Summary. Three salts of penicillin G and one of penicillin V were found to be essentially equal when administered orally in sparing the rat's dietary requirement for thiamine, as determined by rates of growth and thiamine levels in the liver. Growth was not stimulated to a statistically significant degree upon administering these antibiotics intraperitoneally every other day. although thiamine levels in the livers were elevated by such treatment. The effects of penicillin on thiamine levels in ileal and cecal contents were inconclusive.

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Effect of Stimulation of Adrenal Cortex on Blood Pepsinogen in Animals and Man. (22748)

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The level of pepsinogen in blood and urine constitutes an index of gastric activity(1-4). Uropepsinogen excretion reportedly increases following administration of adrenocorticotrophic hormone or adrenal steroids, and after acute emotional and physical stress; therefore it has been suggested as a measurement of adrenal activity. These data and their implications concerning the relationship of gastric activity to stress and the General Adaptation Syndrome have been reviewed recently by Gray *et al.*(5). However, Hirschowitz and his associates were not able to demonstrate increases in blood pepsinogen following ACTH administration(6), and Necheles and his co-workers recently reported failure of cortisone to affect uropepsinogen excretion (7). Similarly, in animal studies, we were unable to demonstrate elevation in blood pepsinogen following either acute or chronic noxious stimulation in cats and rats even when adrenal hypertrophy was observed(8,9). A study was undertaken, therefore, to determine if direct stimulation of the adrenals by exogenous ACTH influenced blood pepsinogen in animals. The effect of a single injection of ACTH on blood pepsinogen in humans was also evaluated and titres in several patients on chronic steroid therapy were measured.

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TABLE I. Response of Blood Pepsinogen to Acute Adrenal Stimulation in Animals.

Animal	Treatment	Blood pepsinogen activity Before	After	Hr after inj.
Cats A	Saline	290	260	24
B	50 units ACTH gel	375	315	24
C	*Idem*	310	335	24
Dogs A	Saline	240	250	6
			260	24
B	40 units ACTH gel	490	350	6
			410	24
C	*Idem*	355	400	6
			405	24
Rats 1	Saline	260	275	24
2	"	250	205	24
3	10 units ACTH gel	265	320	24
4	*Idem*	—	210	24
5	Saline	260	245	48
6	"	285	375	48
7	"	300	255	48
8	30 units ACTH gel	—	325	48
9	*Idem*	320	240	48
10	"	345	340	48

Materials and methods. Acute studies were performed in 3 mongrel cats, 3 mongrel dogs, and 10 male Holtzman rats in whom blood pepsinogen was determined before and after administration of ACTH gel. Treatments and time of measurement of pepsinogen are given in Table I. Acute studies were also performed in 9 healthy human subjects, one patient with pernicious anemia and one with renal disease. In the latter condition, pepsinogen activity may be elevated, as has been demonstrated by Hirschowitz(3) and Spiro (10). In addition, pepsinogen was determined following chronic therapy in one patient with an inactive peptic ulcer and severe pulmonary disease, and in four patients without ulcers, who were receiving large doses of steroids and/or ACTH for a variety of ailments. Diagnoses, dosage schedules and experimental design in the human subjects are presented in Table II. To study adrenal cortical activity and blood pepsinogen levels simultaneously, 42 male Holtzman rats weighing 200-250 g were divided randomly into 4 groups. Blood was obtained by cardiac puncture, and the animals were treated for 5 days as outlined in Table III. All animals were then anesthetized with sodium pentobarbital and rapidly exsanguinated. Adrenals were cleaned of extraneous tissue, weighed to 0.1 mg on a Sartorius balance, and their ascorbic acid content determined by the method of Roe(11). Blood pepsinogen activity was determined by the method of Mirsky *et al.*(12). One unit of pepsinogen represents one microgram of tyrosine released by one ml of plasma or serum; plasma was used for the cats and dogs, serum in the rats and humans.

Results. A consistent increase in pepsinogen activity was not noted in the acute studies (Tables I and II). In 4 patients who were receiving chronic treatment with steroids, blood pepsinogen was within the normal range in our laboratory (*v.i.*) and rose slightly in only one of 3 with further treatment. Activity increased in the ulcer patient after one week of therapy with prednisone alone, but thereafter fell to initial levels despite continued administration of ACTH and prednisone (Table II). The effects of adrenal cortical stimulation on adrenal weight, ascorbic acid content and level of pepsinogen in rats are set forth in Table III. These data were subjected to an Analysis of Variance. The adrenals of the groups which received ACTH and epinephrine were significantly heavier and demonstrated a highly significant depletion of ascorbic acid, but pepsinogen activity was identical in all groups. Comparison of the pepsinogen values before and after treatment in each of the groups suggested a slight fall in the groups given ACTH and epinephrine, but this was not significant statistically. Carbaminoylcholine (Doryl), a parasympathomimetic agent with predominant gastro-intestinal effects, did not stimulate the adrenal nor elevate the average pepsinogen level; however 2 animals in the group displayed titres which were over 500 units. Peptic ulceration was not observed.

Serum pepsinogen levels determined in our laboratory in 18 normal patients ranged from 195 to 445 with a mean of 305 units, and a standard deviation of 78.5. In 24 patients with proven peptic ulcer, the values ranged from 366 to 968, with a mean of 610 and standard deviation of 162. Although there is

TABLE II. Response of Blood Pepsinogen to Adrenal Stimulation in Human Subjects.

Acute studies (11 subjects)

Sex	Treatment (ACTH gel IM), units	Blood pepsinogen activity Before	Blood pepsinogen activity 24 hr after	Sex	Treatment (ACTH gel IM), units	Blood pepsinogen activity Before	Blood pepsinogen activity 24 hr after
♀	40	300	340	♂	40	435	530
♂	40	295	300	♂	40	280	285
♂	40	290	240	♀	40	205	200
♂	40	205	290	♂ *	40‡	50	60
♀	40‡	305	300	♀ †	40‡	735	440
♂	40	250	210				

* Patient with pernicious anemia. † Patient with nephrotic syndrome and blood urea nitrogen of 50 mg %. ‡ Aqueous ACTH IM.

Chronic studies (5 patients)

Diagnosis	Treatment	Pepsinogen activity	Time (day of study)
Inactive peptic ulcer; chronic pulmonary disease	(1) Prednisone daily from day 0 to 60 (total dosage 490 mg)	890	0
		1150	8
		900	16
	(2) ACTH gel IM twice weekly from day 17 through 60 (total dose 260 units)	955	23
		905	30
		905	60
Drug sensitivity	(1) 5 to 10 mg Prednisone daily for 3 mo prior to day 0	400	0
	(2) 40 units ACTH gel IM daily from day 0 to 4	495	4
	(3) No therapy; day 4 to day 8	425	8
Undiagnosed collagen disease	15 mg Prednisone daily for 13 days prior to day 0 (total dosage 195 mg)	275	0
Rheumatoid arthritis, drug-induced Cushing's syndrome with marked hypertension	(1) Cortisone 25 mg daily for 20 days every mo for 3 yr prior to day 0	390	2
	(2) 40 units ACTH gel IM day 0 to day 7; 25 mg cortisone day 0 to day 2; 12.5 mg daily days 3 and 4	220	6
Drug sensitivity	(1) 40 units ACTH gel IM and 10 mg Prednisone; day 0	193	3
	(2) 40 units aqueous ACTH I.V.; day 1 and 2		

TABLE III. Comparison of Blood Pepsinogen Activity and Adrenal Cortical Activity in Rats.

Treatment*	No. of rats	Means and stand. dev. Adrenal wt (mg/100 g body wt)	Adrenal ascorbic acid (mg/100 g adrenal)	Pepsinogen activity (μg tyrosine released/cc serum) Before	After
Control	10	16.7 ± 2.1	455 ± 26.9	256 ± 79	251 ± 163
ACTH	11	22.6 ± 3.7	333 ± 53.9	264 ± 75	210 ± 99
Doryl	11	15.3 ± 1.7	462 ± 41.6	236 ± 85	264 ± 168
Epinephrine	10	19.8 ± 1.8	367 ± 38.9	257 ± 51	198 ± 75
p values Between groups		<.01	<.01	N.S.	N.S.†
Control × ACTH		.001	.001		
Control × doryl		N.S.	N.S.	Grand mean (before) 253 ± 74	
Control × epinephrine		.04	.001		

* Dosage schedules (all treatments of 5 days duration):

Control, 1 ml normal saline daily; ACTH, 10 units of ACTH gel daily; Doryl, 1.5 μg twice daily; Epinephrine, 0.4 mg (in oil) daily.

† Differences between grand mean before treatment and means of individual groups after treatment, including apparent declines in ACTH and epinephrine groups, similarly are not significant.

an overlap in the range from 350 to 450, the differences between means is highly significant ($p = .001$). In 5 patients with pernicious anemia the mean value was 137. The patients in these 3 groups all had normal levels of blood urea nitrogen. In 9 patients with blood urea nitrogen over 20 mg % (range 20 to 135), pepsinogen levels averaged 615 with standard deviation of 213. These data are reported to add our results to the literature confirming the significant elevation of blood pepsinogen in peptic ulcer, its depression in pernicious anemia, and its increase in renal failure.

Discussion. Failure to produce an increase in blood pepsinogen consistently following acute administration of ACTH indicates that the level of this enzyme cannot be considered a sensitive and reliable index of adrenal cortical activation. The studies in rats in which pepsinogen and adrenal cortical activity were measured simultaneously clearly indicate that stimulation of the adrenals sufficiently intense to cause hypertrophy and depletion of ascorbic acid, will not raise blood pepsinogen and may perhaps lower it. Nor was an appreciable elevation of blood pepsinogen noted in human subjects receiving chronic or large doses of ACTH and/or steroids. Accordingly, an elevated level of blood pepsinogen need not indicate adrenal hyperactivity, nor should it be interpreted as evidence that the General Adaptation Syndrome is causally linked to the development of peptic ulcer. These considerations do not obviate the fact that prolonged administration of ACTH or cortical steroids may produce an acute ulcer in some patients, after which pepsinogen may rise(6). However, in such instances the adrenal hormone probably plays a permissive, rather than a causal, role and operates by increasing the sensitivity of the gut to stimuli previously not damaging(13). Direct tissue changes, vascular disturbances, or changes in the character of the mucous secretion of the stomach represent possible mechanisms for this action, which need not then be associated with a prior rise in pepsin secretion.

The increase in *urinary* pepsinogen following ACTH or cortical steroids reported by Gray(5) is not explained by our data. Hirschowitz has demonstrated that gastric pepsin in the human does not increase regularly after ACTH while blood pepsinogen is essentially unchanged, and suggests that the increased urinary output reflects increased renal clearance and not increased production(6). These circumstances should of course result in an actual fall in blood pepsinogen, which is suggested by our data in rats and in the patient with impaired renal function whose blood level declined after ACTH. Accurate delineation of this problem however requires simultaneous measurement of urine, blood, and gastric pepsin under a variety of conditions. The precise meaning of changes in levels in blood and urine might still be obscured because the volume of blood considerably dilutes the small quantities of pepsinogen available for renal clearance, and the methods for measurement carry an inherent error of 5 to 10%. Accordingly, until these arguments are clarified, it would seem well advised to consider blood pepsinogen only as an indicator of gastric activity which is fairly constant in most individuals, elevated in patients with peptic ulcer, and not influenced by acutely stressful situations. Changes which occur in chronic situations with deep-seated psychodynamic dislocations as described by Mirsky(14), probably have no direct relationship to adrenocortical stimulation.

Summary. Blood pepsinogen levels were measured in cats, dogs, rats, and man following acute and chronic administration of adrenocorticotrophic hormone and/or cortical steroids. Despite demonstration of adrenal cortical stimulation, pepsinogen activity did not increase. The data indicate that blood pepsinogen activity does not constitute an index of adrenal cortical activation.

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Chromatographic Separation of Growth Hormone and Other Peptides.* (22749)

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A chromatographic system was devised for the purification of growth hormone which appears, in addition, to be generally useful for the fractionation of polypeptides. The system may be adapted to either of two means of separation. The difference in maximal acetic acid concentration allowing adsorption on carboxymethylcellulose is sufficient to permit separation of some substances. Fractions which have similar adsorbability from acetic acid are separated by elution from carboxymethylcellulose with weak solutions of neutral salts in acetic acid. The resolving power of the salt elution appears to be particularly high when the acetic acid concentration is rather near the maximum strength permitting adsorption. The appropriate acetic acid concentration may be predetermined by small batch experiments, or by gradient elution from a column with the acid.

Results. Experiments with a purified growth hormone preparation indicated that adsorption occurred in acetic acid in strengths up to approximately 3.5 M. Gradient elution with acetic acid separated a minor component, but most of the material appeared with a sharp front at about 3.6 M but with extensive tailing and no further fractionation. Gradient elution with calcium chloride in 1.0 M acetic acid eliminated the tailing and yielded

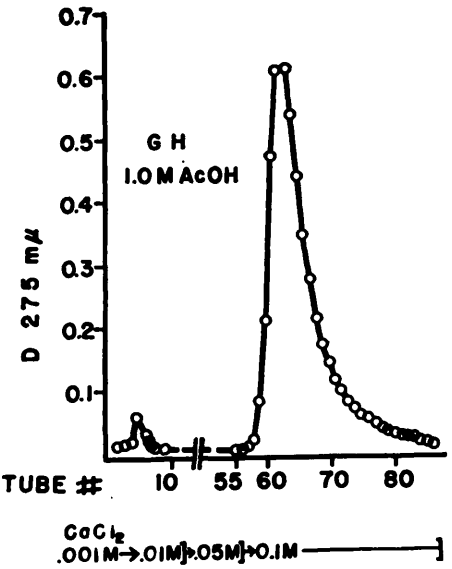

FIG. 1. 30 mg pig growth hormone preparation in 1.0 M acetic acid containing .001 M $CaCl_2$ on 3.5 g carboxymethylcellulose prewashed with same solution and packed under pressure. Column dimensions: 9 × 220 mm. Mixing flask for the gradient elution contained a constant vol of 120 cc. Flow rate, 7.4 ml/hr.

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