reactions under consideration descriptive terms such as "permissive" and "conditioning" will have outlived their usefulness. Indeed, the continued use of these terms without acknowledgment of mechanisms when they are known serves to confound rather than clarify our understanding. An example of this may be found in a recent report(14) which refers to a high sodium intake as conditioning the action of desoxycorticosterone to cause muscular paralysis in dogs. Nowhere does the author acknowledge the well established facts that the paralysis is due to potassium depletion secondary to the influence of DOCA on renal excretion of potassium and that a high sodium intake accentuates potassium depletion by promoting renal loss of potassium.

Summary. The adrenalectomized rat may exhibit a normal hyperglycemic response to trauma if maintained with a constant dose of glucocorticoids while fasting or if untreated but fed. In both cases the normal response is interpreted as being dependent on an availability of liver glycogen to permit an hyperglycemic response while the failure of the fasted untreated adrenalectomized rat to develop hyperglycemia may be due to deficient glycogen stores rather than to hormone lack *per se*. The significance of these findings is discussed in relation to current concepts of the interaction of the adrenal cortex and the stress reaction.

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Influence of Parathyroids on Removal of Citric Acid Administered by Peritoneal Lavage.* (23023)

JOSEPH R. ELLIOTT AND ROY V. TALMAGE
The Rice Institute, Houston, Texas.

Since Dickens(1) discovered that bone contains large quantities of citric acid, there has been much interest in the relationship of this acid to various bone constituents, and in its relation to over-all bone metabolism. Carlsson and Hollunger(2) have shown that citric acid may be produced in bone and reported an increased production of this acid following administration of vit. D. Elliott and Freeman(3,4) reported that citric acid changes parallel those of calcium in plasma of various animals and suggested that plasma increases in citric acid as well as those in calcium were probably supplied by bone. They further noted that either vit D or parathyroid hormone were able to raise plasma levels of both calcium and citrate. Neuman *et al.*(5) reported their belief that bone produces citric acid in response to parathyroid hormone which in turn is complexed with calcium and released into the plasma. It has also been shown(6) that endogenous citric acid, pro-

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duced by blocking the citric acid cycle with fluoroacetate, increased plasma calcium in parathyroidectomized as well as in normal and nephrectomized rats. The evidence of the above reports suggests that citric acid takes an active part in bone metabolism, that citric acid and calcium are capable of forming complexes *in vivo*, and that citric acid may be able to mobilize calcium from the skeleton.

By use of the technic of continual peritoneal lavage, this laboratory has recently demonstrated the interrelation of calcium and endogenous citric acid in the control by the parathyroids of the equilibration between bone and extracellular calcium and citric acid levels(7). While both calcium and citric acid levels in the equilibrated peritoneal wash were reduced by parathyroidectomy in nephrectomized rats, the reduced levels remained fairly constant during the experimental period following parathyroidectomy. The purpose of the following series of experiments was to determine, by similar technics, the effect of exogenous citric acid on the calcium and citrate levels in normal, nephrectomized, and parathyroidectomized-nephrectomized rats, with the hope that these data would give further clarification of bone metabolism and the interrelation of citric acid and the parathyroid hormone.

Methods and materials. Male Sprague-Dawley rats weighing between 225-275 g were used in these experiments; however, for any one experiment the weight range was restricted to 25 g. For most experiments the animals were maintained on a calcium-free diet 3 days prior to use. Nephrectomy was performed through a ventral midline incision. At the same time, a peritoneal plug made from the specifications of Kolff and Page(8) was stitched into the incision. Parathyroidectomy was performed 24 hrs after nephrectomy. Parathyroids were removed individually. All operations were done under ether anesthesia. The peritoneal rinsing fluid was a calcium and phosphate-free isotonic fluid of a composition previously described(7), with the addition of varying amounts of citric acid and the subsequent adjustment of the rinse to a pH of 7.4. For the peritoneal lavage, 30 ml of rinsing fluid were placed in the peritoneal cavity and allowed to equilibrate for 1 hour before removal. Calcium and citric acid analyses were made on the wash after removal. Calcium determinations of the wash were made by precipitation with ammonium oxalate, redissolving in perchloric acid, and reading on a Beckman flame spectrophotometer. Citric acid determinations were made using the Hunter and Leloir modification(9) of the method by Pucher *et al.* (10).

Results. The data obtained in these studies are presented in 3 groups: 1) *Comparison of effect of exogenous citric acid in normal and nephrectomized rats*: Concentrations of citric acid of from .25 to .5% were added to calcium-free rinses administered to normal and 24 hr nephrectomized rats. Calcium and citric acid levels of the wash removed after one hour equilibration are given in Table I. The large part played by the kidney in the metabolism of citric acid is shown by comparison of normal and nephrectomized animals. It is obvious that in the normal animals much of the citric acid was removed by the kidney within the hour. The citric acid remaining in the wash of the nephrectomized animals was proportional to the amount added to the rinse, and for the first rinse appeared to approach a simple dilution with the extracellular fluid. In both the normal and the nephrectomized animal, the exogenous citric acid produced a marked rise in the calcium level of the wash. In each case the calcium level was a reflection of the wash level of citric acid rather than the administered level. Since the rinse used was calcium-free, this rise in calcium in the wash is indicative of the ability of exogenous citric acid to mobilize endogenous calcium.

2) *Comparison of effect of exogenous citric acid in nephrectomized and nephrectomized-parathyroidectomized rats*: Since the parathyroidectomized rats without kidneys were unable to survive a dosage of citric acid larger than 0.2%, this series of experiments was run using citric acid in the rinse in concentrations of 0.025 to 0.2%. The results are summarized in Table I. For comparative

TABLE I. Effect of Exogenous Citric Acid on Calcium and Citrate Content of First Peritoneal Wash.

Citric acid in rinse*	Calculated by dilution‡	Citric acid in wash†			Calcium in wash†		
		Normal	24 hr Neph	24 hr Neph 4 hr PTX	Normal	24 hr Neph	24 hr Neph 4 hr PTX
0		2.34 ± .43 (12)	1.48 ± .36 (16)	.96 ± .27 (17)		7.08 ± .36 (16)	3.49 ± .31 (17)
25	10.9		10.4 ± 2.6 (11)	16.2 ± 2.9 (11)		7.15 ± .45 (11)	3.52 ± .32 (11)
50	20.3		19.4 ± 3.4 (12)	28.8 ± 3.2 (9)		8.32 ± .61 (12)	3.72 ± .51 (9)
100	39.1		47 ± 4.8 (9)	67.1 ± 5 (12)		9.4 ± .72 (9)	5.5 ± .42 (12)
200	76.7		80 ± 5.7 (4)	89.6 ± 4.6 (12)		12.3 ± .86 (4)	5.7 ± .48 (12)
250	95.5	84 ± 8 (6)	96 ± 11 (6)		12.2 ± 1.2 (6)	13.8 ± 1.4 (6)	
400	151.9	89 ± 9 (6)	138 ± 22 (8)		12.4 ± 1.1 (6)	15.6 ± 1.7 (8)	
430	161.8	94 ± 12 (8)	152 ± 19 (14)		12.7 ± 1.2 (8)	16.8 ± 2.1 (4)	
500	189.5	98 ± 11 (7)	193 ± 26 (4)		12.5 ± 1.1 (7)	17.3 ± 2.6 (4)	

* Rinse = Fluid placed in peritoneal cavity. † Wash = Fluid removed from peritoneal cavity after 1 hr equilibration. ‡ Extracellular fluid calculated as 20% body wt.

All values given in mg/100 ml. No. in parentheses are No. of animals in group. Neph = Bilateral nephrectomy. PTX = Parathyroidectomized. Values given with stand. error $\left(\text{S.E.} = \sqrt{\frac{\Sigma d^2}{n(n-1)}} \right)$.

purposes, an earlier experiment utilizing a citrate-free wash was extended. Rats parathyroidectomized approximately 24 hours after nephrectomy showed a small but significant drop in citric acid concentration of the wash after one hour equilibration, accompanied by the marked drop previously noted (5) in calcium concentrations. Following administration of citric acid it can be seen that the rise in the wash values for this ion is greater in the parathyroidectomized animal than in their nephrectomized controls. It must be assumed, therefore, that the parathyroids affect citric acid metabolism at a site other than the kidney. Despite the higher citric acid content of the wash, the calcium content of the wash from the parathyroidectomized rat was lower than its nephrectomized control, though a significant rise in calcium was noted. It would appear, therefore, parathyroidectomy affects the ability of citric acid to remove calcium from bone.

3) *Disappearance of exogenous citric acid administered in successive washes to normal, nephrectomized, and nephrectomized-parathyroidectomized rats*: Successive peritoneal rinses were carried out in each rat. Two rinses were done the first day, followed by 3 on the next day. The data are summarized in Table II. Despite the fact that a constant rinse concentration of citric acid was maintained for each rat, the wash concentration of this ion gradually decreased. This progressive drop in wash concentration of citric acid was seen in all 3 groups. It must, therefore, be assumed that the second site of citrate metabolism is extra-renal and does not require parathyroid hormone. The calcium level also dropped in each successive wash, indicating that the calcium rise was a function of the citric acid concentration of the wash.

Discussion. These experiments clearly demonstrate that while citric acid is able to mobilize calcium even in the absence of the parathyroid gland, this function is much curtailed after parathyroidectomy. This is borne out, first, by the drop in calcium and citric acid following parathyroidectomy and, second, by the smaller increases in calcium

TABLE II. Calcium and Citric Acid Levels in Washes of Successive Peritoneal Rinsings.

Animals	Citric acid in rinse	Citric acid in wash				Calcium in wash			
		2nd	3rd	4th	5th	2nd	3rd	4th	5th
Normal	250	70 ± 7 (6)	57 ± 6 (6)	48 ± 6 (6)	46 ± 5 (6)	11.4 ± .9 (6)	10.9 ± .7 (6)	10.5 ± .6 (6)	10.5 ± .4 (6)
	500	90 ± 13 (7)	73 ± 9 (7)	61 ± 7 (5)	56 ± 6 (5)	12.5 ± 1.1 (7)	10.9 ± .83 (7)	10.6 ± .7 (3)	10.8 ± .9 (5)
Neph	25	8.8 ± 2.1 (11)	6.2 ± 1.6 (11)	5.7 ± 1.1 (11)	5.8 ± 1.4 (11)	6.8 ± .42 (11)	6.91 ± .41 (11)	6.82 ± .37 (11)	6.64 ± .44 (11)
	100	35.9 ± 5.7 (9)	31.4 ± 4.9 (9)	20.2 ± 5.3 (9)	19 ± 6.1 (9)	7.75 ± .69 (9)	8.1 ± .73 (9)	7.6 ± .64 (9)	7.4 ± .67 (9)
	250	74 ± 9 (6)	61 ± 8 (6)	53 ± 7 (6)	51 ± 5 (6)	13.8 ± 1.4 (6)	13.1 ± 1.1 (6)	12.4 ± .9 (6)	12.2 ± 1 (6)
	500	158 ± 24 (4)	142 ± 27 (4)	134 (2)	123 (2)	14.6 ± 18 (4)	12.5 ± 1.3 (4)	11.6 (2)	11.1 (2)
Neph PTX	25	16.2 ± 2.9 (11)	13.7 ± 2.4 (11)	10.2 ± 1.8 (11)	7.3 ± 1.7 (11)	3.44 ± .34 (11)	3.19 ± .29 (11)	3.4 ± .3 (11)	3.23 ± .33 (11)
	100	56.9 ± 6.3 (12)	40.8 ± 4.2 (12)	33.6 ± 5.5 (12)	23.5 ± 4.7 (12)	5.3 ± .38 (12)	5.05 ± .35 (12)	4.8 ± .47 (12)	4.4 ± .38 (12)

For notes—See Table I.

in the equilibrated washes of the parathyroidectomized rats administered citric acid. The lower calciums occurred despite the fact that these washes contained higher citrate levels than their nephrectomized controls.

Of equal interest is the disappearance of exogenous citric acid from the peritoneal wash. Experimental evidence by Martensson(11) and Freeman and Chang(12) has indicated the kidney as the main site of citrate metabolism. The evidence presented here substantiates this renal function. However, it has also been postulated that the liver, in addition to the kidney, may be able to metabolize citrate(13). These data demonstrate that in the presence or absence of the kidneys another site of citrate metabolism was activated or increased its function when citrate was administered via a peritoneal lavage. While investigation of the exact site was not undertaken, the non-effect of the parathyroids on this mechanism would tend to remove the bone from consideration. However, a definite effect of the parathyroids on citrate metabolism is seen in the slower rate of exogenous citrate loss from even the first rinse in the parathyroidectomized animal. Since this slower rate is accompanied by a decreased ability to mobilize calcium, a direct relationship of the parathyroid hormone with calcium and citric acid in bone is indicated.

The authors believe that these findings add strength to the idea that citric acid may use complexing as a means of increasing extracellular calcium. A recent article by Dixon and Perkins(14) on citric acid and bone reviewed and discussed the evidence for a calcium-citrate complex in blood, bone, and extracellular fluid. They expressed belief that such a complex would leave calcium in such a state that it would be unavailable to exert its influence on the transmission of nerve impulses. The evidence in this and other experiments supports such an idea. In the present experiment, it was noted the nephrectomized and parathyroidectomized-nephrectomized rats tolerated progressively smaller amounts of citrate and death was accompanied by violent tetany within 15 minutes of citrate administration. Data on parathyroidectomized rats in tetany from the effects of endogenous citrate acid showed plasma calcium values of 18-20 mg%(6).

While much remains to be done in determining where citric acid fits into the picture of bone metabolism, evidence is accumulating which suggests the possibility that citric acid is involved in the pathway by which bone salts are deposited and resorbed. The evidence also indicates that these processes are influenced or controlled by the parathyroid glands.

Summary. 1. Introduction of exogenous citric acid in a neutralized rinsing fluid into the peritoneal cavities of normal, nephrectomized and parathyroidectomized-nephrectomized male rats was studied. Normal rats survived relatively large amounts of citric acid. Nephrectomized rats were able to survive amounts of citric acid up to 0.50%; parathyroidectomized - nephrectomized rats only up to 0.20%. 2. Normal, nephrectomized, and parathyroidectomized animals showed increases in wash calcium when optimal amounts of citric acid were present in the rinsing fluid. Parathyroidectomized-nephrectomized rats given the same amount of citric acid in the rinsing fluid were found to have higher wash citric acids but lower wash calciums than their nephrectomized controls. In each group of rats the highest calcium and citric acid levels usually occurred in the first wash. Subsequent washes generally showed progressively smaller amounts of calcium and citric acid, even though the amount of citric acid in the rinsing fluid was never changed within a group throughout all the rinsings. Possible explanations are discussed.

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Studies on Thymus of the Mammal. X. Regeneration of Irradiated Mouse Thymus.*† (23024)

CHRISTIANNA SMITH AND DOROTHY ANNE KIEFFER

Department of Zoology, Mount Holyoke College, South Hadley, Mass.

The source of cells which appear in the cortex of a thymus regenerating after accidental involution has been questioned because the number of observed mitoses has been considered insufficient to account for the reconstitution. Murray(1) states that repopulation of the cortex after total-body irradiation proceeds outward from the medulla. Both Murray and Baillif(2), in a study of thymuses involuted after chlorazol black E injections, consider the possibility that new lymphocytes may be derivatives of the epithelial reticulum. However, in comments on epithelial origin of the thymic lymphocytes, Downey(3) suggests that "It is possible that these conclusions can be explained by a failure to distinguish between the epithelial and mesenchymatous portions of the thymic stroma." In the present investigation, the appearance of dividing cells in thymuses regenerating after total-body sublethal dose of gamma rays (400 r) was studied in animals receiving an injection of colchicine previous to their sacrifice. Cells in mitosis appeared all through the cortex and the evidence indicates that they are mesenchymal reticular cells and medium-sized lymphocytes.

Materials and methods. Most of the animals used in these experiments were CAF 1 male mice, 4-5 weeks old from the Roscoe B. Jackson Laboratory. Four were of the BALB/c strain. The series studied included normal animals and mice which had been irradiated 24, 36, 52 hours, 3, 5, and 7 days previously. The dose was 400 r, Cobalt 60: aluminum filter; 38 cm from source. Time of exposure varied from 9′ 3″ at the beginning of experiments to 10′ 3″ at the end. In general, thymuses used for counting cells were fixed in Bouin's fluid and stained in hematoxylin and eosin. May-Grünwald and Giemsa solutions were also used to stain both Bouin and Helly fixed material for the study of cell types. Sections were cut horizontally at 5 μ. All mitotic counts were made from animals injected subcutaneously with 1/20 cc of freshly prepared 0.5% solution of colchicine 6 hours before they were killed by cervical dislocation. As the purpose of these studies was to determine distribution of dividing cells in the regenerating thymus, time of injection had to be determined by the hour of irradiation (9 a. m.). The 24 hour irradiated mice were injected at 3 a. m., the 36 hours, at 3 p. m. and the 52 hours, at 7 a. m. All others were injected at 9 a. m. except three 3-day mice, at 7 a. m. Counts of resting and dividing cells were made with a net micrometer (56,000 cu. μ, 6x7 rows) under oil immersion at a magnification of 970 times.

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