Phosphorus Compounds in the Cell

5. THE COMPOSITION OF THE CYTOPLASMIC AND NUCLEAR RIBONUCLEIC ACIDS OF THE LIVER CELL

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It is well known that ribonucleic acid (RNA) is found in the cell cytoplasm both in the large and in the small particles and in the non-sedimentable supernatant fluid (the cell sap). RNA also occurs in the cell nucleus in small amounts (for review of literature see McIndoe & Davidson, 1952). It is clearly a matter of some interest to know whether variations occur in the RNA from these different sites. In this paper an attempt is made to determine the composition of RNA from different parts of the liver cell using the method of ionophoresis of nucleotides described in part 2 of this series (Davidson & Smellie, 1952*a*). This method is contrasted with the chromatographic procedure in common use after acid hydrolysis of the nucleic acid.

METHODS

Biological. Albino rats from the Department's colony and chinchilla rabbits bred in the Department were used in these experiments. The fractionation of liver tissue into mitochondria, microsomes and cell sap was carried out as described by Smellie, McIndoe, Logan, Davidson & Dawson (1953).

Nuclei were isolated from samples of the same liver tissue by the citric acid method as described by McIndoe & Davidson (1952).

Chemical. RNA was extracted from the cytoplasmic components by 'procedure 2' of Davidson & Smellie (1952*b*). Nuclear RNA was obtained as a solution of mixed ribonucleotides by the modification of the Schmidt & Thann-

hauser (1945) method described by McIndoe & Davidson (1952). Yeast RNA, supplied by British Drug Houses Ltd., was purified by the method of Smith & Markham (1950) followed by precipitation from glacial acetic acid. Whole-liver RNA was isolated by the method of Davidson, Frazer & Hutchison (1951).

The following methods of analysis of RNA were employed:

Method (a). The nucleic acid (10 mg.) was digested to the constituent bases with 0.1 ml. 72% (w/w) HClO_4 at 100° for 1 hr. (or other suitable time) (Marshak & Vogel, 1951; Wyatt, 1951). The hydrolysate was diluted to 1 ml. and 20–30 μl . portions were applied to Whatman no. 1 paper from a micrometer syringe for separation by paper chromatography using 65% aqueous isopropanol 2*N* with respect to HCl. The following R_f values were obtained: guanine 0.33, adenine 0.48, cytosine 0.57, uracil 0.73, but these were subject to slight variation from run to run.

The spots were located by the procedure of Holiday & Johnson (1949), cut out and eluted by shaking vigorously in glass-stoppered test tubes with 5 or 10 ml. HCl of appropriate concentration as indicated in Table 1. The tubes were incubated at 37° overnight and the contents centrifuged. Readings of optical density were taken in the Beckman Model DU spectrophotometer. A separate lane of paper was cut up and eluted for use as blanks. Each digest was run, eluted and read in triplicate.

Method (b). The nucleic acid was hydrolysed to purine bases and pyrimidine nucleotides by heating 20 mg. quantities with 1 ml. *N*-HCl for 1 hr. at 100° (Smith & Markham, 1950). Separation of components of the hydrolysate was carried out as in (a) above, the following R_f values being obtained: guanine 0.33, adenine 0.48, cytidylic acid 0.76, uridylic acid 0.91. Elution and ultraviolet absorption measurements were carried out as in method (a).

Method (c). The nucleic acid (10 mg.) was hydrolysed to the constituent ribonucleotides with 1 ml. 0.3*N*-KOH. The

Table 1. *Ultraviolet absorption data for purine and pyrimidine derivatives showing the millimolecular extinction coefficient ($m\epsilon$) and $E_{1\text{cm.}}^{0.001\%}$*

	Concentration of HCl (N)	Wavelength ($m\mu$.)	$m\epsilon$	$E_{1\text{cm.}}^{0.001\%}$ (found)	$E_{1\text{cm.}}^{0.001\%}$ (literature)	
Adenine	0.1	262.5	13.0	0.96	0.96	(Wyatt, 1951)
Guanine	1.6	249	10.9	0.72	0.73*	(Wyatt, 1951)
Cytosine	0.1	275	11.0	0.99	0.95	(Wyatt, 1951)
Uracil	0.1	259	7.9	0.702	0.705	(Wyatt, 1951)
Adenylic acid	0.01	260	—	—	0.401	(Volkin & Carter, 1951)
Guanylic acid	0.01	260	—	—	0.325	(Volkin & Carter 1951)
Cytidylic acid	0.01	278	—	—	0.393	(Ploesser & Loring, 1949)
Uridylic acid	0.01	260	9.89	0.305	0.305	(Ploesser & Loring, 1949)
Uridylic acid	0.1	260.5	—	—	0.308	(Volkin & Carter, 1951)

* 0.1*N*-HCl.

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digest was acidified in the cold with 60% (w/w) HClO_4 to pH 3.6. Potassium perchlorate was removed by centrifugation and portions of the supernatant fluid dispensed on Whatman 3 MM paper strips 72 cm. long for ionophoresis as previously described by Davidson & Smellie (1952*a*). After the run the papers were dried, cut up and eluted. Phosphorus was determined in each eluate (Davidson & Smellie, 1952*a*).

Method (d). This was a modification of method (c) using strips of Whatman no. 1 paper. Quantities of hydrolysate corresponding to about 200 μg . total nucleotides were applied to the paper. The following relative mobilities were found:

	Relative mobility	Relative net charge/molecule at pH 3.6
Cytidylic acid	0.18	0.16
Adenylic acid	0.36	0.35
Guanylic acid <i>a</i>	0.77	0.83
Guanylic acid <i>b</i>	0.80	
Uridylic acid	1.00	1.00

Elution and ultraviolet absorption measurements were carried out as in method (a). A separate paper strip was run simultaneously to provide the blanks. It was invariably found that guanylic acid appeared as two closely adjacent bands both having the typical ultraviolet absorption spectrum of guanylic acid. We therefore consider them to represent the *a* and *b* isomers of Cohn (1951), but not necessarily respectively. They are labelled in the order indicated by analogy with their behaviour on an anion-exchange column. The relative mobilities of all the nucleotides quoted were calculated after allowance had been made for electro-osmotic flow. Comparison of these mobilities with the relative net charge/molecule calculated from the data of Cohn (1950) shows good agreement.

In the methods (a) and (b) readings of optical density were made at the absorption maximum and also at another appropriate wavelength in order to determine the difference (Δ) in optical density as recommended by Vischer & Chargaff (1948*a*). For purposes of calculation, standard Δ values (Δ_{st}) were obtained from solutions of appropriate

materials at concentrations of 10 $\mu\text{g}/\text{ml}$. in the same solvents as were used for elution (Table 2). The ultraviolet absorption data shown in Table 1 were used as criteria of purity of the bases and in the estimation of the nucleotides separated in method (d). In this last method absorption measurements were made at the maximum only.

The molar extinction coefficients for the nucleotides used are for the equilibrium mixtures of the *a* and *b* isomers formed in acid solution. The same acid was used for elution. This ensures that whatever may be the relative proportions of the *a* and *b* isomers of each of the nucleotides on the paper, the eluates always contain the same equilibrium mixture as that used in determining the molar extinction coefficients. This eliminates any possibility of error which arises out of the reported differences in optical properties of the isomers (Brown, Dekker & Todd, 1952).

Identification of pentose residues. Portions of the hydrolysate obtained in method (b) above were concentrated *in vacuo* and suitable quantities applied to Whatman no. 1 paper, neutralized with ammonia vapour and subjected to descending chromatography using an aqueous ammoniacal ethyl methyl ketone solvent. The solvent was prepared by equilibrating ethyl methyl ketone (100 ml.) with 1% (w/v) NH_4OH (40 ml.) and using the upper layer as the organic phase (cf. Partridge & Westall, 1948). The spots were detected using aniline hydrogen oxalate (Partridge, 1949). The following R_F values were found: arabinose 2.0, xylose 2.5, lyxose 2.9, ribose 3.9.

RESULTS

As has previously been observed (Davidson & Smellie, 1952*a*) the results obtained by ionophoresis (method (c)) show higher values for uracil (and for cytosine) relatively to adenine than are obtained by separation of the bases after perchloric acid digestion (method (a)). This is illustrated in Table 3 for three specimens of RNA from different sources and for a mixture of nucleotides.

Table 2. *Standard values (Δ_{st}) for the difference in extinction at the absorption maximum and at another chosen wavelength for various compounds in concentrations of 10 $\mu\text{g}/\text{ml}$. in HCl solution*

	Concentration of HCl (N)	Absorption maximum (m μ)	Other chosen wavelength (m μ)	Δ_{st} found	Δ_{st} Vischer & Chargaff (1948 <i>a</i>)
Adenine	0.1	262.5	290	0.935	0.900
Guanine	1.6	249	290	0.539	—
Cytosine	0.1	275	290	0.546	0.545
Uracil	0.1	259	280	0.609	0.590
Cytidylic acid	0.01	278	300	0.331	—
Uridylic acid	0.1	260.5	280	0.814	—

Table 3. *Analyses of ribonucleic acids by methods (a) and (c) expressed as molar ratios relative to adenine as 10*

	Mixture of nucleotides		Rat-liver RNA		Rabbit-liver RNA		Chick-liver RNA	
Method	(a)	(c)	(a)	(c)	(a)	(c)	(a)	(c)
Adenine	10	10	10	10	10	10	10	10
Guanine	9.3	9.1	17.5	17.6	16.5	16.9	17.1	17.1
Cytosine	10.7	11.0	13.6	14.3	14.5	14.6	12.4	13.6
Uracil	11.9	13.1	8.1	10.8	7.3	10.3	7.3	10.6

Table 4. *Analyses of two specimens of yeast ribonucleic acid by different methods expressed as molar ratios relative to adenine as 10*

(The results of triplicate analyses are given to illustrate the agreement between replicates.)

Method	Yeast RNA I			Yeast RNA II		
	(a)	(b)	(d)	(a)	(b)	(d)
Adenine	10	10	10	10	10	10
Guanine	11.4	11.6	11.3	11.1	11.3	11.0
	11.2	11.6	11.6	10.7	11.1	11.0
	11.5	11.7	11.6	11.3	11.0	11.0
Cytosine	8.0	9.0*	9.4	7.8	8.5*	9.4
	8.0	8.9*	9.7	7.9	8.6*	9.7
	8.1	9.0*	9.5	7.8	8.6*	9.6
Uracil	7.8	9.5*	10.2	8.8	10.7*	13.3
	7.9	9.1*	10.0	8.5	10.8*	13.2
	7.6	9.5*	9.9	8.8	10.3*	13.2
Calculated recovery of phosphorus (%)	86.2	88.5	97.1	—	—	—

* Corrected as described by Smith & Markham (1950).

Table 5. *Analyses of a specimen of yeast ribonucleic acid by method (a) with variations in the time of hydrolysis*

(Results expressed as molar ratios relative to adenine as 10.)

	Time of hydrolysis (min.)							
	15	30	45	60	120	180	240	360
Adenine	10	10	10	10	10	10	10	10
Guanine	11.2	11.3	11.1	11.4	11.1	11.2	11.3	11.5
Cytosine	5.7	7.1	7.2	8.0	8.8	8.6	8.2	8.9
Cytosine*	5.8	7.2	7.1	8.1	8.8	8.6	8.2	9.1
Uracil	6.2	7.4	7.4	7.8	8.4	7.8	7.9	7.7
Uracil*	10.4	9.9	9.5	8.9	9.2	8.5	8.3	8.3

* Calculated from optical density at absorption maximum and not from Δ values.

The analysis of one specimen of yeast RNA by three different methods is shown in Table 4. Comparison of the first and last columns again reveals the low value for uracil obtained after perchloric acid hydrolysis. When the nucleic acid was hydrolysed with *N*-hydrochloric acid at 100° for 1 hr., the values for uracil and cytosine were considerably raised even without the 5% correction for partial hydrolysis of the pyrimidine nucleotides recommended by Smith & Markham (1950).

In the experiment shown in Table 4, the modification, method (d), of the ionophoresis procedure was used in which ultraviolet absorption measurements were made on the eluates instead of using the phosphorus estimations employed by Davidson & Smellie (1952a). This yielded results essentially the same as those provided by method (c) and had the advantage of being suitable for smaller quantities of material than the original ionophoretic method.

Our results are expressed in terms of molar ratios relative to adenine. It was therefore necessary to determine whether the low figure for uracil obtained by method (a) was in fact related to the uracil itself rather than to the adenine or to both.

The low values for uracil obtained by method (a) appeared to be related to the method of hydrolysis rather than to the method of separation of the hydrolysis products and were obtained for any hydrolysis time between 15 and 360 min. (Table 5).

When yeast RNA was hydrolysed with perchloric acid and the base ratios calculated from the extinction of the paper eluates at the absorption maximum only (Wyatt, 1951), it was observed that the cytosine/adenine ratio was the same as was obtained by the difference method, whereas the uracil/adenine ratio was higher (Table 5).

It was also noted that the material eluted from the uracil spot on chromatograms of perchloric acid hydrolysates of RNA or nucleotide mixtures gave values for the ratio E_{259}/E_{280} in the range 2.0–4.8 rather than the expected 6. This anomaly was not observed when digests of pure bases were used.

It was clear therefore that uracil obtained after perchloric acid digestion of RNA was contaminated. Since the observed R_f value of cytidylic acid (0.76) is close to that of uracil (0.73) it seemed likely that the contamination might be due to traces of unhydrolysed cytidylic acid. Consequently, the material eluted from the uracil spot would give a

relatively high optical density at 280 m μ , since the absorption maximum of cytidylic acid is 278 m μ . The apparent value for uracil would therefore be low owing to reduction of the Δ value.

To test this possibility a sample of radioactive cytidylic acid was prepared by ionophoresis of an alkaline digest of RNA from the liver of a rat which had received ^{32}P . The nucleotide was digested with perchloric acid and subjected to chromatography as described in method (a). The chromatogram was cut up into strips for elution and the eluates examined for optical density at 275 m μ , and for radioactivity.

Fig. 1. Optical density, $\bigcirc$ — $\bigcirc$, and radioactivity (counts/min.), $\times$ — $\times$, of eluates from 1 cm. strips of a chromatogram of a perchloric acid digest of [^{32}P]cytidylic acid. The optical density peak at 31 cm. corresponds to cytosine and the radioactivity peak at 48 cm. to inorganic phosphate. The two peaks at 39 cm. indicate the presence of a component which exhibits the characteristic ultra-violet absorption spectrum of cytidylic acid.

Cytosine was found in the expected position on the chromatogram while inorganic phosphate was detected near the solvent front using the spray reagent of Hanes & Isherwood (1949). In addition, however, that portion of the chromatogram corresponding to the R_f value of uracil contained some unchanged cytidylic acid as shown by radioactivity and optical density measurements (Fig. 1).

In view of these results it was decided to concentrate on method (d) (alkaline hydrolysis followed by ionophoresis of the nucleotides) as being the most reliable available. It was applied to the RNA found in the cytoplasmic components and nuclei of rat

liver with the results shown in Table 6. It is clear that there is no important difference between the RNA's from the three cytoplasmic sources. All show the same pattern of high proportions of guanine and cytosine. Such a pattern is also shown in the specimen of whole rat-liver RNA mentioned in Table 3 and distinguishes liver RNA sharply from yeast RNA in which the bases are present in almost equimolecular proportions (Table 4).

The method of pentose identification was applied to the RNA's from all four morphological components of rat-liver tissue. In each case the sugar was found to be chromatographically identical with ribose. Since ribose is easily distinguished from the other pentose by its high R_f value in the solvent system employed, there can be no doubt that the sugar present in all four liver RNA fractions is ribose. This has been confirmed for RNA from whole liver cytoplasm using the butanol-ethanol-water solvent system of Chargaff *et al.* (1950).

Table 7 shows the molar composition of RNA from maternal and foetal rabbit liver. Again the three cytoplasmic fractions show the same pattern and are apparently identical. Rabbit-liver RNA, like rat-liver RNA, is characterized by high relative proportions of guanine and cytosine, and appears to be the same in the foetus as in the mother.

Figures for the cytoplasmic fractions of regenerating rat liver 26 hr. after partial hepatectomy are also given in Table 7. At this time the turnover of the RNA appears to be near its maximum (Johnson & Albert, 1952; Eliasson *et al.* 1951), but there appears to be no significant change in the relative molar proportions of bases.

The slight difference in composition between the rabbit-liver RNA shown in Table 3 and the pregnant rabbit-liver RNA in Table 7 suggests that liver RNA might vary in composition from one animal to another. This view is supported by the results of analysis of specimens of liver RNA from four different rabbits in which the molar proportions of guanine to adenine as 10 were 19.7, 17.0, 16.5 and 15.4. This divergence is not due to experimental error in the method of analysis since agreement among replicate analyses of the same specimen is satisfactory (Table 4). The composition of rat-liver RNA, on the other hand, appears in our experience to vary only within very narrow limits.

Table 6. Analyses of ribonucleic acid from different morphological fractions of a rat liver, expressed as molar ratios relative to adenine as 10

	Analysis by method (d).			
	Mitochondria	Microsomes	Cell sap	Nuclei
Adenine	10	10	10	10
Guanine	16.9	16.9	16.9	9.0
Cytosine	15.1	14.7	14.6	11.5
Uracil	11.0	10.3	11.3	11.1
Purine/pyrimidine ratio	1.03	1.08	1.04	0.84

Table 7. *Analyses of ribonucleic acid from different morphological fractions of pregnant and foetal rabbit-liver tissue and regenerating rat-liver tissue expressed as molar ratios relative to adenine as 10*

	Mitochondria	Microsomes	Cell sap	Whole cytoplasm
(a) Pregnant-rabbit liver				
Adenine	10	10	10	10
Guanine	15.9	15.6	15.4	15.8
Cytosine	15.6	15.4	15.3	15.2
Uracil	10.3	10.2	10.2	11.1
(b) Foetal rabbit liver				
Adenine	10	10	10	10
Guanine	15.4	16.1	15.4	15.4
Cytosine	15.5	15.5	15.3	15.6
Uracil	10.4	10.4	9.8	10.1
(c) Regenerating rat liver				
Adenine	10	10	10	10
Guanine	17.0	16.7	17.2	16.6
Cytosine	14.2	15.1	14.7	14.1
Uracil	10.3	10.4	10.5	10.2

DISCUSSION

In the analysis of RNA the method of preliminary hydrolysis is of prime importance. Hydrolysis with *N*-hydrochloric acid produces purine bases and pyrimidine nucleotides, but the latter are partially degraded so that a correction has to be applied (Smith & Markham, 1950). Perchloric acid hydrolysis on the other hand yields all four bases, but it is clear from our results that cytidylic acid is not quantitatively hydrolysed under the conditions employed (Fig. 1), with the result that the figures for both cytosine and uracil are unreliable. No improvement results from an increase in the hydrolysis time (Table 5). Vischer & Chargaff (1948*b*) have similarly reported resistance to hydrolysis by formic acid.

These difficulties can be overcome by the use of alkaline hydrolysis provided that the conditions are such as to avoid deamination of cytidylic acid (Davidson & Smellie, 1952*a*). Although a mixture of four nucleotides cannot be completely separated by the paper chromatographic methods of Carter (1950), of Magasanik, Vischer, Doniger, Elson & Chargaff (1950), and of Smith & Markham (1950), adequate separation is easily achieved by ionophoresis on paper which can be adapted to the analysis of very small amounts of material by the use of method (*d*) described above. This is in our opinion the most satisfactory method for the analysis of RNA.

Wyatt (1951) has commented on the low percentage of the total phosphorus of yeast RNA accounted for by calculation from the amounts of bases recovered from chromatograms of perchloric acid digests. We have confirmed this observation; in one experiment, for example, the recovery on this basis was 86 % whereas the corresponding recovery

after separation of the nucleotides by the ionophoretic method (*d*) was 97 % (Table 4).

It is clear from the results in Table 6 that there is no essential difference in composition of the RNA's from the three cytoplasmic sources in rat or rabbit liver. In a brief note, Elson & Chargaff (1951) mention the similarity in composition of the RNA's from mitochondria and submicroscopic particles of rat liver without quoting figures. Both were characterized by high molar proportions of guanine and cytosine such as we have recorded in Table 6. This general pattern has been observed for whole rat- and rabbit-liver RNA by Volkin & Carter (1951) and for the liver tissue of other species by Chargaff *et al.* (1950).

Although nuclear RNA contains the same ribonucleotides as cytoplasmic RNA (McIndoe & Davidson, 1952) it has been found that the proportions may vary widely from animal to animal and are much more readily affected by the conditions of hydrolysis than is the case with cytoplasmic RNA. While relatively low guanine and cytosine values occur in the sample of nuclear RNA shown in Table 6, higher values have been found in many batches of nuclei (McIndoe, unpublished results) although a lower guanine value than is commonly found in cytoplasmic RNA is recorded in the few results available in the literature for the nuclear RNA of rat and calf liver (Elson & Chargaff, 1951; McIndoe & Davidson, 1952; Marshak, 1951). Nuclear RNA can also be distinguished from cytoplasmic RNA by its much more rapid uptake of ³²P (Marshak & Calvet, 1949; Barnum & Huseby, 1950; Jeener & Szafarz, 1950; Davidson, McIndoe & Smellie, 1951; Smellie *et al.* 1953) and of isotopically labelled orotic acid (Potter, Recknagel & Hurlbert, 1951) and of [¹⁴C]formate (Smellie & McIndoe, 1952).

The results of our chromatographic experiments make it clear that the same pentose is found in all three cytoplasmic RNA's and in nuclear RNA, and that it is chromatographically identical with ribose. The pentose of whole liver RNA from pig, sheep, calf and man has been shown to be chromatographically identical with ribose by Chargaff *et al.* (1950). Since the pentose in the whole liver RNA of the sheep has been conclusively proved to be ribose after identification of the *p*-bromophenylhydrazine by Davidson & Waymouth (1944) there would appear to be little doubt that the sugar in liver RNA, from whatever part of the cell, is in fact ribose. There is therefore every justification for using the term ribonucleic acid rather than pentose-nucleic acid when referring to liver tissue.

SUMMARY

1. Three methods for the determination of the proportions of bases in ribonucleic acid (RNA) have been compared; paper-chromatographic separa-

tion after hydrolysis with perchloric acid and after hydrolysis with *N*-HCl, and separation by ionophoresis on paper of the nucleotides obtained by alkaline hydrolysis. It is concluded that the last method is the most satisfactory and a modification for use with small amounts of material is described.

2. The RNA's from mitochondria, microsomes and cell sap of rat liver have been shown to have the same composition in terms of molar proportions of bases, and differ in composition from the RNA of the rat-liver nucleus. The molar proportions of bases in the RNA's from the cytoplasmic fractions of maternal and foetal rabbit liver or of normal and regenerating rat liver have been found to be the same.

3. The pentose sugar in the RNA's from rat-liver mitochondria, microsomes, cell sap and nuclei has been chromatographically identified as ribose.

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REFERENCES

- Barnum, C. P. & Huseby, R. A. (1950). *Arch. Biochem.* **29**, 7.
 Brown, D. M., Dekker, C. A. & Todd, A. R. (1952). *J. chem. Soc.* pp. 2715.
 Carter, C. E. (1950). *J. Amer. chem. Soc.* **72**, 1466.
 Chargaff, E., Magasanik, B., Vischer, E., Green, C., Doniger, R. & Elson, D. (1950). *J. biol. Chem.* **186**, 51.
 Cohn, W. E. (1950). *J. Amer. chem. Soc.* **72**, 1471.
 Cohn, W. E. (1951). *J. cell. comp. Physiol.* **38**, Suppl. 1, 21.
 Davidson, J. N., Frazer, S. C. & Hutchison, W. C. (1951). *Biochem. J.* **49**, 311.
 Davidson, J. N., McIndoe, W. M. & Smellie, R. M. S. (1951). *Biochem. J.* **49**, xxxvi.
 Davidson, J. N. & Smellie, R. M. S. (1952*a*). *Biochem. J.* **52**, 594.
 Davidson, J. N. & Smellie, R. M. S. (1952*b*). *Biochem. J.* **52**, 600.
 Davidson, J. N. & Waymouth, C. (1944). *Biochem. J.* **38**, 375.
 Eliasson, N. A., Hammarsten, E., Riechard, P., Aquist, S., Thorell, B. & Ehrensvar, G. (1951). *Acta chem. scand.* **5**, 431.
 Elson, D. & Chargaff, E. (1951). *Fed. Proc.* **10**, 180.
 Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
 Holiday, E. R. & Johnson, E. A. (1949). *Nature, Lond.*, **163**, 216.
 Jeener, R. & Szafarz, D. (1950). *Arch. Biochem.* **28**, 54.
 Johnson, R. M. & Albert, S. (1952). *Arch. Biochem. Biophys.* **35**, 340.
 McIndoe, W. M. & Davidson, J. N. (1952). *Brit. J. Cancer*, **6**, 200.
 Magasanik, B., Vischer, E., Doniger, R., Elson, D. & Chargaff, E. (1950). *J. biol. Chem.* **186**, 37.
 Marshak, A. (1951). *J. biol. Chem.* **189**, 607.
 Marshak, A. & Calvet, F. (1949). *J. cell. comp. Physiol.* **34**, 451.
 Marshak, A. & Vogel, H. J. (1951). *J. biol. Chem.* **189**, 597.
 Partridge, S. M. (1949). *Symp. biochem. Soc.* no. 3, 57.
 Partridge, S. M. & Westall, R. G. (1948). *Biochem. J.* **42**, 238.
 Ploeser, J. M. & Loring, H. S. (1949). *J. biol. Chem.* **178**, 431.
 Potter, V. R., Recknagel, R. O. & Hurlbert, B. (1951). *Fed. Proc.* **10**, 646.
 Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
 Smellie, R. M. S. & McIndoe, W. M. (1952). *Biochem. J.* **52**, xxii.
 Smellie, R. M. S., McIndoe, W. M., Logan, R., Davidson, J. N. & Dawson, J. M. (1953). *Biochem. J.* **54**, 280.
 Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.
 Vischer, E. & Chargaff, E. (1948*a*). *J. biol. Chem.* **176**, 703.
 Vischer, E. & Chargaff, E. (1948*b*). *J. biol. Chem.* **176**, 715.
 Volkin, E. & Carter, C. E. (1951). *J. Amer. chem. Soc.* **73**, 1516.
 Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.