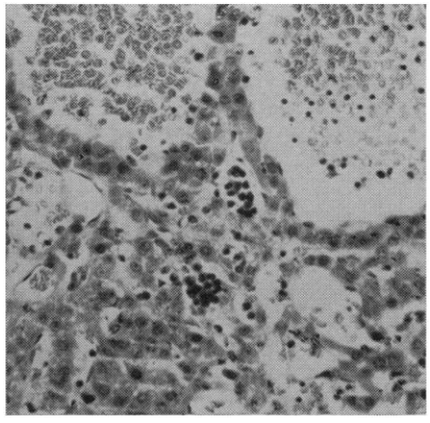

Fig. 2. Details of portions of two fully developed peliotic cavities (top) with degeneration, necrosis, and dissociation of adjacent hepatic cells. Peliotic cavity in process of formation is shown in lower right area. Two foci of extramedullary hematopoiesis are in center. (× 214)

minutes) or extracts of normal rat livers, were not affected.

The clinical and pathological features were identical in all three strains of rats inoculated with the virus; however, there was variation in the degree of severity among individual animals. At the time of onset of clinical symptoms, the animals were pale and inactive. They died a few days later. At autopsy, the livers were pale consistently, but, beneath their capsules and on the cut surfaces, there were scattered, minute, dark, hemorrhagic foci. The livers adhered to the diaphragms. The spleens were usually considerably smaller than those of the control rats and often adherent to the stomach. Hemorrhagic ascites was observed in some of the animals. All other organs appeared normal.

Fig. 3. Section of liver from a W/Fu control rat, showing intact architecture for comparison with Fig. 1. (× 48)

In the sections of liver obtained from rats that either died or were killed, numerous cystic spaces containing blood were observed (Figs. 1 and 2). The spaces had no relation to any particular zone within the liver lobules and were lined by hepatic cells. Mitotic figures were evident in some of the lining cells.

In addition to the blood cells, macrophages and hepatic cells, showing evidence of degeneration or necrosis, were seen in the lumens of some of the cysts. Some of the cystic lesions were separated from adjacent ones by only a single row of hepatic cells. Frequently, cysts communicated with each other in the areas where only remnants of the original partition, consisting of hepatic cells showing varying degrees of degeneration, were seen. In some sections, hemorrhagic cysts extended up to the surface of the liver causing an elevation of the capsule. Areas of fatty metamorphosis, foci of necrosis, and areas of dissociation of hepatic cells were observed in the rest of the hepatic parenchyma. Inflammation was not evident, but there were foci of extramedullary hematopoiesis. As a rule, the spleens of the infected animals showed some evidence of atrophy of the lymphoid tissue. No prominent changes were evident in other tissues (brain was not examined) except foci of extramedullary hematopoiesis. Except for the presence of slight congestion, there was no lesion in the livers of the control animals (Fig. 3). Also, foci of extramedullary hematopoiesis seemed to be less conspicuous than in the infected rats.

The cystic liver lesions in the infected animals of these experiments are characteristic of peliosis hepatis of the parenchymal type in humans (*1*). They are apparently the result of focal hepatic necrosis and hemorrhage after infection with the 9H virus. These findings suggest a possible new etiological factor for consideration in further investigations on peliosis hepatis in other species. They also provide a model system in rats for studies on the pathogenesis of this disease and the nature of the inducing virus.

VICTOR V. BERGS
Variety Children's Research Foundation, Miami, Florida, and *Department of Microbiology, University of Miami School of Medicine, Coral Gables*
THOMAS M. SCOTTI
Department of Pathology, University of Miami School of Medicine

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Potato Spindle Tuber Virus: A Plant Virus with Properties of a Free Nucleic Acid

Abstract. *Infectious entities, extractable, with phosphate buffer, from tissue infected with potato spindle tuber virus and inciting symptoms on tomato that are typical of this virus, have properties incompatible with those of conventional virus particles. The infectious particles sediment in sucrose density gradients at approximately the same rate as particles with a sedimentation coefficient of 10S, are insensitive to treatment with organic solvents, and can be concentrated by ethanol precipitation. Treatment with phenol changes neither their infectivity nor their sedimentation properties. Infectivity is insensitive to deoxyribonuclease, but at low ionic strength it is sensitive to ribonuclease. At high ionic strength, infectivity partially survives incubation with ribonuclease. These properties, as well as elution patterns from columns of methylated serum albumin, suggest that the extractable infectious agent may be a double-stranded RNA.*

Potato spindle tuber has been recognized as an important disease of potatoes for many years (*1*). In nature, the potato spindle tuber virus (PSTV) is spread primarily by mechanical means almost as readily as potato virus X (PVX) is (*2*, *3*). Symptoms incited by PSTV in potato foliage are often very difficult to detect, and study of the causal virus was hampered by the lack of a suitable indicator plant until Raymer and O'Brien (*4*) discovered that this virus also causes distinctive symptoms on tomato plants. In 1964, Allington *et al.* (*5*) reported that potato

spindle tuber was caused by a strain of PVX. Later, Singh *et al.* (*6*) claimed that PSTV was a spherical particle with a predominant size of 25 mμ. Recently, Bagnall (*7*) succeeded in preparing an antiserum specific for PSTV. The antigen particles diffused through agar in a manner that indicated that they were smaller than would be expected for a spherical virus 25 mμ in diameter, and the author suggested that they represent viral protein subunits (*7*). No specific cross-reaction between antiserum to PSTV and PVX-infective sap or between antiserum to PVX and PSTV-infective sap could be obtained (*7*).

In this report, we summarize the results of our efforts to purify and characterize PSTV. Purification of this virus has not been achieved, but the results of our experiments indicate that the extractable infectious agent is not a conventional virus particle. Its properties are compatible with the concept that the agent is a free nucleic acid. We recognize the possibility that infectious entities, different from those extractable by our methods, may exist in infected tissue; but since no evidence for this was found, we consider the extractable infectious agent as the virus and designate it as PSTV. A detailed report of our findings will be presented elsewhere.

Systemically infected leaves of potato (*Solanum tuberosum* L., cv. Saco), free of other known viruses, were used as virus source. Infectivity was assayed on tomato plants (*Lycopersicon esculentum* Mill., cv. Rutgers) in the four-leaf stage (*4*). Tenfold dilutions were made of each inoculum, and each dilution was inoculated onto three plants. Results are expressed as the ratio of the number of plants infected to the number of plants inoculated, or as a dilution end point. As an alternative, we give an "infectivity index" that was derived from the number of plants infected at each dilution, together with the time required for symptoms to appear.

When sap was expressed from infected leaves or when leaves were extracted with phosphate buffer of low ionic strength (0.005*M*), most of the infectious material sedimented at low speed together with the tissue debris. Dilution end point of the infectivity from low-speed supernatants was only 10^{-1}. With phosphate buffer of higher ionic strength (0.05 to 0.5*M*), however, most of the infectivity was found in the supernatants after low-speed centrifugation. Dilution end points were regularly between 10^{-3} and 10^{-4}.

Efforts to concentrate PSTV by high-speed centrifugation failed. Extracts prepared by grinding infected tissue in 0.5*M* K_2HPO_4 (2 ml per gram of tissue) were clarified by low-speed centrifugation, and the resulting supernatant solutions were then centrifuged at 40,000 rev/min (No. 40 rotor, Spinco model L centrifuge). After centrifugation for 4 hours, most of the infectivity was still in the supernatant solution (dilution end points 10^{-3} to 10^{-4}), and little infectivity was in the resuspended high-speed pellets (10^{-1}).

To arrive at a more accurate determination of the sedimentation properties of PSTV, we subjected numerous extracts to rate-zonal centrifugation. In gradients made with clarified extracts, prepared with 0.5*M* K_2HPO_4 or with high-speed supernatants of these extracts, infectivity was mainly in the first four to five fractions taken from the top of the gradients, irrespective of whether the preparations were centrifuged in gradients of low or high ionic strength (*8*) (Table 1).

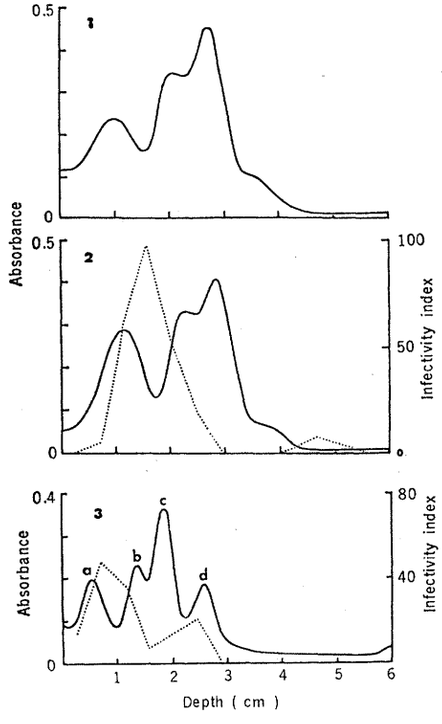
Addition of chloroform and butanol to the extraction medium (½ volume of each per volume of buffer) affected neither the dilution end point nor the sedimentation properties of the infectious material (Table 1), but resulted in preparations with fewer impurities.

Since PSTV could not be concentrated by high-speed centrifugation and since it had sedimentation properties expected of a nucleic acid, the possibility of concentrating the virus by precipitation with ethanol was investigated. This proved feasible, and concentrates with dilution end points of 10^{-6} were regularly achieved (see Table 1).

Concentrates prepared by ethanol precipitation were treated with phenol (three consecutive extractions with 1 volume of phenol each time, mechanical stirring for 20 minutes at 4°C, and removal of residual phenol with ether). No appreciable change in the dilution end point or infectivity distribution (Table 1) could be detected.

Ultraviolet-absorbance profiles of centrifuged gradients showed that high-speed supernatants prepared from either PSTV-infected or healthy leaves had essentially identical absorption profiles (Figs. 1 and 2). Phenol-treated extracts had ultraviolet spectra typical of nucleic acids. Figure 3 shows the absorbance profile and infectivity indices of a phenol-treated concentrate prepared from PSTV-infected tissue. Identically prepared extracts from healthy leaves had essentially identical absorbance profiles; no component was found that could be correlated with infectivity. Comparison of our profiles with those reported for nucleic acid preparations from plant tissues (*9*) indicated a sedimentation coefficient of approximately 10*S* for the major portion of PSTV.

To determine whether PSTV was sensitive to treatment with ribonuclease or deoxyribonuclease, concentrates of the virus were incubated in the presence of these enzymes. As shown



Figs. 1–3. Figs. 1 and 2. Absorbance profiles of centrifuged density-gradient columns containing ethanol-concentrated extracts from healthy (Fig. 1) and PSTV-infected (Fig. 2) potato leaves, and infectivity distribution in the gradient containing the extract from infected tissue. Centrifugation for 16 hours at 24,000 rev/min in a linear 0.2 to 0.8*M* sucrose gradient in 0.005*M* K_2HPO_4 (SW 25 rotor, Spinco model L centrifuge). Fig. 3. Absorbance profile and infectivity distribution of phenol-treated extract from PSTV-infected tissue. Conditions of centrifugation were the same as in Fig. 1, except that gradients contained 0.02*M* phosphate buffer, *p*H 7; *a*, tRNA; *b*, DNA; and *c* and *d*, ribosomal RNA's. Solid lines, absorbance (O.D., 254 mμ); and dashed lines, infectivity index.

Table 1. Infectivity distribution of PSTV in sucrose density gradients. One milliliter of preparation was layered onto a linear gradient (25 ml, 0.2 to 0.8*M* sucrose in the buffer indicated) and centrifuged at 24,000 rev/min for 16 hours (SW 25.1 rotor, Spinco model L centrifuge). Tubes were fractionated in an ISCO density gradient fractionator and ultraviolet analyzer (*22*). Fraction 1 is from the top of the gradient; fraction 12, from the bottom. Pellets were resuspended in 0.5*M* K_2HPO_4. High-speed supernatants are those obtained after centrifugation at 40,000 rev/min for 1 hour (No. 40 rotor, Spinco model L centrifuge). Supernatants to be layered onto gradients in 0.005*M* K_2HPO_4 were dialyzed against 0.005*M* K_2HPO_4 prior to analysis in the gradient. Infectivity is expressed as the number of plants showing symptoms out of the three plants inoculated.

Type of extract	K_2HPO_4 in gradient (molar conc.)	Dilution for assay	Infectivity of consecutive 2-ml fractions from centrifuged gradient tubes, in fraction number												
			1	2	3	4	5	6	7	8	9	10	11	12	Pellet
Extraction medium: 0.5M K_2HPO_4															
High-speed supernatant	0.005	1 : 5	0	3	3	2	3	2	2	0	0	0	0	0	0
High-speed pellet	.005	1 : 5	0	1	0	0	0	0	1	0	0	0	0	0	3
Extraction medium: mixture of K_2HPO_4(0.5M), chloroform, and butanol															
High-speed supernatant	0.005	1 : 10	0	1	2	1	1	0	0	0	0	0	0	0	0
High-speed supernatant	.5	1 : 10	3	3	3	2	2	1	1	1	1	0	0	0	1
Concentrate	.005	1 : 5	3	3	3	2	2	2	1	1	1	1	1	0	0
Phenol-treated concentrate	.005	1 : 10	0	1	3	3	3	2	1	3	0	1	1	1	0
Phenol-treated concentrate	.5	1 : 10	3	3	2	1	2	1	1	1	1	1	0	0	1

in Table 2, when PSTV was suspended in a medium of low ionic strength (0.005*M* K_2HPO_4), incubation with ribonuclease led to complete loss of infectivity, whereas incubation with deoxyribonuclease had no effect on infectivity. When the virus was incubated with ribonuclease in media of high ionic strength (1*M* phosphate buffer, *p*H 7), infectivity partially survived the treatment. In other experiments, sensitivity of phenol-treated concentrates to ribonuclease was compared with that of concentrates not treated with phenol and of high-speed supernatants. No appreciable quantitative differences in sensitivity to ribonuclease were found among the various preparations.

Concentrates from PSTV-infected tissue were also subjected to equilibrium density-gradient centrifugation. Preparations were brought to a density of 1.70 g/cm^3 with cesium chloride or to a density of 1.617 g/cm^3 with cesium sulfate. Each preparation was centrifuged for 72 hours at 35,000 rev/min (SW 39 rotor, Spinco model L-2 centrifuge, temperature 20°C). In the cesium chloride gradient, all infectivity pelleted during centrifugation, whereas in the cesium sulfate gradient, infectivity was mainly distributed just above the center depth of the gradient, indicating that PSTV was an RNA virus, not a DNA one.

To achieve a better separation of nucleic acid components in extracts of PSTV than was possible in sucrose density gradients, we fractionated extracts on columns of methylated serum albumin (MAK) (*10*). No unusual ultraviolet-absorbing components were found. Infectivity eluted mainly in the fractions that contained host DNA (or immediately followed DNA).

Phenol-treated concentrates were subjected to chromatography on cellulose columns, by use of a method recently developed for separation of double-stranded from single-stranded RNA (*11*). As expected, most nucleic acid eluted in the ethanol-containing eluants; with PSTV extracts less than 1 percent of the nucleic acid eluted in buffer alone and even less with extracts from healthy leaves. Bioassays of pooled fractions showed that infectivity was present in all three fractions (the 35-percent, the 15-percent, and the one with no ethanol). No evidence of double-stranded RNA could be found when the melting behavior of fractions eluted in buffer alone was determined.

DNA does not elute as well from cellulose columns as RNA does (*11*). It was conceivable that the unsatisfactory fractionation of PSTV infectivity could have been caused by the association of PSTV with DNA, but when extracts of PSTV were treated with deoxyribonuclease prior to cellulose-column chromatography the elution pattern was only slightly affected.

The most striking characteristic of extracted PSTV is its low rate of sedimentation, which appears to be incompatible not only with a viral nucleoprotein but also with a single-stranded viral RNA. The possibility that PSTV *in situ* might be a conventional nucleoprotein cannot be eliminated conclusively, because at low ionic strength PSTV either is not released from cellular components or it becomes attached to the cell debris. In view of our experiments, however, such a nucleoprotein would have to be very labile, since exposure to relatively low concentrations of salt would be sufficient for complete degradation.

Table 2. Sensitivity of PSTV to treatment with nucleases.

Suspending medium	Enzymatic treatment	Incubation time (hr)	Diluent	Infectivity index
Phenol-treated concentrate				
K_2HPO_4 (0.005*M*)	None	1	K_2HPO_4 (0.005*M*)	183
K_2HPO_4 (0.005*M*)	Ribonuclease (1 μg/ml)	0	K_2HPO_4 (0.005*M*)	12
K_2HPO_4 (0.005*M*)	Ribonuclease (1 μg/ml)	1	K_2HPO_4 (0.005*M*)	0
K_2HPO_4 (0.005*M*)	Deoxyribonuclease (1 μg/ml) *	0	K_2HPO_4 (0.005*M*)	156
K_2HPO_4 (0.005*M*)	Deoxyribonuclease (1 μg/ml) *	1	K_2HPO_4 (0.005*M*)	171
Concentrate not treated with phenol				
Phosphate, *p*H 7 (0.005*M*)	None	1	Phosphate, *p*H 7 (0.005*M*)	78
Phosphate, *p*H 7 (0.005*M*)	Ribonuclease (0.5 μg/ml)	1	Phosphate, *p*H 7 (0.005*M*)	0
Phosphate, *p*H 7 (0.005*M*)	Ribonuclease (1 μg/ml)	1	Phosphate, *p*H 7 (0.005*M*)	0
Phosphate, *p*H 7 (1*M*)	None	1	Phosphate, *p*H 7 (1*M*)	69
Phosphate, *p*H 7 (1*M*)	Ribonuclease (0.5 μg/ml)	1	Phosphate, *p*H 7 (1*M*)	42
Phosphate, *p*H 7 (1*M*)	Ribonuclease (1 μg/ml)	1	Phosphate, *p*H 7 (1*M*)	33

*Incubation medium contained $10^{-2}M$ $MgCl_2$.

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.

T. O. DIENER
W. B. RAYMER*

Crops Research Division,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland 20705

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22. Instrumentation Specialties Co., Lincoln, Neb.

* Present address: Campbell Institute for Agricultural Research, Riverton, N.J.

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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.