series, especially WIN 3800 and WIN 4510, not only possess anesthetic activity considerably greater than that of tetracaine and dibucaine, but the topical anesthetic activity in experimental animals also indicates a greater margin of safety with regard to both irritancy and systemic toxicity.

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Parathyroid and Bone Citrogenase

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It is well known that when the parathyroid glands are removed the serum calcium falls, and, according to Albright and Reifenstein (*1*), the bones tend to become more dense in man, whereas after injection of parathyroid hormone the serum calcium rises and, in rabbits, a reduction of trabeculae of the epiphyses can be shown (*2*). Furthermore, in the experiments of Barnicot (*3*) and Chang (*4*) parathyroid tissue placed in contact with bone *in vivo* caused bone resorption, thus demonstrating a direct effect of the gland upon bone tissue. Therefore, in view of the influence of citrate on the solubility of calcium phosphate precipitated from solutions of physiological ranges of concentrations (*5*) and the presence in bone of enzymes governing the formation and further conversion of citrate (*6*), it seemed of interest to study the citrate content and citrogenase activity of bone in parathyroidectomized and normal rats, and in normal rats injected with parathyroid extract.

All animals were male albino rats and were fed on a stock diet of pellets containing 0.48% P and 0.63% Ca. Parathyroidectomized rats were killed 4–6 weeks after operation. In most cases the serum calcium level was determined immediately before sacrifice, the values obtained on the above Ca and P intakes (between 5 and 6 mg Ca/100 ml) indicating reasonable completeness of extirpation of the glands. Parathyroid hormone[2] was injected intraperitoneally 20 hr before sacrifice. In order to avoid any toxic effects, it was first dialyzed against distilled water until no further phenol reaction was obtained.

The proximal end of the tibia combined with the head and distal end of the femur (in each case comprising the epiphyseal and metaphyseal regions) was examined for citrogenase activity, and in some cases for citrate content, by methods previously described (*6*). The results obtained are given in Table 1.

TABLE 1

BONE CITRIC ACID AND CITROGENASE, AND SERUM CALCIUM IN PARATHYROIDECTOMIZED, NORMAL, AND INJECTED RATS*

Condition	Age in weeks	Bone citrogenase (mg citric acid formed/g tissue/hr)	Bone citric acid (mg/g tissue)	Serum calcium (mg/100 ml)
Normal	7	0.45 (2)		9.7 (2)
	10	0.24 (6)		9.5 (6)
	11	0.19 (7)	4.6 (4)	9.5 (5)
	24	0.09 (2)		—
4–6 weeks after parathyroidectomy	13	0.02 (5)	4.2 (4)	5.5 (3)
	14	0.0 (2)		5.3 (2)
	15	0.02 (3)		5.5 (2)
	18	0.01 (1)		—
4 days after parathyroidectomy	12	0.22 (3)		6.1 (3)
Normal, injected with parathyroid extract (150 u)	10	0.20 (3)		10.2 (3)
4 days after parathyroidectomy, injected with parathyroid extract (300 u)	12	0.21 (2)		12.5 (2)

* Figures in perens indicate the number of animals in the groups.

It can be seen that several weeks after parathyroidectomy the bone citrogenase activity had fallen to a very low level, far lower than that of normal animals of similar age range, whereas in the few determinations made the citrate content of the bones did not differ in the two groups. On the other hand, injection of a massive dose of parathyroid hormone into 10-week-old normals did not lead to any increase of bone citrogenase activity above the normal level for that age. In a few experiments which were performed on animals 4 days after parathyroidectomy, no lowering of the citrogenase activity was observed although the serum calcium had already reached a low level. Administration of parathyroid extract to such animals led to a considerable rise in serum calcium but no change in bone citrogenase activity. The serum cal-

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[2] Eli Lilly, B.P.C. 1934 units.

cium level of operated animals was much more sensitive than that of normal animals to injected parathyroid hormone.

The figures for normal animals show that, as might be expected, increasing age led to lowered bone citrogenase activity. Because the fall in bone citrogenase after parathyroidectomy appears to develop slowly it is probable that the fall in serum calcium produced immediately after parathyroidectomy is not due to change in bone citrogenase. Also, the failure to increase the bone citrogenase by parathyroid hormone injection may have been due to the insufficient time allowed for this effect to develop.

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The Effect of Malonate on *Salmonella typhimurium* Infection in Mice[1]

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The effect of changes in the tricarboxylic acid cycle on *Salmonella typhimurium* infections in mice has been investigated[2] in order to determine the extent to which host metabolism influences response to this pathogen. In a series of experiments over a period of nearly 3 years (*1*), it has been established that mice exposed to the hypoxia of altitude are more susceptible to Salmonellosis than normal control mice. This greater susceptibility, however, is not accompanied by any experimentally demonstrable change in the mechanisms of defense against infectious diseases. A tentative explanation for these observations suggests that the stress of altitude results in some alteration in metabolism which in turn makes the mouse succumb more quickly to the infection. In the absence of any experimental evidence with which to substantiate the hypothesis, the present work was undertaken.

The particular procedure adopted was based on essentially unrelated reports in the literature. Mice subjected to the hypoxia of a simulated high altitude synthesize less influenza A virus in the lungs than control mice at normal pressures (*2*). Similarly, mice injected with sublethal amounts of sodium fluoroacetate synthesize less influenza A virus in the lungs (*3*) and less poliomyelitis virus in the central nervous system (*4*) than normal control mice. This poison, which probably blocks aconitase or isocitric dehydrogenase (*5*), results, *in vivo,* in an accumulation of citric acid in lungs (*3*) and in other organs (*6*). Another metabolic inhibitor, sodium malonate, which blocks succinic dehydrogenase (*7*) and possibly other enzymes (*8*), also produces an accumulation *in vivo* of citric acid in sublethal amounts (*9*). Since sodium malonate is known to be metabolized (*10*) and excreted (*9*) within a comparatively short time, repeated injections are required in order to maintain the block of the citric acid cycle (*9*).

Female mice of the CF-1 strain, weighing 20–25 g, were infected intraperitoneally with 0.5 ml of a saline suspension of *S. typhimurium* containing approximately 250,000 cells. Normal control mice receiving this number of bacteria suffer the first casualty on the third day, except in rare cases, and most of the animals succumb after 6 days. Twenty of these mice were given intraperitoneal injections of 0.5 ml saline at hourly intervals, starting immediately after the bacteria were administered and continuing for a total of 8 injections. The survival data of these animals are shown in Table 1, column 3. Twenty infected mice were treated in similar manner except that they were injected with 20 mg sodium malonate dissolved in 0.5 ml of saline for a total of 8 injections with the results shown in Table 1, column 2. The survival data of 15 mice not infected but given the 8 injections of malonate are given in Table 1, column 4.

It is apparent that mice infected with *S. typhimurium* die much sooner than control mice when their tricarboxylic acid cycle is blocked by sublethal injections of malonate. The malonate alone is not lethal but when it is given to infected mice, many die within a period of 8 hr, long before the control mice show

TABLE 1

NUMBER OF MICE SURVIVING EXPERIMENTAL TREATMENT AT TIMES DESIGNATED

Time in hours from beginning of experiment	Mice inoculated with *S. typhimurium* and injected at intervals of 1 hr with:		Mice not infected but injected at intervals of 1 hr with
	8 × 20 mg malonate in 0.5 ml saline	8 × 0.5 ml saline	8 × 20 mg malonate in 0.5 ml saline
0	20	20	15
4	20	20	15
5	18	20	15
6	15	20	15
7	11	20	15
8	8	20	15
24	1	20	15
48	0	19	15
72		5	15

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