obtained by biophysical techniques. The relationship could be formularized as $y = 0.22x + 1.7$.

The establishment of the EB staining method means that at the level of individual microorganisms or organelles, it is possible to measure a quantity of DNA in organelles such as mitochondria by application of fluorometry.

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Presence of double stranded regions of viral RNA in infected cells

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Summary. Ribonuclease treatment of rhinovirus-infected human embryo lung cells after cell disruption reveals that double stranded RNA is present in the preparation before nucleic acids are extracted with phenol. This shows that the hydrogen bonding between complementary molecules of viral RNA which occurs in infected cells is not a result of the extraction of RNA with phenol.

Virus-specific double stranded RNA (DS RNA) has been found so far in cells infected with bacterial, plant and animal viruses which have RNA (single or double stranded) as genetic material. Although it has been suggested that DS RNA plays an active role in the replication of viral RNA[1] many investigators believe that DS RNA does not exist in the infected cell in vivo, but is formed during the extraction of RNA with phenol[2,3]. There is some experimental evidence to support this for RNA phages[4,5], but there is no definite information in the case of animal RNA viruses. Most RNA viruses are sensitive to the antiviral activity of interferon and this has been shown to be enhanced by DS RNA[6-12]; this is suggestive but not direct evidence for the presence of DS RNA in the infected cell. In this communication we present direct evidence which supports the view that hydrogen bonding between complementary molecules of viral RNA occurs in cells infected with rhinovirus type 2, and that this is not a result of the extraction of RNA with phenol.

We have previously reported the details of the replication of the RNA of rhinovirus type 2 in human embryo lung cells and described the different viral RNA species detectable in this system[13]. Essentially in cells infected with the virus there are 3 RNA species induced which, in order of electrophoretic mobility, are the multi-stranded RNA (MS) which is the replicative intermediate of the replication, double stranded (DS) which is the replicative form, and single stranded RNA (SS), which is identical with the genome of the virus (fig. A). To test whether the replicative form or the replicative intermediate exist in the infected cell in whole or in part in double stranded form, we have tested their sensitivity to RNAse in crude extracts prepared by freezing and thawing the cells before the nucleic acids were extracted with phenol.

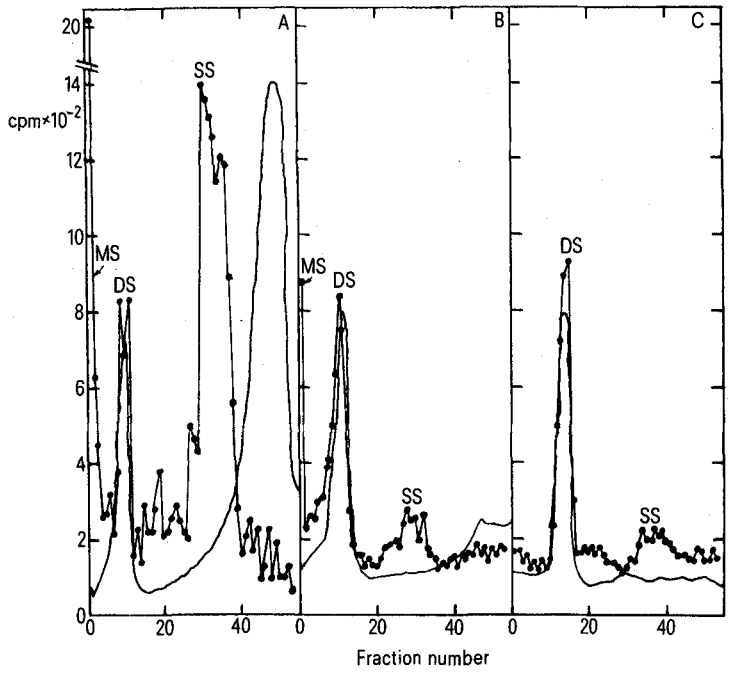
Details of infection, the extraction of nucleic acids and their analysis by polyacrylamide gel electrophoresis have been described before[13]. In brief, 50×10^6 of HEL cells were infected with rhinovirus type 2 and were treated with actinomycin D (1 µg/ml) for 5-9 h p.i. Since we have previously shown that the viral double stranded RNA is accumulated late in the replication cycle[13] we have labelled with 20 µCi/ml of [^{3}H]-uridine for 9-11 h p.i., and then cells were disrupted by freezing and thawing in 2×SSC (300 mM sodium chloride and 30 mM trisodium citrate, pH 8.45). In the broken cells after treatment with RNAse for 10 min at 35 °C with 0.1 or 10 µg/ml of enzyme, SDS was added (1%) and the nucleic acids were extracted with phenol and analyzed by electrophoresis.

If the hydrogen bonding between complementary RNA molecules is formed during the extraction of the RNA with phenol, this treatment of the broken cells with RNAse before the extraction should cause degradation of all single stranded RNA molecules. There would therefore be no DS RNA in the fraction of nucleic acids which are subsequently extracted. This, however, should not rule out the possibility that hydrogen bonding could be formed prior to the extraction with phenol, during the disruption of the cells.

The table and the figure show the results of such an experiment using 2 concentrations of RNAse. In figure A the positions of the MS, DS and SS RNA in 1.8% polyacrylamide gel are presented. Figures B and C show the electrophoretic pattern of the same cell extract after treatment with 0.1 µg RNAse/ml and 10 µg RNAse/ml respectively. The table lists the integrated counts in the 3 peaks of viral RNA corresponding to figures A, B and C. It can be clearly seen that treatment with 0.1 µg/ml of RNAse eliminates the peak of SS RNA and reduces the peak corresponding to the MS RNA. The amount of DS RNA, however, is not reduced but increased. This is probably because some of the replicative intermediate, after the removal of the single nascent strands, is converted to DS cores which are similar to the replicative form. At a concentration of 10 µg/ml of RNAse the SS RNA and the MS RNA are completely degraded but the DS RNA is still present. Finally it should be pointed out that the ribosomal RNA present in the samples was 100% sensitive to both

Treatment with RNAse of infected broken cells

RNAse (µg/ml)	MS RNA cpm	%	DS RNA cpm	%	SS RNA cpm	%
0	2560	100	1920	100	9315	100
0.1	1020	40	3060	159	830	8.9
10	45	1.7	2675	140	520	5.5



Electrophoretic analysis of rhinovirus type 2 RNA treated with RNAse before phenol extraction. *A* Control, *B* and *C* treated with 0.1 and 10μg/ml of RNAse respectively. The continuous line is the OD_{260} trace indicating the position of the marker DNA (which runs together with the DS RNA) and the ribosomal RNA which is present only in the control figure. Direction of electrophoresis from left to right.

concentrations of RNAse whereas cellular DNA was insensitive.

These results show that the DS RNA detected in cells infected with rhinovirus type 2 is present before nucleic acids are extracted with phenol. This is important in view of recent experiments establishing a crucial role for DS RNA in the mechanism of interferon's antiviral action, although the possibility that hydrogen bonding formation occurs before the extraction with the phenol has not been ruled out.

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Endogenous indolyl-3-acetic acid and pathogen-induced plant growth disorders: distinction between hyperplasia and neoplastic development

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Summary. Quantitative analysis of the auxin indolyl-3-acetic acid during the development of neoplastic (crown gall) and hyperplastic (club root) plant growth disorders revealed different physiological mechanisms.

The growth disorders induced in plants by the crown gall agent *Agrobacterium tumefaciens* have many of the characteristics of animal neoplasias[2]. In particular, cells from crown gall tumors continue to proliferate in tissue culture when freed from *A. tumefaciens* and have no requirement for external auxins or cytokinins[3]. The parasitic fungus *Plasmodiaphora brassicae* induces hyperplasias ('club root') in susceptible cruciferous plants which, however, require the continued presence of *P. brassicae* plasmodia for growth on basal media[4]. Since elevated auxin levels have been suggested as causative factors in the development of both plant diseases[5,6], endogenous levels of the major auxin indolyl-3-acetic acid (IAA) were monitored during the early stages of the diseases to clarify the physiological mechanisms involved.

Material and methods. Crown galls were induced on wounded sunflower (*Helianthus annuus* L. var. Russian Giant) with *Agrobacterium tumefaciens* (Smith and Townsend) Conn. strain B6 and seedlings of swede (*Brassica napus* var. Danestone) were infected with *Plasmodiaphora*