ster, and normal human serum were negative, but one serum from a proven case of trichinosis in man was positive at a dilution of 1/20.

Discussion. The role of this immobilization reaction in the pathogenesis of *S. mansoni* infection is unknown. It is possible that it bears some relationship to the ray-like structures surrounding the eggs imbedded in tissue as described by Hoeppli and Li(7).

The time of appearance of the factor in the blood indicates that a possible major stimulus for its production is the initial deposition of eggs. Egg laying activity begins between the 27th and the 34th day of infection with *S. japonica*(8) and presumably during the same period in *S. mansoni* infection. The application of this reaction to studies in man remains to be made. Its possible use in the diagnosis of chronic infections is suggested by the positive reactions in monkeys infected for 5½ years.

Summary. Miracidia of *S. mansoni* were promptly immobilized when placed in heated sera of hamsters and monkeys infected with this parasite. No immobilization occurred in sera from non-infected animals. The factor developed early in the infection and was still present in the sera of monkeys which had been infected with *S. japonica* 5½ years previously. The immobilization of the miracidium appears to result from immobilization of the cilia. Cilia immobilization became less complete in high dilutions of serum and was usually confined to specific areas of the miracidium.

Since this work was done the author has become aware of an abstract by Hiyashi who reported to the Japanese Parasitology meeting in Kyoto in 1950 (Abstract 101) that he had found immobilization of miracidia from the sera of infected animals.

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Influence of Parathyroid Extract on Citric Acid of the Serum. (20524)

MAURICE V. L'HEUREUX AND GILBERT J. ROTH (Introduced by M. B. Williamson.)
From the Department of Biochemistry, Graduate School and The Stritch School of Medicine, Loyola University, Chicago, Ill.

It has long been recognized that the parathyroid glands are intimately concerned with the metabolism of calcium and phosphorus. In recent years, evidence has accumulated for considering the possibility that the active secretion may influence the metabolism of citrate as well. An early observation is that of Sjöström(1) who reported that increased parathyroid function produces a corresponding increase in the citric acid content of the blood. A rough parallelism has been observed between urinary excretion of calcium and of citrate in a patient with hyperparathyroidism and in a subject with hypoparathyroidism given parathyroid extract(2). Alwall(3) demonstrated concomitant increases in serum calcium and serum citric acid following intramuscular injection of parathyroid extract into dogs. The injection of parathyroid extract into puppies has been noted to increase the citrate content of bone(4). Repeated subcutaneous injections of citrate into puppies lead to osseous changes very similar to those produced by large doses of parathyroid extract(5). Freeman and Chang(6) observed, without exception, a decline in both serum calcium and serum citric acid levels following thyroparathyroidectomy in dogs. It thus appears that changes in the level of serum calcium referable to the involvement of the para-

thyroid glands are associated with parallel changes in the citrate concentration, and that the metabolism of citrate is interrelated with other physiological effects of the parathyroid hormone.

In this report are presented observations on the influence of parathyroid extract on the serum citric acid of normal and thyroparathyroidectomized rats. The work was undertaken to explore the extent of the response to varying doses of parathyroid extract and the possibility that the dose-response relationship be of such a character as to provide the basis of a method to estimate the activity of parathyroid hormone preparations.

Materials and methods. Young adult female rats of the Sprague-Dawley strain maintained on Purina rat chow pellets were used. In all experiments, involving a 9-hour experimental period or less, the animals were previously fasted for a period of 16 to 18 hours. In those instances in which the animals were studied for 18 hours, food was removed from the cages 6 to 8 hours before the experiment was begun to insure that the post-absorptive state would be attained. Thyroparathyroidectomy was carried out on fed animals approximately 24 hours prior to the experimental period. Only water was allowed to all animals during the period of observation. Studies were made employing 3 different routes of injection: subcutaneous, intraperitoneal, and intravenous via the external jugular vein. Blood samples were obtained by cardiac puncture. Parathyroid extract (Lilly)* was used as a standard preparation throughout the experiments. For doses of less than 100 U.S.P. units, the extract was diluted with 0.9% sodium chloride solution so that, with the exception of those animals receiving 200 units, the volume injected was 1.0 ml. The method of Natelson, Pincus, and Lugovoy(7) was employed for the microestimation of serum citric acid. Isooctane (Phillips) was employed for the extraction of the pentabromoacetone. The optical density readings were made with a Coleman Model 11 Universal Spectrophotometer at 445 mμ, using rectangular cuvettes with a 4.0 cm light absorption path. The cuvette holder was a shielded side carrier modified for use with the small volume of fluid available for analysis.† For the analysis of serum calcium, the micro method of Rappaport and Rappaport(8) as modified by Biering(9) was employed.

Results and discussion. The subcutaneous injection of 50, 100 and 200 units of parathyroid extract resulted in definite increases in the serum citric acid level within 18 hours after administration. The major increment of this response appears to be elicited no earlier than 9 hours subsequent to the administration of the extract. In this respect, the time-response relationship is quite similar to that reported by many investigators for serum calcium(10). The administration of 200 units was no more effective in influencing the serum citrate levels than was 100 units under these conditions. The expectation that intraperitoneal and intravenous injections might lead to more consistently positive response was not realized in exploratory trials.

Since it is a generally accepted view that relatively smaller amounts of the hormone are required to maintain normal serum calcium levels in the thyroparathyroidectomized animals than are required to obtain increases in the serum calcium level in normal animals, attention was directed to the use of operated animals. The responses of thyroparathyroidectomized female rats 18 hours after the subcutaneous injection of 25, 50, and 100 units are shown in Table I. The greatest degree of uniformity and the most sensitive response to the injected dose was found under the experimental conditions of this study. The mean initial level of serum calcium 24 hours subsequent to bilateral thyroparathyroidectomy was 7.2 mg %. This value is in agreement with that given by Tweedy and Chandler(11) in their study correlating complete parathyroid extirpation with the drop in serum calcium level.

The responses to subcutaneous injections of graded doses of the extract are similar in

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TABLE I. Effect of Graded Doses of Parathyroid Extract on Serum Calcium and Serum Citric Acid of Thyroparathyroidectomized Rat 18 Hours after Subcutaneous Injection.

Dose units	No. of observations	Serum calcium: Mean initial level (mg %)	Serum calcium: Mean* change (mg %)	Serum citric acid: Mean initial level (mg %)	Serum citric acid: Mean* response (mg %)
25	7			4.07	+1.33 ± .28
50	6	6.9	+3.2 ± .30	4.70	+3.33 ± .35
100	6	7.7	+4.3 ± .37	4.88	+2.84 ± .30

* Mean ± stand. error of the mean.

the normal and thyroparathyroidectomized rat in that there is observed in both groups a rise in serum citrate to a maximum after which the administration of larger doses does not produce an increased response. In both groups, the maximal level to which the serum citrate rises was found to be very similar. However, the dose levels at which the phenomenon occurs is different in the 2 groups of animals. Thyroparathyroidectomized rats respond maximally to the administration of only 50 units, whereas normal animals respond maximally to 100 units. It is also observed from Table I that the operated animal is sensitive to the injection of 25 units. No special significance can be assigned at this time to the differences in serum calcium at the 2-dose levels of 50 and 100 units. The mean pre-injection levels of serum citrate in all the thyroparathyroidectomized rats used in this work (4.49 mg %) is significantly lower than that observed in all normal animals (6.75 mg %). There was also observed in the operated animals less variability between individual pre-injection levels and post-injection levels at a given dose than in normal animals after the administration of the same amount of extract.

Although influences exerted by parathyroid extract injections on serum citric acid levels in both normal and thyroparathyroidectomized animals are definite, these experiments do not of themselves fully define the quantitative nature of the observed response. From dose-response data accumulated on the operated animals, a linear relationship appears likely between 0 and 50 units. However, the experiences of this study lead to the conclusion that the use of serum citrate responses in the rat would offer no advantage over existent methods in assessing the potency of parathyroid hormone preparations.

As concerns the source of the increased citrate observed following injections of parathyroid extract no satisfactory explanation can be given. The discovery by Dickens(4) of considerable stores of citrate in bone would suggest that the blood changes after parathyroid extract injections or parathyroid extirpation reflect differences in the degree to which this substance is mobilized from bone.

Summary. The subcutaneous injection of parathyroid extract into normal and thyroparathyroidectomized rats results in definite increases in the serum citric acid level. Dose-response data indicate that the rise in serum citrate varies with the dose of the extract, the route of the injection and the period of observation. The level of serum citrate of thyroparathyroidectomized rats was found to be significantly lower than that of normal animals. Increased sensitivity and greater uniformity of response to the injected dose was observed in the operated animals as compared to normal animals.

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