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The Metabolic Stability of the Nucleic Acids in Cultures of a Pure Strain of Mammalian Cells

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Although in recent years the metabolism of the nucleic acids has been the subject of many experiments with isotopic-tracer techniques, our knowledge of the relative rates at which deoxyribonucleic acid (DNA), nuclear ribonucleic acid (nRNA) and cytoplasmic ribonucleic acid (cRNA) are synthesized and broken down is still far from complete.

The present experiments were designed to provide answers to two particular questions: (1) What inferences about nucleic acid synthesis can be drawn from the manner in which [^{14}C]formate is incorporated into the purines and pyrimidines of DNA, nRNA and cRNA? (2) To what extent are DNA, nRNA and cRNA, once synthesized, metabolically stable in animal cells?

Information of this kind cannot readily be obtained from the intact animal or from tissue slices surviving *in vitro*. Use was therefore made of a pure strain of mammalian cells growing in tissue culture. Brief preliminary reports of some of the results obtained have already been published (Thomson, Paul & Davidson, 1956; Thomson & Paul, 1957).

METHODS

Biological

The cells used in these experiments were adult mouse subcutaneous fibroblasts NCTC strain L, clone 929 (Sanford, Earle & Likely, 1948), hereafter referred to as L cells. These cells were grown directly on the floor of large conical culture flasks having an area of about 140 cm.^{2}. The medium in which they were normally maintained was composed of 20 parts of horse serum, 10 parts of chick-embryo extract and 70 parts of Hanks's balanced salt solution (Hanks & Wallace, 1949). It also contained 50 units of sodium penicillin G/ml. but no other antibiotics.

In most of the experiments to be described, similar vessels were used. In each vessel was placed a glass-covered magnet so that the cells could readily be suspended by placing the culture vessel on a magnetic stirrer. This procedure proved less damaging to the cells than treatment with trypsin. In the conditions described the cells usually reached a maximum population of between 50 and 80 millions/flask, at which level the population became stationary. In all experiments the population was kept near the maximum, the cultures being halved at each transfer and allowed to grow to the maximum once more. The medium, 60 ml./flask, was completely renewed twice a week and on one of these occasions the cells were transferred.

In this way, a slow rate of growth was maintained and the cells remained healthy throughout. In some experiments, where it was necessary to exclude extracellular pools of purines and pyrimidines, the medium used was Eagle's medium (Eagle, 1955) supplemented with 30% (v/v) of dialysed horse serum. Also, in some experiments where the inocula of cells were much smaller than those described above, smaller vessels were used, e.g. 25 ml. Erlenmeyer flasks.

At the commencement of an experiment a suspension of cells was obtained by treating stock cultures of cells with

0·5% (w/v) trypsin (Difco 1/250). After being centrifuged and resuspended in growth-promoting medium, the cells were counted in a haemocytometer chamber and a suitable amount was inoculated into each experimental flask.

The isotope was introduced into the medium as sodium [^{14}C]formate, usually at a final concentration of 2 μc/ml. In two of the long-term experiments, where it was desired to achieve a high degree of labelling initially, this concentration was doubled. In these experiments also 5-amino-4-imidazole carboxamide (AICA) riboside (California Foundation for Biochemical Research) was added in a concentration of 40 μg./ml. during the incorporation phase in order to promote labelling of the purines, as described later.

In some experiments in which retention of isotope in nucleic acids and acid-soluble bases was measured, unlabelled sodium formate was added to the medium, after the withdrawal of the labelled formate, to give a concentration of 22 μg./ml., this being the same concentration of formate as was present during incorporation.

As samples were harvested, they were either fractionated immediately or stored at −70° until it was convenient to carry out the analyses. By gently homogenizing in 0·1 M-citric acid the cells were disrupted. At this point the volume of suspension was carefully noted and a small sample taken for counting of nuclei. For this purpose the nuclei were further diluted with a solution of 0·1% (w/v) crystal violet in 0·1 M-citric acid and transferred to a Fuchs–Rosenthal chamber. Two independent observers each performed a minimum of two counts of each specimen, so that in each case about 1000 nuclei were counted (see Sanford *et al.* 1951). The remaining suspension of disrupted cells was then further fractionated as described below.

Chemical

The chemical treatment of the cells for the separation of nuclear and cytoplasmic fractions and for the determination of the specific activities of the bases in DNA, nRNA and cRNA and of the bases in the acid-soluble nucleotide fraction was carried out as described by Smellie, Thomson & Davidson (1958). cRNA was determined by ultraviolet-absorption measurement (Smellie *et al.* 1958) on the solution made by extracting the dried lipid-free cytoplasmic powder with hot 0·5 N-perchloric acid. A small portion of the nuclear suspension was used for determination of DNA by the method of Schmidt & Thannhauser (1945) as modified by Davidson & Leslie (1951), phosphorus being estimated by the method of Griswold, Humoller & McIntyre (1951).

The total activities of DNA and cRNA were calculated on the assumption that the bases were present in equimolar proportions.

RESULTS

Table 1 shows the pattern of incorporation of [^{14}C]formate into the nucleic acid bases of L cells growing in embryo extract–horse serum medium. As might be predicted on the basis of current theories of purine and pyrimidine biosynthesis (Buchanan *et al.* 1957), the isotope was incorporated into the adenine and guanine of DNA, nRNA and cRNA and into the thymine of DNA. The nucleic acid pyrimidines other than thymine showed no activity. The observation that the DNA thymine was much more heavily labelled than the

Table 1. *Pattern of incorporation of* [^{14}C]*formate into the nucleic acid bases of L cells grown for 2 days in (a) horse serum–embryo extract (control medium), (b) control medium plus liver extract, and (c) control medium plus 5-amino-4-imidazole carboxamide riboside*

Results are expressed in terms of relative specific activities with the value for thymine as 100. The actual specific activity of the thymine in all three cases was of the order of 100 000.

Medium	Base analysed	Relative specific activities (counts/min./μmole)		
		DNA	nRNA	cRNA
(*a*) Control	Adenine	11	18	17
	Guanine	14	23	13
	Thymine	100	—	—
(*b*) Control + liver extract	Adenine	145	238	205
	Guanine	168	326	—
	Thymine	100	—	—
(*c*) Control + 5-amino-4-imidazole carboxamide riboside	Adenine	122	177	160
	Guanine	148	232	188
	Thymine	100	—	—

Table 2. *Retention of* ^{14}C *in deoxyribonucleic acid bases of L cells growing in horse serum–embryo extract medium*

	Total activity (counts/min.)		
	Adenine	Guanine	Thymine
Cells grown for 24 hr. in medium containing [^{14}C]formate	350	1284	91 800
Cells grown for a further 96 hr. in non-radioactive medium	580	1060	108 300

DNA purines is in accord with similar observations made with other tissues *in vitro* (Smellie, Thomson, Goutier & Davidson, 1956). The relatively heavy labelling of thymine might be due either to true DNA synthesis, the poor incorporation into nucleic acid purines being attributable to utilization of existing stocks of unlabelled purines for nucleic acid synthesis, or to a rapid exchange reaction between the DNA thymine and the [^{14}C]formate of the medium. A decision between these alternatives has been made by growing the labelled cells for several days in non-radioactive medium containing inactive formate (Table 2). If the high labelling of the thymine had been due to an exchange reaction, the isotope would have been rapidly released in the inactive medium; on the contrary, the activity of the thymine showed an appreciable increase, presumably as a result of incorporation of labelled precursors formed while the cells were still in the active medium. Consequently, it can be concluded that the incorporation of [^{14}C]formate into thymine is due to true DNA synthesis.

In order to determine whether the behaviour of the cultured cells resembles the known behaviour of normal and neoplastic tissues *in vivo* and *in vitro*, an examination was made of the effect on L cells of mouse-liver extract, which has been shown to stimulate incorporation of [^{14}C]formate *in vitro* into the nucleic acid and acid-soluble purines of several rapidly growing tissues, notably rabbit bone marrow and the Ehrlich ascites carcinoma of the mouse (Smellie *et al.* 1956). The extract produced a pronounced increase in incorporation of [^{14}C]formate into the acid-soluble adenine and nucleic acid purines of the L cells (Table 1) without causing any appreciable difference in incorporation into DNA thymine. The liver extract therefore appeared to have produced a great increase in purine synthesis without affecting the rate of formation of DNA, presumably by supplying either intermediates involved in purine synthesis or the enzymes responsible for their formation. Support for this view is obtained by the observation that the addition of 5-amino-4-imidazole carboxamide riboside, the phosphate ester of which is an intermediate in purine synthesis (Buchanan *et al.* 1957), greatly increases incorporation of [^{14}C]formate into the nucleic acid purines of L cells (Table 1) but not into the DNA thymine. This observation also is in accord with similar results obtained in ascites-tumour and other cells (Smellie *et al.* 1958), and has been made use of in later experiments with L cells in order to obtain good initial labelling of the purines in studies of subsequent isotope loss.

If incorporation of [^{14}C]formate into DNA thymine occurs only in the course of DNA synthesis and not as a result of an exchange reaction, it follows that the disproportionately low incorporation of [^{14}C]formate into DNA purines found in most experiments with L cells must be due, as suggested above, to the utilization by the cells of unlabelled purines from the medium for DNA synthesis. This question was investigated by growing L cells in Eagle's semi-synthetic medium (supplemented with dialysed serum), which contains no purines. Under these conditions the cells incorporated [^{14}C]formate extensively into their nucleic acid purines so that DNA adenine and guanine developed a specific activity twice as great as that of DNA thymine (Table 3). This is, of course, in accordance with the theory that formate contributes two carbon atoms to each molecule of purine and one to each molecule of thymine.

Table 3. *Incorporation of* [^{14}C]*formate into nucleic acid bases and acid-soluble bases of L cells growing for* 48 *hr. in Eagle's medium with dialysed serum and the supplements shown*

Supplement added to medium	Base analysed	Counts/min./µmole of base: DNA	nRNA	cRNA	Acid-soluble bases
1. 0	Adenine	81 600	306 000	121 000	411 000
	Guanine	94 800	236 400	107 500	—
	Thymidine	46 000	—	—	—
2. Adenosine (0·2 µmole/ml.)	Adenine	1 100	20	1 720	7 510
	Guanine	1 700	2 720	1 680	—
	Thymidine	34 800	—	—	—
3. Guanosine (0·2 µmole/ml.)	Adenine	42 400	110 200	66 700	151 600
	Guanine	9 780	18 440	10 920	—
	Thymidine	44 400	—	—	—
4. Thymidine (0·2 µmole/ml.)	Adenine	66 800	243 500	102 700	371 500
	Guanine	60 800	175 700	109 700	—
	Thymidine	3 040	—	—	—
5. Azaserine (10 µg./ml.)	Adenine	28	2 980	1 080	6 160
	Guanine	0	1 840	1 021	—
	Thymidine	17 500	—	—	—

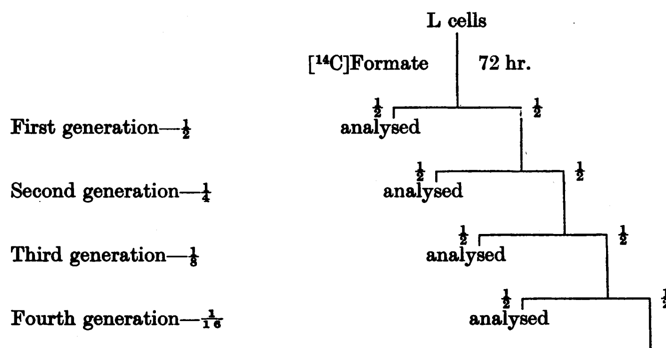


Fig. 1. Design of experiment. For explanation see text.

Addition of adenosine markedly diminished incorporation of [^{14}C]formate into nucleic acid purines and acid-soluble adenine, without greatly affecting incorporation into DNA thymine. Addition of guanosine produced similar but less-extensive effects. Conversely, addition of thymidine diminished incorporation into DNA thymine without greatly affecting incorporation into acid-soluble adenine or nucleic acid purines. Clearly therefore the cells are capable both of synthesizing their own purines and pyrimidines and of utilizing purines and pyrimidines present as nucleosides in the medium.

Table 3 also shows the effects of azaserine $[\overset{-}{N}{:}\overset{+}{N}{:}CH\cdot CO\cdot O\cdot CH_2\cdot CH(NH_2)\cdot CO_2H]$, which is known to inhibit purine synthesis by inhibiting the conversion of formyl glycinamide ribotide into formyl glycinamidine ribotide (Levenberg, Melnick & Buchanan, 1957). As might be expected, azaserine drastically inhibited incorporation into acid-soluble adenine and nucleic acid purines, but incorporation into DNA thymine, although depressed, was still sufficiently pronounced to indicate substantial DNA synthesis. Therefore even when the cells are growing in a purine-free medium and have been prevented from synthesizing purines for themselves, they still continue to synthesize DNA for some time at the expense of their existing stocks of purines in a manner analogous to that found in the Ehrlich ascites carcinoma *in vitro* (Smellie *et al.* 1958).

These experiments were all concerned with the biosynthesis of the nucleic acids. To investigate their metabolic stability it was necessary to adopt a different experimental plan (Fig. 1). L cells were grown in embryo extract–horse serum medium containing 5-amino-4-imidazole carboxamide riboside and [^{14}C]formate until the nucleic acids were heavily labelled in the purines and thymine. These labelled cells were transferred to non-radioactive medium, in which they were grown at a slow rate for eight or nine generations, samples being taken for analysis from time to time. By this means it was possible to determine the degree to which the ^{14}C initially incorporated was retained in the bases of DNA, nRNA and cRNA and hence to estimate their varying degrees of metabolic stability.

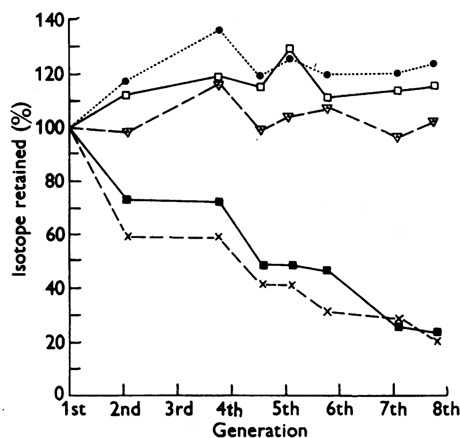


Fig. 2. Total activity of the nucleic acid bases in the DNA and cytoplasmic RNA of L cells grown for several generations in non-radioactive medium after initial labelling with [^{14}C]formate. All values are expressed as percentages of the initial values. ●, DNA guanine; □, DNA adenine; ▽, DNA thymine; ■, cRNA guanine; ×, cRNA adenine.

The plan of the first experiment of this type is shown in Fig. 1. Immediately on removal from the radioactive medium the culture was divided into two equal parts, one of which was analysed. The other was allowed to grow in non-radioactive medium for about one generation, i.e. until the cell number had approximately doubled. Then it in turn was divided into two equal parts, one of which was analysed while the other was allowed to grow for a generation in non-radioactive medium. This process was continued for eight generations. In such an experiment the first sample analysed is equivalent to half the original culture, the second

to one-quarter, the third to one-eighth and so on. From the amounts and specific activities of the DNA, nRNA and cRNA of each sample the degree to which the ^{14}C originally incorporated had been retained in each of the nucleic acid bases could be calculated.

The specific activities of the bases in this experiment (Table 4) showed a steady fall, the drop being more rapid in RNA bases than in DNA bases. It is important to note that the figures for nRNA and cRNA remained remarkably close to each other during the fall. On the other hand, a very different pattern is observed when the total activities of the bases of DNA and cRNA are calculated (Fig. 2). During the first two generations after the transfer to non-radioactive medium, the total amount of ^{14}C in DNA adenine, guanine and thymine tended to increase slightly, presumably because of the incorporation of precursors which had taken up ^{14}C while the cells were in radioactive medium. Thereafter the amount of ^{14}C retained by all three bases tended to remain steady or to fall slightly.

In striking contrast with the behaviour of the DNA, the amount of ^{14}C retained in cRNA adenine and guanine fell rapidly and continuously. The conclusion suggested by these results is that whereas DNA once formed may be metabolically stable or only very slowly broken down, some RNA is continually being broken down and resynthesized at a fairly rapid rate. These results have been confirmed by subsequent experiments of the same type.

Table 4 demonstrates that at the beginning of the experiment the acid-soluble adenine had a specific activity about twice as high as that of the nRNA and cRNA adenine. This fell very sharply during the first generation in non-radioactive medium but throughout the remainder of the experiment remained at a level only slightly lower than that of the nRNA and cRNA adenine.

The very high degree of retention of ^{14}C in the DNA bases in this experiment might not have been entirely due to the stability of the DNA molecule since it is quite possible that DNA broke down slowly to give labelled products, but that these were completely, or almost completely, utilized for the synthesis of new DNA, so that there was no overall loss of ^{14}C from the DNA of the culture as a whole. For kinetic reasons such reutilization of labelled breakdown products would be most extensive in rapidly growing tissues. In the present experiment the rate of growth was relatively slow with a mean generation time of 4–7 days. By this means reutilization of labelled-breakdown products could be reduced, but not completely eliminated. Theoretically, it could be reduced still further if the cells were flooded with unlabelled nucleic acid precursors such as nucleosides and nucleotides.

Table 4. *Specific activity of nucleic acid bases at each generation during the growth of* ^{14}C*-labelled L cells in non-radioactive medium*

Approx. generation no.	Cell population (millions)	Base analysed	Specific activities (counts/min./µmole) DNA	nRNA	cRNA	Acid-soluble bases
1	138	Adenine	182 000	192 000	200 000	446 000
		Guanine	149 200	144 000	183 400	—
		Thymine	77 000	—	—	—
2	288	Adenine	76 700	47 950	48 800	30 200
		Guanine	67 200	55 500	56 000	—
		Thymine	28 700	—	—	—
3	1 008	Adenine	30 800	12 460	18 700	10 360
		Guanine	29 625	19 900	20 400	—
		Thymine	12 920	—	—	—
4	1 840	Adenine	15 900	5 610	6 420	2 360
		Guanine	13 520	6 320	6 960	—
		Thymine	5 780	—	—	—
5	2 624	Adenine	11 800	3 415	4 480	2 470
		Guanine	9 495	4 000	4 740	—
		Thymine	3 970	—	—	—
6	4 352	Adenine	5 300	1 860	1 650	755
		Guanine	4 750	2 004	2 332	—
		Thymine	2 175	—	—	—
7	10 752	Adenine	2 200	560	628	462
		Guanine	1 940	650	543	—
		Thymine	800	—	—	—
8	17 920	Adenine	967	202	188	165
		Guanine	856	220	194	—
		Thymine	362	—	—	—

To investigate this point, a second experiment was set up to determine whether the retention of ^{14}C in DNA thymine and nucleic acid adenine was affected if the cells were supplied with unlabelled thymidine and adenosine. If the observed retention were due to absence of breakdown of DNA, it would obviously be unaffected by the presence of the thymidine and adenosine. On the other hand, if it were attributable to the reutilization of products of DNA breakdown for the synthesis of new DNA, the flooding of the cells with unlabelled thymidine would greatly dilute the labelled thymidylic acid and thymidine liberated by DNA breakdown and thus reduce the degree to which they were incorporated into new DNA. Addition of unlabelled adenosine might similarly diminish reutilization of any labelled deoxyadenylic acid or deoxyadenosine liberated by DNA breakdown.

The design of this experiment (Fig. 3) closely resembled that of its predecessor. As before, the cells were labelled by growing them in the presence of [^{14}C]formate and 5-amino-4-imidazole carboxamide riboside. Half of the culture was then taken for analysis and the remainder allowed to grow for about a generation in non-radioactive medium. Then it in turn was divided into two equal parts, one of which (equivalent to one-quarter of the original culture) was analysed. The other was allowed to grow for a further generation. The cells, which by this point had presumably utilized all the [^{14}C]formate which they had taken up, were then suspended in inactive medium and eight portions, each equal to 1/64 of the total culture and equivalent to 1/256 of the original culture, were dispensed into flasks. The cells in each flask were maintained for a further six generations: four flasks in ordinary non-radioactive medium; two in non-radioactive medium containing adenosine; two in non-radioactive medium containing thymidine. They were then analysed. The remainder of the culture (equivalent to 56/256 of the original culture) was analysed immediately. The degree of retention of ^{14}C in each of the nucleic acid bases was calculated as before.

The results are shown in Table 5. During the earlier part of the experiment covering approximately the first two generations in non-radioactive medium, retention of ^{14}C in the adenine, guanine and thymine of the DNA was almost complete. But in the latter part of the experiment, the degree of retention was dependent on the nature of the medium in which the cells were grown. Thus in

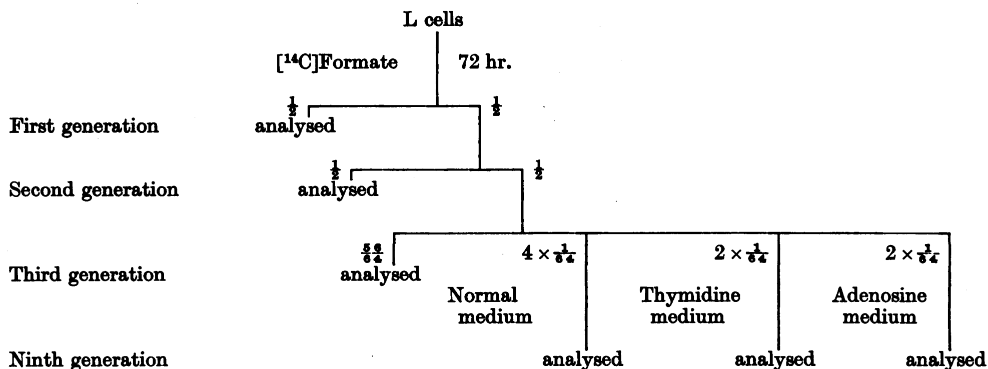


Fig. 3. Design of experiment. For explanation see text.

Table 5. *Percentage of initial radioactivity retained in bases of deoxyribonucleic acid and cytoplasmic ribonucleic acid of L cells growing in embryo extract–horse serum medium with and without addition of adenosine and thymidine*

Figures represent the means of several samples.

	DNA			cRNA	
	Adenine	Guanine	Thymine	Adenine	Guanine
Initial sample	100	100	100	100	100
Second sample	80·4	92·7	83·0	74·1	80·6
Third sample	83·1	93·9	93·1	47·2	51·5
Final sample (ordinary medium)	58·1	70·2	72·7	21·4	13·2
Final sample (ordinary medium + thymidine)	58·9	68·2	51·9	4·7	8·4
Final sample (ordinary medium + adenosine)	63·8	71·5	70·9	8·63	9·4

ordinary medium 60% of the ^{14}C initially incorporated into the DNA adenine and 70% of that initially incorporated into DNA guanine and thymine were retained at the end of the experiment. In the cells grown in the thymidine-containing medium the percentages of ^{14}C retained in DNA adenine and guanine were very similar to those found in ordinary medium. This suggests that the presence of thymidine had no effect in accelerating DNA breakdown. On the other hand, retention in DNA thymine was substantially lower (about 50% as opposed to about 70%).

It appears therefore that if the argument set out above is valid, the high degrees of retention of ^{14}C in DNA in this experiment and its predecessor were due in part to reincorporation of labelled breakdown products. The presence of adenosine in the medium had no apparent effect on retention of ^{14}C in DNA purines, possibly because the medium contains some purines so that the addition of more does not alter the situation greatly. As in the previous experiment, the purines of cRNA retained ^{14}C to a much smaller extent than did those of DNA.

The most important result of this experiment, namely that retention of ^{14}C in DNA thymine is depressed if unlabelled thymidine is present in the medium, implying as it does a measurable breakdown of DNA, appeared to merit confirmation. Similar results were obtained in a further experiment on the same lines.

DISCUSSION

It is clear from the results presented in Table 1 that L cells growing in culture present the same pattern of incorporation of [^{14}C]formate as is found with Ehrlich ascites carcinoma and certain other cells under conditions *in vitro* (Smellie *et al.* 1956, 1958). The most satisfactory explanation of the relatively low activities of the DNA adenine and guanine in comparison with thymine is that incorporation into the thymine is a measure of true DNA synthesis and that when preformed purines are available in the medium they are preferentially utilized for nucleic acid synthesis (Smellie *et al.* 1958). Confirmation of this view is obtained from the observation that in a purine-free medium incorporation into the purines is higher than that into thymine. Clearly the L cells can synthesize purines if the need arises, but they will utilize preformed adenosine, guanosine or thymidine if any of these nucleosides are provided (Table 3). Moreover, if purine synthesis is blocked by azaserine nucleic acid synthesis can still take place, albeit on a much reduced scale, presumably at the expense of limited existing stocks of purines in the cells.

It is also clear from Table 3 that adenosine may act as a precursor of nucleic acid guanine and guanosine of nucleic acid adenine, although our findings are in agreement with those of Brown & Balis (1957) in showing that the conversion of guanosine into adenosine is not so readily achieved as the reverse reaction.

The results of the retention experiments reported here indicate quite clearly that DNA is metabolically much more stable than nRNA or cRNA and thus confirm the generally accepted opinion on this question (see Davidson, 1957). However, the observations might conceivably be the result of the release from dead or dying cells of nucleic acid breakdown products which are subsequently reutilized from the culture medium. An explanation along these lines seems rather unlikely since it would be necessary to assume that the breakdown products of DNA were more extensively reutilized than those from RNA. Furthermore, the cells were grown in a monolayer under a thin layer of fluid and their mean generation time was of the order of 3 or 4 days. It is therefore probable that breakdown products of dying cells would readily diffuse throughout the medium. Since the volume of the medium was never less than 250 times the volume of the cells and since it was totally changed at least 15 times during the experiment, it is probable that any labelled nucleic acid breakdown products released by dying cells would be so extensively diluted as to prevent appreciable reincorporation taking place from the medium.

The fact that the activities of the three labelled bases in DNA keep in parallel with each other argues against the possibility that loss of isotope is due merely to an exchange reaction similar to the reversible exchange of thymine for 5-bromouracil, which has been observed in the DNA of non-dividing cells of *Escherichia coli* by Zamenhof, Reiner, de Giovanni & Rich (1956). In any case, the results reported in Table 2 provide additional evidence for the absence of such exchange reactions in our system.

Nevertheless, a change in the ratios of the activities of the three labelled bases in DNA was obtained by introducing a large pool of thymidine to the system (Table 5). Its effect in diluting the activity of DNA thymine indicates that there may be some degradation of DNA within the cell with subsequent reutilization of the breakdown products.

The metabolic stability of DNA has been the subject of several other investigations. Brues, Tracy & Cohn (1944) have shown that ^{32}P incorporated into the DNA of rat liver during the regeneration after partial hepatectomy is extensively retained for 2 weeks after the operation. Similar results are reported by Barton (1954). Hecht & Potter (1956) and Takagi, Hecht & Potter (1956) have also carried out similar experiments

with [6-^{14}C]orotic acid and found that isotope incorporated into DNA of the liver during regeneration is almost completely retained during the 50–100 days after the operation. Fresco, Bendich & Russell (1955) labelled the DNA of rat liver regenerating after partial hepatectomy with [1:3-$^{15}N_2$]adenine and [2-^{14}C]glycine and observed the disappearance of ^{15}N and ^{14}C during the 3 months after the thirtieth post-operative day. They found a very small loss of activity which could be accounted for by an equivalent small gain in liver weight during the experimental period. Extensive retention of isotope has also been found in the DNA of the livers of young growing rats (Swick, Koch & Handa, 1956; Kihara, Amano & Sibatani, 1956; Takagi *et al.* 1956). It should, however, be noted that in several of the experiments on liver there appeared to be some breakdown comparable with that which we have found in the present studies (Hecht & Potter, 1956; Takagi *et al.* 1956; Swick *et al.* 1956). For example, Swick *et al.* (1956) noted a 50% loss of DNA activity between the third and sixth weeks after incorporation, which they attributed to the death of short-lived cells.

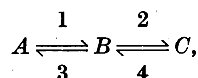
Our present finding that there is a more rapid loss of isotope from nRNA and cRNA than from DNA is also in agreement with the results obtained from rat liver (Brues *et al.* 1944; Furst & Brown, 1951; Hecht & Potter, 1956; Takagi *et al.* 1956; Kihara *et al.* 1956; Swick *et al.* 1956). The fact that the activities of the nRNA and cRNA decrease in parallel is in agreement with the results obtained from regenerating rat liver by Takagi *et al.* (1956), who suggested that both might be in reversible exchange with a pool of acid-soluble precursors which is at least partly common to both. The observation that in the present experiments the specific activity of the acid-soluble adenine was of the same order as that of the nRNA and cRNA adenines supports this suggestion.

The observations summarized in Fig. 2 demonstrate that RNA is broken down in the living cells, and the fact that some breakdown products can be utilized is evident from Table 3. At the same time there is a tendency for the cells to retain even small molecules, as shown by the fairly high activity retained in the acid-soluble adenine throughout the experiments illustrated in Table 4. This suggests that, when RNA is degraded, the breakdown products are reincorporated into new RNA. It is possible that similar kinetic considerations may apply to all or part of the DNA. If the nucleic acids are partially broken down and the products subsequently reutilized *within the cell*, it is clear that any leakage to the medium will be greatest if the cells are grown slowly over a long period of time.

The retention of isotope in the nucleic acids of L cells in tissue culture has also been investigated by Siminovitch & Graham (1956*a*), Healy, Siminovitch, Parker & Graham (1956) and Graham & Siminovitch (1957), who found that $^{32}PO_4^{3-}$ ion incorporated into the nucleic acids of cells growing logarithmically was completely retained in both DNA and RNA. This result is in marked contrast with the loss of activity from RNA in the present experiments and in the experiments on rat liver cited above. It is, however, in agreement with similar observations on logarithmically growing *E. coli* (Hershey, 1954; Fujisawa & Sibatani, 1954; Siminovitch & Graham, 1956*b*). It would therefore appear that isotope may be retained in rapidly growing cells to a much greater extent than in cells growing more slowly.

This retention of label in both RNA and DNA during logarithmic growth is presumably due to the inevitably rapid incorporation of all precursors into cell constituents, with the result that some constituents, such as RNA, are increasing so quickly that breakdown products, if any, would immediately be reincorporated and no demonstration of turnover would be possible. The degree to which such reincorporation of breakdown products takes place will obviously be greatest where the rate of synthesis is much greater than that of breakdown. The situation in a system such as we employ, in which the cells grow more slowly, is quite otherwise and the difference between the results reported in this paper and those reported by Siminovitch & Graham (1956*a*), by Healy *et al.* (1956) and by Graham & Siminovitch (1957) can be adequately explained as being due to the differences in the kinetics of the two experiments.

In the system described in the foregoing experiments the reactions can be summarized thus



$$A \underset{3}{\overset{1}{\rightleftharpoons}} B \underset{4}{\overset{2}{\rightleftharpoons}} C,$$

where A represents the extracellular pool of precursors of B and C, B represents the intracellular pool of precursors of C, and C represents the end products, in this case DNA, or RNA. The most favourable conditions for observing diffusion exchange of B between the inside and the outside of the cell are (1) a high steady-state concentration of B in the cell ($K_4 > K_2$); (2) a rapid exchange between inside and outside as compared with removal by synthesis ($K_3 > K_2$); and (3) a sufficient time of experiment. In the present case these conditions are best satisfied by observing slow growth over a long period as we have done.

We are therefore led to the conclusion, in the light of the results reported here, that RNA has a detectable rate of turnover in growing cells, and the possibility of turnover in DNA is not altogether excluded. Since the necessary information is not

available it is impossible to state whether this turnover involves all the RNA or DNA, or what the absolute rate may be in either case. The results do not exclude the possible existence of a transmissible block of DNA such as has been demonstrated to occur in bacteriophage by Levinthal (1956), but the existence of such a block remains to be proved and its size remains to be defined.

SUMMARY

1. The pattern of incorporation of [^{14}C]formate into the nucleic acids of strain L mouse fibroblasts was studied. The metabolic stability of the nucleic acids was also investigated by measuring the loss of ^{14}C from deoxyribonucleic acid, nuclear ribonucleic acid and cytoplasmic ribonucleic acid of labelled cells.

2. The cells are capable of synthesizing purines and thymine, but preferentially utilize adenosine, guanosine and thymidine when these are present in the medium. Adenosine is more readily incorporated than guanosine and conversion of adenine into guanine takes place more readily than does the reverse reaction.

3. Like some other tissues which have been studied *in vitro*, the cultured cells show a higher degree of incorporation of [^{14}C]formate into deoxyribonucleic acid thymine than into deoxyribonucleic acid purines when the medium contains plentiful amounts of purine and pyrimidine precursors. Incorporation into purines of both deoxyribonucleic acid and ribonucleic acid can be enhanced by addition to the medium of a mouse-liver extract or of 5-amino-4-imidazole carboxamide riboside. In the absence of preformed purines and pyrmidines in the medium twice as much [^{14}C]formate is incorporated into the deoxyribonucleic acid purines as into thymine.

4. When labelled cells are allowed to grow slowly in non-radioactive medium, activity is progressively lost from ribonucleic acid (in excess of the loss due to dilution). Cytoplasmic ribonucleic acid and nuclear ribonucleic acid behave similarly and some degree of turnover in both is suggested.

5. Little loss of ^{14}C from deoxyribonucleic acid is observed in similar conditions. However, when a large amount of thymidine is added to the medium the loss of activity from deoxyribonucleic acid thymine exceeds that found in control cultures, whereas the activities of adenine and guanine are unaffected. These observations suggest that there may be some turnover of deoxyribonucleic acid also but that this is obscured by reincorporation of breakdown products.

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