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ON THE METABOLIC STABILITY OF NUCLEIC ACIDS IN MITOTICALLY INACTIVE ADULT TISSUES LABELED DURING EMBRYONIC DEVELOPMENT

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SUMMARY

[8-^{14}C]adenine was administered to pregnant mice in order to incorporate isotope into DNA and RNA of dividing fetal tissue (brain and liver) that subsequently becomes mitotically inactive in the adult animal. The radioactivity in the DNA and RNA purines of these and other tissues from the offspring were determined at periods from nine days to one year after birth. During this period there was no loss of ^{14}C from the DNA purines of brain and liver, whereas the RNA of these tissues did lose radioactivity. These results further confirm the metabolic stability of DNA and indicate that radioactive atoms incorporated into DNA of the liver and brain of embryos may remain fixed for the life of the animal.

INTRODUCTION

The results of many studies in a variety of biological systems have pointed to the metabolic stability of deoxyribonucleic acid (DNA). In bacteria[1,2], in mammalian cells in tissue culture[3], and in the intact animal[4–9], convincing evidence has been obtained that DNA is synthesized only for cell division and, once synthesized, is meta-

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bolically inert in both rapidly dividing and in resting cells. The stability of DNA in resting mammalian cells has been emphasized in studies with liver[6,7] in which it was shown that DNA, labeled with an isotope during regeneration following partial hepatectomy, showed no significant loss of activity after regeneration was complete.

In the work with liver cited above, the stability of nucleic acids was examined over periods up to three months. The present paper extends this type of study to longer periods of observation and to tissues other than liver. For this study, the developing embryo appeared to be the system of choice. In the embryo, there is rapid cell division and synthesis of DNA in all tissues, and it would therefore be expected that if an appropriate precursor were administered, then DNA in all tissues would be extensively labeled. In those tissues in which cells are being renewed following cellular sloughing or expulsion, it would be expected that labeled nucleic acids would be lost at a rate proportional to renewal. However, in other tissues, such as brain and liver, which increase in cell number and mass during embryonic development but have little or no mitotic activity in the mature animal, it would appear that DNA synthesized by the embryo might be retained for the life of original cells and perhaps, in some instances, for the life of the animal. The experiments reported here were designed to study the long-term retention of ^{14}C in the DNA and in the ribonucleic acid (RNA) of mouse tissues labeled during embryonic development by [8-^{14}C]adenine and, at the same time, to see if retention of isotope is such that animals so labeled might be used profitably in future studies of the effects of ^{14}C on somatic cells.

METHODS AND RESULTS

Swiss mice were mated for a 24 h period. Fourteen days later each of five pregnant animals was given a single intraperitoneal injection of [8-^{14}C]adenine at a level of 10 μC/25 g. The animals delivered at the expected time. A group of twenty of the offspring, selected randomly, were sacrificed nine days after birth. After weaning, the remaining offspring (a total of 20 animals) from all litters were pooled, and groups of four to six animals were sacrificed for radioassays at periods from forty days to one year after birth.

The livers, spleens, whole intestines, brains, and (in one group) lungs from each group were pooled separately and homogenized. The homogenate was then extracted three times with cold 5 % trichloroacetic acid (TCA), and the resulting residue was washed with alcohol and ether. For determination of total activity in the crude TCA extracts, measured portions were oxidized, and the resulting $^{14}CO_2$ was counted by a modification of the BERNSTEIN-BALLENTINE method[10]. From the residue, DNA and RNA were separated by the SCHMIDT-THANNHAUSER method, and adenine and guanine from both nucleic acid fractions were isolated on paper chromatograms and assayed for radioactivity by procedures that have been described in detail elsewhere[11]. The results are presented in Table I. In this table are also presented calculations of the ratio of the specific activity of DNA adenine to that of DNA guanine. This ratio provides an additional index of the degree of stability of a nucleic acid. As shown by BENNETT AND KARLSSON[12], if a precursor is used that gives unequal labeling of nucleic acid adenine and guanine, then a change of this ratio with time is a reflection of the breakdown of the nucleic acid, re-equilibration of the adenine and guanine fragments, and reutilization of the equilibrated products. In the particular case of DNA in a

TABLE I

SPECIFIC ACTIVITIES OF ADENINE AND GUANINE FROM DNA AND RNA OF VARIOUS ORGANS OF THE PROGENY OF MICE THAT HAD RECEIVED [8-^{14}C]ADENINE DURING GESTATION

Twenty animals were used in the 9-day group. The other groups each consisted of 4–6 animals. The specific activity values preceded by a "<" sign indicate the presence of some activity in the sample isolated, but so little above background that it could not be assayed accurately.

Organ	*Time after birth (days)*	*Average wt. of organ (mg)*	*Specific activities (counts/sec/μg of purine)*				*Ratio of specific activities*
			RNA		*DNA*		*DNA adenine / DNA guanine*
			Ad	*Gu*	*Ad*	*Gu*	
Brain	9	185	0.40	0.23	0.90	0.20	4.5
	40	360	0.14	0.051	0.54	—	—
	71	413	< 0.09	< 0.04	0.53	0.12	4.4
	270	445	0.083	ca. 0.02	0.50	0.11	4.5
	365	452	ca. 0.14	< 0.08	0.48	0.11	4.4
Liver	9	130	0.37	0.17	0.77	0.24	3.2
	40	1520	< 0.02	< 0.02	0.12	ca. 0.04	ca. 3.3
	71	1900	< 0.02	< 0.04	0.093	0.036	2.6
	270	2200	< 0.02	< 0.01	0.058	0.021	2.8
	365	2000	< 0.007	< 0.009	0.050	0.016	3.1
Intestine	9		0.078	0.042	<0.019	—	—
	40		0.011	< 0.02	0.012	< 0.008	
	71		—	—	0.011	—	
	270		< 0.005	< 0.006	0.0088	0.0043	
	365		< 0.006	< 0.002	0.0069	0.0032	
Lung	365	—	< 0.2	< 0.1	0.16	< 0.07	
Spleen *	9	—	0.034	< 0.04	< 0.04	< 0.02	

* There was no measurable radioactivity in spleen purines after nine days.

TABLE II

TOTAL ACTIVITIES PRESENT IN THE VARIOUS FRACTIONS FROM ORGANS OF THE PROGENY OF MICE THAT HAD RECEIVED [8-^{14}C]ADENINE DURING GESTATION

Organ	*Time after birth (days)*	*Total activities ($\mu C \times 10^{-4}$) in TCA extract**	*Specific activities corrected for growth***			
			RNA		*DNA*	
			Ad	*Gu*	*Ad*	*Gu*
Brain	9	7.90	0.40	0.23	0.90	0.20
	40	0.20	0.26	0.10	1.05	—
	71	0.39	—	—	1.14	0.27
	270		0.20	0.048	1.27	0.27
	365		approx. 0.34	—	1.09	0.26
Liver	9	4.28	0.37	0.17	0.77	0.24
	40	1.62			1.39	0.46
	71				1.36	0.53
	270				0.99	0.36
	365				0.77	0.25

* Where no values are given, there was insufficient radioactivity for accurate assay.

** The corrected specific activities at each time period were calculated by multiplying the specific activity at that time by the ratio of the organ weight at that time to the weight at nine days. Where no values are given the specific activities were too low for accurate assay (see Table I).

growing tissue, this ratio obviously may change without breakdown of DNA as a result of turnover of RNA and utilization of RNA breakdown products for synthesis of DNA. The observed ratios (Table I) are invariant for brain; the ratios for liver are more variable, but there is no consistent decline. The greater variability in the liver ratios is probably the result of the lower individual specific activities of the purines, and consequently greater inaccuracy of the assays, as compared to assays of the purines from brain.

Since the tissues were increasing in mass during the earlier part of the experiment, the total activities rather than the specific activities are a measure of the retention of radioactivity in the DNA and RNA purines. On the basis of the observed average weights of each tissue (Table I), the specific activities at the nine-day period were corrected for growth and additional nucleic acid synthesis from inactive or almost inactive precursors, so that the resulting values are a measure of total radioactivity. These corrected specific activities are presented in Table II. No growth corrections were made for intestine because of the very low specific activities (Table I) and the unreliability of a growth correction in tissues known to slough and renew cells at a considerable rate. Table II also contains values for the total activities in the crude TCA extract; it should be noted that the values are given for this crude fraction in absolute microcuries, whereas those for the DNA and RNA purines are specific activities corrected for growth. Because of the crudeness of the samples, the values for the TCA extract are of limited interest. It is perhaps significant that in some tissues

TABLE III

RADIATION DOSAGE PER ORGAN IN THE PROGENY OF MICE THAT HAD RECEIVED [8-^{14}C]ADENINE DURING GESTATION

Tissue	*Time after birth (days)*	*Radiation dosage in mRem/week*		
		RNA	*DNA*	*Total*
Brain	9	95	100	195
	30–365	21	58	79
Liver	9	311	185	496
	30–71	—	26	26
	71–365	—	13	13

TABLE IV

NUMBER OF TRANSMUTATIONS OCCURRING IN DNA OVER A TWO-YEAR LIFESPAN OF PROGENY OF MICE THAT HAD RECEIVED [8-^{14}C]ADENINE DURING GESTATION

Tissue	*Number of transmutations**		
	Per million molecules of DNA	*Per cell*	*Per tissue*
Brain	11.5	66.0	$3.7 \cdot 10^9$
Liver	1.25	8.18	$5.1 \cdot 10^9$
Lung	2.80	18.4	$4.0 \cdot 10^9$

* The molecular weight of DNA[18] was taken as $5 \cdot 10^6$. Literature values for DNA content[9,14] were used in the calculations.

radioactivity was present in soluble form two months after birth of the animal. This radioactivity may come from the turnover of RNA, which did lose some activity during this period. In experiments with adult mice, HENDERSON AND LEPAGE[13] found significant radioactivity in acid-soluble adenine of a number of tissues nine days after administration of [^{14}C]adenine.

Since the results of Table II showed that ^{14}C was retained indefinitely in the DNA of the brain and liver, it was of interest, from the viewpoint of possible biological effects of the resulting radiation, to calculate the radiation dosage resulting from the ^{14}C present in the DNA (and also in the RNA when the activity was significant). Table III presents the results of these calculations in terms of mRem* per week. Table IV presents the number of transmutations theoretically occurring in the various organs over the assumed two-year life span of the animal calculated on the assumption that the DNA would be conserved for the last year of life as it was during the first. In calculating the values of Tables III and IV, pool sizes of DNA and RNA for the various tissues were taken from the literature[9,14] and, in some instances, were for animals other than the Swiss mice used in this experiment. However, the values calculated can be taken as indicating the correct orders of magnitude.

DISCUSSION

The data of Tables I and II indicate clearly that the ^{14}C present in the DNA purines of liver and brain of the nine-day old animal is retained without loss for the next twelve months—the limit of these experiments. During the first two months of this period, these tissues were increasing in mass and thereafter remained essentially constant in weight; thus, the DNA was conserved in both dividing and in resting cells. Earlier studies have shown the retention of ^{32}P in the liver of weanling rats[8] and of ^{14}C and ^{15}N in adult liver[6,7]; the studies with ^{32}P were carried out for periods up to ten days and those with ^{14}C and ^{15}N for periods up to three months. From the data of the present experiments, which covered half of the life span of the mouse, it would appear that the isotope incorporated into DNA of the brain or liver may be retained for the life of the animal.

Evidence for the stability of DNA in these tissues lies both in the retention of total activity when corrections are made for increases in mass of tissue (Table II) and in the invariance of the ratio of the specific activity of adenine to that of guanine (Table I). Although these results do not preclude the possibility that DNA may be in equilibrium with, or exchange molecules with soluble precursors, they strongly suggest that this does not happen. If there is any such exchange or equilibrium, then any breakdown products of DNA would have to be completely reutilized for synthesis of DNA and in such a manner that the magnitude of the Ad:Gu specific activity ratio would be unchanged. This is unlikely, since the pool size of acid-soluble adenine derivatives of liver and other tissues that have been studied is much greater than the pool size of soluble guanine derivatives[9].

The biochemical inertness of DNA and the parallel between DNA synthesis and cell division now appear to be generally accepted. Nevertheless, PELC has recently

* mRem = milliroentgen-equivalent-man, defined as that quantity of any type of radiation which, when absorbed by man, produces an effect equivalent to the absorption of 0.001 R of X- or γ-radiation.

obtained evidence that in the seminal vesicles of the mouse there is synthesis of DNA that is unrelated to cell division and has cited evidence indicating that the DNA of liver is being renewed at a rate of about 1 % per day[15–17]. The data of Table II provide no evidence of any significant renewal of DNA in brain or liver over a one-year period. However, in view of the heterogeneity of DNA, these data cannot be interpreted as indicating anything more than that certain DNA fractions that were labeled in the embryo are conserved completely. There could well be other DNA fractions that were not labeled under these conditions and that are renewed in the adult animal.

The behavior of RNA was different from that of DNA. In the liver, there was such an increase in weight between nine days and forty days (Table I) that the specific activities of the RNA purines would be lowered by new synthesis alone to very low values. The observed specific activities after nine days were, in fact, too low for accurate assay, and therefore the data do not permit an estimate of the degree of stability of RNA. However, although a quantitative comparison cannot be made, the specific activities of the RNA purines and DNA purines were of the same order at the nine-day period, and the fact that the specific activities of the RNA purines were consistenly lower than those of the DNA purines at the later periods does indicate some loss of activity from RNA. This finding is consistent with those from studies of regenerating liver and the liver of young animals which showed extensive loss of label from RNA without significant loss from DNA[7,8]. In contrast to the liver, the increase in the mass of the brain was very small, and activity could be measured in the RNA purines at all periods of the experiment. The loss of activity from brain RNA appears to be slow and most of the loss occurred between nine and forty days. In considering these results, it should be borne in mind that RNA, like DNA, is a heterogenous material, and it is therefore possible that the RNA fractions that are being rapidly renewed have already lost most of their activity by the time the first measurement was made nine days after the animals were born. If this were so, then the labeled RNA that was studied during the experiment would represent those RNA fractions that were being renewed at a very slow rate.

Among the other tissues studied, intestine and spleen rapidly lost radioactivity from the nucleic acids as is to be expected of tissues of organs that are dividing rapidly and expelling or sloughing cells in order to maintain equilibrium compatible with the host. As compared with liver and brain, the nucleic acids of the intestine and spleen contained little measurable activity at nine days; this fact suggests that most of the activity initially incorporated by these actively dividing tissues had already been lost by this time. The radioactivity present in the intestine at nine days declined slowly; it is probable that his activity is present in the DNA of muscle, which has been shown to have essentially no turnover[15,16]. The lungs were assayed only at the twelve-month period and, hence, retention of isotope could not be determined; however, the specific activity of DNA adenine of lung at this period was intermediate between those of liver and brain.

The retention of isotope in liver and brain is of interest for studies on possible radiation damage, particularly for study of the long-term effects of ^{14}C, localized in the DNA, on somatic cells. The calculations of Table III show that, as a result of the ^{14}C taken up by the embryo, the nucleic acids of the liver and brain are exposed for a period extending from nine days to thirty days after birth of the animal to radiation in excess of the 100 mRem/week recommended by the U.S. Atomic Energy Commission as the

maximum permissible level for continuous exposure. After thirty days, the radiation has dropped below this level as a result both of loss of ^{14}C from RNA and increase in tissue mass. It should be noted that the suggested safe radiation level of 100 mRem/week is for total body radiation and hence may not apply to the present situation, because the bulk of the ^{14}C is present as an integral part of the DNA, a site presumed to be particularly sensitive to damage by radiation. Of equal interest with regard to biological effects is the number of transmutations occurring in the DNA (Table IV). The number of transmutations per million molecules of DNA is small; however, when calculations are made on a per cell or per tissue basis, it is apparent that over the life span of the animal the number of transmutations occurring in liver, brain, and lung is appreciable. Since the β-radiation originates in, and the transmutations occur in, the genic chemicals, it is possible that ^{14}C thus incorporated and retained might produce effects on somatic cells that could be detected.

A long-term study is underway to evaluate any such effects of ^{14}C incorporated by the intact animal under these extreme experimental conditions—intraperitoneal administration to pregnant animals of a specific labeled precursor of nucleic acids—in which incorporation and retention of isotope in DNA of certain tissues are maximal.

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