tubular reabsorption. This higher value in newborn infants may be more apparent than real, since the tubules presumably have presented to them a relatively smaller load in the glomerular filtrate. Since clearance periods in this age period are admittedly inadequate, no definite conclusions on this point can be drawn.

Summary. 1. The clearance of endogenous inorganic phosphate is relatively low in the newborn period. 2. The clearances of phosphate in older infants, children and adolescents approximate those of the adult. 3. The tubular reabsorption of phosphate was found to average 87.7% of the glomerular filtrate in children and adolescents. 4. The clearances of phosphate at all ages have a wide variation.

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Fate and Excretion of Adrenocorticotrophic Hormone.*† (18687)

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The fate and excretion of adrenocorticotrophic hormone (ACTH) have assumed practical as well as academic significance with the extensive use of the hormone in the treatment of disease. Porcine ACTH disappears rapidly from the blood of man following its intravenous injection(1). Two hours after its administration, only 2% of the biological activity of the injected hormone remains in the vascular compartment; after 3 hours, ACTH activity is no longer detectable in the blood. The hormone is not excreted in appreciable quantities in the urine. Essentially the same results have been obtained in the rat following the administration of porcine ACTH(2) and sheep ACTH(3).

It is not possible to decide from the results of the above experiments whether the rapid disappearance of ACTH activity from the blood stream is a characteristic of the endogenously secreted hormone or is due to the fact that in each instance a protein, foreign to the recipient species, was injected. In order to exclude the latter possibility the present experiments were designed to determine the fate and excretion of rat ACTH injected intravenously into the rat.

Methods. Rat ACTH was prepared as follows. Adult male rats from the Sprague-Dawley farm, weighing 250 to 400 g, were decapitated and the pituitaries carefully removed from the sella turcica. The anterior lobes were dissected away from the posterior lobes and ground in a 0.9% sodium chloride solution made acid to 0.1 M with hydrochloric acid. The mixture was centrifuged and the supernatant solution of ACTH placed in a boiling water bath for one hour. The final concentration of the solution of rat ACTH was 2 anterior pituitaries per ml. Each 0.5 ml of extract had the biological activity equivalent to approximately 100 μg of the ACTH standard, La-1-A. All potencies and doses are expressed as μg equivalents of La-1-A. The confidence limits of the bioassays to be presented below are based on a p value of 0.67. The male rats employed for the study of the distribution of injected ACTH were obtained from the Sprague-Daw-

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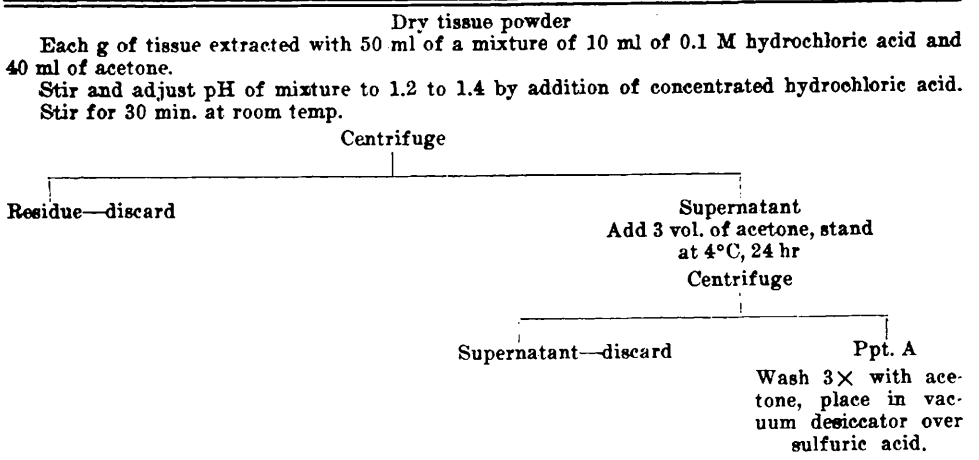
FIG. 1. Extraction and Precipitation of ACTH Activity from Tissue.



ley farm and weighed 250 to 350 g. The animals were lightly anesthetized with ether, injected intravenously (saphenous vein) with a solution of rat ACTH (0.5 ml per 100 g body weight) and allowed to recover from anesthesia. At 5 or 15 minutes following injection of hormone the animals were again lightly anesthetized with ether and blood was withdrawn from the abdominal aorta into a heparinized syringe. The animals were then sacrificed and the liver, kidneys and adrenals prepared for analysis. About 0.25 ml of 0.1 M hydrochloric acid was injected into the bladder and the contents of the organ were withdrawn and stored in a refrigerator until bioassayed. The tissues were frozen in dry ice immediately after removal from the animal, lyophilized and ground to a fine powder. Aliquots of the powder were extracted for ACTH according to the flow sheet presented in Chart 1. Precipitate A, the ACTH concentrate, was dissolved in 0.9% sodium chloride solution made acid to 0.1 M with hydrochloric acid and injected into the test animals for the determination of ACTH potency. Blood, urine and tissue concentrates were assayed for adrenocorticotrophic activity by the adrenal ascorbic acid-depletion test in hypophysectomized rats(4). The experiments to be reported below are designated by numbers which represent the dates on which they were performed. This is done advisably because each assay requires its own reference standard; in quantitative work it is not permissible to compare the individual adrenal ascorbic acid-depletion figures of an assay done on one day with those obtained on a different day.

Results. Blood. The following experiments were conducted in order to determine the stability of rat ACTH in rat blood *in vitro*. Heparinized blood was collected from several untreated rats and pooled. When injected into test assay animals in a dose of 3.0 ml per 100 g body weight, this blood induced no decrease in adrenal ascorbic acid. Rat ACTH was dissolved in the pooled blood to a final concentration of 1.0 μg per 0.5 ml or 4.0 μg per 0.5 ml. The 2 samples were incubated at 37°C for 1 hour. At the end of incubation they were cooled to 4°C and maintained at this temperature until bioassayed. Two dose levels, 1.0 and 4.0 μg, were employed in the assay, *i.e.*, each test rat was injected with 0.5 ml of the appropriate blood sample per 100 g body weight. The reference standard employed was rat ACTH incubated in phosphate buffer, pH 7.5, at 37°C for one hour at concentrations of 1.0 and 4.0 μg per 0.5 ml. The results are presented in Table I. Exp. 4-25-50 indicates that rat ACTH is relatively stable in phosphate buffer. Rat ACTH incubated in rat blood was estimated to be 1.10 times as potent as the hormone

4. Sayers, M. A., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, v42, 379.

TABLE I. Stability of Rat ACTH in Rat Blood *in Vitro*.

Exp. 1950	Preparation	Dose μg	Adrenal ascorbic acid depletion mg per 100 g tissue*	Estimate of potency with confidence limits % of specific control
4-25	Rat ACTH unincubated acid extract	2 .5	113, 113, 120 57, 98, 37, 56, 75	—
	Rat ACTH incubated in phosphate buffer, pH 7.5, 4 hr at 37°C	2 .5	113, 81, 122, 76, 104 52, 35, 56, 68	77 (60 to 101)
5-2	Rat ACTH incubated in phosphate buffer, 1 hr at 37°C	4 1	101, 131, 116, 79, 99 57, 58, 49, 58, 65	—
	Rat ACTH incubated in rat blood, 1 hr at 37°C	4 1	113, 156, 136, 145, 108, 104 17, 39, 53, 79, 21, 55	110 (93 to 131)

* Each figure in this column represents the difference between the concentration of ascorbic acid in the left adrenal, which is removed immediately prior to injection of the test material in the assay rat, and the right adrenal which is removed one hour after injection of the test substance.

TABLE II. Adrenocorticotrophic Activity of Blood from Rats Injected with Rat ACTH.

Exp. 1950	Preparation	Dose	Adrenal ascorbic acid depletion mg per 100 g tissue*†	Estimate of potency in relation to specific control,—with confidence limits
5-9 Intact rats	Rat ACTH in phosphate buffer	4 μg 1 μg	132, 111, 126, 114, 117, 114 36, 23, 51, 52	—
	Blood 5 min. after ACTH inj.	3 ml .75 ml	81, 120, 161, 95, 101, 121 61, 39, 29, 77, 43	1 ml of blood equivalent to 1.23 (1.09 to 1.39) μg
	Blood 15 min. after ACTH inj.	3 ml	5, –6, 26, 11	1 ml of blood equivalent to less than 0.10 μg
6-13 Intact rats	Rat ACTH in phosphate buffer	4 μg 1 μg	96, 160, 131, 176, 188, 135, 137 78, 90, 79, 75, 59	—
	Blood 5 min. after ACTH inj.	3 ml .75 ml	109, 127, 94, 161, 135 68, 20, 21, 67, 46, 39	1 ml of blood equivalent to .81 (.66 to 1.00) μg
6-15 Adrenalectomized rats	Rat ACTH in phosphate buffer	4 μg 1 μg	149, 149, 107, 113, 119 111, 41, 94, 76, 100, 69	—
	Blood 15 min. after ACTH inj.	3 ml	12, –3, –14, 6	1 ml of blood equivalent to less than .10 μg

* See footnote Table I.

† Negative values indicate a greater conc. of ascorbic acid in the right than in the left adrenal.

incubated in phosphate buffer (Exp. 5-2-50). The confidence limits (0.93 to 1.31) are such that this increase is not significant and it may be concluded that rat ACTH is stable *in vitro* in rat blood for at least 1 hour at body temperature. Recipient rats were injected intravenously with 0.5 ml of rat pituitary extract (equipotent with 100 μg of standard), per 100 g of body weight. The duration of injection was 15 seconds. Blood was withdrawn 5 or 15 minutes after ACTH administration. The blood of 6 rats, the number used in each group, was pooled and injected into hypophysectomized assay animals. The results are presented in Table II. After 5 minutes the concentrations of ACTH in the

blood were equal to 1.23 (Exp. 5-9-50) and 0.81 (Exp. 6-13-50) μg per ml. These values are in satisfactory agreement with each other when one considers that ACTH disappears rapidly from the blood and that the interval between ACTH injection and blood withdrawal may have varied by plus or minus one minute.

If it is assumed that injected ACTH is not excreted or inactivated, and that it does not enter cells, the concentration of the hormone in the extracellular fluid should equal 5 μg per ml (100 μg, the amount injected per 100 g of rat, divided by 20 ml, the estimated extracellular fluid volume per 100 g of rat). Five minutes after administration of the hormone, the actual concentration of ACTH in whole blood was equal to 1.0 μg per ml (average of 1.23 and 0.81 μg per ml). If one assumes a hematocrit of 50% and zero concentration of ACTH in erythrocytes, the concentration in plasma may be estimated to be about 2.0 μg per ml; this means that only 40% of the injected hormone remained in the extracellular fluid compartment. Obviously, the assumption that the hormone is not excreted, inactivated or taken into cells is not permissible. Fifteen minutes after ACTH was injected, biological activity could not be detected in the blood. In terms of the sensitivity of the assay method each ml of blood had less than 0.10 μg of ACTH.

The adrenal cortex is the only tissue known to respond to ACTH. Inasmuch as some investigators assume that the site of action of a hormone may also be the locus of degradation, it was decided to ascertain whether the presence of the adrenals is concerned with the rapid disappearance of the hormone from the blood. The results are presented in Table II. Fifteen minutes after the administration of ACTH to adrenalectomized rats, biological activity was not detectable in the blood. Thus the adrenal glands do not appear to be responsible for the rapid disappearance of ACTH from the circulation, but the results do not rule out the possibility that injected ACTH has a slightly longer sojourn in the blood of adrenalectomized rats.

Tissues. The results are presented in Table III. The kidney of uninjected rats contains a substance which depletes the adrenal ascorbic acid content of the hypophysectomized rat (Exp. 9-5-50 and 9-7-50). A very approximate estimate indicates that concentrate (Ppt. A, Chart 1) obtained from 0.2 g of dried kidney tissue elicited a response equal to that of 0.5 μg of standard. A dose of 0.05 g of kidney tissue evoked a negative response (Exp. 9-7-50). No quantitative assay has been performed to determine the exact potency of kidney tissue from normal uninjected rats. Furthermore, it is not possible to state with certainty the nature or the origin of the kidney substance. The animals were decapitated and there was, therefore, no opportunity for stress to induce an acceleration of ACTH discharge from the pituitary. If the substance is of pituitary origin, it must represent an accumulation in the kidney prior to sacrifice. Liver tissue from uninjected rats in a dose of 0.2 g did not deplete adrenal ascorbic acid (Exp. 9-5-50).

It was of some importance to determine the percentage recovery to be expected with the method presented in Chart 1 for extracting and precipitating ACTH activity from rat tissues. Rat ACTH was added to liver tissue obtained from normal uninjected rats. The mixture was processed according to the scheme presented in Fig. 1. From the data of Exp. 9-7-50 it appears that the method is capable of recovering most if not all of the ACTH activity. The recovery of added ACTH was 146%. Unfortunately, the assay was relatively inaccurate (confidence limits 67 to 321%). However, even the low figure of 67% represents an acceptable recovery of added hormone. Kidney removed 5 or 15 minutes after injection of ACTH was remarkably potent in biological activity. Such tissue in a dose of 0.1 g evoked a response greater than that to 2 μg of standard (Exp. 9-13-50). Even 0.025 g of kidney tissue elicited a marked response (Exp. 9-20-50). In Exp. 10-5-50, 0.02 and 0.005 g dose levels caused responses of a magnitude suitable for statistical analysis. Each g of the dry 5-minute kidney tissue was equal in potency to 112 (86 to 147) μg of ACTH and each g of the dry 15-minute kidney tissue was equal

TABLE III. Adrenocorticotrophic Activity of Tissues from Rats Injected with Rat ACTH.

Exp. 1950	Preparation	Dose	Adrenal ascorbic acid depletion mg per 100 g tissue*	Estimate of potency in relation to specific control
9-5	Rat ACTH	4 μg	116, 155, 118, 125	—
	Kidney from uninj. rats	.2 g†	66, 89, 89, 95	.2 g = .5 μg (very approx.)
	Liver from uninj. rats	.2 g	−12, −15, −1, −2, 8, −18	.2 g<.25 μg
9-7	RAT ACTH	2 μg .5 μg	107, 148, 132, 118, 127, 108 101, 121, 93, 106, 111, 121	—
	ACTH + liver from uninj. rats	4 μg 1 μg	116, 193, 209, 103, 151 122, 137, 85, 120, 119	146 (67 to 321) % recovery
	Kidney from uninj. rats	.2 g .05 g	44, 70 1, 14, −28, 10	.2 g = .25 μg (very approx.)
9-13	Rat ACTH	2 μg .5 μg	141, 131, 107, 117, 109 26, 49, 10, 29, 70	—
	Kidney 5 min. after inj. of ACTH	.1 g	157, 162, 196, 162, 143, 153	.1 g>2 μg
	Kidney 15 min. after inj. of ACTH	.1 g	97, 157, 122, 126, 144, 159	.1 g>2 μg
	Liver 15 min. after inj. of ACTH	.2 g	−3, 13, 4, 5	.2 g<.25 μg
9-15	Rat ACTH	2 μg .5 μg	132, 152, 108, 160, 128 88, 32, 68, 41, 101	—
	Liver 5 min. after inj. of ACTH	.2 g	52, −20, −6, −1, 0	.2 g<.25 μg
	Adrenal 5 min. after inj. of ACTH	.035 g	138, 98, 121	.035 g = 2 μg (very approx.)
	Adrenal 15 min. after inj. of ACTH	.033 g	34, 81	.033 g = .5 μg (very approx.)
9-20	Rat ACTH	2 μg .5 μg	139, 90, 107, 160, 122, 102 83, 100, 81, 48, 62, 96	—
	Kidney 5 min. after inj. of ACTH	.1 g .025 g	147, 153, 161, 136, 178, 154 173, 132, 204, 193, 92, 192, 124	.025 g>2 μg
	Kidney 15 min. after inj. of ACTH	.1 g .025 g	149, 124, 199, 144, 147 158, 107, 100, 141, 167, 151	.025 g = or>2 μg
9-22	Rat ACTH	2 μg .5 μg	133, 137, 105, 97, 133 71, 99, 82, 43, 40	—
	Liver 5 min. after inj. of ACTH	.2 g	0, 51, −23, 4	.2 g<0.25 μg
	Liver 15 min. after inj. of ACTH	.2 g	0, 10, 30, −32	.2 g<0.25 μg
	Adrenal from uninj. rats	.033 g	−12, 0, 43	.033 g<0.25 μg
10-5	Rat ACTH	2 μg .5 μg	183, 177, 135, 132, 100 112, 70, 60, 92, 32, 42	—
	Kidney 5 min. after inj. of ACTH	.02 g .005 g	210, 148, 140, 116, 110 108, 67, 78, 68, 77, 71	1 g kidney tissue = 112 (86 to 147) μg
	Kidney 15 min. after inj. of ACTH	.02 g .005 g	172, 151, 114, 120, 140 102, 41, 62, 60, 42	1 g kidney tissue = 89 (71 to 111) μg

* See footnote Table I.

† Dry wt tissue from which concentrate was obtained.

TABLE IV. Distribution of Injected Rat ACTH Throughout Tissues and Body Fluids of Rat.

Amt. ACTH inj., μg	Time after ACTH inj., min.	Total quantity of ACTH μg in: Extracellular fluid	Kidneys	Liver	Adrenals	Urine
100	5	40*	20	<1	.2	0
100	15	<4*	15	<1	.05	0

Calculated per 100 g rat; extracellular fluid 20 ml; dry wt of kidneys, 0.167 g; dry wt of liver, 1.0 g; dry wt of adrenals, 0.0033 g.
* Based on assumption that there is no ACTH in erythrocytes (see text).

in potency to 89 (71 to 111) μg of ACTH.

In contrast to kidney tissue, the liver contained no appreciable ACTH activity at 5 or 15 minutes after ACTH injection (Exp. 9-13-50, 9-15-50, 9-22-50). A dose of 0.033 g of adrenal from normal uninjected rats did not produce a decrease in adrenal ascorbic acid (Exp. 9-22-50). On the other hand, the adrenals of rats injected with ACTH contained appreciable ACTH activity. The limited amount of tissue available has made it impossible to obtain an accurate estimate of the ACTH activity of this tissue. The assay of single doses of adrenal gland (0.035 g from the 5-minute animals and 0.033 g from the 15-minute animals) indicates that the concentration of ACTH in the adrenal was definitely less, on a weight basis, than that in the kidney. Furthermore, the data suggest that the activity in the adrenal was less at 15 than at 5 minutes. An estimate of potency—a very approximate one—indicates that 5 minutes after the injection of 100 μg ACTH per 100 g of body weight, 0.035 g of dry adrenal tissue contains 2 μg of ACTH and that after 15 minutes, 0.033 g contains 0.5 μg of ACTH.

Urine. The urine which had accumulated in the bladder during the 15 minutes following ACTH injection contained no detectable ACTH activity. This is of particular interest because of the large concentration of the hormone in the kidney. Urine from rats in our laboratory is alkaline, a medium in which ACTH is relatively unstable. Rat ACTH incubated in rat urine (pH 8.0) for 15 minutes at 37°C lost ½ to ¾ of its biological potency. In a separate experiment rat ACTH was injected into rats which had been given ammonium chloride so that the pH of the urine was reduced to less than 6. Rat ACTH was demonstrated to be stable in urine at this pH. The urine which had accumulated in the bladder during the 15 minutes following ACTH injection into ammonium chloride-treated rats contained no detectable ACTH activity. It may be concluded that ACTH, under the conditions of our experiments, is not excreted in the urine of rats in a form detectable by the assay method used.

Discussion. The results presented in Table III have been used to calculate the distribution of injected ACTH throughout the organism. These calculations are presented in Table IV. It is to be emphasized that the assay data will allow only approximate estimates to be made. However, there can be no doubt as to the significance of the gross differences which exist between the tissues and body fluids which were analyzed. The studies were conducted in intact rats and hence the possibility must be considered that endogenous ACTH discharge contributed to the quantities found in tissues and blood following injection of exogenous ACTH. Experiments are now being carried out to determine the distribution of injected ACTH in hypophysectomized rats. The rapid disappearance of hormone activity from the blood is not due to destruction of ACTH in this fluid. *In vitro* studies indicate that ACTH is relatively stable in blood at 37°C.

The results demonstrate that the kidney has a remarkable ability to trap injected ACTH. Since rat ACTH was injected into rats it is unlikely that the rapid accumulation of hormone activity in the kidney represents the removal of a foreign protein by this organ. It will be of interest to know whether ACTH, discharged in response to stress, accumulates in the kidney.

No unequivocal data have appeared in the

literature regarding endogenous ACTH titers in the blood. However, it is certain that the concentration of ACTH in blood is very low, even under stressful circumstances. In this connection the authors would like to suggest to clinical investigators that the bioassay of kidney rather than of blood for ACTH may provide a useful tool for elucidating the etiology of adrenocortical dysfunction. For example, kidney biopsy could be performed during the course of an exploratory laparotomy for the purpose of determining the status of the adrenals. In hypercorticism with primary involvement of the adrenals, one would expect to find a lower than normal concentration of ACTH in the kidney because of suppression of ACTH release by the high concentration of cortical hormone in the body fluids. In contrast, in hypercorticism secondary to pituitary over-activity, one would expect to find a higher than normal concentration of ACTH in the kidney if it proves to be true that the renal material is of pituitary origin.

Summary. Rat ACTH is a dose equivalent to 100 μg La-1-A per 100 g body weight, injected intravenously into rats, disappears rapidly from the circulating blood of intact and adrenalectomized rats. Five minutes after injection of the hormone into intact rats about 40% of the injected dose is present in extracellular fluid and 20% in the kidneys; after 15 minutes a negligible quantity is present in extracellular fluid and 15% in the kidneys. The adrenals have a relatively high concentration of the hormone although the concentration is lower than in the kidney. It is estimated that less than 1/500th and 1/2000th of the injected dose is present in the adrenals at 5 and 15 minutes after injection, respectively. Liver contains no detectable quantity of the injected ACTH either 5 or 15 minutes after injection of hormone. Injected ACTH could not be detected in the urine in these experiments.

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Observations on a Relationship Between Vitamin B_{12}, Folic Acid and the Citrovorum Factor.* (18688)

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The presence of a growth factor for *Leuconostoc citrovorum* 8081, present in natural products was reported by Sauberlich and Baumann(1). That this factor is closely related to folic acid (PGA) has been established beyond all reasonable doubt. It has been shown that *Leuconostoc citrovorum* factor (LCF) possesses *Streptococcus faecalis* R activity(2,3). Broquist *et al.*(3) have reported that LCF may replace the PGA requirement of the chick. Drysdale *et al.*(4) have observed that the administration of vit. B_{12} enhances the urinary excretion of LCF in

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