. 208 of reference (1) for pertinent data on patient reported). A preliminary report has been made of this work(2).

Methods. The data reported are for a 29-year-old schizophrenic female who was studied for 4 days prior to administration of the hormone, 10 days during the administration of a daily dose of 150 mg of ACTH, and for 5 days after the cessation of the treatment. The hormone was administered 6 times daily in 25 mg doses via the intramuscular route. The various *nitrogenous constituents* listed in Table I-A were determined by customary procedures. The microbiological determinations shown in Table I-B were performed on urine dialysates by previously described methods(3). For *2-dimensional paper chromatography* samples of urine were dialyzed to equilibrium against 3 ml of glass-distilled water in a rocking dialyzer for 18-24 hours at 4°C. Samples corresponding to 1 x 10^{-4} of the 24-hour excretion were employed for chromatography. Aliquots of the dialysate were hydrolyzed for 18-24 hours in 6 N HCl in a sealed tube at 110°, and the acid removed *in vacuo* prior to placing the samples on paper. Two-dimensional chromatograms were made of the protein-free dialysates before and after acid hydrolysis by the method of Consden *et al.*(4), as extended by Dent(5,6). By visual inspection it was possible to compare the 24-hour excretion of the major free and bound amino acids from day to day. Urine

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TABLE I. Influence of ACTH on Distribution of Urinary Nitrogenous Constituents. Results in g/Hr under A; mg/Hr under B.

			ACTH (150 mg/day)			
			Day			
Constituent		Control	4	8	10	Recovery*
A						
Total N		10.0	11.4	18.1	17.9	11.1
α-amino N		.10	.41	.44	.43	.08
Ammonia N		.28	.63	.99	.77	.23
Urea N		8.72	9.00	14.56	14.90	9.88
Uric acid N		.20	.29	.43	.41	.19
Creatinine N		.32	.32	.30	.31	.29
Undetermined N		.38	.75	1.38	1.08	.44
B						
Aspartic acid	F†	4	11	22	19	7
	C†	133	210	340	285	120
Glutamic acid‡	F	30	31	46	50	23
	C	322	460	660	530	373
Threonine	F	23	61	287	313	22
	C	22	58	26	0	21
Histidine	F	158	305	526	520	100
	C	tr§	tr	tr	tr	tr

* Second day after last inj. of ACTH.

† F = free; C = combined. Values for "free" amino acids represent microbiological response before acid hydrolysis, while "combined" values represent the difference between results obtained after and before acid hydrolysis.

‡ Prior to analysis the samples were autoclaved under conditions leading to virtually complete destruction of glutamine.

§ tr = trace.

samples obtained during the entire period of study were examined by these methods.

Results. The results in Table I-A show the nitrogen distribution in the urine during typical control and recovery days and for 3 days during the period of daily administration of 150 mg of ACTH. All of the substituents studied except creatinine increased during the administration of the hormone. The proportionate increases in α-amino nitrogen and ammonia were greater and occurred before marked elevations were noted in the excretion of total nitrogen, urea, or uric acid. It was found that the total α-amino acid nitrogen of post-absorptive plasma was not significantly increased by hormone administration. The levels were 5.1 and 5.2 mg % on the 4th and 12th days of hormone treatment, respectively, as compared to the control value of 4.8 mg %. The possibility is not ruled out that during hormone treatment the plasma levels of amino acids might have been significantly higher during active absorption of protein split-products after a meal, with the consequent excretion of those substances for which the renal threshold was exceeded for a short time. It has been shown by Dent and Shilling(7) that after the ingestion of proteins large rises occur in the concentrations of many amino acids in the portal blood of dogs and that there can be an accompanying amino aciduria. The increase in ammonia excretion occurred at the time that sodium retention began to exceed chloride retention, and could presumably be a result of the response of the kidney to increased acidity of the urine. During the first 4 days of ACTH administration there was a retention of 280 meq. of sodium and a loss of 40 meq. of potassium, or a net retention of 240 meq. of base, while the chloride retention was only 100 meq. On the 4th day only 10% of the ingested sodium was excreted, while 90% of the potassium and 70% of the chloride appeared in the urine. At this time the pH of the blood had increased from 7.3 to 7.6 and the pH of the urine had dropped from 6.8 to 5.0.

Chromatograms of a urine sample before and after hydrolysis for a typical control day are shown in Fig. 1 and 2. Glycine was present in the highest concentration in the free form. Serine, alanine, glutamine, taurine,

Key to numbers on chromatograms: Leucines, 1; tyrosine, 2; valine, 3; α-amino-n-butyric acid, 4; taurine, 5; cystine (cysteic acid), 6; histidine, 7; alanine, 8; threonine, 9; β-alanine, 10; glutamine, 11; glycine, 12; serine, 13; asparagine, 14; glutamic acid, 15; aspartic acid, 16; lysine, 17; X_1–X_5, unidentified substances. Some substances discussed in text were excreted in such small quantities that the spots did not appear on photographs, although they were easily detectable on original chromatograms.

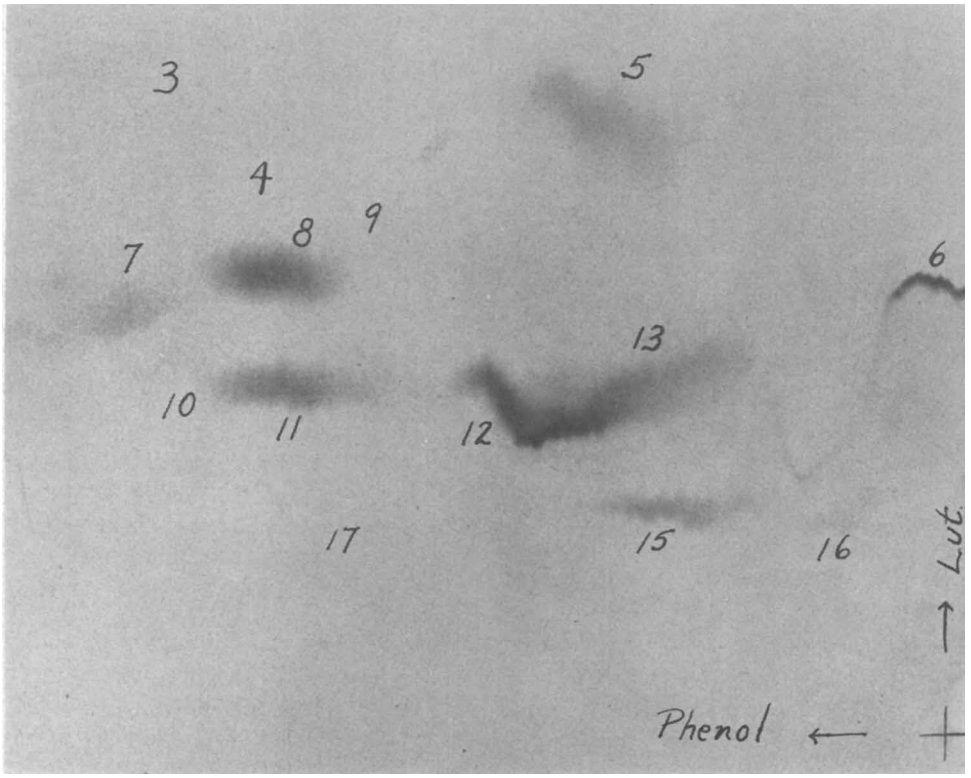

FIG. 1.

cystine, and glutamic acid were present in appreciable, but smaller amounts. In some of the samples there were detectable quantities or traces of histidine, α-aminobutyric acid, γ-aminobutyric, valine, the leucines, tyrosine, lysine, threonine, β-alanine, and aspartic acid. Upon acid hydrolysis there were large increases in the amounts of glutamic and aspartic acids, glycine, and β-alanine. Increases in these amino acids on hydrolysis of urine have been reported by Dent(6,7).

During the period of administration of the hormone there were increases in the excretions of a number of the constituents in the free form. This effect was noted even in the first sample collected after the beginning of administration of the hormone, but appeared to reach its maximum intensity at 3 to 4 days after beginning of treatment, with the high level of free amino acid excretion continuing throughout the entire period of administration of the hormone. A typical experimental day is represented by the chromatograms obtained from the urine on the fifth day after the first administration of the hormone (Fig. 3 and 4). A comparison of Fig. 1 and 3 reveals that especially marked increases were found in free glycine, serine, alanine, taurine, glutamine, threonine, and lysine. The contents of cystine, histidine, and glutamic and aspartic acids were also somewhat increased. Of particular interest was the appearance of relatively large amounts of asparagine, an amino acid not found in the control samples. This amino acid was identified by the characteristic orange-brown color, the position on the chromatogram, the destruction on acid hydrolysis, and by the finding that known samples of

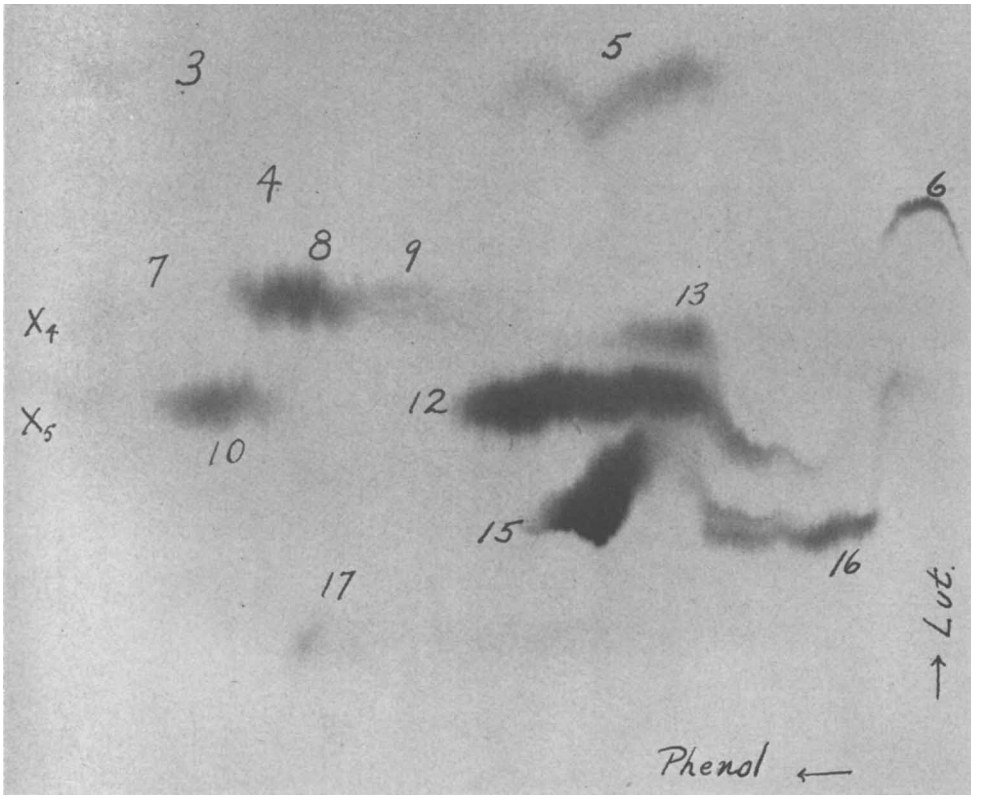

FIG. 2.

FIG. 1 and 2. Chromatograms of unhydrolyzed and hydrolyzed urine samples obtained during a control day.

asparagine, when added to the urine samples, migrated to the same position as the substance in urine and intensified the color. As in the controls (Fig. 2), there were marked increases in the content of glutamic and aspartic acids and β-alanine after acid hydrolysis of the urine obtained during the hormone treatment (Fig. 4). However, almost all of the glycine was now found in the free form, little or no increase in glycine being found on acid hydrolysis. It is interesting in this connection that there was a decreased utilization of the nitrogen of N^{15}-labeled glycine for protein synthesis in normal and adrenalectomized rats receiving cortisone(8) and a decreased incorporation of C^{14}-labeled glycine in hypophysectomized rats receiving ACTH(9). There was a precipitous drop in free amino acid excretion on the first day after cessation of administration of the hormone to levels slightly below those found during the control period (Fig. 5), together with a disappearance of asparagine from the urine. After acid hydrolysis (Fig. 6) the pattern of amino acids was very similar to that of the controls. Chromatograms made of samples obtained on the following 4 days showed patterns virtually identical with those found during the control period, both before and after acid hydrolysis. It thus appears that the influence of the hormone on amino acid excretion is rapidly reversible.

The large increase in the glutamine content of the urine and the appearance of relatively large amounts of asparagine during the administration of the hormone are particularly interesting. Although there is much information concerning the metabolism of glutamine in animal tissues(10,11) there is little comparable information about asparagine. Dent (6) has reported the presence of asparagine in "pathological urine", although the nature of the pathological states was not specified. The

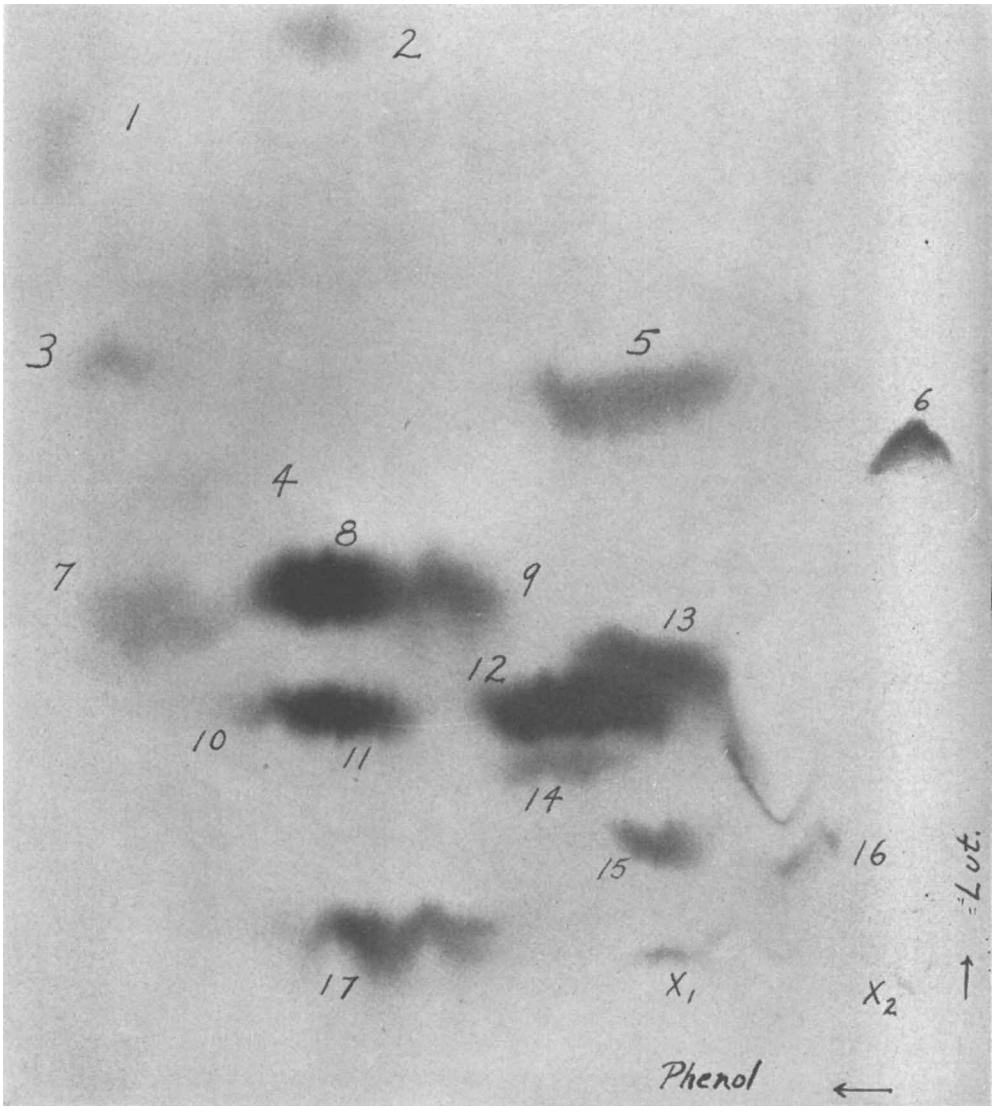

FIG. 3.

recent finding that glutamine and asparagine play a significant role in transamination in liver(12) is of particular interest in this connection, and a study of the influence of the administration of ACTH and cortisone on these processes is suggested. It does not appear likely that the increased excretion of glutamine in the present instance is caused by a diminished rate of its utilization for the formation of urea and ammonia, since there was actually an increased urinary excretion of both of these substances during administration of the hormone (Table I-A).

The microbiological determinations of 4 amino acids (Table I-B) are in agreement with the chromatographic findings. Increases of threonine and histidine were almost entirely in the free form, while bound forms of glutamic and aspartic acids accounted for the chief increment in the latter amino acids. Consideration could not be given to the microbiological availability of the bound forms of the amino acids, since their chemical nature was not known. However, the above results indicate that the bound forms of aspartic and glutamic acids are largely not available to the

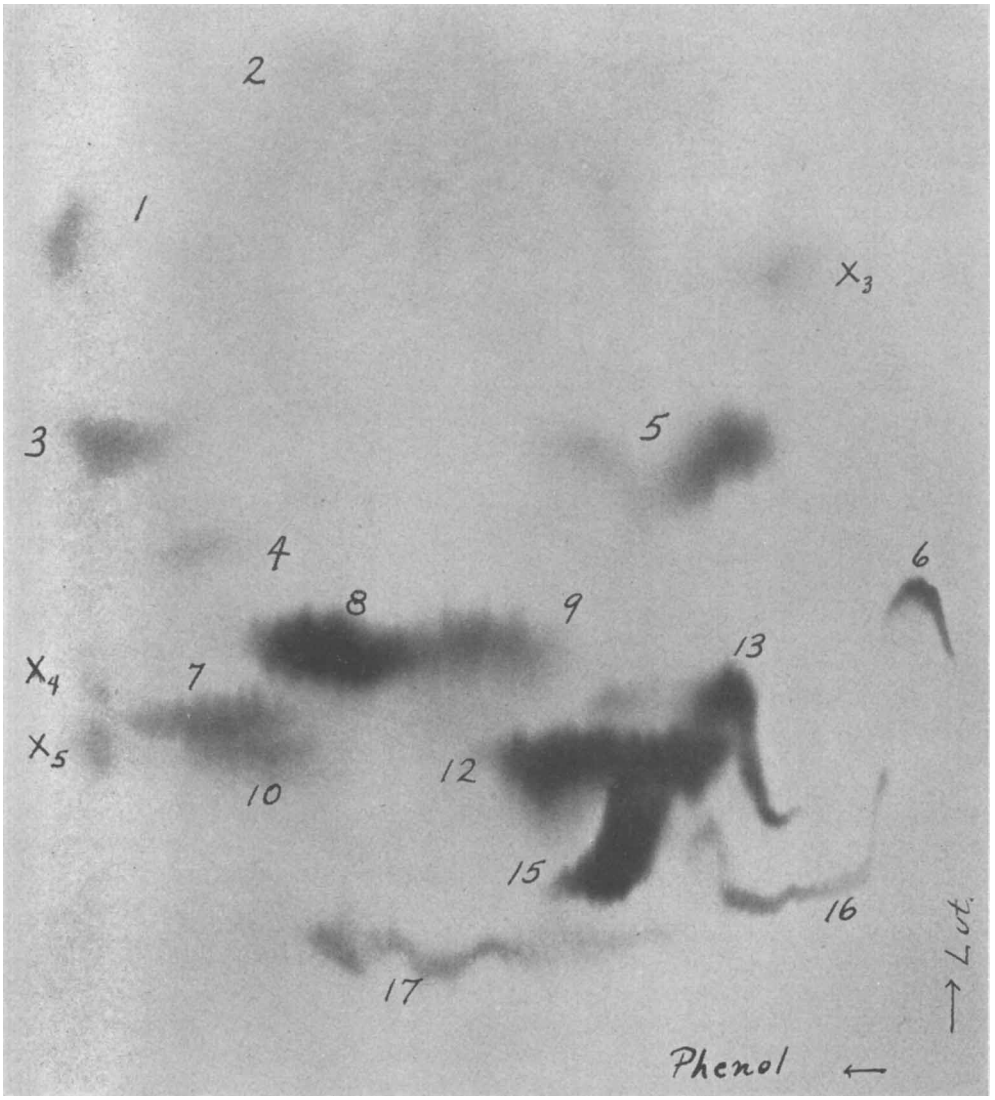

FIG. 4.

FIG. 3 and 4. Obtained on fifth day after first administration of ACTH.

test organisms. The values for free threonine and histidine found during the control period are in close agreement with those previously reported for normal individuals(13). However, the maximal increases in the excretion of these amino acids upon administration of ACTH were much greater in the present study than in the one cited above(13). This may possibly be attributable to the fact that a 40 mg daily dose of ACTH was employed in the latter study as compared to 150 mg in the present experiments.

Discussion. The results reported herein are in keeping with those previously cited(8,13, also see bibliography in these references) which indicate that the administration of ACTH or cortisone can produce profound alterations in nitrogen metabolism. The multitude of alternative pathways, both physiological and biochemical, which might be affected prevents fruitful speculation at the present time about the mechanism of action.

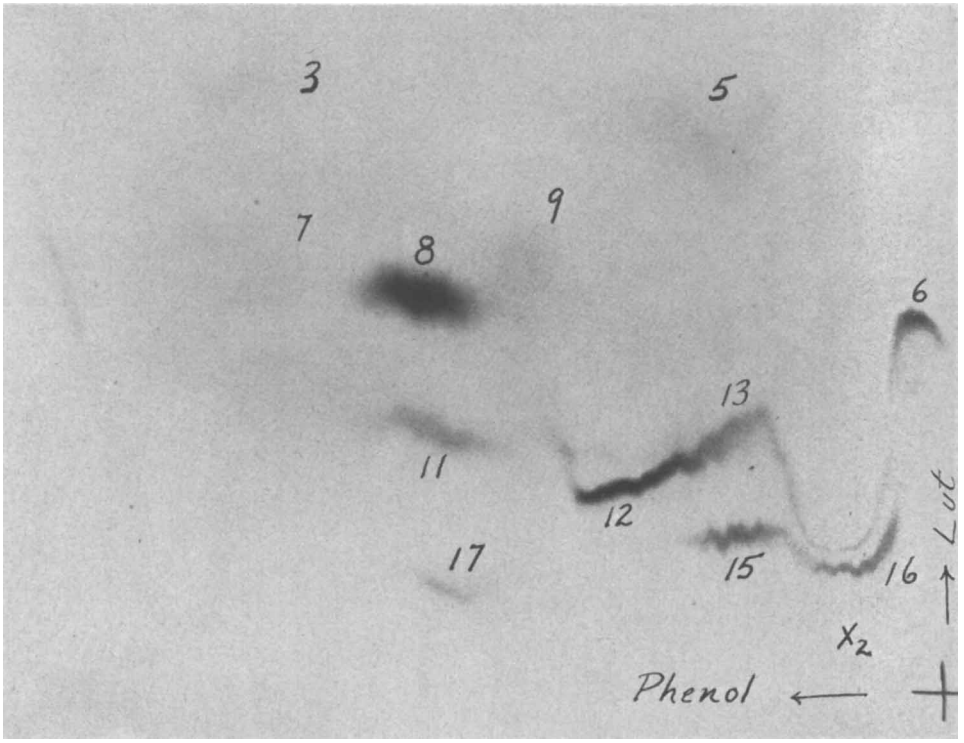

FIG. 5.

Considerable evidence(14) has been adduced for the conclusion that optimal utilization of amino acids for protein synthesis "requires the simultaneous availability of adequate amounts of all of the essential and non-essential amino acids participating in the make-up of the given protein". It would be expected that losses of essential and non-essential amino acids such as were found in the present study would decrease the potentialities for protein synthesis at various sites in the body.

A study of the influence of cortisone on the patterns of free amino acids in the tissues of the developing chick embryo showed that the chief abnormality produced by the hormone was an accumulation of free hydroxyproline (15). In the present study the latter amino acid was not detected in the urine either during the control period or during the administration of ACTH.

Summary. Typical results are presented showing the influence of ACTH administration in the human on the urinary excretion of nitrogenous substances. The hormone evoked notable increases in the excretion of amino acids, ammonia, urea, uric acid, and some undetermined nitrogenous constituents. Two-dimensional chromatograms revealed some of the detailed changes in the excretion of both free and bound amino acids. Microbiological determination of some of the amino acids gave results which were concordant with the chromatographic findings. All of the urinary nitrogenous constituents returned to normal levels shortly after cessation of the treatment. The possible significance of some of the findings is indicated.

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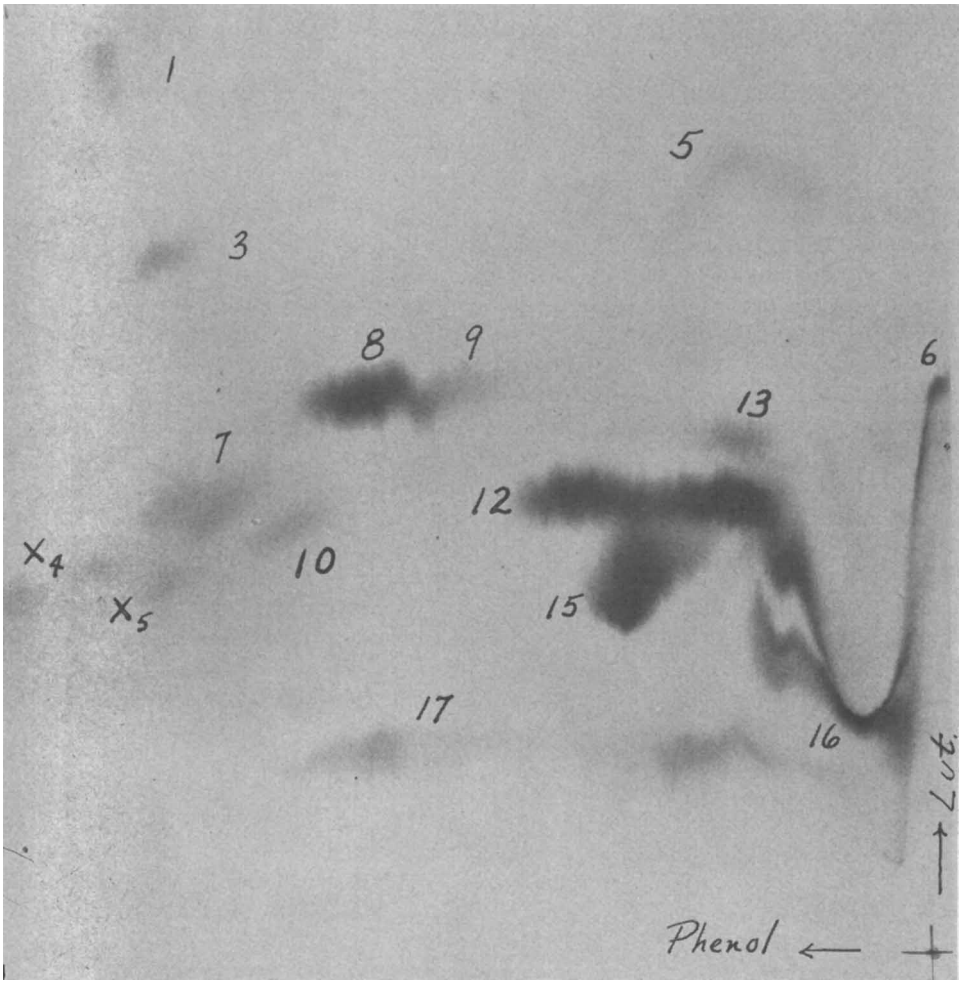

FIG. 6.

FIG. 5 and 6. Obtained on first day after cessation of administration of ACTH.

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