REFERENCES

- Buchel, L. & McIlwain, H. (1950). *Nature, Lond.*, **166**, 269.
 Butler, T. C. (1950). *Pharm. Rev.* **2**, 121.
 Conway, E. J. (1947). *Micro-diffusion Analysis and Volumetric Error*. London: Crosby Lockwood.
 Conway, E. J. & Cooke, R. (1939). *Biochem. J.* **33**, 479.
 Dawson, R. M. C. (1950). *Biochem. J.* **47**, 386.
 Dawson, R. M. C. & Richter, D. (1950a). *Proc. roy. Soc. B*, **137**, 252.
 Dawson, R. M. C. & Richter, D. (1950b). *Amer. J. Physiol.* **160**, 203.
 Dickens, F. (1946). *Biochem. J.* **40**, 145.
 Elliott, K. A. C. & Libet, B. (1942). *J. biol. Chem.* **143**, 227.
 Elliott, W. H. (1948). *Nature, Lond.*, **161**, 128.
 Etsten, B. & Himwich, H. E. (1946). *Anesthesiology*, **7**, 536.
 Fowden, L. & Penney, J. R. (1950). *Nature, Lond.*, **165**, 846.
 Fuhrman, F. A. & Field, J. (1943). *J. Pharmacol.* **77**, 392.
 Gerard, R. W. (1947). *Anesthesiology*, **8**, 453.
 Haber, C. & Saidel, L. (1948). *Fed. Proc.* **7**, 47.
 Himwich, W. A., Homburger, E., Maresca, R. & Himwich, H. E. (1947). *Amer. J. Psychiat.* **103**, 689.
 Jowett, M. & Quastel, J. H. (1937). *Biochem. J.* **31**, 565.
 Kerr, S. E. (1942). *J. biol. Chem.* **145**, 647.
 Krebs, H. A. (1935). *Biochem. J.* **29**, 1951.
 Krebs, H. A. (1948). *Biochem. J.* **43**, 51.
 Mann, P. J. G. & Quastel, J. H. (1946). *Biochem. J.* **40**, 139.
 Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
 Quastel, J. H. & Wheatley, A. H. M. (1932). *Proc. roy. Soc. B*, **112**, 60.
 Richter, D. & Dawson, R. M. C. (1948). *J. biol. Chem.* **176**, 1199.
 Stone, W. E. (1940). *J. biol. Chem.* **135**, 43.
 Stern, J. R., Eggleston, L. V., Hems, R. & Krebs, H. A. (1949). *Biochem. J.* **44**, 410.
 Waelsch, H. (1949). *Lancet*, **2**, 1.

The Composition of some Microbial Deoxypentose Nucleic Acids

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Recent advances in nucleic acid chemistry, principally through the application of chromatographic techniques, have led to the abandonment of the tetranucleotide hypothesis and the discovery that both deoxypentose nucleic acids (DNA) and ribonucleic acids (RNA) show a range of compositions characteristic of their source. Because of the close association of DNA with the chromatin material of the nucleus, comparative studies on the structure of DNA from different organisms have a special biological significance. Analyses by Chargaff and co-workers have led to the conclusion that, among animals, the composition of DNA (i.e. the molar proportions of the purine and pyrimidine bases) is characteristic of the species source and constant for different tissues of one species, and that DNA from bacteria also shows characteristic differences in composition (Chargaff, 1950; Chargaff, Zamenhof, Brawerman & Kerin, 1950). Results obtained in this laboratory with improved chromatographic techniques and methods of hydrolysis, while in general agreement with these conclusions, have also led to the recognition of the pyrimidine, 5-methylcytosine, as a constituent of DNA from animals and higher plants; this is apparently lacking from nucleic acids of microbial origin (Wyatt, 1950, 1951a, b). It is

apparent that in molecules so large as those of the nucleic acids many possibilities exist for structural differences which do not involve differences in nucleotide composition; nevertheless it is only through such quantitative analysis that an approach may at present be made to the comparison of different nucleic acids. We have therefore extended these analyses to include some bacterial and virus deoxypentose nucleic acids, and shall now describe the determination of the purine and pyrimidine composition of DNA from *Mycobacterium tuberculosis*, *Escherichia coli*, the two *E. coli* bacteriophages T₂ and T₆, and the virus causing polyhedral disease in gipsy-moth (*Lymantria dispar* L.) caterpillars.

It is now recognized that all bacteria which have been examined contain both RNA and DNA, and there is evidence that the DNA is confined to the bacterial nucleus (Robinow, 1945). *E. coli*, which contains about 4% (dry wt.) of DNA, was chosen in order to permit comparison of the nucleic acids in a virus-host system. The tubercle bacillus was of particular interest because of the reported presence of 5-methylcytosine in its nucleic acid (Johnson & Coghill, 1925), although Vischer, Zamenhof & Chargaff (1949) had been unable to confirm this claim.

The bacteriophages and the polyhedral viruses of insects are convenient for nucleic acid analysis

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because of their relatively high content of DNA, and are, incidentally, the only viruses which have been shown conclusively to contain only nucleic acid of the deoxypentose type. The corresponding claim for certain animal viruses (e.g. vaccinia; Hoagland, Lavin, Smadel & Rivers, 1940) has been based on the properties of nucleic acid isolated with the use of hot 5% (w/v) aqueous sodium hydroxide, which would eliminate by hydrolysis any RNA that had been present. There has been controversy over the possible presence of RNA in the bacteriophages T_2 and T_4 ; recently, however, Cohen & Arbogast (1950) have shown by serological methods that RNA in T_2 , T_4 and T_6 preparations is associated with material antigenically related to the host and that RNA is almost certainly not a constituent part of these viruses. The virus which may be isolated from gipsy-moth polyhedral bodies has been reported to contain about 14% of DNA, sufficient to account for its total phosphorus content (Bergold, 1947, 1950; Bergold & Pister, 1948).

MATERIALS

Mycobacterium tuberculosis

Samples of dried bacterial cells of human and bovine strains were obtained through the courtesy of the Wellcome Research Laboratories.

Escherichia coli

The mutant of the strain sensitive to the T series of bacteriophages designated as B/r (Witkin, 1946) was grown on nutrient broth (Lemco, 3 g.; peptone, 10 g.; NaCl, 5 g.; distilled water, 1 l.; pH 7.5) and on a synthetic medium of the following composition: sodium lactate, 10 g.; NH_4Cl , 1 g.; K_2HPO_4 , 0.7 g.; KH_2PO_4 , 0.3 g.; Na_2SO_4 , 0.1 g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g.; distilled water, 1 l. Stock cultures were subcultured daily on each medium. For the nucleic acid preparations 36 or 48 hr. cultures, grown on agar slopes in Roux flasks at 37° and in the stationary phase of growth, were rapidly washed three times in 0.9% (w/v) aqueous NaCl, twice in 90% (v/v) aqueous ethanol and in ethanol, suspended in ether for 30 min., centrifuged, and dried at 105° for 6 hr.

Bacteriophages T_2 and T_4

The bacteriophages T_2 and T_4 (Demerec & Fano, 1945) were grown on the B/r strain of *E. coli*. Stock suspensions were titrated and kept at 4°. The usual plaque-counting method of assay was used throughout the experiments. The procedure for growth and purification of the bacteriophages followed closely that used by Putnam, Kozloff & Neil (1949) for the isolation of the bacteriophage T_6 . Broth lysates were

prepared by inoculating a young aerated culture at 37° with a small number of bacteriophage particles and incubating for 8–16 hr. Here several cycles of adsorption and lysis were involved. Synthetic-medium lysates were prepared by 'single-stage growth'. To an aerated culture, containing about $1-3 \times 10^8$ bacteria/ml., bacteriophage was added to give 1–5 particles/bacterium and incubation continued for about 12 hr. The bacteriophage was isolated by differential centrifugation. The lysate was centrifuged at 3000 rev./min. to remove bacterial debris, and the supernatant passed through the Sharples supercentrifuge at 24,000 rev./min. sufficiently slowly to sediment over 95% of the virus. The pellet was suspended in a small volume of 0.85% (w/v) aqueous NaCl, centrifuged at low speed (15 min. at 3000 rev./min.) to remove insoluble material, and the virus again sedimented on the Sorvall SS1 angle centrifuge at 12,000 rev./min. for 1 hr. After resuspension in saline, more debris and aggregated material were removed by low-speed centrifuging.

As the purification of bacteriophage T_6 has not been described in the literature we record here some of the characteristics of our preparations. The ultraviolet absorption spectrum in neutral solution shows a peak at 260 $\mu\mu$. and trough at 240 $\mu\mu$, and is very similar to the absorption curves of T_2 and T_4 bacteriophages. The preparations, both from broth and the synthetic medium, were analysed for total N and P and assayed for infective particles. These results are shown in Table 1. The quantity of N/infective particle is higher than that reported for T_2 and T_4 bacteriophages (1.3×10^{-14} g. N/infective particle) and corresponds with the greater diameter of T_6 particles as shown by measurements on electron micrographs (Putnam, 1950). The mean diameter of T_6 given by Putnam is 90 $\mu\mu$, while that for a dry particle calculated from the ratio N/infective particle, assuming an N content of 16% and a specific volume identical with that of T_2 and T_4 (0.66), is 80–85 $\mu\mu$. Considering the errors involved in the various measurements the agreement is reasonably good.

Gipsy-moth polyhedral virus

Viruses causing the polyhedral diseases of insects were first shown by Bergold (1947) to have the form of rod-shaped particles which can be liberated from the polyhedra by dissolving the surrounding 'polyhedral protein' in dilute alkali. These polyhedra are obtained from infected caterpillars, in which they are freed in great abundance on lysis of the tissues at death, and can be purified and washed by centrifugation. The diseased gipsy-moth caterpillars (*Lymantria dispar* L.) which provided polyhedra for the present work were collected from an infestation in Slovakia in June, 1949. The polyhedra were purified centrifugally, and repeatedly washed in 0.9% (w/v) aqueous NaCl until a white suspension was obtained which showed very few bodies other than polyhedra on examination with the dark-field microscope. After the final sedimentation from saline, the polyhedra were suspended in distilled water and stored at –10° until needed.

Table 1. Characteristics of the T_6 bacteriophage preparations

	g. N/infective particle ($\times 10^{-14}$)	N/P ratio (by wt.)	Extinction coefficient at 260 $\mu\mu$. of a solution containing 1 g. atom N/l.
Broth preparations (mean of two preparations)	3.3	3.55	1280
Synthetic-medium preparation	3.9	3.35	1180

Virus was prepared from the purified polyhedra by the following procedure, which is essentially that of Bergold (1947). Polyhedra were suspended at a concentration of about 5 mg./ml. in a solution containing Na_2CO_3 , 0.008 M, and NaCl , 0.05 M, and the mixture was allowed to stand at room temperature for about 3 hr. Examination with the dark-field microscope then showed that almost all of the polyhedra had dissolved, leaving free virus particles and empty skins or ghosts which represent the insoluble surface layer of the polyhedral bodies. The suspension was centrifuged at 6000 rev./min. for 6 min. on the Sorvall angle centrifuge to remove undissolved polyhedra, most of the skins, and any bacteria or other gross impurities. The yield of virus could be slightly increased by suspending and re-sedimenting this material. The supernatant liquid was then centrifuged at 9600 rev./min. for 60 min., and the virus sedimented as a translucent, bluish-white, birefringent pellet, on top of which was a less compact brownish layer consisting mainly of polyhedral skins. This impurity could be washed off, with the loss of some virus, by a carefully directed stream of water from a pipette. The virus was suspended in distilled water (about one-eighth of the volume of the original solution) and again sedimented for 60 min. at 9600 rev./min. It was then suspended in a small volume of water, and centrifuged briefly (about 2 min. at 5000 rev./min.) to remove larger particles and aggregated material.

It has not been possible to test the infectivity of such preparations in this laboratory. Examination of several of them with the electron microscope (by Dr V. E. Cosslett in the Cavendish Laboratory, Cambridge) showed that they consisted mainly of rod-shaped particles about $30 \times 300 \mu\text{m}$. in size, occurring both singly and in bundles, and similar to those which Bergold has identified as the virus. Analysis of several preparations gave mean values N, 15.1% and P, 1.77% of the dry wt. (drying at 110°). The P content is rather higher than that reported by Bergold (1.33%) and appears to reflect differences in the DNA content of his preparations and ours.

METHODS

The isolation of the nucleic acids

The procedures described by Mirsky & Pollister (1946) for the isolation of deoxyribonucleoproteins from animal and plant tissues are often ineffectual with bacteria, possibly because of the impermeability of the cell wall. It has been shown in this laboratory (Wyatt, 1951*b*) that treatment with N-NaOH at 37° does not change the purine and pyrimidine composition of DNA, although RNA is completely hydrolysed to nucleotides (Schmidt & Thannhauser, 1945). This method was therefore used to prepare the nucleic acids from both bacteria and viruses. The material was incubated for 16–20 hr. at 37° in a small volume of N-NaOH . Insoluble material was removed by centrifuging, the supernatant liquid brought to pH 4 with acetic acid, and one volume of ethanol added. The precipitate, containing DNA, was collected by centrifugation, dissolved in dilute NaOH or alkaline m-NaCl , and protein removed by gel formation on shaking with a mixture of 8 parts of CHCl_3 and 1 part of commercial octanol (v/v) (Sevag, Lackman & Smolens, 1938). A further precipitation with acetic acid and ethanol completed the purification. Nucleic acid thus prepared was free of RNA and contained very little protein, but when derived from bacteria usually contained considerable extraneous material apparently polysaccharide in nature.

Analysis of the nucleic acids

The purine and pyrimidine composition of the nucleic acids was determined by techniques which have already been fully described (Markham & Smith, 1949; Wyatt, 1951*b*). Following hydrolysis either with pure formic acid (175° for 30 min.) or with 72% (w/w) aqueous HClO_4 (100° for 60 min.), the bases were separated on paper chromatograms using as the solvent 65% (v/v) aqueous isopropanol 2.0 N with respect to HCl . They were then eluted and estimated spectrophotometrically.

For the bacterial nucleic acids, which were contaminated with carbohydrate, hydrolysis with HClO_4 was found advantageous, since the use of formic acid leaves extraneous absorbing materials which interfere with chromatography. These are largely destroyed by HClO_4 , and a cleaner chromatogram results.

With viruses such as bacteriophages and insect viruses (which contain a high proportion of nucleic acid all of one type, and little or no carbohydrate other than that of the nucleic acid) it was found that good purine-pyrimidine chromatograms can be obtained without isolation of the nucleic acid, by hydrolysis of the whole virus. Although such chromatograms are heavily loaded with amino-acids, these do not interfere appreciably with estimation of the nucleic acid bases. Tryptophan is destroyed by acid hydrolysis and thus only tyrosine has sufficiently intense ultraviolet absorption to show on the prints of the chromatograms; with the solvent used it moves in a position between cytosine and uracil, where it does not interfere. This method was used in some of the analyses of the polyhedral virus, and gave proportions of the bases similar to those found from purified virus nucleic acid.

In these analyses the information considered of interest was the relative proportions of the purine and pyrimidine components of the nucleic acids. As the reliability of the analytical procedures had already been established, N and P analyses were made only on those preparations of which sufficient was available.

Elementary analyses

N was estimated by a micro-Kjeldahl technique (Markham, 1942), and P by the colorimetric method of Allen (1940).

RESULTS

All the bacterial and virus DNA's analysed contained the purines adenine and guanine and the pyrimidines cytosine and thymine. 5-Methylcytosine could not be detected in any of these nucleic acids, even when the appropriate bands from large chromatograms were eluted, concentrated and run in a different solvent, a method which is capable of detecting a pyrimidine occurring in amounts as small as 0.05% of a nucleic acid (Wyatt, 1951*a*).

The molar proportions of the four bases in each nucleic acid are presented in Table 2. Each analysis is based on three or more replicate sets of spots on the chromatogram. The number of preparations of each nucleic acid analysed and the method of hydrolysis used are stated and the mean results given.

Table 2. *Proportions of purines and pyrimidines in deoxypentose nucleic acids from microbial sources*

Source of nucleic acid	No. of preparations analysed	Method of hydrolysis	Molar proportions calculated to total 4.00				Purines/ pyrimidines (mol. proportions)
			Adenine	Guanine	Cytosine	Thymine	
Bacteria:							
Tubercle bacilli							
Human strain	1	H.COOH	0.72	1.14	1.34	0.80	0.87
Bovine strain	1	H.COOH	0.71	1.17	1.35	0.76	0.89
<i>Escherichia coli</i> (mutant B/r):							
Synthetic medium	1	HClO ₄	0.90	0.98	1.03	1.09	0.89
Broth	2	HClO ₄	0.93	0.94	1.02	1.10	0.88
Viruses:							
<i>E. coli</i> phages							
T ₅ synthetic medium	1	HClO ₄	1.36	0.85	0.43	1.37	1.23
T ₅ broth	1	H.COOH	1.34	0.82	0.41	1.44	1.17
T ₂ broth	2	{ H.COOH } * { HClO ₄ }	1.33	0.73	0.505	1.44	1.08
Gipsy-moth polyhedral virus	4	{ HClO ₄ } * { H.COOH }	0.86 ± 0.01	1.20 ± 0.01	1.12 ± 0.04	0.81 ± 0.04	1.07

* Mean results given.

The DNA preparations from *Escherichia coli* grown on broth and on the synthetic medium do not differ significantly in composition. The same is true of DNA isolated from bacteriophage T₅ from bacteria grown on the two media. Similarly, the differences between the human and bovine strains of tubercle bacilli may probably be accounted for by systematic error, although from the few analyses that have been done, the possibility of small real differences cannot be excluded. The differences between T₂ and T₅, on the other hand, we consider too great to be accounted for by analytical error, and represent a real difference between the compositions of the two nucleic acids.

The T₅ bacteriophage preparation isolated from bacteria grown on broth was also examined for the presence of RNA. A suspension of the virus containing 0.9 mg. nitrogen was precipitated with acetic acid at pH 4, dried, and incubated with *n*-sodium hydroxide at 37° for 12 hr. in order to convert any RNA to mononucleotides. The solution was made acid with hydrochloric acid and the precipitate containing DNA was removed on the centrifuge. The supernatant liquid was concentrated, placed on a chromatogram, and run with the *isopropanol*-hydrochloric acid solvent. In this solvent the ribonucleotides from RNA give two spots, one containing uridylic acid and the other a mixture of adenylic, guanylic and cytidylic acids. Both these run clear of the purines adenine and guanine. No well defined spots were distinguishable in these positions from the alkaline hydrolysate of bacteriophage; the amount of non-specific material present would have allowed the detection of RNA if present in an amount equivalent to more than 1% of the DNA content of the virus.

Chromatograms of the gipsy-moth virus hydrolysed whole, without isolation of its nucleic acid, showed no uracil, thus confirming the absence of RNA from this virus. A polynucleotide integrated from the found amounts of the DNA bases would be equivalent to 15.2% by weight of the virus, and account for 88% of the total virus phosphorus. Since similar purine-pyrimidine analyses of purified DNA's have usually succeeded in accounting for only about 90% of nucleic acid phosphorus (Wyatt, 1951*b*), it is probable that almost all of the virus phosphorus is included in its DNA. In one preparation, DNA was estimated by the diphenylamine reaction (Dische, 1930), which indicated the presence of 16.6% DNA after a downward correction of 4% had been applied to allow for the different purine/pyrimidine ratios in virus DNA and the thymus DNA standard (see Discussion).

DISCUSSION

Although the deoxypentose nucleic acids of only a small number of bacteria and viruses have been examined, these included organisms representative of several unrelated groups, and some generalizations are possible. Whereas DNA from a number of animals and at least one higher plant has a common pattern of composition, with more adenine and thymine than guanine and cytosine, a relatively small content of 5-methylcytosine, and a purine/pyrimidine ratio very close to unity (Wyatt, 1951*b*), this pattern is not shared by the nucleic acids of microbial origin. These are marked by the absence of 5-methylcytosine, by wide diversity in the relative proportions of the other bases, and by considerable

variation in the purine/pyrimidine ratio. There is some evidence that the composition of DNA varies less between related than between unrelated organisms, but many more nucleic acids will have to be analysed before we can state how closely these variations may parallel phylogenetic relationship.

Chargaff *et al.* (1950) have observed that DNA's from avian tubercle bacilli, *Serratia marcescens* and *Bacillus Schatz*, unlike those of higher animals, all contain an excess of guanine and cytosine over adenine and thymine, and on the strength of this they have proposed that DNA's may be divided into two main classes, the 'AT type' characteristic of animals and the 'GC type' of bacteria. According to this system, the bacteriophages would have the AT type and the gipsy-moth virus the GC type; *E. coli*, however, would have to be classified as 'transitional'. Again, only a more extensive survey of DNA composition can show whether this apparent grouping is more than fortuitous.

The variations in purine/pyrimidine ratio, whatever biological significance they may have, are of considerable practical importance, for the diphenylamine reaction commonly used for estimating DNA depends on the colour produced by purine-bound deoxypentose (Dische, 1930). If thymus nucleic acid, with a purine/pyrimidine ratio very close to 1.00, is used as the reference standard, estimations of DNA having a different purine/pyrimidine ratio will require correction by a corresponding factor. For example, estimations of DNA from phage T₂ will require downward correction by 3%—not, in this case, a very large error, but one which may possibly account for the recent finding by Cohen & Arbogast (1950) of enough DNA, estimated with diphenylamine, in phage T₂ to account for up to 105% of the total phosphorus of the virus. The error with phage T₂ or with *E. coli* would be greater still.

The compositions of DNA from host and bacterio-

phage are strikingly different and it is apparent that the bacterial nucleic acid could not be transferred directly in virus synthesis. This accords with the observations of Cohen (1948) and of Kozloff & Putnam (1950) that about 70–80% of the virus phosphorus is derived from inorganic phosphorus taken up from the medium after infection of the host. Kozloff & Putnam, however, consider that the remaining 20–30% of virus phosphorus comes from the bacterial DNA although this transfer may proceed via nucleotides.

SUMMARY

1. Deoxypentose nucleic acids have been isolated from the human and bovine strains of *Mycobacterium tuberculosis*, *Escherichia coli* (strain B/r), the bacteriophages T₂ and T₅ and the gipsy-moth polyhedral virus. Using paper chromatography the purine and pyrimidine compositions of these nucleic acids have been determined quantitatively.

2. Adenine, guanine, cytosine and thymine are present in all the nucleic acids, but 5-methylecytosine could not be detected.

3. The nucleic acids from different micro-organisms show a wide range of nucleotide composition differing from the general structural pattern found among the deoxypentose nucleic acids of higher organisms.

4. The compositions of deoxypentose nucleic acids from the bacteriophages T₂ and T₅ are distinct, although similar, but differ greatly from that of the host (*Escherichia coli*).

We wish to express thanks to the Wellcome Research Laboratories for the gift of dried tubercle bacilli, and to Prof. J. Komárek, Charles University, Prague, who facilitated the collection of diseased gipsy-moth caterpillars as a source of polyhedra. One of us (G. R. W.) was engaged on this project while on transfer of research from the Division of Entomology, Department of Agriculture, Ottawa, Canada.

REFERENCES

- Allen, R. J. L. (1940). *Biochem. J.* **34**, 858.
 Bergold, G. (1947). *Z. Naturforsch.* **2b**, 122.
 Bergold, G. (1950). *Viruses* 1950, ed. M. Delbruck, p. 60. California Institute Technology, Pasadena.
 Bergold, G. & Pister, L. (1948). *Z. Naturforsch.* **3b**, 406.
 Chargaff, E. (1950). *Experientia*, **6**, 201.
 Chargaff, E., Zamenhof, S., Brawerman, S. & Kerin, L. (1950). *J. Amer. chem. Soc.* **72**, 3825.
 Cohen, S. S. (1948). *J. biol. Chem.* **174**, 295.
 Cohen, S. S. & Arbogast, R. (1950). *J. exp. Med.* **91**, 607.
 Demerec, M. & Fano, U. (1945). *Genetics*, **30**, 119.
 Dische, Z. (1930). *Mikrochemie*, **8**, 4.
 Hoagland, C. L., Lavin, G. I., Smadel, J. E. & Rivers, T. M. (1940). *J. exp. Med.* **72**, 139.
 Johnson, T. B. & Coghill, R. D. (1925). *J. Amer. chem. Soc.* **47**, 2838.
 Kozloff, L. M. & Putnam, F. W. (1950). *J. biol. Chem.* **182**, 229.
 Markham, R. (1942). *Biochem. J.* **36**, 790.
 Markham, R. & Smith, J. D. (1949). *Biochem. J.* **45**, 294.
 Mirsky, A. E. & Pollister, A. W. (1946). *J. gen. Physiol.* **30**, 117.
 Putnam, F. W. (1950). *Science*, **111**, 481.
 Putnam, F. W., Kozloff, L. M. & Neil, J. C. (1949). *J. biol. Chem.* **179**, 303.
 Robinow, C. F. (1945). Addendum to *The Bacterial Cell*, p. 353. By Dubos, R. J. Cambridge, Mass.: Harvard University Press.
 Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
 Sevag, M. G., Lackman, D. B. & Smolens, J. (1938). *J. biol. Chem.* **124**, 425.
 Vischer, E., Zamenhof, S. & Chargaff, E. (1949). *J. biol. Chem.* **177**, 429.
 Witkin, E. M. (1946). *Proc. nat. Acad. Sci., Wash.*, **32**, 59.
 Wyatt, G. R. (1950). *Nature, Lond.*, **166**, 237.
 Wyatt, G. R. (1951a). *Biochem. J.* (in the Press).
 Wyatt, G. R. (1951b). *Biochem. J.* (in the Press).