The Liver Nucleic Acid Incorporation of Adenine-8-C^{14} during Azo Dye Carcinogenesis*

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Brown and associates (2, 9) conclusively demonstrated that isotopically labeled adenine was incorporated into the purines of the nucleic acids of normal tissues. The results of these and other investigators indicated that the uptake of the labeled compound into the cytoplasmic ribonucleic acid (RNA) was considerably greater than that into the desoxyribonucleic acid (DNA). In regenerating liver or in tumors, however, the incorporation into DNA was enhanced. Several investigators have found that the nuclear RNA had a rapid turnover of the isotope when labeled compounds were injected or fed in the diet. Barnum and Huseby (1) reported that the rate of incorporation of P^{32} into the nuclear RNA of mouse liver was extremely high. The specific activity of this fraction was greater than that of phospholipids and also that of the microsomal RNA. Davidson and McIndoe (6) observed that the RNA of nuclei isolated after the administration of P^{32} had a higher specific activity than the DNA. Following administration of P^{32} to rats, Marshak (21) isolated from the nuclei of liver, a "pentosenucleic acid" with a specific activity 8–13 times that the cytoplasmic RNA. Jeener and Szafarz (15), employing chromatographic technics for fractionation, found that the nuclear RNA of rat liver had a phosphorus specific activity 2 or 3 times that of the cytoplasmic RNA fraction.

Recently, Hurlbert and Potter (14) have obtained further evidence confirming the metabolic activity of the nuclear RNA. Labeled orotic acid was rapidly incorporated into the pyrimidines of this fraction. Tyner, Heidelberger, and LePage (28) observed that glycine-2-C^{14} was incorporated into guanine and adenine of both liver DNA and RNA at nearly the same rate. Furst and Brown (8) have also made similar observations in nongrowing tissues indicating that DNA may be synthesized by different pathways. In subsequent studies Tyner, Heidelberger, and LePage (29) found that specific activities of P^{32} and glycine-C^{14} were higher in nuclear RNA fractions of liver and carcinoma transplants than in other nucleic acid fractions. In this laboratory (11, 30), P^{32} was administered to normal, azo dye-fed and tumor-bearing rats. The nuclear RNA from the liver of each group showed an initially high specific activity of phosphorus. The present study was initiated to obtain further information concerning the metabolic activities of the nucleic acid fractions of normal and precancerous liver and tumor tissues. Adenine labeled with C^{14} was used, since it is readily incorporated into the tissue nucleic acids, and with adenine it is possible to avoid certain of the difficulties encountered with P^{32} in the complete fractionation of the phosphorus-containing compounds. A preliminary report has been made (12).

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METHODS

Adenine labeled with C^{14} in position 8 was synthesized essentially by the procedure of Clark and Kalckar (5). C^{14}-labeled sodium formate, when mixed with 4,5,6-triaminopyrimidine in a hydrochloric acid solution, failed to yield crystals of 4,6-diamino-5-formylaminopyrimidine hydrochloride. The procedure was therefore modified by taking the reaction mixture to dryness in a vacuum desiccator. The crystalline residue obtained was then used for cyclization without purification. The yield of adenine from 4,5,6-triaminopyrimidine was 40 per cent. Two fractions of adenine of different isotopic activity were obtained: the crystalline product of the reaction (which was later diluted with nonisotopic adenine) and a less active fraction resulting when the mother liquor was "washed" with nonisotopic adenine. Both products were checked chromatographically against known adenine samples. An Rf value of 0.33 was obtained with Schleicher and Schull No. 597 filter paper and a butanol-water solvent. The products and the eluates from the chromatograms showed

an adsorption peak of 261 mμ which result checks with the findings of Cavalieri *et al.* (3).

Albino male rats of the Holtzman-Sprague Dawley strain were maintained on a semisynthetic control diet for 6–7 weeks (13). At this time they weighed approximately 250 gm. Each animal was injected intraperitoneally with 3 μc. of the isotopic adenine (1 μc/mg adenine) in 2 ml. of 0.1 M phosphate buffer at pH 7. Two rats were sacrificed at the end of 3 and 24 hours and at the end of 7 days. Another group of rats was maintained for approximately 7 weeks on the control diet containing 0.06 per cent 3′-methyl-4-dimethylaminoazobenzene. This group was designated as the precancerous group and was injected and sacrificed like the above control group. At the time of sacrifice, the livers of these animals were cirrhotic and exhibited the precancerous changes usually evident at this time. For the tumor study, seven rats of the same strain were maintained on the control diet plus the dye for 12 weeks and were then fed the dye-free diet for an additional 5 weeks. Liver tumors could be palpated in these animals at this time. These animals were injected with 2.5 μc. of adenine and sacrificed as described. The tumors from two or three animals were pooled for the subsequent fractionations.

In the normal and precancerous groups, the livers were perfused, removed, and immediately frozen. In the tumor group, the tumors were freed of surrounding liver tissue and from any necrotic tissue. The liver or tumors were homogenized with cold 2 per cent citric acid (1 gm. tissue made up to 10 cc. with citric acid) for 3 minutes in a Waring Blendor at half speed. This homogenate was termed fraction I. On centrifugation at 2,000 r.p.m. in a Servall angle centrifuge (Model SS1), the nuclei from the homogenate remaining after removal of fraction I were separated. The nuclei were resuspended and washed twice more with cold 2 per cent citric acid, essentially as described by Dounce (7). Microscopic examination revealed that the nuclei were apparently free of cytoplasmic contamination.

The nuclei and aliquots of fraction I were separately extracted with cold 10 per cent TCA (trichloroacetic acid) and then with cold 5 per cent TCA. The combined acid-soluble extracts constituted fractions II and N-II. The letter *N* was used to designate the fractions from nuclei. The residue from the acid extraction was then extracted once with ethanol, twice with hot 3:1 ethanol-ether, and once again with ethanol. These combined extracts were designated as the lipid fractions, III and N-III.

The method of Schneider (27) was used to separate the total nucleic acids. The residue of the ethanol extraction was heated for 20 minutes at 90° C. in 5 per cent TCA. After being centrifuged, the supernate containing the nucleic acids was decanted off (fractions IV and N-IV), and a residue designated as phosphoprotein (fractions V and N-V) remained. In the case of the nuclear material, and in some cases with the whole liver material, fractionation with the methods of Schmidt and Thannhauser (26) was employed on the residue following the lipid extraction. This residue was hydrolyzed at 37° C. for 24 hours in 1 M KOH. The resulting clear solution was neutralized with HCl, and the DNA and protein were precipitated by the addition of 5 per cent TCA. The precipitate was washed and then heated at 90° for 20 minutes with 5 per cent TCA. This mixture was centrifuged, and the supernate constituting the DNA fraction (VII and N-VII) was decanted from the protein residue (VIII and N-VIII). The supernate from the KOH hydrolysis constitutes the RNA and nuclear RNA fractions (IX and N-IX).

DNA was determined on fractions IV and N-IV by the diphenylamine color reaction (27). Phosphorus (17) was determined in all fractions in order to check the efficiency of the fractionation procedures. Aliquots of each of the fractions were pipetted onto aluminum disks and counted with a Tracerlab autoscaler equipped with an automatic sample changer and windowless tube constructed in this laboratory (23). Samples containing TCA were neutralized with NH_4OH to stop any reactions with the aluminum. When the samples were nearly dry, a few drops of a very dilute solution of monomethylaniline in acetone were put on the plate. After complete drying under infrared lamps, the TCA salt was completely dispelled. Graphs of C^{14} self-adsorption were prepared by plating varying weights of tissue to which known amounts of the radioisotope had been added. The corrections for most of the samples were negligible; however, the IX and N-IX samples required salt self-absorption corrections because of the KCl present. The results were calculated for each fraction as disintegrations per second per gram of fresh liver or tumor tissue. Results were also calculated in terms of phosphorus as C^{14} disintegrations/second/mg of phosphorus.

RESULTS

Total recovery of C^{14} in the constituent fractions was good, and the accuracy of the fractionation procedure was also checked by phosphorus determinations. There was no evidence of C^{14} activity in the lipid or protein fractions. The normal liver DNA ranged from 300 to 360 mg. per cent. Livers from azo dye-fed animals contained from 470 to 520 mg. per cent and the tumor tissues from 553 to 798 mg. per cent of DNA. The percentage recovery of DNA in the nucleic preparations was from 77 to 90 per cent of the total.

Three hours following administration of the adenine, the livers from the control and azo dye rats and the liver tumors all exhibited essentially the same concentration of C^{14} (Table 1). The concentration of isotope in these tissues was reduced at each subsequent time interval. There were no significant differences among the normal liver, precancerous liver, and the tumor tissues in the acid soluble isotope activity.

From Table 1 it may be observed that the nuclear RNA fraction has a high rate of incorporation and turnover of the labeled adenine. Three hours following the administration of this compound the nuclear RNA fraction C^{14} activity was equal to or greater than the cytoplasmic RNA isotope activity. The DNA fractions, N-VII, had relatively little activity at this time, however; the tumor tissues had the highest activity, and the normal tissue the least activity in this respect. At subsequent time intervals the cytoplasmic RNA contained more of the labeled ade-

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nine while the activity of the nuclear RNA diminished. The DNA C^{14} activity increased at the later periods following the labeled adenine injection. In Chart 1 the relative uptakes of C^{14} in the various nucleic acid fractions were plotted with respect to the total nucleic acid uptake. The cytoplasmic RNA values were obtained by difference. Several of the cytoplasmic RNA fractions were checked by direct isotope determinations. The results were in essential agreement with the values obtained by difference. Shortly after the administration of adenine-8-C^{14}, the nuclear RNA contained a relatively high percentage of all the isotope present in the total nucleic acid fraction. This was especially evident in the tumor tissues. At subsequent time intervals the nuclear RNA decreased while the isotope activities in the DNA and the cytoplasmic RNA were increasing.

Another expression of the isotope incorporation was obtained by calculation of the ratio of C^{14} uptake to phosphorus. It may be observed from Table 1 that the C^{14} uptake relative to phosphorus is higher in the nuclear RNA than any other nucleic acid fractions 3 and 24 hours following the administration of the adenine. This was especially true for the precancerous liver and the tumor tissue. After an interval of 7 days, however, the C^{14} phosphorus values in the DNA and the cytoplasmic RNA were increasing.

TABLE 1

INCORPORATION OF C^{14} BY FRACTIONS DERIVED FROM NORMAL, PRECANCEROUS, AND CANCEROUS LIVER TISSUE FOLLOWING ADMINISTRATION OF LABELED ADENINE

		Time after administration of adenine								
		3 hours			24 hours			7 days		
	Fraction	Control	3′-Me-DAB*	Tumor	Control	3′-Me-DAB	Tumor	Control	3′-Me-DAB	Tumor
I	Homogenate									
	Carbon-14†	1,277	1,400	1,200	765	920	670	340	590	234
	Phosphorus‡	330	338	250	345	375	260	274	323	278
	C^{14}/P§	385	415	480	223	246	258	125	183	84
II	Acid-soluble									
	Carbon-14	915	1,030	1,040	473	485	324	101	194	63
	Phosphorus	87	101	84	74	105	65	73	92	78
	C^{14}/P	1,055	1,020	1,230	632	461	498	138	212	81
IV	Total nucleic acids									
	Carbon-14	68	114	153	250	304	230	194	360	126
	Phosphorus	76	92	86	102	103	83	66	87	90
	C^{14}/P	89	124	176	246	295	277	293	414	140
N-IV	Total nuclear nucleic acids									
	Carbon-14	33	63	136	100	108	96	53	108	53
	Phosphorus	31	35	50	50	51	45	35	40	47
	C^{14}/P	106	180	273	200	212	214	150	270	114
N-VII	Desoxyribonucleic acid									
	Carbon-14	0	3	20	1	30	41	5	50	30
	Phosphorus	20	32	29	17	36	33	15	27	30
	C^{14}/P	0	9	70	6	83	124	33	185	100
N-IX	Nuclear ribonucleic acid									
	Carbon-14	35	49	160	92	65	60	48	68	33
	Phosphorus	13	9	20	20	19	14	18	14	19
	C^{14}/P	270	544	800	460	342	428	266	480	174

* 3′-Me-DAB (3′-methyl-4-dimethylaminoazobenzene) fed in diet for 7–8 weeks.

† Disintegrations/sec/gm fresh tissue.

‡ Total phosphorus expressed as mg/100 gm of fresh tissue.

§ Disintegrations/sec/mg P.

All C^{14} values for fractions derived from tumors were multiplied by a factor of 1.2 to correct for smaller quantity of isotope administered.

DISCUSSION

The rapid incorporation of the labeled adenine by the nuclear RNA is in agreement with the observations of several other groups employing P^{32} (1, 11, 15, 21), labeled glycine (19, 29), and orotic acid (14). Calculations made from the data of Price and associates (25) indicated that the nuclear RNA fraction constitutes approximately 25 per cent of the total nucleic acid of the nucleus or 9 per cent of the nucleic acid of normal whole rat liver. For animals fed diets containing 3′-Me-DAB, the values are 20 and 12 per cent, respectively. Histochemical observations also indicate that the nucleolus, in which most of the nuclear RNA is found, constitutes a relatively small percentage of the total cellular volume. In the present study the nuclear RNA, or at least the alkali soluble fraction of isolated liver cell nuclei, contained 50 per cent of the total C^{14} activity of the tissue nucleic acid fraction 3 hours following administration of the adenine. On the basis of a relative activity (C^{14}/P), this fraction exhibited

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the highest uptake of the labeled adenine of all the nucleic acid fractions, at this time period. At subsequent periods the nuclear RNA activity was decreasing while the DNA and the cytoplasmic RNA were increasing in C^{14} activity, indicating that the nuclear RNA is the most active of the cellular nucleic acid fractions and may possibly be the precursor of other components of the cell. Other investigators have proposed this possibility (15, 21). The nuclear RNA of normal liver also has a high uptake of the C^{14}, which fact suggests that the fraction may be involved in functional metabolic activities as well as in cellular growth and development. Marshak (22) analyzed the nuclear RNA and other cellular nucleic acid fractions of several tissues for their constituent purine and pyrimidine content. The nuclear RNA was different in composition from other nucleic acid fractions and was characterized by a low pyrimidine content.

There were no appreciable differences between normal liver and liver tumors in their total uptake to the C^{14}-labeled adenine. In a previous study involving labeled glycine (10), the proteins of liver

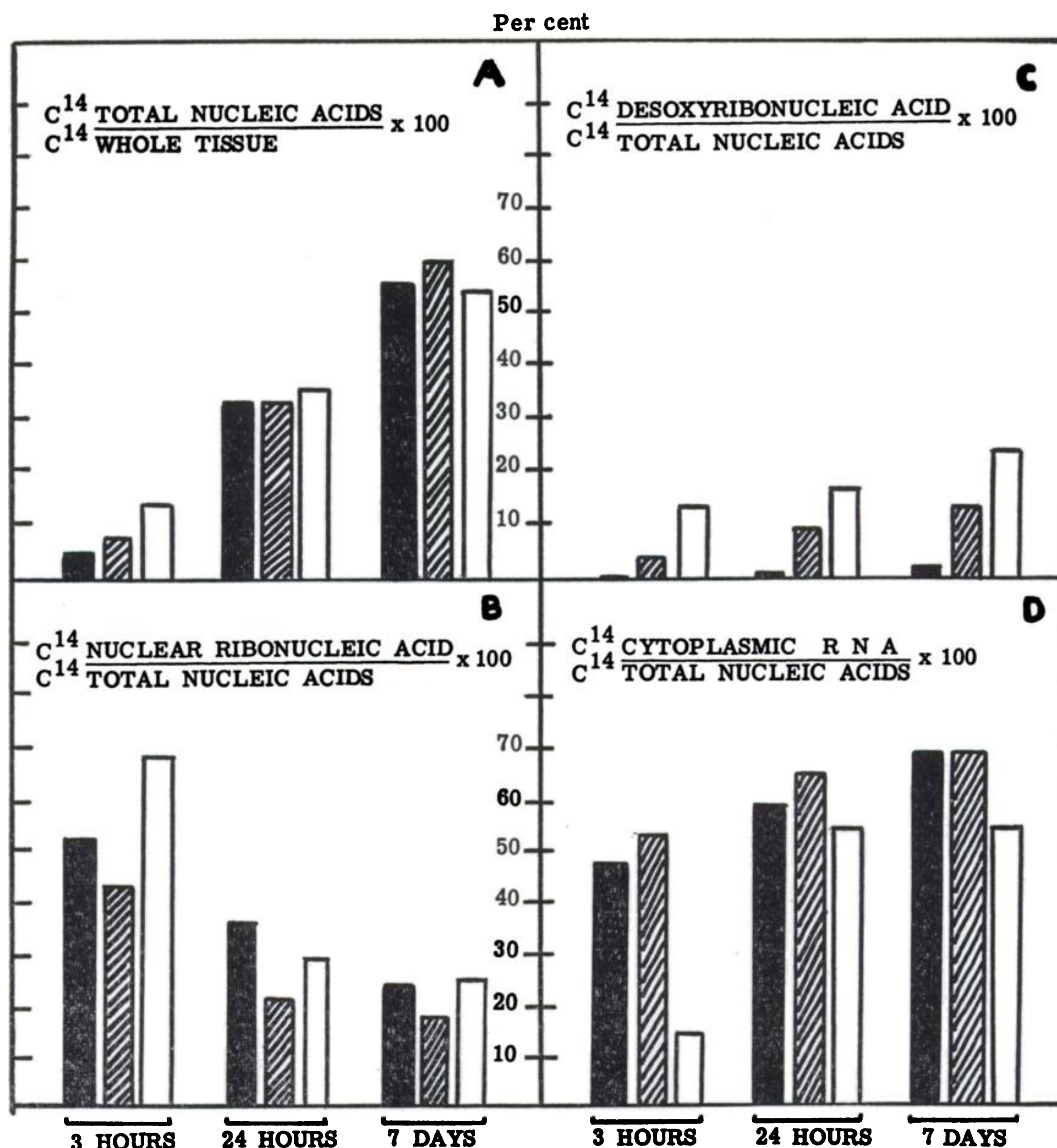

CHART 1.—Per cent uptake C^{14} (adenine) in various nucleic acid fractions with respect to whole tissue or total nucleic acids. Solid, shaded, and unshaded areas represent normal, precancerous, and tumor tissues, respectively.

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exhibited a faster incorporation of isotope than the proteins of liver tumor. Zamecnik *et al.* (31) obtained similar results with C^{14}-labeled glucose and amino acids. In studies with glycine, Tyner *et al.* (28) also found that the protein of liver incorporated the isotope faster than the protein of Flexner-Jobling carcinoma. In contrast, however, the nucleic acids of the tumor incorporated the glycine faster than the nucleic acids of liver. *In vivo* studies involving slice technics have demonstrated that proteins of hepatoma tissue have a far greater uptake of labeled isotope than proteins of liver (31). The difference in the incorporation by protein of isotopes between the *in vivo* and *in vitro* studies, according to Zamecnik, may be attributed to a circulatory impairment of the tumor in the living animal and perhaps the nonuniformity of tumor tissues. LePage (19) incubated cell suspensions of mouse liver and also ascites tumors in a medium containing labeled glycine. The proteins and purines of the tumor cells were labeled to a greater extent than those of liver cells.

Kelly and associates (16) have reported that the noncancerous tissues of tumor-bearing mice have a greater incorporation of administered P^{32} in the DNA than the corresponding tissues from normal animals. A decrease in the P^{32} incorporation into nuclear RNA of liver in mice bearing carcinoma was noted (24). Cerecedo *et al.* (4) found an increase in the content of DNA and RNA in the liver and lung of mice bearing sarcomas. It is not immediately evident why the nuclear RNA in normal tissues of tumor-bearing animals should show a decrease in the incorporation of P^{32} while the incorporation into DNA is increased. These findings do not support the hypothesis that RNA is necessarily a precursor of DNA. The glycine studies of Furst and Brown (8) and Tyner and associates (28) indicate that the DNA of normal tissues is being synthesized at a greater rate than is indicated from studies with P^{32} and labeled adenine. As had been pointed out, DNA may be synthesized by different pathways. Recently, Litt, Monty, and Dounce (20) and Krakaur and associates (18) have reported methods whereby nucleoli may be isolated from tissue homogenates. These direct procedures should facilitate studies involving the composition as well as the metabolic and growth significance of the nuclear ribonucleic acid.

SUMMARY

1. Normal albino rats, rats fed diets containing the carcinogenic azo dye 3′-methyl-4-dimethylaminoazobenzene, and rats with liver tumors were given intraperitoneal injections of solutions containing adenine-8-C^{14}. Animals were sacrificed from each group at 3 hours, 24 hours, and 7 days following the administration of the isotope, and the liver and tumors were fractionated into acid-soluble, total nucleic acid, DNA and nuclear RNA fractions. C^{14} activity and phosphorus were determined on all fractions.

2. The incorporation of the isotope by DNA was greatest in the tumor tissue, intermediate in the liver from the dye-fed animals, and least in the normal liver. The concentration of isotope for each tissue increased at each time interval following the administration of the labeled adenine.

3. The isotope was incorporated into the nuclear RNA at a greater rate than into the other nucleic acid fractions. This was especially true for the tumor tissue; however, there was also considerable activity in this fraction obtained from the livers of the normal and the dye-fed animals. At subsequent periods following the administration of the labeled adenine, the activity of this nuclear RNA decreased while the DNA and the cytoplasmic RNA were increasing.

REFERENCES

1. BARNUM, C. P., and HUSEBY, R. A. The Intracellular Heterogeneity of Pentose Nucleic Acid as Evidenced by the Incorporation of Radiophosphorus. Arch. Biochem., **29**:7–26, 1950.
2. BROWN, G. B. Precursors of Nucleic Acids. J. Cell. & Comp. Physiol., Suppl. 1, **38**:121–41, 1951.
3. CAVALIERI, L. F.; BENDICH, A.; TINKER, J. F.; and BROWN, G. B. Ultraviolet Absorption Spectra of Purines, Pyrimidines, and Triazolopyrimidines. J. Am. Chem. Soc., **70**:3875–80, 1948.
4. CERECEDO, L. R.; REDDY, D. V. N.; PIRCIO, A.; LOMBARDO, M. E.; and TRAVERS, J. J. Nucleic Acid Changes in Tumor-bearing Mice. Proc. Soc. Exper. Biol. & Med., **78**:683–87, 1951.
5. CLARK, V. M., and KALCKAR, H. M. A Synthesis of Adenine Labeled with C^{14}. J. Chem. Soc., pp. 1029–30, 1950.
6. DAVIDSON, J. N., and McINDOE, W. M. Phosphorus Compounds in the Cell Nucleus. Biochem. J., **45**: xvi, 1949.
7. DOUNCE, A. L. The Desoxyribonucleic Acid Content of Isolated Nuclei of Tumor Cells. J. Biol. Chem., **151**:235–40, 1943.
8. FURST, S. S., and BROWN, G. B. On the Role of Glycine and Adenine as Precursors of Nucleic Acid Purines. J. Biol. Chem., **191**:239–47, 1951.
9. FURST, S. S.; ROLL, P. M.; and BROWN, G. B. On the Renewal of the Purines of the Desoxypentose and Pentose Nucleic Acids. J. Biol. Chem., **183**:251–66, 1950.
10. GRIFFIN, A. C.; BLOOM, S.; CUNNINGHAM, L.; TERESI, J. D.; and LUCK, J. M. The Uptake of Labeled Glycine by Normal and Cancerous Tissues in the Rat. Cancer, **3**:316–20, 1950.
11. GRIFFIN, A. C.; CUNNINGHAM, L.; BRANDT, E. L.; and KUPKE, D. W. Effect of a Carcinogenic Azo Dye on Radio-Phosphorus Turnover in Rat-Liver Nuclei and Cytoplasm. Cancer, **4**:410–15, 1951.
12. GRIFFIN, A. C.; DAVIS, W. E.; and TIFFT, M. O. The Liver Nucleic Acid Uptake of C^{14} Adenine during Azo Dye Carcinogenesis. Cancer Research, **12**:267, 1952.
13. GRIFFIN, A. C.; NYE, W. N.; NODA, L.; and LUCK, J. M.

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Tissue Protein and Carcinogenesis. I. The Effect of Carcinogenic Azo Dyes on Liver Proteins. J. Biol. Chem., **176**:1225–35, 1948.

14. Hurlbert, R. B., and Potter, V. R. A Survey of the Metabolism of Orotic Acid in the Rat. J. Biol. Chem., **195**: 257–70, 1952.
15. Jeener, R., and Szafarz, D. Relations between the Rate of Renewal and the Intracellular Localization of Ribonucleic Acid. Arch. Biochem., **26**:54–67, 1950.
16. Kelly, L. S.; Payne, A. H.; White, M. R.; and Jones, H. B. The Effect of Neoplasia or Pregnancy on the Tissue Desoxypentosenucleic Acid. Cancer Research, **11**:694–96, 1951.
17. King, E. J. The Colorimetric Determination of Phosphorus. Biochem. J., **26**:292–97, 1932.
18. Krakaur, R.; Graff, A. M.; and Graff, S. Isolation of Cell Particles. Cancer Research, **12**:276, 1952.
19. LePage, G. A. Studies on the *in Vitro* Synthesis of Glycine into Purines and Proteins. Cancer Research, **12**: 277, 1952.
20. Litt, M.; Monty, K. J.; and Dounce, A. L. Isolation and Properties of Rat Liver Cell Nucleoli. Cancer Research, **12**:279, 1952.
21. Marshak, A. Evidence for a Nuclear Precursor of Ribo- and Desoxyribonucleic Acid. J. Cell. & Comp. Physiol., **32**:381–406, 1948.
22. ———. Purine and Pyrimidine Content of the Nucleic Acids of the Nuclei and Cytoplasm. J. Biol. Chem., **189**: 607–15, 1951.
23. Nye, W. N., and Teresi, J. D. Automatic Sample Changer for Windowless Gas Flow Counters. Anal. Chem., **23**:643–46, 1951.
24. Payne, A. H.; Kelly, L. S.; and White, M. R. Effect of Neoplastic Tissue on the Turnover of Liver Nucleic Acids. Cancer Research, **12**:65–68, 1952.
25. Price, J. M.; Miller, E. C.; Miller, J. A.; and Weber, G. M. Studies on the Intracellular Composition of Livers from Rats Fed Various Aminoazo Dyes. II. 3′-Methyl-, 2′-Methyl-, and 2-Methyl-4-dimethylaminoazobenzene, 3′-Methyl-4-monomethylaminoazobenzene, and 4′-Fluoro-4-dimethylaminoazobenzene. Cancer Research, **10**:18–27, 1950.
26. Schmidt, G., and Thannhauser, S. J. A Method for the Determination of Desoxyribonucleic Acid, Ribonucleic Acid, and Phosphoproteins in Animal Tissues. J. Biol. Chem., **161**:83–89, 1945.
27. Schneider, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid. J. Biol. Chem., **161**:293–303, 1945.
28. Tyner, E. P.; Heidelberger, C.; and LePage, G. A. *In Vivo* Studies on Incorporation of Glycine-2-C^{14} into Proteins and Nucleic Acid Purines. Cancer Research, **12**:158–64, 1952.
29. ———. Intracellular Distribution of Radioactivity in Nucleic Acid Nucleotides and Protein Following Simultaneous Administration of P^{32} and Glycine-2-C^{14}. Fed. Proc., **11**:300, 1952.
30. Ward, D. N.; Brandt, E. L.; and Griffin, A. C. The Effects of Nitrogen Mustard and a Pyrimidine Compound on the $Phosphorus^{32}$ Turnover in Rat Liver during Azo Dye Cancer Induction. Cancer, **5**:625–30, 1952.
31. Zamecnik, P. C.; Loftfield, R. B.; Stephenson, M. L.; Steele, J. M. Studies on the Carbohydrate and Protein Metabolism of the Rat Hepatoma. Cancer Research, **11**: 592–602, 1951.

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