vations of the MR of vaccinia virus in the cells of the CAM. In fact it remains a most plausible explanation of the results.

Summary. Irradiated vaccinia virus preparations increase in pock titer when the virus particles are subsequently induced to aggregate. Measurements by electron microscopy of the degree and mode of aggregation indicate the operation of a highly efficient multiplicity reactivation of the virus in the cells of the CAM. There is evidence for penetration of the cell and pock production by groups of 5 or more virus particles.

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Effects of Porcine Thyrocalcitonin on Serum Calcium, Phosphorus And Magnesium in the Monkey and in Man.* (31417)

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Thyrocalcitonin is a polypeptide hormone which acts to lower the serum calcium(1-3). It has been isolated from the thyroid glands of a number of animal species including man (4).

In the present work studies are presented to show that porcine thyrocalcitonin is active in the monkey and in normal human subjects.

Materials and methods. A crude extract of thyrocalcitonin was prepared from porcine thyroid glands by the method of Tenenhouse, Arnaud and Rasmussen(3). The trichloroacetic acid (TCA) precipitate was suspended in 0.1 M acetic acid and the TCA was removed by titrating to pH 3.4 with an excess of Dowex 1X2 acetate resin. The mixture was stirred for one hour, filtered and lyophilized. In the studies in monkeys, the powder was dissolved in 0.15 M sodium chloride and the pH was adjusted to 2.5 with 0.1 N hydrochloric acid. In the studies with human subjects, the powder was washed in a sterile funnel with acetone, and taken up in a sterile 0.15 M solution of sodium chloride with 0.25% phenol and acidified by addition of hydrochloric acid. Before use, it was shown to be free of bacterial contamination. The extract was assayed in young male Sprague-Dawley rats weighing between 100 and 120 g. Before the assay they were fed a low calcium diet for 4 days and fasted for 16 hours. Five or six rats were used to assay each dose. The mean fall in serum calcium 1½ hours after subcutaneous injection was 1.8 mg % for 1.0 mg of the extract and 0.8 mg % for 0.1 mg.

Studies in monkeys. One male and 5 female rhesus monkeys, *Macaca mulatta,* were used. Weights ranged from 3.0 to 5.9 kg. The monkeys were fasted overnight and were given phenobarbital, approximately 30

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mg/kg subcutaneously before the study. Thyrocalcitonin was given subcutaneously in a volume which varied from 0.2 to 1.2 ml. Blood samples were obtained before injection of the extract and at intervals up to 24 hours after administration and were analyzed for calcium(5) and for phosphorus(6) and magnesium(7) with methods modified for the Auto Analyzer. The monkeys were kept anesthetized with intravenous injections of phenobarbital until after the last blood sample, were returned to their cages and allowed to eat and drink *ad libitum*. Blood samples were obtained the next morning with the animals awake.

Studies in human subjects. The subjects were 3 normal adult male volunteers ranging in age from 34 to 39 years. Their weights ranged from 73 to 86 kg. Before administration they were tested with an intradermal injection of 0.1 ml of a 1:100 dilution of the extract. The tests were negative. The subjects were fasted overnight and were allowed to drink tap water throughout the study.

Thyrocalcitonin was given subcutaneously in a volume which varied from 0.2 to 0.3 ml. Blood samples were obtained before injection and at intervals of up to 6 hours after injection and were analyzed for calcium, phosphorus and magnesium. No systemic symptoms were noted.

Control studies in which the vehicle alone was injected were carried out in both the monkeys and normal subjects.

Results. In 4 monkeys thyrocalcitonin, 3 mg/kg given subcutaneously, produced decreases in serum calcium which were maximal 3 to 6 hours after injection and which, in 2 of them, lasted as long as 24 hours (Fig. 1). In 2 monkeys thyrocalcitonin, 15 mg/kg given subcutaneously, produced decreases in serum calcium which were maximal 2 and 6 hours after injection and lasted as long as 8 hours (Fig. 2).

In 3 normal human subjects, thyrocalcitonin, 0.15 mg/kg given subcutaneously, produced transient decreases in serum calcium which were maximal between ½ and 2 hours (Fig. 3).

Thyrocalcitonin produced no change in serum magnesium (Fig. 1-3). In control studies, serum calcium varied very little (Fig. 2a and 3a) and the changes in serum phosphorus were the same as those observed after administration of the hormone.

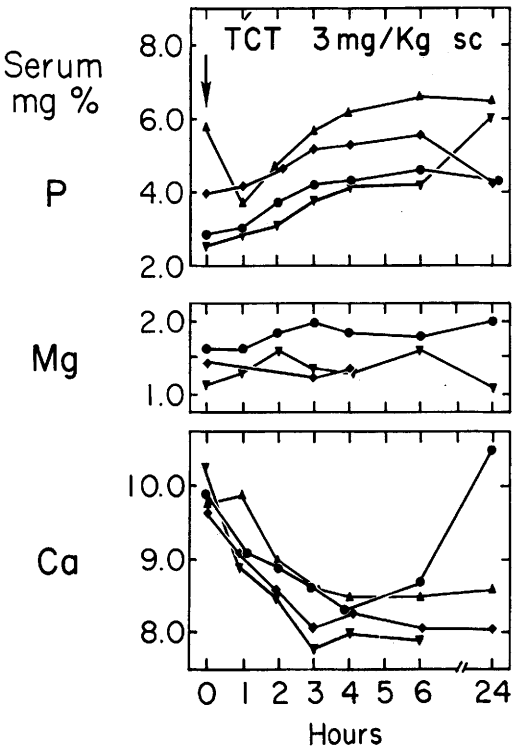

Fig. 1. Effects of porcine thyrocalcitonin (TCT), 3 mg/kg, on serum calcium (Ca), phosphorus (P) and magnesium (Mg) in 4 monkeys.

Discussion. The results demonstrate that a crude extract of porcine thyrocalcitonin is effective in decreasing the serum calcium in man and in the rhesus monkey. There appears to be a dose-response relationship: the largest decreases occurred in the monkeys given 15 mg/kg, whereas the smallest decreases were in the human subjects given 0.15 mg/kg. Serum phosphorus and magnesium were not consistently altered. It appears likely that the rhesus monkey provides a suitable means for testing preparations of thyrocalcitonin before administration to human subjects.

In previous studies, extracts of porcine thyrocalcitonin lowered the serum calcium in the dog(8,9), goat(10), rat(1-3) and man (4,11). Activity has been demonstrated in

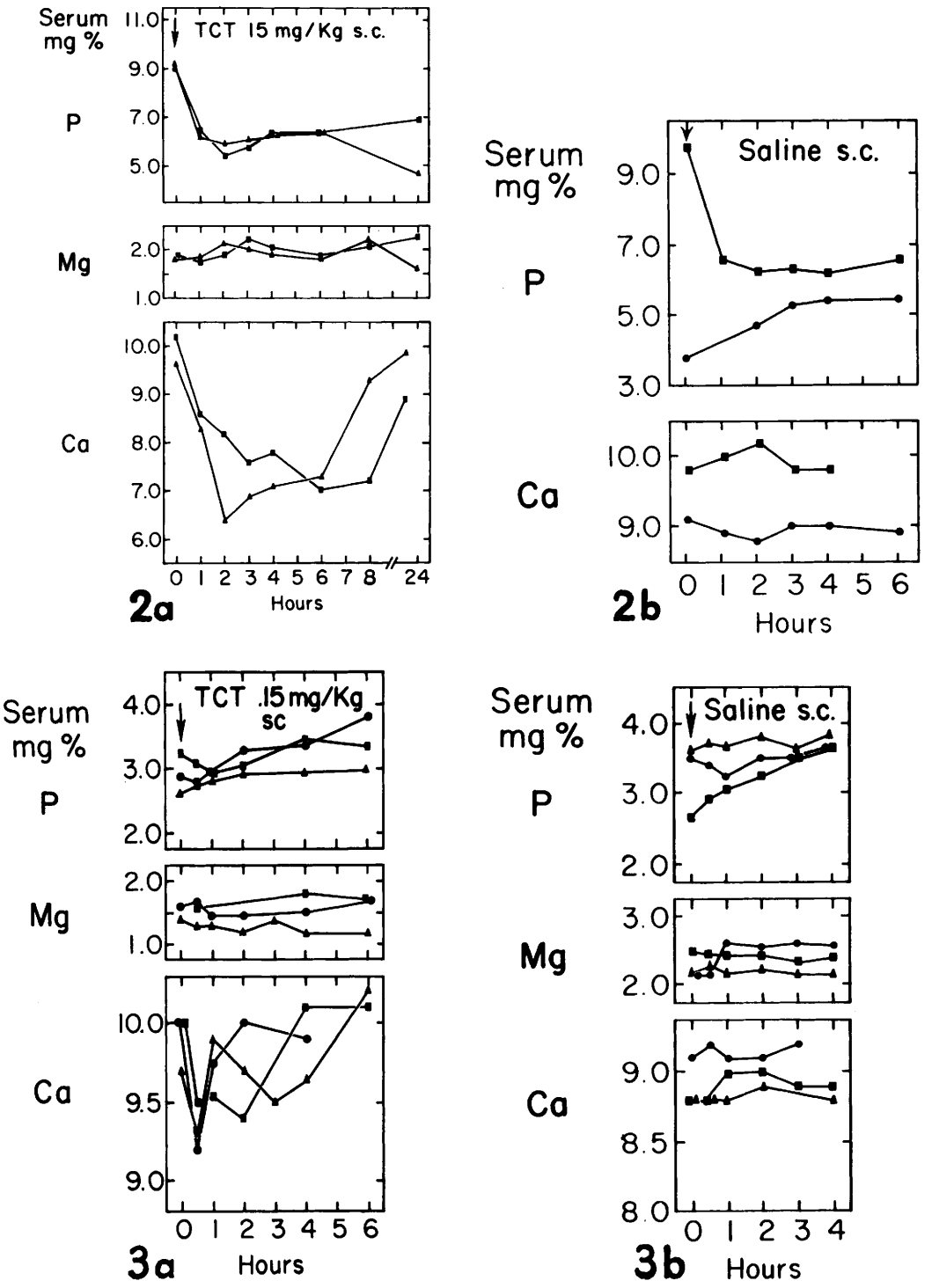

FIG. 2. Effects of porcine thyrocalcitonin (TCT), 15 mg/kg (a), and of saline vehicle (b) on serum calcium (Ca), phosphorus (P) and magnesium (Mg) in normal monkeys.

FIG. 3. The effects of porcine thyrocalcitonin (TCT), 0.15 mg/kg (a), and of saline vehicle (b) on serum calcium (Ca), phosphorus (P) and magnesium (Mg) in three normal men.

extracts of thyroid glands from rat, rabbit, pig, dog, ox, monkey, goat(1-3,8-10) and man(4). That extracts of human thyroid tissue, obtained at post mortem, prepared as described in *Methods,* lower serum calcium in the rat has been confirmed in this laboratory. Five mg of crude extract produced a mean fall of 1.8 mg % in rats. One mg of material was inactive.

While the present investigation was in progress, 2 studies of the effects of extracts of porcine thyroid glands in man were reported. Milhaud and associates(4) noted a maximum mean decrease of 0.4 mg % in four human subjects. The decreases were noted at one-half hour, were maximal at one hour and lasted as long as 2 hours after intravenous injection of 160 "units" (approximately 0.16 MRC units, see below) of thyrocalcitonin. Their material contained about 200 "units" per mg (a "unit" is defined as the quantity which will lower the serum calcium of a rat 0.1 mg % 50 minutes after intravenous injection). Foster and associates(11) administered purified pig thyrocalcitonin containing from 1 to 22 MRC units(12) intravenously to 3 subjects with hypercalcemia due to metastatic carcinoma to bone and to one adult human subject. In the 3 patients, decreases of from 1.2 to 1.6 mg % in serum calcium were noted. The decreases were maximal 3 to 10 hours after injection and lasted as long as 18 hours. In the normal subject, serum calcium fell 0.5 mg %. Serum magnesium was not altered appreciably and, in 2 subjects, serum phosphorus tended to fall. It was suggested(11) that thyrocalcitonin is more active in the absence of parathyroid hormone since the patients with hypercalcemia, in whom secretion of the hormone was presumably inhibited, showed a more dramatic response.

It is of interest that the onset of action of thyrocalcitonin was just as rapid when given subcutaneously in the present studies as when given intravenously(4).

Johnston and Deiss(13) have determined the effects of thyrocalcitonin on serum calcium and serum radioactivity in rats a) 3 hours and b) 2 weeks after administration of Ca^{45}. In the 3-hour study (in which most of the Ca^{45} was in the extracellular fluid), serum calcium decreased but serum radioactivity did not change with thyrocalcitonin. Failure of the hormone to produce decreases in both stable calcium and radioactive calcium was interpreted to mean that the reduction in serum calcium resulted not from enhanced deposition but from diminished mobilization of calcium from bone. In the 2-week study (at which time most of the labeled calcium was in bone), both serum calcium and serum radioactivity decreased with thyrocalcitonin. These tests were also taken to mean that the hormone inhibited release of calcium from bone.

These investigations also demonstrated that the effects of parathyroid hormone to increase the rate of release of stable and radioactive calcium from bone in rats given Ca^{45} two weeks previously and to increase glucose uptake and lactate production by bone were inhibited by thyrocalcitonin. They have postulated that the hormone acts by inhibiting bone resorption. From studies with Ca^{45} in rats, Milhaud and associates(14) have drawn the same conclusion.

Summary. The effects of subcutaneous administration of a crude extract of porcine thyrocalcitonin on serum calcium, phosphorus and magnesium were studied in 5 rhesus monkeys and in 3 normal human subjects. Thyrocalcitonin lowered serum calcium in each of the monkeys and normal subjects but did not change serum magnesium. Changes in serum phosphorus were the same as those in control studies. Extracts from human thyroid glands showed calcium-lowering activity in rats. These findings together with those from other laboratories support the view that thyrocalcitonin plays a role in the physiological control of calcium metabolism in man.

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The Effect of Host Age, Virus Dose and Route of Inoculation on Inapparent Infection in Mice with Japanese Encephalitis Virus.* (31418)

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The pathogenesis of Japanese encephalitis (JE) and other arbovirus encephalitides is poorly understood. The relationships between pathogenicity and infectivity of the encephalitic arboviruses are not clear; and it is unknown, for example, why man is rarely diseased though often infected by JE virus (1,2). The following factors may be important considerations: 1) the route of inoculation of virions by vector mosquitoes, *i.e.*, into the lumina of blood vessels for immediate transport to the central nervous system or into subcutaneous tissues where virus proliferates before entering the circulatory system; 2) the quantities of infectious virus inoculated by different mosquitoes; 3) variations in host resistance mechanisms, *e.g.*, related to age, that might affect interferon or antibody production; and 4) differences in virulence among JE virus strains in nature.

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Previous studies have described how the route of inoculation and the dose of virus may be related to the *lethal effects* of JE and other arboviruses(3-6), but the relationship of *infectivity* to pathogenicity has not been defined. This study was undertaken to evaluate the possible effects of route of inoculation and dose of virus on the ability of JE virus to produce disease as opposed to inapparent infection. A single strain of JE virus in low suckling mouse passage was used to avoid variation among strains and to minimize the possible effects of passage on pathogenicity and infectivity of naturally occurring virus. Mice were chosen as hosts because of their known susceptibility to JE virus and because of the ease of making observations upon large numbers of animals.

Materials and methods. Virus. The JE virus strain M5/596 used for infection as well as antibody tests had been recovered from a suspension of mosquitoes collected in Japan in 1956(7) and passaged 6-8 times intracranially in suckling mice. Virus suspensions were kept in sealed glass ampoules on dry ice as 10% suckling mouse brain suspensions in Hanks' balanced salt solution (H) containing 40% rabbit serum, and penicillin, 100 units,