# INCORPORATION OF $^{14}$C-FORMATE AND (2-$^{14}$C)-GLYCINE INTO NUCLEIC ACID BASES OF THE RAT LIVER *IN VIVO* AND *IN VITRO*

A. SIBATANI*

*Biochemistry Department, University of Glasgow (Scotland)*

Studies on the incorporation of $^{14}$C-formate into the bases of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in various animal tissues *in vivo* and *in vitro* have revealed several interesting features. Unlike adenine, formate is incorporated into RNA of the normal rat liver only very slightly[1], and the specific activity ratio of RNA/DNA for normal liver is usually much lower than the ratios obtained with most other precursors such as labelled adenine, orotic acid, or inorganic phosphate[2]. This property of formate is shared by the labelled glycine[3,4]. It has also been reported that $^{14}$C-formate is actively incorporated not only into RNA but also into DNA in liver slices of normal rats *in vitro*[5]. Under certain conditions a striking difference has been observed in the extent of formate incorporation into the purines and into thymine. Thus, the incorporation of $^{14}$C-formate into nucleic acid purines is greatly reduced in such tissues as bone marrow or Ehrlich ascites carcinoma cells under *in vitro* conditions[6,7], or in thymus *in vivo* after X-irradiation[8], while incorporation into DNA thymine is not affected under the same conditions.

In order to obtain more information about the nature of the $^{14}$C-formate incorporation into the nucleic acid bases, experiments were conducted using normal and regenerating rat livers *in vivo* and *in vitro* with and without X-ray irradiation. In addition, (2-$^{14}$C)-glycine was also employed for comparison.

In planning the experiments of this type, it was realised that formate and glycine, unlike other precursors of nucleic acids, are readily incorporated into proteins. Even slight contamination of final samples of nucleic acid bases by amino acids may thus lead to erroneous results, especially when the uptake of these precursors by nucleic acid bases is very slight. These considerations led to the development of a rapid and convenient method which eliminates the possibility of such contamination.

## EXPERIMENTAL

### *Animals*

Young adult rats weighing 175 ± 25 g were used. Partial hepatectomy was conducted by removing the central and left lateral lobes of the liver under ether anaesthesia. The operations were carried out between 9.00 and 11.00 A.M. unless otherwise stated. Isotopic experiments were started 17–24 hours after operation.

* British Council Scholar, 1955–56. Present address: Cytochemistry Laboratory, Yamaguti Medical School, Ube, Japan.

*References p. 599.*

*Irradiation*

The animals received either 100 r or 500 r of whole body X-irradiation, unfiltered, at 200 r.p.m. from an Aeromax 12 tube (87 kV, 5 mA) at various times before or after partial hepatectomy.

*Procedures for isotopic incorporation*

For *in vivo* experiments 100 $\mu$c of $^{14}$C-formate or (2-$^{14}$C)-glycine were injected intraperitoneally into normal and partially hepatectomised rats. Four or 24 hours later, the animals were killed and the livers perfused with physiological saline and frozen in solid $CO_2$-ethanol mixture. Each liver was processed separately in subsequent treatment. Usually duplicate animals were employed for one observation.

For *in vitro* studies, slices of about 0.3 mm thickness were prepared from non-perfused livers with the aid of the tissue chopper of McILWAIN AND BUDDLE[9]. Usually 2–3 livers were pooled for one observation. The slices were incubated essentially in accordance with the method of REICHARD[10]. Portions weighing 1.5 g were transferred in duplicate to 50 ml conical flasks with 5 ml of Krebs-Ringer bicarbonate buffer (without $CaCl_2$) containing $^{14}$C-precursors at the final concentration of 5 $\mu$c/ml. The flasks were shaken at 37° for 7·8 hours while oxygen-carbon dioxide (95 % : 5 %) mixture was slowly passed through them. No serious bacterial contamination occurred if the buffer solution was prepared freshly each time. At the end of the incubation the medium was filtered off, the slice washed with small volumes of 0.1 *M* citrate buffer, pH 4.5, suspended in the same buffer and frozen immediately.

*Separation of nucleic acids*

The frozen samples were thawed, homogenised briefly with a Potter-Elvehjem glass homogeniser with perspex pestle in the citrate buffer mentioned above. The homogenate was centrifuged and the sediment washed with ice-cold buffer and resuspended in 10 ml of 0.025 *M* disodium phosphate-HCl buffer, pH 7.4, containing 0.9 % NaCl. One ml of sodium dodecylsulphate solution (5 % in 45 % ethanol) was added and the nucleic acids were extracted at 100° for 10 min. 0.5 g NaCl was added and the mixture was centrifuged. The supernatant fluid was then shaken briefly with 2 ml of chloroform and centrifuged for 20 min at 3000 r.p.m. The water layer was recovered, treated with 4 vol. of ethanol and left at least 2 hours. The flocculent precipitate was centrifuged, the sediment dissolved in 3 ml of distilled water to yield a water-clear solution. Nucleic acids were reprecipitated with 7 ml of ethanol and washed with ethanol and ether. The dried nucleic acids were taken up in 4 ml of distilled water, which was then acidified with 1 ml of 1 *N* $HClO_4$ in an ice bath. The precipitate was immediately centrifuged and washed first with 6 ml of absolute ethanol. The dried nucleic acids were then incubated in 1 ml of 0.3 *N* KOH at 37° overnight.

The alkaline hydrolysate was acidified with 10 *N* $HClO_4$ and centrifuged in the cold. The supernatant (RNA fraction) was dried in a vacuum desiccator. The sediment was washed once with 6 ml of ice-cold 0.5 *N* $HClO_4$, centrifuged and resuspended in 4 ml of ice-cold distilled water. The suspension was made alkaline with a few drops of 1 *N* NaOH until the precipitate of $KClO_4$ and DNA was completely dissolved. One ml of chilled 1 *N* HCl was added to precipitate the DNA which was washed once with 5 ml of cold ethanol.

*Separation of the bases*

The RNA and DNA fractions were hydrolysed with 0.1 ml 12 *N* $HClO_4$ at 100° for 1 hour. They were then treated with 0.15 ml 10 *N* KOH and 0.1 ml 6 *N* HCl, and if necessary, diluted with 0.1 ml *N* HCl, and the sediment was spun down. The water-clear supernatant was used for paper chromatography with butanol–$NH_4OH$[11] for 40–48 hours on a sheet of Whatman No. 1 filter paper. Thymine spots were eluted from the paper directly with 0.1 *N* or 1.6 *N* HCl. Transverse strips containing guanine and adenine spots were rechromatographed in the second dimension with distilled water as a developing agent for 1 hour (18 cm). Any contaminating amino acids travelled up to the solvent front in this system. Occasionally overlapping pyrimidines, with an $R_F$ of about 0.8 in distilled water, could be eliminated at this step from the purines whose $R_F$ values lay around 0.4. A portion of guanine (probably the free base) remained at the origin in the first run with alkaline solvent. Before running in distilled water the two guanine spots were moistened with a drop of 1 *N* HCl, dried, exposed to $NH_4OH$ vapour and then again dried. Unless this was done, guanine did not move satisfactorily in distilled water. Paper chromatography of nucleic acid bases in water was attempted by LEVENBOOK using distilled water at pH 10 (cited by WYATT[12]). His listed $R_F$ values are very close to those obtained in this laboratory with plain distilled water.

Adenine and guanine were eluted from the chromatogram with 0.1 *N* (or 1.6 *N*) and 1.6 *N* HCl, respectively.

*Separation of acid-soluble purines*

A portion of the liver samples in *in vivo* experiments was homogenised in 0.2 *N* $HClO_4$ and centrifuged in the cold. The supernatant was neutralised with 10 *N* KOH, the precipitate of $KClO_4$

*References p. 599.*

centrifuged off, and the supernatant evaporated to dryness over a steam bath. The residue was hydrolysed with 12 $N$ $HClO_4$, and then processed as in the case of nucleic acid bases. The bases were extracted repeatedly with butanol from the hydrolysate in HCl, the combined butanol layers were concentrated over the steam bath, and the bases separated by two-dimensional paper chromatography with *iso*propanol–HCl[13] and butanol–$NH_4OH$. Each spot was cut out and re-chromatographed with distilled water on to a filter paper strip attached by stapling. Adenine and hypoxanthine spots, which could be detected easily in the ultraviolet, were eluted as in the case of nucleic acid bases, 1.6 $N$ HCl being used for the latter.

*Protein*

The precipitate of the tissue homogenate in 0.2 $N$ $HClO_4$ was used as the starting material. Nucleic acids were removed by hot trichloroacetic acid extraction. The protein residue was then extracted with lipid solvents and dried.

*Measurement of specific activity*

Samples were plated in infinitely thin layers on nickel planchets and counted in end-window counters attached to conventional scaling units. Specific radioactivity was expressed in c.p.m./$\mu$mole of the bases or c.p.m./mg protein, and then converted to relative specific activity which is given by:

$$\text{Relative specific activity} = \frac{\text{Specific activity of the bases or protein}}{\text{c.p.m./}\mu\text{mole of the radioactive precursor used}} \times 10^6$$

## RESULTS

### *Incorporation into DNA and RNA*

Results of the *in vivo* and *in vitro* experiments of formate incorporation using normal and regenerating livers are shown in Table I. The very slight amounts of $^{14}$C-formate incorporated both *in vivo* and *in vitro* into the bases of DNA suggest a very low rate of synthesis of DNA in normal liver. The high specific activities of DNA bases obtained for regenerating liver *in vivo* and *in vitro* are by no means unexpected since in this tissue there is an extensive net synthesis of DNA. It should be noted, however, that $^{14}$C-formate was poorly incorporated by DNA *in vitro* in 17-hour regenerating liver, whereas a slight but significant incorporation of $^{14}$C-formate was observed *in vivo* for 17–21 hours after hepatectomy. This observation suggests, in confirmation of the results of HECHT AND POTTER[14], that the production of a cellular mechanism for DNA biosynthesis was not completed until some time between 17 and 21 hours

TABLE I

INCORPORATION OF $^{14}$C-FORMATE INTO NUCLEIC ACID BASES IN NORMAL AND REGENERATING LIVER

| Condition | Number of observations | Period of regeneration* h | Duration of incorporation h | Relative specific activity | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | DNA | | | RNA | | Ratio RNA/DNA | |
| | | | | *Ad* | *Gu* | *Th* | *ad* | *gu* | *ad*/*Ad* | *gu*/*Gu* |
| *In vivo* | 2 | 0 | 4 | (1.5) | (2) | (0.5) | 9 | 6 | 6 | 3 |
| *In vivo* | 1 | 0 | 24 | (9) | (13) | (5) | 205 | 96 | 22 | 7.2 |
| *In vitro* | 2 | 0 | 7–8 | (18) | (9) | (9) | 443 | 88 | 25 | 9.8 |
| *In vivo* | 1 | 17** | 4 | 27 | 109 | 19 | 144 | 498 | 5.3 | 4.6 |
| *In vitro* | 1 | 17 | 8 | (4) | 0 | (4) | 255 | 164 | 64 | >164 |
| *In vivo* | 2 | 24 | 4 | 112 | 276 | 63 | 211 | 451 | 1.9 | 1.6 |
| *In vitro* | 10 | 24 | 7–8 | 242 | 102 | 228 | 933 | 286 | 3.9 | 2.8 |

*Ad*, *Gu* and *Th* denote adenine, guanine and thymine of DNA and *ad* and *gu* denote adenine and guanine of RNA. Figures in parentheses are derived from counts less than 50% of the background.

* Normal liver is listed as 0 hour.

** Partial hepatectomy was carried out between 2.00 and 4.00 p.m.

*References p. 599.*

TABLE II

INCORPORATION OF (2-$^{14}$C)-GLYCINE INTO NUCLEIC ACID BASES IN NORMAL AND REGENERATING LIVER

| *Condition* | *Number of observations* | *Period of regeneration h* | *Duration of incorporation h* | *Relative specific activity* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *DNA* | | | *RNA* | | *Ratio RNA DNA* | |
| | | | | *Ad* | *Gu* | *Th* | *ad* | *gu* | *ad Ad* | *gu Gu* |
| *In vivo* | 1 | 0 | 4 | (2) | (5) | (2) | 27 | 34 | 17 | 6.8 |
| *In vivo* | 1 | 0 | 24 | (5) | (10) | (3) | 121 | 91 | 24 | 9.1 |
| *In vitro* | 1 | 0 | 8 | (6) | (10) | (1) | 357 | 52 | 55 | 5.2 |
| *In vivo* | 1 | 24 | 4 | 61 | 152 | (18) | 130 | 272 | 2.2 | 1.8 |
| *In vitro* | 2 | 24 | 7 8 | 303 | 99 | 138 | 1175 | 202 | 3.9 | 2.0 |

See Table I for explanation of symbols etc.

after hepatectomy, and that, once the liver was removed from the organism, this process could not be continued *in vitro* even under the conditions which permit of actual DNA synthesis.

On the other hand, $^{14}$C-formate was extensively incorporated into the RNA purines of normal liver *in vitro* and of regenerating liver *in vivo* and *in vitro*. The incorporation into RNA bases in normal liver *in vivo* was extremely poor in 4-hour experiments, although it attained a significant level 24 hours after injection of the isotope.

It should be noted that *in vivo* and *in vitro* $^{14}$C-formate was incorporated only into the purines and thymine of the nucleic acids. No incorporation into cytosine and uracil could be detected.

Table II shows the results of similar experiments using (2-$^{14}$C)-glycine. It is clear that glycine showed a trend more or less similar to that of formate, although the picture was at times less clear cut than that with formate.

Because of the lack of uniformity in conditions of *in vivo* and *in vitro* experiments, no direct comparison of the figures obtained in the two systems is feasible but it is possible to compare relative values. With both formate and glycine the specific activity ratios of RNA/DNA for adenine and guanine showed some tendency to be higher *in vitro* than *in vivo*. In the formate experiments lasting 4 hours *in vivo*, the incorporation into RNA purines was so low that reasonable values for the RNA/DNA ratio could not be expected. Otherwise the RNA/DNA ratio was higher than 20 for adenine in normal liver and also in 17-hour regenerating liver *in vitro*, while 24-hour regenerating liver gave much lower values. The RNA/DNA ratio for guanine was usually lower than that for adenine.

*Incorporation into acid-soluble purines and protein*

In one experiment (Table III) measurements were made of the specific activity of acid-soluble purines and of protein. Both normal and regenerating livers showed a considerable variation among individuals, but the specific activity ratios of acid-soluble adenine/RNA adenine and of protein/RNA adenine were in general surprisingly uniform in duplicate animals of their respective groups. It should be noted that in regenerating liver there was only a slight decrease in the ratio of acid-soluble adenine/RNA adenine for both precursors, while the ratio of protein/RNA adenine was reduced markedly in the regenerating liver as compared to the normal liver especially in the formate experiment. The specific activity of hypoxanthine was

*References p. 599.*

TABLE III

INCORPORATION OF $^{14}$C-FORMATE AND (2-$^{14}$C)-GLYCINE INTO RNA, INTO ACID-SOLUBLE PURINES, AND INTO PROTEIN *in vivo* (4 hours)

| *Liver* | *Precursor* | *Relative specific activity* | | | | *Ratio Acid-soluble adenine / RNA adenine* | *Ratio Protein / RNA adenine* |
|---|---|---|---|---|---|---|---|
| | | *RNA Adenine* | *Acid-soluble* | | *Protein* | | |
| | | | *Adenine* | *Hypoxanthine* | | | |
| Normal | Formate | 9 | 59 | 106 | 31 | 6.6 | 3.4 |
| | | 16 | 107 | 182 | 36 | 6.7 | 2.3 |
| Regenerating | Formate | 159 | 769 | 1130 | 65 | 4.8 | 0.41 |
| | | 66 | 274 | 394 | 47 | 4.1 | 0.71 |
| Normal | Glycine | 42 | 322 | 933 | 183 | 7.7 | 4.4 |
| | | 12 | 85 | 250 | 302 | 7.1 | 25 |
| Regenerating | Glycine | 168 | 1010 | 1610 | 641 | 6.0 | 3.8 |
| | | 92 | 470 | 770 | 282 | 5.1 | 3.1 |

Individual figures represent determinations on separate animals.

consistently higher than that of adenine under different conditions tested. Thus it seems probable that, *in vivo*, the *de novo* synthesis of purines in general is relatively inactive in the normal liver and enormously enhanced in the regenerating liver.

*Difference between* in vivo *and* in vitro *systems*

The relative magnitudes of the specific activities of the individual bases of the nucleic acids *in vitro* are markedly different from those *in vivo*. This can be seen clearly from Figs. 1 and 2. Thus, except in DNA in normal liver *in vivo* and *in vitro* and in RNA in normal liver *in vivo* (4 hours), in which the specific activities were very low, the specific activity of the individual bases in a number of independent experiments decreased consistently in the order adenine $\cdot =$. thymine $>$ guanine *in vitro* (7–8 hours) and guanine $>$ adenine $>$ thymine *in vivo* (4 hours). In a 24-hour experiment *in vivo* with normal liver, the specific activity of RNA adenine was higher than that of RNA guanine as was also found *in vitro*. In general the results with glycine agreed with those for formate, but the specific activity of thymine was lower than that of adenine.

*Effect of X-irradiation*

Table IV shows the reduction of formate incorporation into DNA and RNA bases *in vitro* in slices of the regenerating liver taken from rats receiving 500 r at different times before and after hepatectomy. The results of some preliminary tests with 100 r are also included. In all cases the animals were killed and the experiment started 24 hours after hepatectomy.

The general effect is a reduction of incorporation of $^{14}$C-formate into DNA bases in all cases except where irradiation immediately preceded killing. Incorporation into RNA purines was also affected in most cases but to a lesser extent. The effect of irradiation with 100 r seems to be of shorter duration and perhaps more specific for DNA. These results are in agreement with those of CATER *et al.*[15] using $^{32}$P *in vivo*, and suggest that the production of the DNA synthesising mechanism rather than the synthesis of DNA *per se* is affected by irradiation. The incorporation of formate into different bases was uniformly depressed by X-rays, the agreement between adenine and thymine of DNA being especially good. The specific activity of guanine was much lower than that of adenine and must be subject to larger errors.

*References p. 599.*

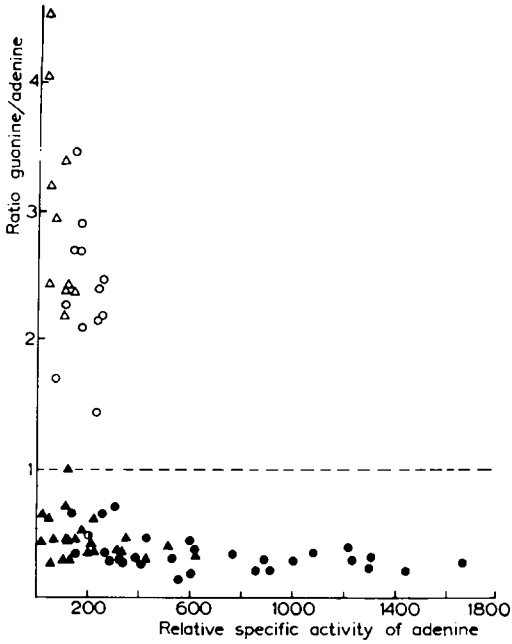


Fig. 1. The specific activity ratio of guanine/adenine in experiments with $^{14}$C-formate *in vivo* and *in vitro*. Individual plots represent separate analyses on single animals *in vivo* or the mean of duplicate flasks *in vitro*. △ DNA of regenerating liver *in vivo* – duration: 4 hours; ○ RNA of normal and regenerating liver *in vivo* – duration: 4 hours; ◑ RNA of normal liver *in vivo* – duration: 24 hours; ▲ DNA of regenerating liver *in vitro* – duration: 6–8 hours; ● RNA of normal and regenerating liver *in vitro* – duration: 6–8 hours.

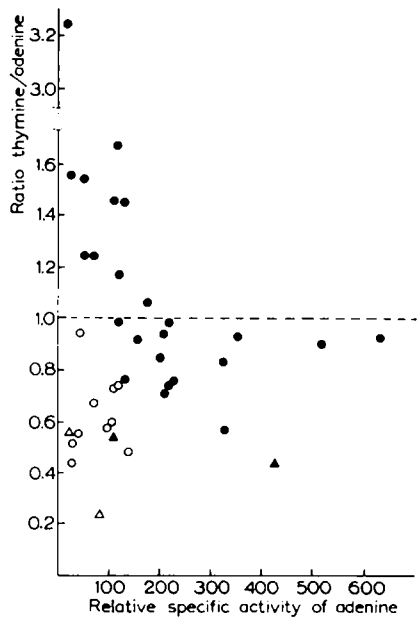


Fig. 2. The specific activity ratio of thymine/adenine in DNA of regenerating liver *in vivo* and *in vitro*. Details as in Fig. 1.

△ with (2-$^{14}$C)-glycine } *in vivo*, duration:
○ with $^{14}$C-formate } 4 hours.

● with (2-$^{14}$C)-glycine } *in vitro*, duration:
▲ with $^{14}$C-formate } 6–8 hours.

TABLE IV

THE EFFECT OF TOTAL BODY X-IRRADIATION ON THE *in vitro* INCORPORATION OF $^{14}$C-FORMATE INTO LIVER REGENERATING 24 HOURS AFTER PARTIAL HEPATECTOMY

| *Irradiation* | | *Incorporation as per cent of control* | | | | |
|---|---|---|---|---|---|---|
| | | *DNA* | | | *RNA* | |
| *Dose r* | *Time* h* | *Adenine* | *Guanine* | *Thymine* | *Adenine* | *Guanine* |
| 500 | −6** | 48 | 50 | 48 | 83 | 113 |
| 500 | 0 | 51 | 65 | 46 | 83 | 91 |
| 500 | 0 | 29 | - | 26 | 85 | |
| 500 | 6 | 68 | 52 | 79 | 88 | 59 |
| 500 | 7 | 48 | 44 | 40 | 65 | 47 |
| 500 | 12 | 81 | 87 | 78 | 92 | 113 |
| 500 | 12 | 92 | 52 | 86 | 109 | 70 |
| 500 | 17** | 5 | 15 | 5 | 21 | 31 |
| 500 | 17 | 50 | 38 | 56 | 93 | 96 |
| 500 | 24 | 133 | 76 | 102 | 157 | 120 |
| 100 | 0 | 129 | - | 143 | 122 | - |
| 100 | 6 | 68 | 83 | 63 | 103 | 87 |

* Figures in this column refer to the time of hepatectomy. Irradiation preceding hepatectomy is indicated by a negative sign; that immediately before hepatectomy by 0; and that after hepatectomy by positive values.

** Hepatectomy in these cases was conducted between 4.00 and 5.00 p.m.

*References p. 599.*

## DISCUSSION

The observation that $^{14}$C-formate and (2-$^{14}$C)-glycine are poorly taken up by DNA of the resting liver but extensively incorporated into DNA of regenerating liver 24 hours after hepatectomy is in line with the results obtained with other precursors[3,16,17]. Significant values for the incorporation of glycine and of formate into normal rat liver DNA *in vivo* or *in vitro* have been reported[4,5,31], and in most of the previous reports with normal rat liver, labelled formate and glycine gave specific activity ratios for RNA/DNA ranging from 1 to 5. These values were considerably lower than those obtained with other labelled precursors such as adenine, orotic acid or orthophosphate, which usually gave values higher than 20[2], and they were assumed by some workers[3,4] to indicate a metabolic instability of DNA purines without the integrity of the polynucleotide backbone being affected.

In the present study, however, RNA/DNA ratios higher than 20 were obtained both *in vivo* and *in vitro* for adenine in normal liver with both $^{14}$C-formate and (2-$^{14}$C)-glycine. These values are similar to those obtained with $^{32}$P-orthophosphate *in vivo* under the same conditions[18]. The corresponding values for guanine were significantly lower than those for adenine, but were nevertheless higher than most of those reported by previous workers[2]. It is possible that low values for the RNA/DNA ratio as obtained by some authors with labelled formate and glycine might be due in part to contamination of DNA base samples by radioactive amino acids. The present results strongly indicate the stability of DNA purines in resting liver, and lend support to the conclusions of other workers[18-21] on the metabolic inertia of the DNA molecule.

The systematic change in the relative magnitude of the specific activities of adenine, guanine and thymine, *in vivo* and *in vitro*, as obtained with $^{14}$C-labelled formate and glycine has so far been little noticed, but the present results are in agreement with such fragmentary information as is available[4,5,22-27,30-32], although some authors[3,27,29] have also published conflicting views. The observed changes may only partly be explained by the diluting effect of pre-existing non-labelled acid-soluble adenine compounds or by the higher sensitivity to *in vitro* conditions of the enzyme system, which catalyses formate incorporation into guanine, than of the corresponding enzyme for adenine[5]. It has also been observed that the incorporation of glycine into adenine and guanine *in vivo* is independently modified by dietary conditions[28].

The behaviour of the irradiated regenerating liver *in vitro* can be readily explained on the assumption that the production of the DNA-synthesising mechanism is arrested by X-irradiation as well as by *in vitro* conditions. In thymus, on the other hand, it is possible that X-irradiation produces a situation very similar to that prevailing in bone marrow and some ascites tumours under *in vitro* conditions. The enhanced incorporation of formate into thymine and of adenine into purines of DNA might therefore be merely of a compensatory nature.

## ACKNOWLEDGEMENTS

It is a pleasure to express my sincere gratitude to Professor J. N. DAVIDSON, Dr. R. M. S. SMELLIE and Dr. R. Y. THOMSON for valuable advice, encouragement and help in this work and for being responsible for the partial hepatectomies and other

*References p. 599.*

animal manipulations. The carbon labelled compounds were provided from a grant made to Professor DAVIDSON by the British Empire Cancer Campaign.

## SUMMARY

1. Incorporation of $^{14}$C-formate and (2-$^{14}$C)-glycine into nucleic acid bases was studied using normal and regenerating livers of adult rats *in vivo* and *in vitro*. The base samples were carefully freed of contaminating amino acids, which may cause serious errors in experiments of this type.

2. Formate and glycine were poorly incorporated into DNA of normal liver, and the specific activity ratio of RNA/DNA for adenine was usually higher than 20, suggesting a remarkable metabolic stability of DNA purines.

3. Incorporation into RNA and acid-soluble purines was relatively inactive in the normal liver *in vivo*, but was markedly enhanced by partial hepatectomy.

4. In the liver, formate incorporation into nucleic acid purines is not abolished under *in vitro* conditions or by X-irradiation, but the relative magnitude of the specific activities of adenine, guanine and thymine varied systematically *in vivo* and *in vitro* with both precursors.

## REFERENCES

[1] P. DROCHMANS, D. H. MARRIAN AND G. B. BROWN, *Arch. Biochem. Biophys.*, 39 (1952) 310.
[2] G. B. BROWN AND P. M. ROLL, in E. CHARGAFF AND J. N. DAVIDSON, *Nucleic Acids*, Vol. 2. Academic Press, Inc., New York, 1955, p. 341.
[3] S. S. FURST AND G. B. BROWN, *J. Biol. Chem.*, 191 (1951) 239.
[4] G. A. LEPAGE AND C. HEIDELBERGER, *J. Biol. Chem.*, 188 (1951) 593.
[5] W. A. MANNELL AND R. J. ROSSITER, *Biochem. J.*, 61 (1955) 418.
[6] R. Y. THOMSON, R. M. S. SMELLIE, R. GOUTIER AND J. N. DAVIDSON, *Biochim. Biophys. Acta*, 22 (1956) 585.
[7] H. HARRINGTON AND P. S. LAVIK, *Cancer Research*, 17 (1957) 43.
[8] H. HARRINGTON AND P. S. LAVIK, *Arch. Biochem. Biophys.*, 54 (1955) 6.
[9] H. MCILWAIN AND R. L. BUDDLE, *Biochem. J.*, 53 (1953) 412.
[10] P. REICHARD, *J. Biol. Chem.*, 197 (1952) 391.
[11] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 45 (1949) 294.
[12] G. R. WYATT, *Biochem. J.*, 48 (1951) 584.
[13] G. R. WYATT, in E. CHARGAFF AND J. N. DAVIDSON, *Nucleic Acids*, Vol. 1. Academic Press, Inc., New York, 1955, p. 243.
[14] L. I. HECHT AND V. R. POTTER, *Federation Proc.*, 15 (1956) 271.
[15] D. B. CATER, B. E. HOLMES AND L. K. MEE, *Acta Radiol.*, 46 (1956) 655.
[16] L. I. HECHT AND V. R. POTTER, *Cancer Research*, 16 (1956) 988.
[17] O. NYGAARD AND H. P. RUSCH, *Cancer Research*, 15 (1955) 240.
[18] H. K. KIHARA, M. AMANO AND A. SIBATANI, manuscript in preparation.
[19] J. R. FRESCO, A. BENDICH AND P. J. RUSSELL, *Federation Proc.*, 14 (1955) 214.
[20] R. W. SWICK, A. L. KOCH AND D. T. HANDA, *Arch. Biochem. Biophys.*, 63 (1956) 226.
[21] H. K. KIHARA, M. AMANO AND A. SIBATANI, *Biochim. Biophys. Acta*, 21 (1956) 489.
[22] K. MOLDAVE AND C. HEIDELBERGER, *J. Am. Chem. Soc.*, 76 (1954) 679.
[23] R. ABRAMS, *Arch. Biochem. Biophys.*, 33 (1951) 430.
[24] D. A. GOLDTHWAIT AND A. BENDICH, *J. Biol. Chem.*, 196 (1952) 841.
[25] A. BENDICH, *Exptl. Cell Research*, Supplin 2 (1952) 181.
[26] P. REICHARD AND S. BERGSTRÖM, *Acta Chem. Scand.*, 5 (1951) 190.
[27] L. K. MEE, in J. S. MITCHELL, B. E. HOLMES AND C. L. SMITH, *Progress in Radiobiology*, Oliver and Boyd, Edinburgh and London, 1956, p. 12.
[28] C. M. CLARK, D. J. NAISMITH AND H. N. MUNRO, *Biochim. Biophys. Acta*, 23 (1957) 587.
[29] E. P. TYNER, C. HEIDELBERGER AND G. A. LEPAGE, *Cancer Research*, 12 (1952) 158.
[30] J. A. HAYDAR, F. CHERNIGOV, A. KHATCHADOURIAN AND S. E. KERR, *J. Biol. Chem.*, 224 (1957) 707.
[31] E. HAMMARSTEN, S. ÅKVIST, E. P. ANDERSON, N. A. ELIASSON AND B. THORELL, *Acta Chem. Scand.*, 10 (1956) 1508.
[32] E. P. ANDERSON AND S. ÅKVIST, *Acta Chem. Scand.*, 10 (1956) 1576.

Received April 27th, 1957