phase during the day, irrespective of meal hours and sleep. This theory was supported by Gerritzen(3) who reported some observations on healthy young adults, some of whom drank hourly and had meals at the usual hours while others drank and ate the same amount of food hourly.

From observations on children (5-10 years of age) receiving 4 meals at 6-hourly intervals, Manchester(4) found the maximum excretion of N to be between 6 A.M. and 12 noon and the minimum between 12 midnight and 6 A.M. Examination of the data reported by these authors showed that the variation in N excretion was always accompanied by a corresponding variation in urine volume. The so-called rhythm of N excretion may be simply a rhythm of water excretion which depends not only on food and water intake but on a host of other factors which impinge on the complex nervous and hormonal control of water metabolism. Unless the rate of water excretion is fairly uniform, it is impossible to locate the real crest and trough of N metabolism or indeed to decide whether there is a cycle or rhythm of N metabolism independent of feeding and other routine of living.

Although the present experiment was originally conceived as a test of the influence of feeding schedule on N utilization through its effect on the amino acid concentration in the metabolic pool, it is realized that N balance experiments can reveal not this effect alone but rather the net effect on a whole series of processes from digestion to excretion. Furthermore, as the present experiment has been performed on a single subject and the number of observations is relatively small, the conclusions must be regarded as tentative. However, the result does indicate that feeding schedule is a factor to be considered in metabolism experiments.

Summary. A normal adult in N equilibrium was given the same diet divided into 2, 3, and 4 approximately equal meals in different periods. With a liberal protein intake (1.14 g per kg), there was a definite indication of a higher efficiency of N utilization with 4-meal than with 2-meal schedule, but the difference was not appreciable to be of practical importance. With a lower protein intake (0.73 g per kg), no significant difference in N utilization was observed.

With the same total intake of food, the pattern of N excretion differed with different feeding schedules and with total N and water intakes. This finding casts doubt on the existence of a diurnal cycle or rhythm of N metabolism irrespective of water intake and feeding schedule.

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3. Gerritzen, F., *Acta Med. Scand.*, 1936, v89, 101.

4. Manchester, R. C., *J. Clin. Invest.*, 1933, v12, 995.

Effect of Adrenocorticotropic Hormone and Cortisone Therapy on Human Plasma Aminopeptidase Activity.* (17816)

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Recent reports have indicated that a considerable increase in serum peptidase activity occurred following the injection of ACTH or adrenal cortical extract in mice(1) and that much less striking increases in various serum peptidases appeared after the administration

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TABLE I.
Plasma LGGase Activity of Normal Subjects.†

Subject	Age	Sex	Date	$K°_{LGG}$* 60 min.	$K°_{LGG}$* 120 min.
T.	32	M	1/3/50	.067	.067
			5	.073	.070
			6	.067	.067
			11	.073	.067
J.	23	M	2/17	.053	.054
			3/16	.055	.052
C.	23	F	16	.061	.055
M.	33	M	16	.041	.039
L.	26	M	16	.059	.063

* Enzyme-substrate mixtures consisting of 0.5 ml of 0.1 M LGG, 0.5 ml plasma, 0.5 ml of 0.02 veronal buffer (pH 7.8), and 0.5 ml 0.85% NaCl were maintained at 37.8°. $K°_{LGG}$ (see text) was calculated from the per cent hydrolysis of LGG of samples withdrawn from enzyme-substrate mixtures at 60 and 120 minutes after the start of hydrolysis.

† This group will be expanded considerably when greater quantities of LGG become available.

of ACTH to normal men(2). We have been unable to confirm these observations in the rat and mouse(3), and have extended these investigations to include patients treated with ACTH and Cortisone.‡

Methods. Ten ml of venous blood, from patients usually fasted, were mixed with 0.1 ml of heparin solution,§ centrifuged and the clear *non-hemolyzed* plasma removed. Enzymic hydrolysis of L-leucylglycylglycine (LGG) by human plasma (LGGase activity) was followed essentially as described elsewhere(3). Per cent hydrolysis was determined using a ninhydrin photometric procedure(4). Plasma LGGase activity was represented by the constant, $K°_{LGG}$, which is defined as the per cent hydrolysis of LGG per minute for 0.1 ml plasma per ml of hydrolysis mixture.

Results. In a small series of controls it was found that plasma $K°_{LGG}$ for normal subjects, fasted or fed, varied from approximately 0.04 to 0.08, as indicated in Table I.

In Figs. 1 and 2 are shown changes in

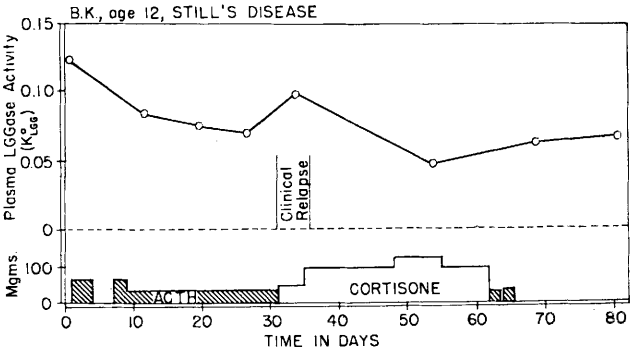

FIG. 1.
Changes in plasma LGGase activity occurring with ACTH and Cortisone therapy in a patient with Still's disease.

1. Holman, H. R., White, A., and Fruton, J. S., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 196.

2. Sayers, G., Burns, T. W., Tyler, F. H., Jager, B. V., Schwartz, T. B., Smith, E. L., Samuels, L. T., and Davenport, H. W., *J. Clin. Endocrin.*, 1949, v9, 593.

‡ More complete metabolic studies on these patients await future publication.

§ The small dilution error thus introduced has been ignored.

3. Schwartz, T. B., and Engel, F. L., *J. Biol. Chem.*, 1949, v180, 1047.

4. Schwartz, T. B., and Engel, F. L., *J. Biol. Chem.*, 1950, v184, 197.

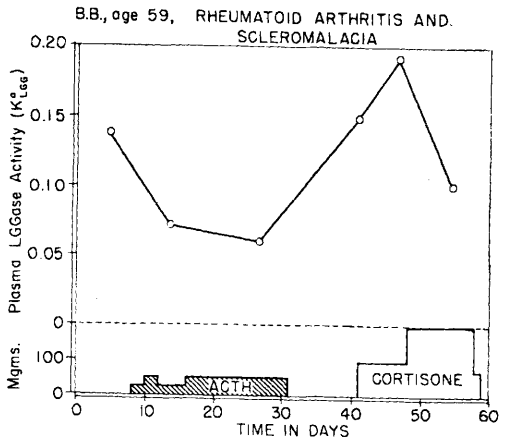

FIG. 2.
Changes in plasma LGGase activity occurring with ACTH and Cortisone therapy in a patient with rheumatoid arthritis and scleromalacia.

plasma K^o_{LGG} which occurred during treatment with ACTH and Cortisone. Note that pre-treatment levels are high and fall with adequate hormonal therapy. These results are, in part, similar to those of Stern and associates(5) who have shown in patients with fractures that cobalt-activated serum LGGase activity falls from high to normal levels with healing.

The data shown in Table II reflect other observations of interest. In patients Y., F., H., and N. no clinical improvement was manifested during adequate therapy with potent hormonal preparations and plasma LGGase activities did not change significantly from pre-treatment values.

That intense chronic adrenal hypersecretion may not increase plasma LGGase activity is illustrated by patient C. with Cushing's syndrome, whose plasma K^o_{LGG} was repeatedly normal. Similarly, in patient A. with chronic adrenal cortical insufficiency plasma K^o_{LGG} was within the normal range. Finally, in another patient (L.) with Addison's disease complicated by a migrating polyarthritis, plasma LGGase activity was significantly elevated. It should be mentioned that LGGase activity of human erythrocytes have been studied, normal values ranging from 4.8 to 6.2. No consistent or profound

5. Stern, K., Cullen, A. M., and Barber, V. T., *J. Clin. Invest.*, 1949, v28, 419.

TABLE II. Observations on Human Plasma LGGase Activity.

Subject	Age	Sex	Diagnosis	Hormonal therapy	Duration of therapy, days	K^o_{LGG}* 60 min.	K^o_{LGG}* 120 min.	Clinical status
Y.	8	F	Acute leukemia	0	—	.056	.058	Essentially unchanged.
				ACTH	2	.049	.051	
				,,	6	.052	.050	
				,,	11	.033	.034	
				,,	14	.057	.055	
F.	50	M	Intestinal lipodystrophy	0	—	.132	.132	,, ,,
				Cortisone	14	.122	.121	
H.	49	M	Malignant hypertension with uremia	0	—	.30	.30	Died 5 days after start of therapy.
				ACTH	3	.36	.31	
N.	6	M	Acute leukemia without uremia	0	—	.28	.26	Died 8 days after start of therapy.
				Cortisone	2	.32	.30	
				,,	6	.30	.30	
C.	28	F	Cushing's syndrome	0	—	.075	.075	Unchanged (4 day interval between samples)
				0	—	.077	.074	
A.	45	F	Addison's disease	Desoxycorticosterone	Continuous	.072	.067	
L.	42	M	,, ,,	,,	,,	.103	.100	Acute migrating arthritis.

* See footnote, Table I.

changes in erythrocyte K^{o}_{LGG} occurred in this group of patients during ACTH or Cortisone therapy.

Summary and conclusions. 1. In a small control series the normal range of human plasma L-leucylglycylglycine-splitting (LGGase) activity has been established.

2. In 2 patients who responded to ACTH and Cortisone therapy, the pre-treatment LGGase activities were abnormally *high* and, concurrent with clinical remissions, plasma LGGase activities fell to normal levels only to rise again with onset of clinical relapses after cessation of therapy.

3. In 4 patients who did not respond to hormonal therapy, no change in plasma LGGase activity occurred irrespective of whether pre-treatment levels were high or normal.

4. Plasma LGGase activity was normal in a patient with Cushing's syndrome and in a patient with Addison's disease but was significantly elevated in a second patient with Addison's disease complicated by arthritis.

5. These studies lend credence to the hypothesis that changes in plasma LGGase activity reflect changes in clinical status and are not necessarily mediated through the adrenal cortex.

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Application of the Feulgen Reaction to the Laboratory Diagnosis of Smallpox.* (17817)

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The elementary bodies of smallpox were first described by Buist, and then independently by Paschen(1). Methods for microscopical diagnosis of these bodies have been proposed by several investigators. Van Rooyen and Illingworth(2) were able to differentiate variola from varicella by staining the elementary bodies with basic fuchsin as proposed by Paschen. Herzberg(3) recommended victoria-blue for the same purpose. Recently Nagler and Rake(4) reported on the use of electron microscopy for the diagnosis of smallpox. Phase contrast microscopy has also been suggested for this purpose (Barer)(5). None of these methods provides means of distinguishing elementary virus bodies from other small particles. Bland and Robinow(6) have shown that the elementary bodies of vaccinia, and the corresponding inclusion bodies react positively to Feulgen's method of staining desoxyribonucleoproteins. In view of the great similarity, or possible identity, of the viruses of vaccinia and variola, we applied the Feulgen reaction to the study of the variola virus. The present report deals with the use of the Feulgen reaction as a quick diagnostic test for smallpox.

Technic. Skin lesions, preferably fresh vesicles or papules are opened with a sharp instrument, laying free the base of the lesion.

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1. Smadel, J. E., Smallpox and Vaccinia in: Viral and Rickettsial Diseases, edited by Thomas M. Rivers. Lippincott, Philadelphia, 1948.

2. VanRooyen, C. E., and Illingworth, R. S., *Brit. Med. J.*, 1944, v2, 526.

3. Herzberg, K., *Klin. Wchnschr.*, 1936, v15, 1385.

4. Nagler, F. P. O., and Rake, G., *J. Bact.*, 1948, v55, 45.

5. Barer, R., *Nature*, 1948, v162, 251.

6. Bland, J. O. W., and Robinow, C. F., *J. Path. and Bact.*, 1939, v48, 381.