duce a plethora is not helpful when vascular muscle has lost tone.

Conclusion. Antiadrenergic therapy by coeliac blockade or phenoxybenzamine injected *via* the upper abdominal aorta prevents death from otherwise lethal endotoxic or hemorrhagic shock if administered within 30 minutes after onset of the shock state. Phenoxybenzamine intravenously is not effective in twice the dose that is effective when it is administered *via* the upper abdominal aorta. The failure of antiadrenergic therapy can be accounted for by the speed with which severe ischemia inflicts irreversible injury to vascular muscle and to the endotoxin-detoxifying mechanisms in the splanchnic tissues.

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Effect of Thyrocalcitonin, Administered During Peritoneal Lavage, on Removal of Bone Salts and Their Radioisotopes.*‡ (31809)

D. C. KLEIN, H. MORII,† AND ROY V. TALMAGE
Rice University, Houston, Texas

The existence of thyrocalcitonin, a hypocalcemic agent produced in the thyroid of all mammals studied, has been thoroughly confirmed(1-3). This hormone has been extracted in relatively pure form and is known to be a peptide of low molecular weight, similar in some respects to parathyroid hormone(1,4-7). While several investigators have explored the possibility of an extraosseous site of action of this principle(1,8), it is generally assumed that bone is its primary target(9). Experiments have demonstrated that increased secretion of thyrocalcitonin occurs during conditions of hypercalcemia(10). Whether or not it is also secreted at lower rates in normal or hypocalcemic conditions has not yet been established. Such information will be of importance in determinining the physiological significance of this newly identified hormone, since in the normal mammal the problem of hypocalcemia tends to be more acute than that of hypercalcemia.

A major question concerning the action of thyrocalcitonin is whether its hypocalcemic effect is accomplished through increasing bone accretion, or by decreasing the processes removing calcium from bone, or both. It has been pointed out by Gaillard that the hormone "can nearly abolish the effect" of parathyroid extract on his organ culture of mouse radii(11). The hormone's effect on inhibiting bone resorptive processes has also been demonstrated by Friedman and Raisz(12) and by Aliapoulios *et al*(9), using *in vitro* systems. Recent *in vivo* studies by Johnston and Deiss(13) have also led to the suggestion that thyrocalcitonin inhibits metabolic bone resorption. Since the peritoneal lavage technique employed in our laboratories exaggerates bone resorptive processes by removing an average

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† Present address: Dept. of Biochemistry, Univ. of Wisconsin, Madison.

of 1.6 mg of calcium/100 g body wt/hr from bones of normal rats, and 1.3 mg of calcium/100 g body wt/hr from bones of parathyroidectomized rats(14), experiments were designed to test the effect of thyrocalcitonin on calcium removal from bone, and to compare this effect with the reduction in bone resorption due to parathyroidectomy.

Materials and methods. Thyrocalcitonin preparation. Thyrocalcitonin was prepared from fresh porcine thyroid tissue according to the procedures described by Hirsch *et al* (1). Active fractions from the eluent of a Sephadex G-50 chromatographic column were pooled and lyophilized. Activity was assayed according to the method of Hirsch *et al*(1). A standardized dose of 2-4 units containing 25 μg protein(15) was used in all experiments unless otherwise indicated. All thyrocalcitonin administered was obtained from a single extraction. Analysis of blood (1.5 ml) obtained by serial cardiac punctures indicated a drop of 20% in serum calcium values one hour after injection of 2-4 units in 180-200 g Holtzman male rats, with the calcium levels returning to normal within 3 hours.

Peritoneal lavage. Details of the technique of peritoneal lavage have been described(16). For these experiments a buffered, isotonic rinse maintained at a pH of 7.4 and containing 2.0% glucose was used. A continuous resorption of bone was stimulated by keeping both normal and parathyroidectomized rats on a calcium-free diet for 24 hours and using a lavage rinse devoid of calcium and phosphate ions. Thirty ml of the rinse were introduced into the peritoneal cavity of each animal through stainless steel plugs and were replaced hourly. Each wash was subjected to the desired analyses. In all but one experiment, thyrocalcitonin was injected in a single subcutaneous dose after the third hour of an 8-hour lavage. Fig. 1 shows the similarity of effect of thyrocalcitonin on the serum calcium of normal animals and on the lavage calcium of animals undergoing peritoneal lavage.

In addition to the studies in which a single dose of thyrocalcitonin was administered, a supplementary experiment was performed to determine the effects of continuous stimulation on the removal of the inorganic ions. Thyrocalcitonin, at twice the dosage level described above, was administered subcutaneously at the start, and after the second and fifth hours of lavage. Otherwise, protocol was as described above.

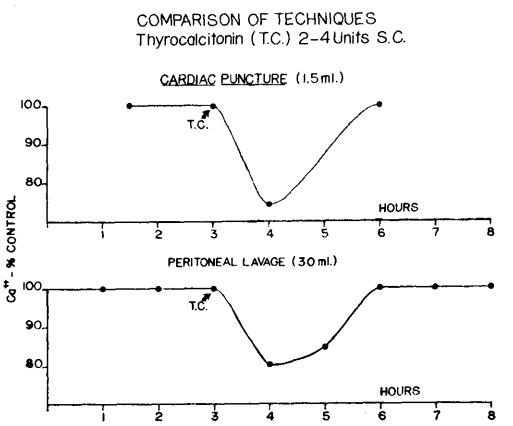

FIG. 1. Comparison of standard dose of thyrocalcitonin when given to normal animals and animals undergoing peritoneal lavage. Both figures give calcium values expressed as percent reduction in treated animals. Maximum drop in each case is significant with $P<.001$. Each point represents the average of 18 more animals.

Radioisotope administration. For these experiments Ca^{45} and P^{32} with a negligible amount of carrier were employed. In one group of animals, approximately 50 μc of either of the isotopes were administered in 3 daily doses, starting 3 weeks before the scheduled lavage. Other groups of animals received a single injection of approximately 15 μc of either of the isotopes 18 hours prior to lavage. The peritoneal washes removed from the animals were analyzed for these 2 isotopes by standard radiometric procedures.

Parathyroidectomy. The parathyroids were removed surgically with fine forceps, 9 hours prior to start of lavage.

Analyses. Calcium and magnesium were determined fluorometrically using the automated methods of Hill for the Antoanalyzer (17,18). Phosphorus was analyzed as phosphate by the method of Allport and Keyser (19).

Results. Effects of thyrocalcitonin on removal of calcium and phosphate by peritoneal lavage. The effects of a single injection of thyro-

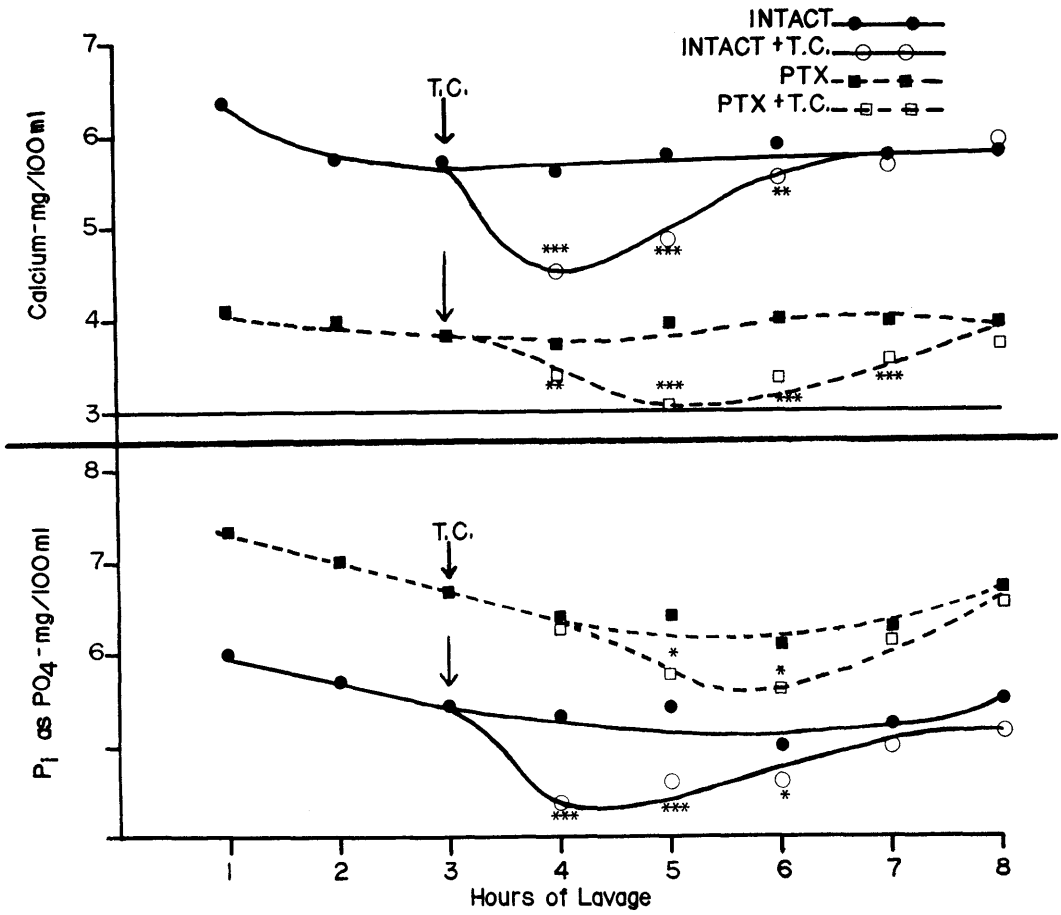

FIG. 2. Effect of a single dose of thyrocalcitonin (2-4 U) on removal of calcium and phosphate by peritoneal lavage. Note: Each point in the graph is a mean of 10-36 values. PTX = parathyroidectomized; TC = thyrocalcitonin injected (2-4 U). Statistical significance of the drop in removal rate is shown by asterisks at each point: * = $P<.05$; ** = $P<.01$; *** = $P<.001$.

calcitonin administered after the third hour of peritoneal lavage on calcium and phosphate removal from the extracellular fluids are summarized in Fig. 2. Because of previous investigations and the design of this experiment, it is assumed the source of most of these 2 ions was bone(20,21). It should be noted that the degree of suppression of removal of phosphate and calcium was similar. The only differences noted in the absence of endogenous parathyroid secretion were the delay of the maximum hypocalcemic effect of thyrocalcitonin until the second hour after injection and the extended time necessary for recovery from the resulting hypocalcemia.

Effects of thyrocalcitonin on removal of magnesium by peritoneal lavage. The relationship of magnesium to calcium metabolism(22) and bone(23) prompted the study of effects of thyrocalcitonin on the levels of magnesium in lavage fluid. Since no clear cut effect of thyrocalcitonin on magnesium removal had been observed in experiments in which a single dose (2-4 units) of the hormone had been administered during the third lavage hour, an experiment was designed in which thyrocalcitonin (4-8 units) was injected 3 times, as described above. This was done to maintain the hormonal effect continuously over an 8-hour period. Results appear in Table I. It is apparent that while multiple thyrocalcitonin stimulation resulted in the expected suppression in the removal of bone calcium and phosphate, there was

TABLE I. Effect of Continual Stimulation by Exogenous Thyrocalcitonin on Removal of Calcium, Phosphorus and Magnesium by Peritoneal Lavage.

	Average ion concentration in hourly lavage fluid*		
	Calcium (mg/100 ml)	Phosphorus (mg/100 ml)	Magnesium (meq/l)
Experimental group			
Control	5.8 ± .1	5.3 ± .1	.77 ± .02
Thyrocalcitonin	4.2 ± .1	3.7 ± .2	.75 ± .02
Treatment differences	1.6 ± .1†	1.6 ± .2†	.02 ± .02‡

* Each value represents mean ion concentration in lavage fluid from 4 animals over 8 hours for calcium and magnesium. Only 5 hours are used for phosphorus. Values reported as means ± standard error.
† $p < .001$.
‡ Not statistically significant.

no accompanying suppression in the removal of magnesium ions. In contrast, 9-hour parathyroidectomy resulted in reduced magnesium levels in lavage fluids collected during an 8-hour lavage (Table II).

Effect of thyrocalcitonin on long term isotope removal. In this series of experiments, thyrocalcitonin was administered to animals which had received injections of radioisotopes 3 weeks before lavage. After this period of time, these isotopes are sufficiently incorporated into bone so that their removal is influenced by parathyroidectomy to the same degree as the removal of stable calcium and phosphate(20,24,25).

The effect of thyrocalcitonin on the removal of Ca^{45} and P^{32} is summarized in Fig. 3. The removal of both radiocalcium and radiophosphorus, administered 3 weeks prior to experimental use, was decreased by thyrocalcitonin, as was that of total calcium and phosphate. This effect was noted both in parathyroid-intact and parathyroidectomized rats. The failure of the radiocalcium values to return to normal in the parathyroidectomized animals by the end of the eighth hour of lavage was the only major difference produced by the concurrent absence of endogenous parathyroid secretion.

TABLE II. Magnesium in Lavage Fluid of Intact and Parathyroidectomized Animals.

	Avg magnesium concentration (meq/l) in hourly lavage fluid*
Experimental group	
Control	.74 ± .01
Parathyroidectomized	.65 ± .02
Treatment difference	.09 ± .02†

* Each value represents mean ± standard error of magnesium concentration of lavage fluid from 8 animals for 8 hours.
† $p < .001$.

Effect of thyrocalcitonin on short term radioisotope removal. The experiments described above were repeated in animals given the isotopes only 18 hours prior to the start of the lavage. After this short period, parathyroidectomy has little influence on the removal of recently deposited isotope(24,25). These data are also summarized in Fig. 3. The only difference between the short and long term isotope experiments was the magnitude of the suppression of radioacticity removal by thyrocalcitonin. The reduction in isotope removal in these short term experiments was considerably less than for their stable counterparts. Because of the small statistical differences ($P<0.05$) observed with short term Ca^{45}, these experiments were repeated using twice (4-8 units) the standard dose. The larger dose essentially doubled the suppression of the removal rate for the isotope.

Discussion. In an experimental system which produces a high rate of bone resorption, thyrocalcitonin has a marked inhibitory effect on net calcium removal in both control and parathyroidectomized rats. This is demonstrated by the suppression in removal rates of both stable calcium and phosphate and their radioactive isotopes in animals undergoing peritoneal lavage. While these studies do not rule out an effect of the hormone on bone formation or bone salt deposition, they strongly support the

premise that the primary action of the hormone is through its inhibitory effects on one or more bone resorptive processes(9,12, 26). This is illustrated by the following: (1) the hormone suppresses equally serum calcium and calcium removed by peritoneal lavage; (2) thyrocalcitonin suppresses calcium and phosphate removal in calcium-phosphate ratios which suggest this is due to an inhibition of the breakdown of apatite crystal; (3) the effect of thyrocalcitonin on previously administered radioisotopes is greater than that on those recently deposited; and (4) degree of suppression of removal of previously administered radioactivity is the same as that for stable calcium and phosphate. If the major effect had been on bone accretion processes, the changes in the removal rate of recently deposited isotopes, rather than those previously administered, would have more nearly mimicked changes in stable calcium and phosphate.

Since parathyroid hormone stimulates bone resorption, it is of interest to compare the effects of parathyroidectomy to those produced by injection of thyrocalcitonin. First, as noted above, thyrocalcitonin is

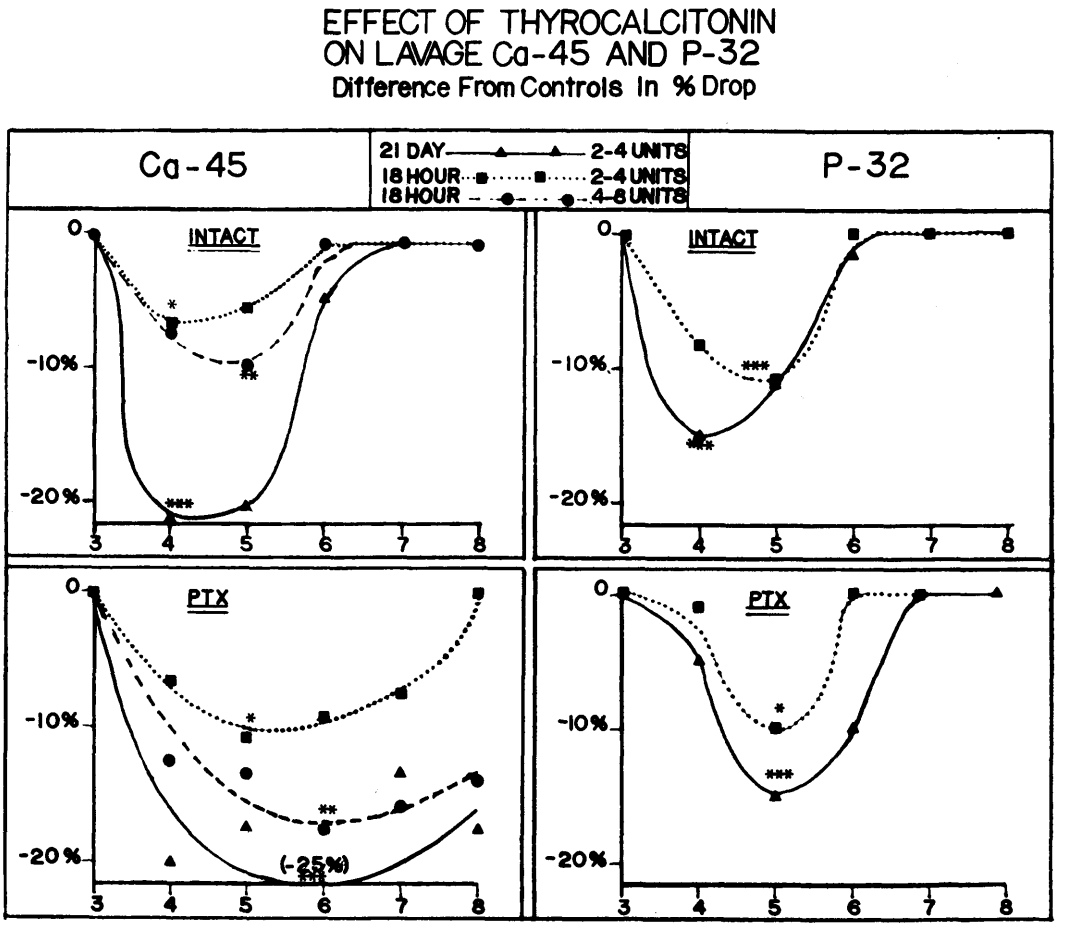

FIG. 3. Effect of thyrocalcitonin on removal of radioisotopes administered 18 hours and 3 weeks prior to lavage. 1) Data are presented as percent reduction from control value during 3rd to 8th hours of peritoneal lavage. 2) All points are averages of values taken from 6 or more animals except for the top line, (■) of the Ca^{45}-PTX group which is the average of 4 values. 3) PTX = parathyroidectomized. 4) Legend on graph refers to time of administration of radioisotopes and amount of tyhrocalcitonin given. 5) Statistical significance of the drop in removal rate is shown by asterisks at each point: * = $P<.05$; ** = $P<.01$; *** = $P<.001$.

equally effective in parathyroidectomized and control rats. Secondly, while a drop in serum phosphate or the removal rate of this ion during peritoneal lavage can be demonstrated following parathyroidectomy in nephrectomized rats(21), these changes are hidden in animals with functional kidneys by the over-riding parathyroid effects on renal handling of phosphate. In contrast, phosphate changes are marked following thyrocalcitonin injection in rats with functional kidneys.

It is obvious that while thyrocalcitonin appears to negate, at least temporarily, the effects of parathyroid hormone on increasing calcium and phosphate transport from bone, its effects appear to be more comprehensive. If one assumes there is only one major bone resorptive process, one might conclude thyrocalcitonin acts only at the same biochemical sites as does parathyroid hormone, but antagonistically to it. In such a system, thyrocalcitonin would negate not only the stimulation produced by parathyroid hormone, but would further reduce the activity of this resorptive process. An alternative explanation is that there are two or more processes for the resorption of bone salts, only one of which is stimulated by parathyroid hormone. However, both or all are suppressed by thyrocalcitonin. In the light of current concepts of bone breakdown (16,27,28,29), we feel the present evidence favors the second explanation.

Summary. The effect of porcine thyrocalcitonin has been studied in the rat using an experimental system which produces a high rate of bone resorption. The technique utilized was that of peritoneal lavage with a calcium- and phosphate-free rinse. The hormone extracted from thyroid glands was administered either as a single injection following the third hour of lavage, or as multiple injections administered at intervals of 2 to 3 hrs. Thyrocalcitonin produced a marked suppression in removal rates of calcium, phosphate and their radioisotopes. It was also effective in animals parathyroidectomized 12 hours prior to lavage, though the recovery in these animals was slower than in those with intact parathyroid glands. Also, in all cases, the removal of radioactive isotopes administered 2 or more weeks prior to use of the animals was suppressed to a greater degree than those recently administered. Magnesium removal was not affected by administration of thyrocalcitonin. These studies support the conclusion that the primary action of the new hormone is related to its ability to rapidly reduce bone resorption. It is also suggested that while thyrocalcitonin inhibits parathyroid action, the two hormones are not solely antagonistic to each other.

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Chemical Characterization of Inbred Strain Mouse Milk. II. Total Fatty Acids and Fatty Acid Analyses.* (31810)

H. MEIER, W. G. HOAG, J. J. MCBURNEY, AND D. D. MYERS
(Introduced by N. Kaliss)
The Jackson Laboratory, Bar Harbor, Maine

Chemical analysis of inbred strain mouse milk is now possible through the development of a simple reliable milking apparatus and the recent progress in micro-analytical methods(1). A previous paper reported on certain gross compositional observations and amino acid patterns of milk from DBA/2J and C57BL/6J mice(2). The same 17 amino acids were present in both strains, but quantitative differences were evident especially in the free amino acid fraction. Since both strains were fed an essentially similar diet except for a small difference in fat content, the deviations in amino acids could be assumed to be due to the genotypic differences between the strains.

The purpose of this study was to characterize the fatty acid composition of mouse milk regarding number of different fatty acids, their levels, and possible differences between mouse genotypes.

Materials. The 2 inbred strains studied were DBA/2J and C57BL/6J. Both are non-agouti (gene symbols, *aa*), and they differ at the brown (*b*) and dilute (*d*) loci; C57BL/6J is wild type (+) at these 2 loci and DBA/2J is *bb dd*. F_1 hybrids, $B6D2F_1$, were obtained by mating C57BL/6J with DBA/2J females. $B6D2F_1$ females were backcrossed to DBA/2J males, yielding several kinds of offspring with respect to coat color. They were dilute black (*b*+ *dd*), brown (*bb d*+), and dilute brown (*bb dd*). The $B6D2F_1$ is identical with respect to coat color to the black backcross mice. Female offspring from each of the backcrosses were mated with DBA/2J males. The number of black backcross mice obtained was not sufficient to provide adequate milk for analysis. The milk was collected between the first and second week of lactation, and milk samples from 5 to 10 mice were pooled. Lactating mice were separated from their young for 16 hours, *i.e.*, overnight, and milked in the morning. The females had free access to food and water. The mouse diet contained 2% fiber, 19% protein from skim milk and wheat, and 11% fat from corn oil for all mice except DBA/2J whose diet contained only 8% fat. Ash content was 4.7%, calcium 1.0% and phosphorus 0.6%. The amounts of vitamins per kg were as follows: vit A 13640 USP, thiamine 8.2

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