this ratio were very crude by modern standards. What is more surprising is that this nucleic acid approximates so closely to a tetranucleotide, the maximum discrepancy being only 20%. It is evident, however, that, if all the nucleic acid particles in the preparation have a similar composition, the minimum 'molecular weight' of a polynucleotide must be at least 100,000. The inference is that the particles, as they exist in commercial yeast nucleic acid, are not all alike.

SUMMARY

A simple chromatographic method for the quantitative analysis of the bases of ribonucleic acids is described. The amount necessary for one analysis is about 0·1 mg. One hydrolysis only is needed and all the bases are recovered from single spots after chromatography on filter paper. Analyses of a specimen of yeast nucleic acid are given.

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Chromatographic Studies on Nucleic Acids

3. THE NUCLEIC ACIDS OF FIVE STRAINS OF TOBACCO MOSAIC VIRUS

By R. MARKHAM and J. D. SMITH
Agricultural Research Council, Plant Virus Research Unit, Molteno Institute, University of Cambridge

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The tobacco mosaic virus was the first of several plant viruses to be shown to be nucleoprotein by the work of Bawden, Pirie, Bernal & Fankuchen (1936) and that of Bawden & Pirie (1937). The importance of the nucleic acid, although originally disputed by workers in America, is now accepted as a matter of course, and it seems probable that nucleic acid is an essential factor for the multiplication of many viruses. In their original work Bawden & Pirie (1937) isolated the nucleic acid in several ways and showed that it resembled the ribose nucleic acid of yeast. Later Loring (1939) showed that its constituents were at least similar to those in yeast nucleic acid. Recently we have developed an accurate quantitative method for the analysis of ribonucleic acids (Smith & Markham, 1950), and it seemed desirable to see whether the virus nucleic acids resembled yeast nucleic acid in their quantitative composition, and to what extent, if at all, the various viruses differ in the make-up of their nucleic acids. In this paper we discuss the results obtained with five different strains of virus, all of which have serological groupings in common and all of which are similar morphologically.

EXPERIMENTAL

Materials

The viruses used in this investigation were all related strains belonging to the tobacco mosaic virus group. All were passed through single lesions on *Nicotiana glutinosa* (excepting cucumber virus 4 which does not go to this plant) in order to avoid any possibility of strain mixtures. The purification method used followed the lines of that of Bawden & Pirie (1937).

The strains used were as follows:

Tobacco mosaic. The ordinary type strain, which has been maintained here for many years, has from time to time been put through single lesions to maintain its purity. It does

produce a small percentage of yellow and necrotic yellow mutants. It was multiplied on White Burley tobacco plants.

Tomato mosaic 844 *A*. This virus, obtained from Dr R. E. Taylor of the National Agricultural Advisory Service, is the commonest mosaic disease of glasshouse tomatoes in this country. It resembles in its symptoms on tomato the type virus, but differs in that it does not cause a systematic disease in tobacco. It was grown on Potentate tomatoes.

Tomato aucuba mosaic. The original 'Aucuba' mosaic of tomato (Henderson Smith, 1928) appears to differ from that so named by American workers. This virus is a frequent cause of disease in commercial tomato plants in this country. It gives a very characteristic symptom pattern in tomatoes and causes only local necrotic lesions in tobacco plants. It was obtained through Dr R. E. Taylor and was grown on Potentate tomato plants.

Rib-grass virus. This strain of tobacco mosaic was found on plantain by Holmes (1941). It was obtained through Dr G. Oster, and was grown on Kawala tobacco plants.

Cucumber virus 4. This virus, which is apparently confined to cucurbitaceous plants, was first described in this country, where it now no longer seems to occur naturally (Ainsworth, 1935). It was obtained through Dr R. E. F. Matthews and grown on Bedfordshire Ridge cucumber plants.

Preparation of the virus nucleic acids. The nucleic acids from all the virus strains were isolated in an identical manner. A solution of the virus at pH 7–8 containing a trace of salt was heated at 100° for about 10 min. when the virus protein is denatured and collects as a flocculent precipitate, while the nucleic acid remains in solution. As the solution often became slightly acid on boiling, the pH was readjusted to 7–8 before the protein was centrifuged off. The nucleic acid was precipitated from the supernatant fluid by the addition of an equal volume of ethanol and a few drops of N-HCl. This precipitate was collected by centrifugation and dissolved in the minimal quantity of very weak NH_3 and centrifuged at 12,000 rev./min. to remove traces of protein. The nucleic acid was finally precipitated a second time with acidic ethanol in a small tube, and collected by centrifugation as a pellet which could then be hydrolysed directly in the centrifuge tube.

Methods

Nucleic acid analysis. The technique employed has been described previously (Markham & Smith, 1949; Smith & Markham, 1950). The virus nucleic acid was hydrolysed for 1 hr. at 100° in sufficient N-HCl to give a concentration of about 20 mg. of nucleic acid/ml. We have shown that this hydrolysis is quantitative, and as the bases are removed quantitatively from the chromatograms, it is unnecessary to use large quantities of nucleic acid in cases like this where only knowledge of the relative proportions of the bases is sought. The hydrolysate containing the purine bases and the pyrimidine nucleotides was placed in measured quantities (10–20 μl.) on the chromatogram paper. This was then run in 70% (v/v) *tert.*-butanol-water, 0·8 N with respect to HCl, the absorbing areas were located by our ultraviolet photographic technique, cut out together with adjacent blank areas, eluted in 5 ml. of 0·1 N-HCl and estimated on the Beckman photoelectric spectrophotometer making use of the characteristic ultraviolet absorption. The same spectrophotometric data were used as in our analysis of yeast nucleic acid (Smith & Markham, 1950).

Chromatography of the sugar component. The purine-bound pentose was liberated from the nucleic acid by hydrolysis at 100° in N-H_2SO_4. Sulphate was removed from the hydrolysate by neutralization with $Ba(OH)_2$ followed by centrifugation. The solution, which contained pentose, was concentrated by vacuum distillation and placed on a paper chromatogram together with suitable markers (arabinose, rhamnose and D-ribose from yeast nucleic acid). This chromatogram was run in *n*-butanol-water-NH_3, a solvent which clearly resolves ribose from other known pentoses (Partridge, 1948). The sugars were located on the paper by spraying with aniline oxalate and heating for 10 min. at 100° (Partridge, 1949).

RESULTS

Analysis of nucleic acids

Ultraviolet absorption of the nucleic acids. The absorption curves of the nucleic acids in the ultraviolet region were plotted from measurements made using the Beckman photoelectric spectrophotometer. These curves were obtained as a routine check on the strength of the nucleic acid preparations and were used as a guide to the quantity necessary for a hydrolysis. As shown in Fig. 1, these curves have a

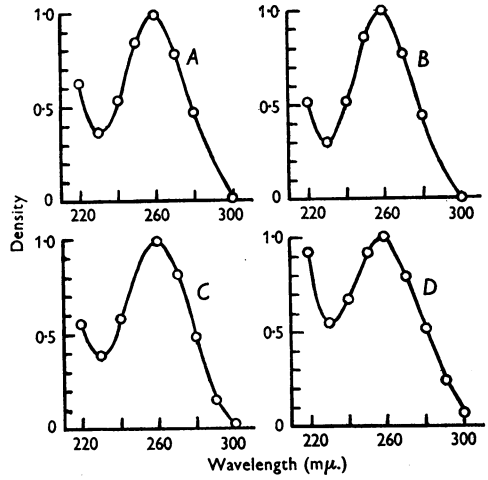

Fig. 1. Ultraviolet absorption curves of the nucleic acids from four strains of tobacco mosaic virus. Density plotted against wavelength in mμ. The concentration is approximately 35 μg. of nucleic acid/ml. in a 1 cm. cell. Strains: *A*, tobacco mosaic; *B*, rib-grass; *C*, tomato mosaic; *D*, cucumber 4.

similar although not identical shape. All show a well defined peak at 260 mμ. and a trough at about 230 mμ. It has been suggested that the ratio peak density/trough density can be used as a criterion of purity of nucleic acids, but we consider that this ratio is also largely dependent on the degree of aggregation of the particles which affects their light scattering in this region.

Identification of the components of the nucleic acids. On hydrolysis with N-HCl at 100° for 1 hr. the nucleic acids from all the tobacco mosaic virus strains yield only adenine, guanine, cytidylic and uridylic acids, which were identified by their relative positions on the chromatograms, by absorption spectra and by hydrolysis of the nucleotides to the free bases. Like all other ribonucleic acids so far investigated these, therefore, contain only the purines adenine and guanine and the pyrimidines cytosine and uracil. The purine-bound pentose from the type strain and that from tomato mosaic were identified by paper chromatography and found to be identical with ribose from yeast nucleic acid.

Quantitative composition. Using our technique we have determined the molar ratios of the four purine and pyrimidine bases in each of the nucleic acids. These ratios are given in Table 1. As the tomato mosaic virus nucleic acid most nearly approached the mean values for all five strains, comparisons are made with the values obtained from this nucleic acid. Those figures which appear to differ significantly from the tomato mosaic values are printed in heavy type. (The significances of the values were computed by means of the *t* test. In three cases the value of *P* was less than 0·01 and in the fourth (the uracil of cucumber 4) *P* was between 0·01 and 0·02.)

Table 1. *Purine and pyrimidine composition of the nucleic acids from five strains of tobacco mosaic virus*

(The molar proportions of the four bases (mean = 1) together with their S.E.M. are given. The figures in heavy type differ significantly from the corresponding values for tomato mosaic virus nucleic acid.)

Strain	Adenine	Guanine	Cytosine	Uracil	No. of determinations
Aucuba mosaic	1·20	0·95	0·78	1·05	5
Type tobacco mosaic	1·24±0·026	**1·17**±0·023	**0·62**±0·021	0·96±0·019	4
Rib-grass	1·17	1·08	0·69	1·05	6
Cucumber 4	**1·04**±0·009	1·03±0·009	0·74±0·006	**1·19**±0·012	4
Tomato mosaic	1·18±0·007	1·04±0·014	0·73±0·004	1·05±0·037	4
Mean of all strains	1·17	1·05	0·71	1·06	23
Yeast nucleic acid	1·03	1·25	0·80	0·93	11

Serological and biological tests

As the tomato virus differed from the tobacco mosaic virus in the composition of its nucleic acid we made some serological cross-absorption experiments. The antisera used were produced by injecting into the ear veins of each rabbit a single quantity of 1–2 mg. of purified virus in a volume of 1 ml. The rabbits were bled after a fortnight, and the sera were titrated against the heterologous strain by the optimal proportions method (i.e. antitobacco mosaic against tomato mosaic virus and antitomato mosaic against tobacco mosaic virus). A quantity of each serum was then incubated at 50° with an excess of the heterologous virus strain and the precipitate centrifuged off. The absorbed sera were then tested against more of the absorbing virus for precipitins to check the completeness of the absorption and finally against the homologous virus to detect the presence of antigenic components not shared by the pair of viruses. Absorbed sera were also tested against cucumber virus 4, because the latter is probably the most atypical virus of this group. It will be seen from the results shown in Table 2 that the tobacco mosaic and cucumber 4 viruses have antigenic components which the tomato mosaic virus lacks. Tomato mosaic also has an antigenic component which is not present in tobacco mosaic, but which is shared by cucumber virus 4.

Infection with one strain of a virus protects a plant from subsequent infection with a related strain (Salaman, 1933). This phenomenon of 'cross-protection' has been made the basis of a method which is in general use among plant virus workers for determining whether two viruses are strains of the same virus or are not related to each other; e.g. a plant infected with a virus suspected of being a strain of cucumber mosaic virus might be inoculated with a strain of the latter which has easily distinguishable symptoms. Failure of the authentic strain to enter the plant is regarded as proof of a strain relationship between the two. Recently it has been found that this cross-protection fails in some cases where serological and other tests have definitely established a relationship, and in these cases it seems likely that the relationship is fairly remote. A similar case exists here since the difference in antigenic constitution between tomato mosaic and tobacco mosaic is paralleled by the fact that the

Table 2. *Serological cross absorptions*

(+ indicates precipitation; − indicates absence of precipitation.)

Serum	Tobacco mosaic	Tomato mosaic	Cucumber 4
Antitobacco mosaic	+	+	+
Antitobacco mosaic absorbed with tomato mosaic	+	−	+
Antitomato mosaic	+	+	+
Antitomato mosaic absorbed with tobacco mosaic	−	+	+

former is able to infect tomato plants already systematically infected with tobacco mosaic virus (Hitchborn, private information).

On the other hand, Holmes (1941) found that there was a cross-protection relationship between his rib-grass virus and tobacco mosaic virus. These facts suggest that the English tomato mosaic viruses may be even more distantly related to tobacco mosaic virus than is the rib-grass virus, which because of its differences in host range and symptomatology is generally regarded as a rather exceptional strain.

DISCUSSION

The nucleic acid of the tobacco mosaic virus has been investigated by Loring (1939) and Schwerdt & Loring (1947), who from acid hydrolysates obtained and identified adenine, guanine and cytosine, and after alkaline hydrolysis isolated guanylic, cytidylic and uridylic acids which were identified by their chemical composition, optical rotations and decomposition points. Although no direct evidence was offered to show that the sugar was actually ribose, their results indicated that the virus nucleic acid was similar to that of yeast from a qualitative point of view.

As nucleic acid of some kind has been found as an invariable constituent of all authentic virus preparations, and is probably responsible in part for the biological activity of these parasites, it is of some interest to determine whether any specific differences can be found in the nucleic acids of viruses which could be correlated with the biological differences of their nucleoproteins. In the case of the protein component of various strains of tobacco mosaic virus it was found by Knight and others (Knight, 1942, 1943, 1947, 1949; Knight & Stanley, 1941; Gaw & Stanley, 1947) that appreciable differences in the amino-acid composition can be detected between the more distantly related strains. In the case of the nucleic acids there are fewer factors that can vary, and as the components which we have found seem to be the same as those of yeast nucleic acid, there is only the possibility of measuring the variation in the relative amounts of the four nucleotides present. Possibly at a later date information about the arrangement of the nucleotides relative to each other may be obtained. It is striking that the relative proportion of the four nucleotides is so similar in all five strains, only four small differences having been noticed. It is possible that more refined measurements on a very large number of preparations may show up more differences of an even smaller magnitude. The general composition of the nucleic acids of the tobacco mosaic group, however, is considerably different both from that of a yeast nucleic acid, which we have investigated (Smith & Markham, 1950) and from that of several preparations of the nucleic acid of the turnip yellow mosaic virus (Kenneth Smith, Markham & Smith, unpublished). It may be found that in general closely related viruses have very similar nucleic acids and we intend to investigate this point further.

We should point out here that there is little probability that, apart from the rib-grass strain and cucumber 4, our viruses are the same as those with similar names on which amino-acid determinations have been made. Indeed our type strain of virus has a glutamic acid content (Wiltshire, unpublished) which is entirely different from that of the type strain investigated by Knight. This state of affairs is inevitable as there would appear to be an almost infinite number of strains of this virus, most of which are in a state of flux, and there is little one can do to prevent changes due to mutations becoming dominant in the host plants. It is for this reason that we have been careful to ensure that our experimental material originated from single necrotic lesions and was therefore relatively homogeneous.

SUMMARY

1. The nucleic acids from five distinct strains of tobacco mosaic virus have been isolated and their composition studied by means of paper chromatography.

2. The purine-bound sugar from the nucleic acids of two of these strains has been shown by paper chromatography to be identical with ribose from yeast nucleic acid.

3. All the nucleic acids have been shown by our chromatographic technique to contain only the four bases, adenine, guanine, cytosine and uracil.

4. The nucleotide compositions of the nucleic acids from the five strains were found to be very similar. Small significant differences were detected among three of the strains.

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Effect of Acetate on Glycogen Synthesis and Glucose Utilization in the Isolated Diaphragm of Rats

By I. PARNES and E. WERTHEIMER
The Laboratory for Pathological Physiology, The Hebrew University, Jerusalem

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Central metabolic products are believed to be basic regulators of cellular metabolism, the influence of the hormones on metabolism being only secondary, or superimposed on these primary regulatory mechanism. It may be of interest therefore to investigate the influence of such products on certain metabolic processes *in vivo* and *in vitro*. Bloch (1947) has pointed out the central position of acetic acid in metabolism. In this paper we made a study of the influence of acetate on glycogen synthesis and glycogenolysis and glucose utilization by isolated rat diaphragms, using the technique of Gemmill (1940*a*, *b*).

EXPERIMENTAL

Male albino rats of laboratory stock weighing 80–100 g. were used. Preparation of diaphragm was carried out according to Gemmill (1940*a*), using diaphragms from rats fasted for 20 hr. The medium used for incubation was 'phosphate-buffered saline' containing 0·74, NaCl; 0·015, Na_2HPO_4 and 0·003% NaH_2PO_4. All the added substrates were given as Na salts. The vessels containing the diaphragms in the medium were gassed for 2 min. with O_2 and then placed in a water bath at 37°. The flasks were shaken 120 times/min.

Glycogen synthesis. This was measured according to Tuerkischer & Wertheimer (1948).

Glucose utilization. For measurement of the glucose utilization by the diaphragm the methods of Krahl & Cori (1947) and Perlmutter & Greep (1948) were used.

Measurements of the glycogen synthesis by the isolated diaphragm of acetate-fed rats. The rats were given the following diet during 2 days: 58% carbohydrate, 12% Na acetate, 20% casein and 10% olive oil, with the usual addition of vitamins and salts. After fasting for 24 hr. the animals were killed, the diaphragms excised and the experiments carried out in the usual medium with 0·05% glucose.

Measurement of respiration. Warburg's direct method was used for the determination of the O_2 uptake (Dixon, 1943) in phosphate Ringer solution. The diaphragms were halved, one half was incubated in the medium containing 0·05% glucose and 0·1% acetate and the other half with glucose alone. Measurements were made over periods of 3 hr. and the average value for 1 hr. was recorded.

Chemical procedures. Determinations of glycogen in the diaphragm were carried out by a micromodification of Pflüger's method (Good, Kramer & Somogyi, 1933). Glucose was estimated according to Somogyi (1937). Determination of acetic acid was carried out by steam distillation.

RESULTS

Glycogen synthesis by isolated rat diaphragm, with acetate in the medium

Incubation of rat diaphragm in the usual medium was followed by a marked glycogenolysis (Tuerkischer & Wertheimer, 1948). On addition of lactate (0·1%), citrate (0·1%) and succinate (0·1%) there was glycogenolysis of the same order of magnitude as in the medium without additional substrate. α-Ketoglutarate decreased, while acetoacetate markedly increased the glycogenolysis. Acetate in a concentration of 0·2% caused glycogenolysis of the same order of magnitude as was observed in the medium without substrate ($-0·062 \pm 0·009$ in three experiments), but no glycogenolysis was observed in the presence of 0·05% acetate ($-0·002 \pm 0·010$ in four experiments). Addition of 0·1% acetate gave rise to a small but significant net glycogen increase. Pyruvate caused net increase in glycogen, though not to the same extent as glucose (Table 1).

Table 1. *Glycogen synthesis by normal rat diaphragm in presence of acetate, pyruvate, lactate, etc.*

(The diaphragms were incubated at 37° for 3 hr. Glycogen synthesis is expressed as mg. glucose/100 mg. wet tissue.)

No. of experiments	Medium containing (0·1%)	Glycogen synthesis (mg./100 mg.)
14	Acetate	$0·016 \pm 0·008$
11	Pyruvate	$0·037 \pm 0·013$
7	Lactate	$-0·062 \pm 0·023$
5	Citrate	$-0·060 \pm 0·026$
5	Succinate	$-0·069 \pm 0·024$
7	α-Ketoglutarate	$-0·020 \pm 0·019$
4	Acetoacetate	$-0·115 \pm 0·024$