If extracted PSTV is a nucleoprotein, it would have to be very small or contain a component of low density which reduces its rate of sedimentation. The smallest plant virus known, tobacco necrosis satellite virus, sediments at 50*S* (*12*); and as is well known (*13*), it can multiply only in the presence of tobacco necrosis virus. Since PSTV is able to replicate independently, it is most unlikely that the necessary genetic information could be contained in a nucleoprotein of a size consistent with the sedimentation rate observed. If PSTV were larger, but contained a component of low density (presumably a lipid), its resistance to lipid solvents would be difficult to understand. Also, in this case, its sedimentation properties would likely change upon treatment with organic solvents, but we observed no such change. Furthermore, treatment of PSTV with phenol materially affected neither its sedimentation rate nor its infectivity.

Two properties of PSTV appear to rule out a single-stranded RNA. The RNA would have to be much smaller than any known infectious viral RNA; and its resistance to ribonuclease in media of high ionic strength is contrary to the properties of single-stranded RNA's.

Our data on sedimentation and sensitivity to nuclease are, however, compatible with the hypothesis that PSTV is a nucleic acid of double-helical structure, at least one strand of which is composed of RNA. The nucleic acids of several viruses are known to be double-stranded (*14*), and double-stranded RNA [sometimes referred to as replicative form (RF)] is known to occur in tissue infected with RNA viruses (*15–21*). Comparison of the properties of RF with PSTV discloses a number of similarities. Sedimentation coefficients of RF range from 8*S* (*8*) to 20*S* (*17*), and PSTV sediments in this general range; RF is soluble in 1 or 1.5*M* NaCl (*11, 15, 17*), and PSTV is soluble in media of high ionic strength; RF elutes in MAK columns at 0.7*M* NaCl (*18*), together with host DNA (*19*), and PSTV elutes in the same area; RF is sensitive to treatment with ribonuclease at low ionic strength and insensitive at high ionic strength (*15*), and PSTV has similar properties.

Although the chemical nature of the infectious agent is still a matter of conjecture, the concept that PSTV is a double-stranded, infectious RNA agrees most closely with our data; and the aberrant elution pattern from cellulose columns might be explained by some special property of this RNA, such as a conformational difference or association of non-nucleic acid constituents with the molecule.

Whatever the chemical nature of PSTV, our experiments demonstrate that it is a most unusual viral pathogen. When one considers the nucleic-acid-like properties of PSTV, the ease with which it is transmitted, its remarkable stability, and its high specific infectivity are astounding. Concentrates with infectivity dilution end points of 10^{-6} or 10^{-7} gave no recognizable ultraviolet absorption peaks in sucrose gradients or in eluates from MAK columns; and fractions eluted from cellulose columns, which contained only 4 to 5 μg of nucleic acid per milliliter, had infectivity dilution end points of 10^{-3} or 10^{-4}. Thus, solutions containing as little as 5.10^{-4} μg of total nucleic acid per milliliter (undoubtedly mostly host RNA) were infectious.

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Iodine Determined in Purified Thyrocalcitonin

Abstract. *Thyrocalcitonin has been suspected of containing iodine, because its analytical behavior and method of extraction are similar to that of iodinated peptides. The iodine content of increasingly pure thyrocalcitonin extracts was determined by two methods, Kolthoff's procedure and activation analysis, with good agreement. A possible link between calcium and iodine metabolism is suggested by these findings.*

Calcitonin, a hypocalcemic factor, was demonstrated by Copp (*1*), who obtained it by the perfusion of the thyroparathyroid system with blood rich in calcium. Hirsch (*2*) presented evidence that calcitonin was of thyroid rather than parathyroid origin, as first supposed, and named this hypocalcemic substance thyrocalcitonin. Although experiments in dogs yielded some conclusive evidence, the definite proof of thyroid origin of thyrocalcitonin resulted from work with goats, in which the parathyroid and thyroid glands are separate (*3*). From our own research with groups of rats—normal, parathyroidectomized, thyro-parathyroidectomized, and hypophysectomized — we found that injection in rats of 2 ml of parathyroid extract (Para-Thor-Mone, Lilly) caused an increase in the concentration of plasma calcium. The fact that this increase was significantly greater in the thyro-parathyroidectomized group also confirmed the thyroid origin of the hypocalcemic factor (*4*). In that we have worked on thyroid metabolism for a number of years, we are especially interested in substances of thyroid origin, and we have observed a similarity in method of extraction and analytical behavior between iodinated peptides and thyrocalcitonin (*5*). This led us to search for iodine in thyrocalcitonin, which we have now demonstrated in extracts of various purities. Our finding suggests a possible close link between the metabolism of thyroid hormones and that of calcium.

Purified extracts of thyrocalcitonin have been produced since 1964 when Hirsch and co-workers (*2*), and Baghdiantz *et al.* (*6*) first achieved it. MacIntyre and Tenenhouse and their teams produced extracts of increased purity. All methods are based on extraction with hot HCl before the purification procedures.

We first identified thyrocalcitonin in crude extracts of thyroid treated with HCl, according to the method of MacIntyre (*3*). The resulting fraction, F_1, was then purified further with buffer, *p*H 4.6, giving fraction F_2. Descending one-dimensional chromatography was done on the two extracts on Whatman paper No. 1, with Partridge solvent. After isolation and elution of the different substances that give a positive ninhydrin reaction, biological tests were performed on each of the revealed bands by injecting the buffered eluate into the tail vein of a normal rat. Fifty minutes after injection blood was drawn from the aorta. Plasma calcium determinations were made by flame spectrophotometry. There was a very strong hypocalcemic activity for the band corresponding to R_F 0.28 and a lighter activity for R_F 0.11. All other bands were inactive.

Table 1. Comparison of ninhydrin-positive substances present in fractions F_1, F_2, and F_3.

F_1 (R_F)	F_2 (R_F)	F_3 (R_F)
0.03		
.06		
.09		
.11		
.18	0.18	
.21	.21	0.21
.28	.28	.28
.40	.40	.40
.60	.60	.60

Afterward we studied the substance corresponding to R_F 0.28. This band was cut and treated with 1*N* HCl for 12 hours in order to elute the substances fixed on the paper. One part of the liquid was used as control; the other part was hydrolyzed by heating it at 120°C for 72 hours at a final concentration of 6*N* HCl. A portion of the liquid corresponding to the band R_F 0.28, before and after hydrolysis, was evaporated to dryness and again dissolved in a citrate buffer (*p*H 2.75) containing an international standard of norleucine. Separation was then made on ion-exchange resin, according to the method of Moore *et al.* (*7*) with a Technicon autoanalyzer.

We have compared the two diagrams before and after hydrolysis and found that the substance R_F 0.28, which is hypocalcemic, is very rich in cysteic acid, aspartic acid, threonine, serine, isoleucine, tyrosine, phenylalanine, lysine, histidine, and arginine. The concentration of some of these amino acids increases after acid hydrolysis of the extract, an indication of a peptide molecule.

However, because our thyrocalcitonin was only partially purified (completely pure thyrocalcitonin has not yet been isolated), we realized that a number of the isolated amino acids might be impurities instead of parts of the molecule. Therefore we repeated the experiment with a purer extract, F_3, arrived at by treatment of F_2 with 2*M* and 3*M* NaCl; the precipitate was deposited on a Sephadex column G100 and eluted with water. Thus we established a comparative table of the different ninhydrin-positive substances present in each fraction F_1, F_2, and F_3 with their R_F (Table 1).

Simultaneously we studied these three fractions with the autoanalyzer for amino acids and peptides, isolating the constituents of each fraction before and after hydrolysis. We found that most of the free amino acids disappeared, and that only a small number remained in the extract. In contrast, the concentration of peptides increased as the purification progressed.

In a supplementary set of experiments on hydrolyzates of thyroid glands from rats that had received tracer doses of I^{131}, we found that the fractionation of the hydrolyzates on Dowex 1X2 led to the isolation of several radioactive peaks, the first being of peptide nature, namely that of iodinated peptides. Thyrocalcitonin at stage F_3 of purification gave a very similar diagram.

We demonstrated the presence of iodine in F_3 either by determination of I^{127} with the autoanalyzer or by intraperitoneal injection of I^{131} into rats. The animals were killed 24 hours later, the thyroids were removed, and the extracts were prepared by the method of MacIntyre. The R_F 0.28 band of the paper chromatography is radioactive when one operates with thyrocalcitonin F_1 or F_2.

In further experiments to ascertain that iodine was not an impurity we studied the iodine in two extracts, F_2 and F_3T, further purifications (*8*), by the catalytic method of Kolthoff. In F_2 we found 200 μg of iodine per gram, and in F_3T iodine was pres-

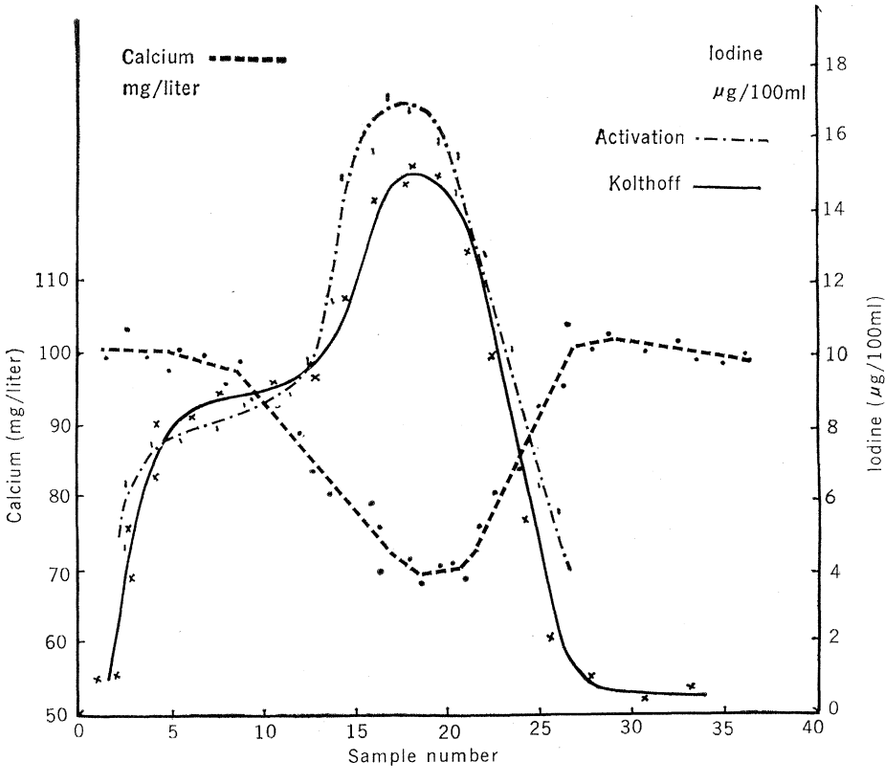

Fig. 1. Iodine and plasma calcium. Maximum hypocalcemic activity corresponds to maximum iodine content in the sample examined.

ent in the amount of 175 μg/g. In that F_2 is about ten times more concentrated than F_3T and the concentration of iodine remains approximately the same in both, iodine appears to be really part of the thyrocalcitonin molecule.

We have purified the F_2 fraction still further by precipitating it with 20 percent trichloroacetic acid and then passing it twice through Sephadex column G100 and eluting with 0.1*N* formic acid. In one typical experiment, 35 samples were analyzed by the Kolthoff method and by activation analysis (*9*). The good agreement between the results derived by the two methods (Fig. 1) supports the earlier evidence that iodine is a component of thyrocalcitonin. Plasma calcium was determined by biological assay by injecting into the tail vein of a rat 0.5 ml of the elution liquid from each 10-ml sample. In order to avoid any alterations of its biological properties, we did not evaporate the eluate.

There is a correlation between the iodine content and the potency of the hypocalcemic effect. When the hypocalcemic effect is not present, iodine is not measurable. The maximum amount of iodine is found (samples 17 and 18) simultaneously with a maximum hypocalcemic effect (Fig. 1). The maximum of the iodine curve coincides roughly with the minimum of the plasma calcium curve. This also seems to indicate that the substance containing the hypocalcemic hormone contains iodine in its molecule.

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Australian Desert Mice: Independence of Exogenous Water

Abstract. *Certain Australian desert mice can survive and may gain weight on a diet of dry seed without drinking water. Urine concentrations for two of the three species studied are the highest recorded for mammals. The kidneys appear to be the major avenue of water conservation.*

Deserts contain as conspicuous faunal elements nocturnal, fossorial rodents. These represent rather divergent familial lines with heteromyids and cricetids predominating in North America, cricetids predominating in Afro-Eurasia, and only murids present in Australia. There is a remarkable degree of morphological and behavioral convergence among these desert rodents. In addition, North American heteromyids and Afro-Eurasian cricetids are physiologically convergent, extreme degrees of water conservation imparting virtual independence of exogenous water (*1*). Since the little-known Australian desert rodents inhabit some of the hottest, driest regions on earth, they too might exhibit unique physiological adaptations in their water economies. We have studied the water economies of Australian hopping mice *Notomys alexis* and *Notomys cervinus* and of the sandy inland mouse *Leggadina hermannsburgensis*.

The *N. alexis* and *L. hermannsburgensis* (mean weight 28.9 ± 5.5 and 12.6 ± 0.9 g, respectively) were collected near Yuendumu Settlement, 296 km west-northwest of Alice Springs, Northern Territory; *N. cervinus* (mean weight 34.7 ± 2.9 g) was collected on Sandringham Station, 160 km north of Birdsville, Queensland. All are from regions of less than 25.4 cm mean annual rainfall; all were collected at the end of a severe drought of 10-year duration.

In the laboratory, animals were housed individually in cylindrical wire-mesh cages; they were provided an excess of mixed bird seed for food (10 percent preformed water by weight). In studies of water deprivation, animals were previously hydrated on fresh apple as a water source and then deprived of water altogether. Initial responses to water deprivation were determined under natural photoperiod at an ambient temperature (T_A) of 19° to 25°C and a relative humidity of 55 to 75 percent, after which animals were transferred to a constant-temperature room on a 12-hour photoperiod at a T_A of 25°C and a relative humidity of 30 to 40 percent; urine, blood, and feces were collected from the animals under the latter conditions. Urine was collected at night in petri dishes placed under the cages and filled with mineral oil to prevent evaporation. Blood was collected in heparinized capillary tubes from a vascular area in the anteriodorsal aspect of the orbit, without permanent injury to the animal. Fecal water content was determined on pellets removed as they were voided by animals held in hand. The pellets were then dried to a constant weight at 100°C. Feces obtained from different mice at the same time were pooled in a single sample.

Concentrations of urea in whole blood and urine were determined by the Conway microdiffusion technique. Osmotic pressures of plasma and urine were calculated from the freezing point depressions measured with a Kalber Biological Cryostat. The rates of pulmocutaneous water loss and oxygen consumption were measured simultaneously for 1-hour periods at a T_A of 28°C. Oxygen consumption was measured in an open-air system at a flow rate of 692 cm^3/min with a Beckman Model E2 oxygen analyzer; pulmocutaneous water loss was determined gravimetrically in expired air passed through tubes containing calcium chloride. While the rates of pulmocutaneous water loss reported herein are for hydrated animals, less complete data for animals deprived of water indicate they were no lower. Measure-

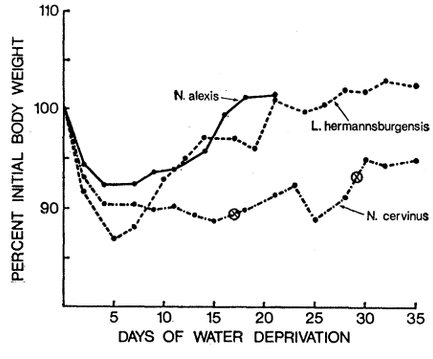

Fig. 1. The mean responses of body weight to water deprivation in ten *Notomys alexis*, eight *N. cervinus*, and nine *Leggadina hermannsburgensis* on a diet of air-dried seed (T_A = 19° to 25°C; relative humidity, 55 to 75 percent). ⊗, day of death of each of two *N. cervinus*.