Evidence for Ethylation of Rat Liver Deoxyribonucleic Acid after Administration of Ethionine

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1. Administration of a large dose (500 mg/kg body wt.) of ^3H -labelled L-ethionine to rats resulted in the incorporation of a small amount of radioactivity into the liver DNA. Considerable evidence that this radioactivity was not due to contamination of the isolated DNA with labelled protein, RNA, S-adenosyl-L-ethionine or L-ethionine was obtained. 2. After acidic hydrolysis of the DNA isolated from the livers of rats treated with labelled L-ethionine, virtually all of the radioactivity present in the DNA was found in a fraction with similar chromatographic properties to 7-ethylguanine. 3. Treatment of rats with comparable doses of L-methionine did not lead to the formation of 7-methylguanine in the liver DNA. 4. These results are discussed in relation to the induction of liver tumours by ethionine.

Prolonged feeding of the amino acid, ethionine, to rats has been shown to result in the production of hepatomas (Farber, 1963). The discovery that ethylation of RNA and protein takes place in the livers of rats treated with ethionine (Farber & Magee, 1960; Stekol, Mody & Perry, 1960; Natori, 1963; Farber *et al.* 1967a; Rosen, 1968; Friedman, Shull & Farber, 1969) supports the possibility of a common mechanism for the induction of cancer by ethionine and compounds as chemically dissimilar as nitrosamines, polycyclic hydrocarbons and aromatic amines which share the ability to produce intermediates reacting with components of the tissue in which they produce cancer (Miller, 1970). Although many of these carcinogens are known to interact with DNA, reports on the reaction between ethionine and DNA are conflicting. The ethylation of DNA in the livers of rats treated with ethionine was reported by Stekol *et al.* (1960) and by Stekol (1965), but both Farber, McConomy & Frumanski (1967b) and Ortwerth & Novelli (1969) failed to confirm this finding. The experiments reported here show that administration of large doses of ethionine to rats produced a small but definite ethylation at the 7-position of guanine in liver DNA but that comparable doses of methionine did not produce 7-methylguanine.

EXPERIMENTAL

Materials. L-[Me- ^3H]methionine was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. and L-[Et-1- ^3H]ethionine from New England Nuclear Corp.,

Boston, Mass., U.S.A. The radioactive amino acids were diluted to the required specific radioactivity with L-methionine and L-ethionine obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. S-Adenosyl-L-[Et-1- ^{14}C]ethionine (4.2 mCi/mmol) was prepared as described by Pegg & Williams-Ashman (1970). 7-Ethylguanine was synthesized by using an appropriate modification of the procedure of Jones & Robins (1963) for the preparation of 7-methylguanine.

Conduct of animal experiments. Male rats of a non-inbred Wistar stock maintained in the Courtauld Institute were used. Each of a group of five rats (150 g) was injected intraperitoneally with 4 ml of water containing 120 mg of L-[Me- ^3H]methionine (1.24 mCi/mmol). The animals were allowed free access to water but no food after the injection. After 7 h from the time of injection, they were killed and the livers rapidly removed and used for the preparation of nucleic acids. Another five rats (200 g) were each given 5 ml of water containing 100 mg of L-[Et-1- ^3H]ethionine (1.63 mCi/mmol) by intraperitoneal injection. The solution of ethionine utilized in this experiment was found to contain an appreciable amount of ethionine sulfoxide (15% of the total) but no other radioactive impurities by paper chromatography (solvents 1 and 2). Throughout the period after administration of the labelled amino acid the rats were starved but allowed free access to water. After 18 h from the time of injection, the animals were killed and nucleic acids isolated from the liver. This experiment in its essential details was repeated three times.

Preparation of nucleic acids. RNA and DNA were extracted from homogenates of the livers of the treated animals and purified by the procedures described by Swann & Magee (1968).

Chromatography of nucleic acid hydrolysates on Dowex 50. Nucleic acids were hydrolysed to pyrimidine nucleotides and free purines by dissolving in 1M-HCl and heating at 100°C for 1 h. The hydrolysate was then cooled and applied to a column (20 cm $\times$ 1 cm) of Dowex 50 (X12; H⁺ form) that had previously been equilibrated with

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1M-HCl. A small amount (0.5mg in most cases) of authentic 7-methylguanine or 7-ethylguanine was mixed with the sample before application to the column. The column was then eluted with an exponential gradient of either 1-3M-HCl or 1-4M-HCl at a flow rate of 24ml/h. Fractions of approx. 5ml or 10ml were collected and $E_{260}^{1\text{cm}}$ was determined for each fraction. The radioactivity present was then determined after transference of the material to a scintillation vial, evaporating to dryness, dissolving the residue in 0.5ml of 1M-Hyamaine in methanol and adding 10ml of scintillation fluid.

Paper chromatography. Paper chromatograms were run in the descending manner on strips (4cm×60cm) of Whatmann 3MM paper. The solvents employed were: 1, butan-1-ol-acetic acid-water (25:4:10, by vol.); 2, 2-methylpropan-2-ol-butan-2-one-diethylamine-water (10:5:1:10, by vol.); 3, butan-1-ol-aq. NH_3 (sp.gr. 0.88)-water (86:5:9, by vol.); 4, propan-2-ol-conc. HCl-water (65:18:17, by vol.). Solvents 1 and 2 (Ortwerth & Novelli, 1969) were used to investigate the purity of the samples of ethionine used in these experiments. Solvents 3 and 4 were used for the separation of alkylated purines and pyrimidines.

Determination of radioactivity. All radioactivity measurements were made in a scintillation counter (Packard Instrument Co., La Grange, Ill., U.S.A.). The scintillation fluid contained 0.6% 2,5-diphenyloxazole in toluene (w/v). The counting efficiency was determined either by the addition of known amounts of labelled toluene to the samples or by the method of pulse height shift (Baillie, 1960).

Chromatograms of radioactive materials were cut into

segments 1 cm across and the segments were covered with 15ml of scintillation fluid and the radioactivity was determined. Other samples were dissolved in 0.5ml of 1M-Hyamaine in methanol and the radioactivities counted in the presence of 10ml of scintillation fluid.

RESULTS

DNA isolated from the livers of rats that had received a large dose (500mg/kg body wt., 1mCi/animal) of L-[Et-1- ^3H]ethionine 18 h before death was found to contain a small amount of radioactivity. The total yield of DNA from the five rats in this experiment was 58mg and the specific radioactivity was 9.7 d.p.m./mg (two other experiments in which the dose of ethionine was 750mg/kg body wt. gave similar results). Half of the sample of DNA was hydrolysed to free purine bases and pyrimidine nucleotides and chromatographed on Dowex 50. As shown in Fig. 1 all of the radioactivity present in the sample was eluted from the column in a region coincident with a sample of 7-ethylguanine added to the DNA sample after hydrolysis. No radioactivity was found in the pyrimidine nucleotide fractions or in the fractions corresponding to guanine and adenine. The other half of the DNA sample was also hydrolysed and separated on the Dowex 50 column, but in this case the fractions containing 7-ethylguanine were combined, evap-

Fig. 1. Ion-exchange chromatography of hydrolysed DNA from rats given L-[Et-1- ^3H]ethionine (500mg/kg body wt., 1mCi/rat). About 0.3 mg of 7-ethylguanine was added to 28mg of DNA (9.7 d.p.m./mg) and the mixture heated at 100°C for 60 min in 2 ml of 1M-HCl before chromatography on Dowex 50 (X12; H^+ form) with an exponential 1-3M-HCl gradient. ▲, $E_{260}^{1\text{cm}}$; ●, radioactivity; Py, pyrimidine nucleotides; G, guanine; E, 7-ethylguanine; A, adenine.

orated to dryness at 60°C under reduced pressure, taken up in 0.1 ml of 0.01 M-hydrochloric acid and subjected to paper chromatography with solvent 3. The chromatogram was run for 48 h (the solvent runs off the end of the paper during this time and the liquid which did so was collected in a beaker). The paper was dried, examined under u.v. light to locate the 7-ethylguanine and then cut into strips and the radioactivity counted. More than 80% of the radioactivity that had been applied to the Dowex column was found to be present in the region of the paper corresponding to 7-ethylguanine. No radioactivity was present in any other fraction from the Dowex column or in any other region of the paper chromatogram (including the solvent that had run from the end of the paper) except at the origin. The radioactivity found at the origin amounted to approx. 15% of the total radioactivity present in the DNA added to the column. This

material has not been identified but could represent a degradation product formed from 7-ethylguanine after the evaporation to dryness of the acidic samples from the column. Whatever the nature of this material, the DNA extracted from rats that had received a single dose of 500 mg/kg body wt. of ethionine contained some material with the properties of 7-ethylguanine. Assuming that the specific radioactivity of the 7-ethylguanine was similar to that of the administered ethionine, the 7-ethylguanine present in the rat liver DNA amounted to 2.5 pmol/mg of DNA or 1/230 000 guanine residues.

Analysis of a hydrolysate of DNA isolated from rats that had received a large dose (800 mg/kg body wt., 1 mCi/rat) of L-[Me-³H]methionine by chromatography on Dowex 50 columns is shown in Fig. 2. There was considerable incorporation of radioactivity into the fractions corresponding to

Fig. 2. Ion-exchange chromatography of hydrolysed DNA from rats given L-[Me-³H]methionine (800 mg/kg body wt.; 1 mCi/rat). About 5 mg of 7-methylguanine was added to 25 mg of DNA and the mixture heated for 60 min at 100°C in 1 M-HCl before chromatography on Dowex 50 (X12; H⁺ form) with an exponential 1-4 M-HCl gradient. ▲, $E_{260}^{1\text{cm}}$; ●, radioactivity; Py, pyrimidine nucleotides; G, guanine; M, 7-methylguanine; A, adenine.

pyrimidine nucleotides, guanine and adenine but there was no label present in 7-methylguanine. This finding is in agreement with earlier reports that no 7-methylguanine could be detected in normal rat liver DNA (Shank & Magee, 1967; Craddock, Villa-Trevino & Magee, 1968; Swann & Magee, 1968) and also shows that within the limits of detection (0.5 pmol/mg of DNA) there was no 7-methylguanine formed after the administration of a very large dose of methionine to normal rats.

As the amount of alkylation of liver DNA detected after the administration of ethionine was very small, it was of great importance to rule out artifacts due to contamination of the DNA with other cellular constituents. Such compounds that would be expected to be present in the liver and to become labelled after the administration of L-[Et-1-³H]ethionine are RNA, protein, *S*-adenosyl-L-ethionine and ethionine itself. The experiments described below provide strong evidence that contamination with these compounds is not responsible for the observed labelling of DNA.

It is unlikely that ethionine would remain bound to the DNA throughout the precipitation steps involved in the preparation and ethionine is separated from 7-ethylguanine by the chromatographic systems used for the analysis of the hydrolysed DNA. However, *S*-adenosyl-L-ethionine is known to accumulate in large concentrations in rat liver after the administration of ethionine (Farber, 1963, 1967; Stekol, 1965) and since *S*-adenosyl-L-ethionine has a strong positive charge at neutral pH it is

possible that it could become bound to the DNA and remain with it throughout the preparative procedure. After acidic treatment, this bound *S*-adenosyl-L-ethionine could be degraded to labelled material which co-chromatographs with 7-ethylguanine. This possibility was examined by adding 0.05 μ mol of *S*-adenosyl-L-[Et-1-¹⁴C]ethionine (0.21 μ Ci) to 20 mg of rat liver DNA, hydrolysing the mixture by heating in 1 M-HCl at 100°C for 1 h and separating the radioactive products by chromatography on Dowex 50. As shown in Fig. 3, after this treatment 75% of the radioactivity was present in unchanged *S*-adenosyl-L-ethionine which was removed from the column only by washing with 5 M-HCl. *S*-Adenosyl-L-methionine is known to be much more stable to acid hydrolysis than most nucleosides (Parks & Schlenk, 1958) and the observation that the ethyl analogue is also resistant to acidic hydrolysis is therefore not unexpected. Four other radioactive products were formed from the degradation of the *S*-adenosyl-L-[Et-1-¹⁴C]ethionine and separated on the Dowex column but none of these coincides with 7-ethylguanine (Fig. 3).

A third possibility is that the isolated DNA is contaminated with protein. Liver proteins are known to contain ethionine and certain ethylated amino acids after the administration of ethionine (Stekol *et al.* 1960; Natori, 1963; Farber *et al.* 1967a; Friedman *et al.* 1969; Ortwerth & Novelli, 1969). However, it is unlikely that the mild acid hydrolysis utilized in these experiments would be sufficient to degrade such contaminating proteins to free amino

Fig. 3. Ion-exchange chromatography of a mixture of 20 mg of rat liver DNA (unlabelled), 0.5 mg of 7-ethylguanine and 0.05 μ mol of *S*-adenosyl-L-[Et-1-¹⁴C]ethionine (4.6×10^5 d.p.m.). The mixture was heated at 100°C for 1 h in 2 ml of 1 M-HCl; applied to Dowex 50 (X12; H⁺ form) and eluted with an exponential gradient of 1–3 M-HCl. After 1 litre of effluent had been collected, the column was eluted with 100 ml of 5 M-HCl as shown. Symbols as for Fig. 1.

Fig. 4. Ion-exchange chromatography of hydrolysed RNA extracted from the livers of rats given L-[Et-1-³H]-ethionine (500 mg/kg body wt., 1 mCi/rat). About 0.5 mg of 7-ethylguanine was added to 40 mg of RNA (1277 d.p.m./mg) and the mixture heated for 60 min at 100°C in 2 ml of 1 M-HCl before chromatography on Dowex 50 (X12; H⁺ form) with an exponential 1-3 M-HCl gradient. Symbols as for Fig. 1.

acids or small peptides. Large polypeptides would not be expected to move as a discrete band corresponding to 7-ethylguanine in chromatography on Dowex and paper. Further, no labelled material which did not correspond to known components of DNA was present in the DNA extracted from rats treated with L-[Me-³H]methionine although very highly labelled protein was present in the livers of these animals suggesting that there was no significant contamination of the DNA with protein.

The RNA extracted from the livers of the ethionine-treated rats had a specific radioactivity of 1277 d.p.m./mg which is greater than 100 times that of the DNA. Therefore, although the purified DNA had been treated with ribonuclease and was apparently free of RNA, a contamination with only 1% RNA could account for the radioactivity found in the DNA. The following two experiments suggest that this was not the case and that contamination with RNA was not responsible for the 7-ethylguanine found in the DNA sample. First, when 20 mg (26000 d.p.m.) of RNA isolated from the livers of rats treated with L-[Et-1-³H]ethionine was added to an homogenate obtained from the livers of two untreated rats and DNA was then purified from this mixture, the resultant DNA preparation (11 mg) had less than 3 d.p.m. above background. Second, Fig. 4 shows that the RNA isolated from rats injected with 100 mg of L-[Et-1-³H]ethionine

(1.63 mCi/mmol) could be resolved into four radioactive peaks after hydrolysis and analysis on Dowex 50. In addition to considerable radioactivity in 7-ethylguanine there were radioactive peaks corresponding to the pyrimidine nucleotide fraction and two other regions preceding 7-ethylguanine. [These peaks appear to correspond to 5-ethylcytidylic acid, 5-ethyluridylic acid and ethylated ribose, N²N²-diethylguanine and an unidentified purine, and N²-ethylguanine respectively (Rosen, 1968; A. E. Pegg, unpublished work).] It is most unlikely that the isolated DNA could be contaminated with an RNA species containing only one of these ethylated products and since there was no trace of radioactivity in any fraction from the chromatography of hydrolysed DNA except that corresponding to 7-ethylguanine it is very probable that the observed 7-ethylguanine did originate in the liver DNA.

DISCUSSION

The experiments reported in this paper confirm earlier observations that after the administration of ethionine to rats the liver RNA becomes ethylated to a much greater extent than the DNA (Natori, 1963; Farber *et al.* 1967a; Ortwerth & Novelli, 1969). However, the present work strongly suggests that as a result of a single dose of ethionine there

was a small but definite ethylation of DNA. This finding and that of Stekol (1965) appear to be in contradiction to the negative results of Farber *et al.* (1967b), and of Ortwerth & Novelli (1969). However, there is a major, and perhaps crucial, difference in the experimental conditions of the two groups of studies. The experiments in which negative results were obtained used only small tracer doses of ethionine (2mg or less per dose per animal). The experiments yielding positive results used either a single large dose (500–750mg/kg body wt.) in the present study or the prolonged administration of carcinogenic amounts (Stekol, 1965). With the large dose, *S*-adenosylethionine, a known metabolic ethyl donor, accumulates in large amounts in the liver (Farber, Shull, Villa-Trevino, Lombardi & Thomas, 1964; Smith & Salmon, 1965; Shull, McConomy, Vogt, Castillo & Farber, 1966).

The mechanism by which 7-ethylguanine was formed in liver DNA after the administration of ethionine is obscure. Since large amounts of *S*-adenosyl-L-ethionine accumulate in the liver under these conditions (Farber *et al.* 1964; Smith & Salmon, 1965; Shull *et al.* 1966), it has been suggested that the ethylation of RNA and proteins is catalysed by the action of enzymes that normally utilize *S*-adenosyl-L-methionine as a donor of methyl groups to methylate these macromolecules (Farber, 1967; Hancock, 1968; Stekol, 1965). If this is correct, ethyl groups should only be found at the same sites of the macromolecules as those that normally contain methyl groups. 7-Methylguanine was not detected in normal rat liver DNA (Shank & Magee, 1967; Craddock *et al.* 1968; Swann & Magee, 1968) and 7-methylguanine was not found in rat liver DNA after the administration of a large dose of methionine in the present work. Further, no enzyme catalysing the methylation of DNA at the 7-position of guanine and utilizing *S*-adenosyl-L-methionine as a methyl donor has been described. These results suggest that the ethylation of DNA by ethionine may not be mediated by the action of a methyltransferase acting with *S*-adenosyl-L-ethionine as a donor of alkyl groups. Ortwerth & Novelli (1969) have suggested that the ethylation of RNA *in vivo* by ethionine is not mediated through the RNA methyltransferases and that new and as yet unknown mechanisms are used. The presence, in the liver of rats treated with ethionine, of an ethylating agent of sufficient power to act directly on the DNA would explain the predominance of ethylation on the N-7 position of guanine since this position is the most favoured for direct chemical alkylation of polynucleotides (Lawley, 1966), and may also explain the difference between the effects of methionine and ethionine in alkylation of DNA. However, it should be emphasized that in

experiments with rats with methionine conditions can never be entirely analogous to those with large doses of ethionine. When a large dose of ethionine is given, *S*-adenosyl-L-ethionine accumulates in large amounts in the liver (Farber *et al.* 1964; Smith & Salmon, 1965; Shull *et al.* 1966), whereas in the rat the amount of *S*-adenosyl-L-methionine cannot be raised to the same degree by administration of a large dose of methionine (Farber *et al.* 1964; Shull *et al.* 1966). If sufficiently high intracellular concentration of a methylating intermediate could be achieved it is conceivable that methylation on the 7-position of guanine could occur in mammalian DNA. Culp, Dore & Brown (1970) have reported the presence of several methylated purines, including 7-methylguanine, in the DNA of HeLa cells.

It is noteworthy that there was no incorporation of label from L-[*Et*-1-³H]ethionine into the pyrimidine nucleotide fraction of liver DNA when rats were treated with ethionine in the present experiments. 5-Methyleytosine is known to be present in DNA from mammalian cells (Borek & Srinivasan, 1966; Kappler, 1970) and enzymes catalysing the transfer of methyl groups from *S*-adenosyl-L-methionine to certain cytosine residues in DNA have been isolated from bacterial and mammalian cells (Gold & Hurwitz, 1964; Borek & Srinivasan, 1966; Oda & Marmur, 1966; Kaye, Fridlender, Salomon & Bar-Meir, 1967; Kalousek & Morris, 1969). Our results therefore imply that the enzyme responsible for this reaction in rat liver does not utilize the *S*-adenosyl-L-ethionine formed after ethionine administration for the formation of 5-ethylcytosine. This finding is in agreement with the observation that *S*-adenosyl-L-ethionine could not substitute for *S*-adenosyl-L-methionine in the formation of 5-alkylcytosine in DNA by a purified enzyme from rat spleen (Kalousek & Morris, 1969). However, this result is not conclusive since it is known that formation of 5-methyleytosine in DNA of mammalian cells takes place shortly after synthesis of the DNA (Burdon & Adams, 1969; Kappler, 1970) and the effect of the large dose of ethionine on the rate of DNA synthesis in the liver was not determined.

The demonstration that ethionine does react with the DNA of the tissue in which it can produce tumours implies that DNA cannot be ruled out as the 'target molecule' of this carcinogen. However, the significance of the formation of 7-ethylguanine in liver DNA in the hepatocarcinogenic process cannot be evaluated at present. The degree of ethylation produced by a single injection of ethionine was very much less than that found when other carcinogenic ethylating agents such as di-ethylnitrosamine and *N*-ethyl-*N*-nitrosourea were administered (Swann & Magee, 1970) but these com-

pounds are more potent and versatile carcinogens than ethionine (Druckrey, Preussmann, Ivankovic & Schmahl, 1967; Magee & Barnes, 1967). The procedure normally used to produce hepatomas in rats with ethionine requires a diet containing 0.25% ethionine to be fed for 4 months (Farber, 1963). During this time a more substantial degree of ethylation may occur, and other sites of ethylation that were not detected in the present experiments may also be attacked. The 7-position of guanine is known to be the most reactive site in nucleic acids towards attack by alkylating agents *in vitro* (Lawley, 1966), but there is poor correlation between the carcinogenic activity of methylating agents and the amount of 7-methylguanine which each produces *in vivo* (Swann & Magee, 1968). Other minor products of this attack may be of more importance in affecting the biological activity of nucleic acids (Singer & Fraenkel-Conrat, 1969; Loveless, 1969; Ludlum, 1970*a,b*). The production of 7-ethylguanine in the DNA of rats treated with ethionine may, therefore, be of importance only in revealing that formation of an intermediate capable of reacting with DNA has taken place.

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REFERENCES

- Baillie, L. A. (1960). *Int. J. appl. Radiat. Isotopes*, **8**, 1.
 Borek, E. & Srinivasan, P. (1966). *A. Rev. Biochem.* **35**, 275.
 Burdon, R. H. & Adams, R. L. P. (1969). *Biochim. biophys. Acta*, **174**, 322.
 Craddock, V. M., Villa-Trevino, S. & Magee, P. N. (1968). *Biochem. J.* **107**, 179.
 Culp, L. A., Dore, E. & Brown, G. M. (1970). *Archs Biochem. Biophys.* **136**, 73.
 Druckrey, H., Preussmann, R., Ivankovic, S. & Schmahl, D. (1967). *Z. Krebsforsch.* **69**, 103.
 Farber, E. (1963). *Adv. Cancer Res.* **7**, 383.
 Farber, E. (1967). *Adv. Lipid Res.* **5**, 119.
 Farber, E., McConomy, J., Franzen, B., Marroquin, F., Stewart, G. A. & Magee, P. N. (1967*a*). *Cancer Res.* **27**, 1761.
 Farber, E., McConomy, J. & Frumanski, B. (1967*b*). *Proc. Am. Ass. Cancer Res.* **8**, 16.
 Farber, E. & Magee, P. N. (1960). *Biochem. J.* **76**, 58*p*.
 Farber, E., Shull, K. H., Villa-Trevino, S., Lombardi, B. & Thomas, M. (1964). *Nature, Lond.*, **203**, 34.
 Friedman, M., Shull, K. H. & Farber, E. (1969). *Biochem. biophys. Res. Commun.* **34**, 857.
 Gold, M. & Hurwitz, J. (1964). *J. biol. Chem.* **239**, 3858.
 Hancock, R. L. (1968). *Cancer Res.* **28**, 1223.
 Jones, J. W. & Robins, R. K. (1963). *J. Am. chem. Soc.* **85**, 193.
 Kalousek, F. & Morris, N. R. (1969). *J. biol. Chem.* **244**, 1157.
 Kappler, J. W. (1970). *J. cell. Physiol.* **75**, 21.
 Kaye, A. M., Fridlender, B., Salomon, R., & Bar-Meir, S. (1967). *Biochim. biophys. Acta*, **142**, 331.
 Lawley, P. D. (1966). *Prog. nucleic Acid Res. molec. Biol.* **5**, 89.
 Loveless, (1969). *Nature, Lond.*, **223**, 206.
 Ludlum, (1970*a*). *J. biol. Chem.* **245**, 477.
 Ludlum, (1970*b*). *Biochim. biophys. Acta*, **213**, 142.
 Magee, P. N. & Barnes, J. M. (1967). *Adv. Cancer Res.* **10**, 163.
 Miller, J. A. (1970). *Cancer Res.* **30**, 559.
 Natori, Y. (1963). *J. biol. Chem.* **238**, 2075.
 Oda, K. & Marmur, J. (1966). *Biochemistry, Easton*, **5**, 761.
 Ortwerth, B. J. & Novelli, G. D. (1969). *Cancer Res.* **29**, 380.
 Parks, L. W. & Schlenk, F. (1958). *J. biol. Chem.* **230**, 295.
 Pegg, A. E. & Williams-Ashman, H. G. (1970). *Archs Biochem. Biophys.* **137**, 156.
 Rosen, L. (1968). *Biochem. biophys. Res. Commun.* **33**, 546.
 Shank, R. & Magee, P. N. (1967). *Biochem. J.* **105**, 521.
 Shull, K. H., McConomy, J., Vogt, M., Costillo, A. & Farber, E. (1966). *J. biol. Chem.* **241**, 5060.
 Singer, B. & Fraenkel-Conrat, H. (1969). *Prog. nucleic Acid Res. molec. Biol.* **9**, 1.
 Smith, R. C. & Salmon, W. D. (1965). *Archs Biochem. Biophys.* **111**, 191.
 Stekol, J. A. (1965). In *Transmethylation and Methionine Biosynthesis*, p. 231. Ed. by Shapiro, S. K. & Schlenk, F. Chicago: University of Chicago Press.
 Stekol, J. A., Mody, U. & Perry, J. (1960). *J. biol. Chem.* **235**, 59.
 Swann, P. F. & Magee, P. N. (1968). *Biochem. J.* **110**, 39.
 Swann, P. F. & Magee, P. N. (1970). *Abstr. 10th int. Cancer Congr., Houston*, p. 1.