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Microdetermination of Nucleic Acid Phosphorus by Neutron Activation Analysis*

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ABSTRACT: Neutron activation analysis was standardized for the determination of DNA phosphorus down to 0.1 ng. The method was based upon the transformation of stable polynucleotide phosphorus into ^{32}P by capture of thermal neutrons and it included four steps: (1) irradiation of DNA in a nuclear reactor; (2) mineralization of the organic material in Teflon tubes by a HNO_3–$HCOOH$–H_2O_2 mixture changing all ^{32}P species into inorganic radiophosphate; (3) isolation of the $[^{32}P]PO_4^{3-}$ from contaminating radionuclides; (4) assay of ^{32}P by counting its Cerenkov effect in 6 M HF with the aid of a conventional apparatus for liquid scintillation spectrometry. For specimens with more than 100 μg of total phosphorus, oxidative acid digestion of the nucleotides, and separation of the ^{32}P from other radioelements were not always necessary. When such samples were prepared and activated under controlled conditions, the β^- emission of ^{32}P was measured directly in irradiated DNA without interferences after 1 week of decay. Except for neutron activation, all manipulations can be carried out in a normal biochemical laboratory. Some of the auxiliary techniques developed for the neutron activation analysis method may be applied in chemical assays of nucleic acid phosphorus for improving accuracy and sensitivity.

The biochemical characterization of nucleic acids rests primarily on the quantitative measurement of their phosphorus content (Fiske and Subbarow, 1925). The established methods involve mineralization of the sample and colorimetric assay of the released inorganic phosphate. At very low phosphorus concentrations, the overall procedure embodies several sources of error. As a matter of fact, the 1.0 or 2.0 μg of total phosphorus recommended as the smallest amounts for reliable

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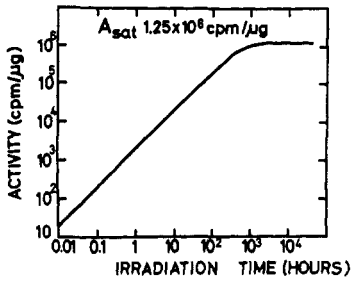

FIGURE 1: Counting rates per microgram of phosphorus at different irradiation times. A_{satn} = maximal counts per migrogram of irradiated phosphorus at saturation and decay time 0. The samples were exposed to a flux of 5×10^{12} neutrons cm^{-2} sec^{-1}. For details, see Results.

analyses exceed the ultimate sensitivity of the spectrophotometric determination of phosphate at least by two orders of magnitude (Ames and Dubin, 1960; Morrison, 1964). The possibility to detect nucleotides at the nanogram level would allow to trace minute quantities of these often rare materials in biochemical preparations carried out in microscale.

The present communication describes a reliable method for measuring down in this investigation, down to 0.0001 µg of nucleic acid phosphorus by neutron activation analysis. This technique was already successfully applied in paper chromatography and electrophoresis to locate spots of organophosphorus compounds such as phosphatides (Yagi *et al.*, 1963; Strickland and Benson, 1960) and oligonucleotides (Murray and Offord, 1966; Rushizky and Miller, 1967). It served also to detect DNA and RNA contents in cell extracts (Akaboshi *et al.*, 1967).

The reported method is simple and all the operations required before and after irradiation of the sample are within the capacity of most biochemists and molecular biologists.

Material and Methods

Biochemicals and Reagents. $[^{32}P]PO_4^{3-}$ was purchased from Amersham Radiochemical Centre; acid aluminum oxide from M. Woelm, Eschwege, West Germany; highly polymerized calf thymus DNA from Worthington. All other chemicals were reagents of analytical grade purity. Aqueous solutions were prepared with quartz bidistilled water.

Glassware and Plastics. Test tubes and pipets were of plastic polyallomers and the glassware of Pyrex brand. The irradiation vials were of polyethylene or quartz.

Radioactivation of Phosphorus. Neutron activation of phosphorus was performed by irradiating the samples in the Ispra 1 reactor which is moderated and cooled by heavy water and operates at a steady 5-MW power. We use an exposure facility allowing the irradiation of six capsules of magnesium–aluminum alloy (14 mm diameter × 60 mm length) for periods up to 21 days, in a flux of 5×10^{12} neutrons cm^{-2} sec^{-1} at temperatures of about 100°. The transfer of the capsules from the reactor to the radiochemical laboratory was done pneumatically.

This facility is particularly suited to the neutron activation analysis determination of phosphorus, since the neutron flux has a very low fraction of high-energy neutrons, and the interference due to nuclear reactions on chlorine and sulfur leading to ^{32}P is minimal (see Discussion). Details on the reactor characteristics and on the applied procedures for neutron activation analysis have been published (Bresesti and Neumann, 1964).

Spectrophotometric Assays. Inorganic phosphate was also assayed according to a conventional method (Morrison, 1964). A chemical determination of nucleotide phosphorus will be reported under Results.

The concentration of calf thymus DNA solutions was estimated from their ultraviolet extinction coefficient (cm^{-1}) for P-DNA of 6.66 at 260 nm.

Chromatographic Methods. The various chemical species of phosphorus were chromatographically differentiated on Whatman No. 1 paper by using a 1-butanol–dioxan–1 N NH_4OH mixture as solvent (Bonnin and Süe, 1952). Ascending chromatograms were developed for 20 hr at 22°, dried, and stained with the phosphomolybdic acid reagent or analyzed for ^{32}P radioactivity. According to preliminary tests, DNA was retained at the origin, inorganic phosphate had an R_F of 0.14, and less oxidized forms of phosphorus such as phosphite and hyphophosphite were spotted at 0.33 and 0.60 R_F's, respectively. A portion of the sampled materials (5–10%) was always retained at the opposition site.

^{32}P-labeled compounds were detected in chromatograms by testing the radioactivity of 0.5-cm paper strips cut parallely to the moving boundary throughout the length of the solvent run. The ^{32}P on the paper was measured by the Cerenkov effect produced by the strips immersed in 6 M HF. The counting method was identical with that reported under Results for ^{32}P sources dissolved in HF.

Results

Neutron Activation Analysis. Neutron activation analysis for nucleic acid phosphorus takes advantage of the formation of radioactive ^{32}P from the stable isotope ^{31}P of natural nucleotides by the neutron capture reaction: $^{31}P + n \rightarrow {}^{32}P + \gamma$ rays. The procedure, here developed, consists of four different steps (irradiation, mineralization of organic material, chemical separation of $[^{32}P]PO_4^{3-}$, and counting of ^{32}P) which will be sequentially described.

Irradiation. DNA was dissolved in 10 mM Tris-acetate (pH 8.0) and extensively dialyzed against the same buffer in order to lower the concentrations of chlorine and sulfur down to undetectable levels (see Discussion).

Aliquots (0.2–1.8 ml) of the dialyzed solution with 1 ng to 3.8 mg of nucleic acid were collected into small tubes of either polyethylene or silica and freeze-dried. Each sample was paired with a similarly treated standard known to contain 40–100 µg of $(NH_4)_2HPO_4$. Two or three vial pairs were placed into an irradiation capsule and subjected to neutron activation. Relative measurements of ^{32}P formed in the specimens and its comparator provided a quantitative calibration of the phosphorus content.

The activation time was varied according to the expected amount of phosphorus in the samples since the production of ^{32}P increased primarily by prolongating the irradiation. Figure 1 records the counts per minute calculated for 1 µg of DNA phosphorus *vs.* the exposure time. In general, DNA aliquots with more than 30 µg of phosphorus were irradiated less than 6 min and those with 0.1–1.0 ng of phosphorus were let 7 days in the neutron flux.

Many short-lived radionuclides were produced by neutron activation of metal impurities present in the sample and the resulting hazard was eliminated by storing the capsules withdrawn from the reactor in a shielded hood of the radiochemical laboratory. A week of decay was always sufficient to

lower the surrounding radiation field to less than a few millireontgens per hour.

Eventually, the vials of samples and standards were externally washed with 1 M HNO_3 and carefully opened. Their contents were dissolved in 0.5–1.0 ml of 1 M HF and quantitatively transferred into mineralization bottles with 100 μg of carrier PO_4^{3-}.

Mineralization. Due to the interaction between phosphorus atoms and radiation-induced radicals, the neutron activation of esterified phosphates gives rise to various phosphorus species, including phosphite, hypophosphite, phosphonic, and phosphinic acid derivatives (Yagi *et al.*, 1963).

Paper chromatography was used to check the occurrence of multiple phosphorus forms in our neutron activated samples and the conversion of ^{32}P into a single chemical species as required for its further separation from other contaminating radionuclides. Figure 2 records the distribution of ^{32}P in chromatograms of DNA irradiated for 10 hr as compared with the profile obtained for a reference $[^{32}P]PO_4^{3-}$ aliquot. Indeed, most of the radioactivated phosphorus was associated with the polynucleotide material at the origin of the chromatogram but a notable portion of ^{32}P was found in various unidentified substances having R_F's equal as or even higher than the one of inorganic phosphate (A). Following a treatment with 1 N HCl at 100° and for 1 hr, the ^{32}P radioactivity decreased largely in DNA and augmented conversely in the mobile fractions (B). Mineralization of the specimens in strong acids and under oxidative conditions was necessary for transforming all the ^{32}P of neutron activated DNA into a unique class of compounds which behaved chromatographically like $[^{32}P]PO_4^{3-}$ (C and D).

Combustive acid hydrolyses for mineralizing DNA were tested for completeness of nucleic acid digestion and for experimental losses of phosphorus by volatilization (Charlot, 1961) or physicochemical adsorption on glassware (Hassenteufel *et al.*, 1963). Small aliquots of nucleic acid in solution were supplemented with a known amount of irradiated DNA which served as a tracer. The combined mixtures containing 2.8–3.8 mg of total DNA and corresponding exactly to 5×10^4 cpm of ^{32}P actually measured by Cerenkov effect were mineralized in vials of Teflon and Pyrex glass according to the following procedures: (1) H_2SO_4–$HClO_4$ method of Fiske and Subbarow (1925); (2) $Mg(NO_3)_2$–C_2H_5OH method of Ames and Dubin (1960); and (3) HNO_3–HCOOH–H_2O_2 method. To the sample (0.01–2.0 ml) 1 ml of nitric acid and 1 ml of formic acid were added; the vial was placed on a heater at 150°. When the development of the red fumes had ceased, 1 ml of H_2O_2 was slowly supplemented, and the mixture was boiled up to almost dryness. The procedure was repeated when a dark residue was obtained.

The digests were finally diluted with 5 ml of various acids and the ^{32}P content of the resulting clear solutions was counted. The yields of ^{32}P from the different assays are reported in Table I. From these data and from complementary circumstantial observations, the foregoing conclusions could be drawn. (1) Mineralizations in glass gave lower per cent recoveries than those obtained from digestions in Teflon test tubes. Residual radioactivity on the used crushed vials showed that a relevant uptake of ^{32}P on glassware occurred even in the presence of large amounts of added PO_4^{3-} carrier. (2) The H_2SO_4–$HClO_4$ method requiring high temperatures (300°) for the development of the white SO_3 fumes caused loss of radioactivity which was detected in the condensed fumes. (3) The 0.5 M or more concentrated acids were adequate to dissolve quantitatively the DNA digests. The 7.0 M HNO_3

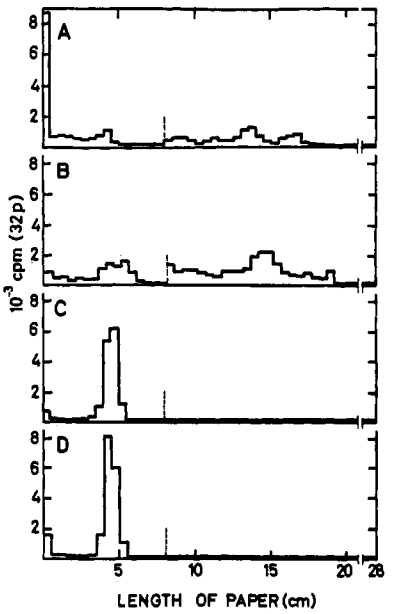

FIGURE 2: Chromatographic profiles obtained for 2 μg of neutron activated DNA before (A) and after acid hydrolysis (B) or oxidative mineralization in HNO_3–HCOOH–H_2O_2 mixture (C). (D) Chromatogram shows the profile of 2 μg of $[^{32}P]PO_4^{3-}$. After the dotted line the ordinate scale was expanded ten times. For details, see Material and Methods, and Results.

was, however, preferred since it allowed to process directly the sample for the subsequent radiochemical separation of $[^{32}P]PO_4^{3-}$. (4) The highest and most reproductible yields were those of DNA digested by the HNO_3–HCOOH–H_2O_2 solution in the Teflon vials. Therefore, method 3 in Teflon was adopted as a standard procedure.

Radiochemical Separation of $[^{32}P]PO_4^{3-}$. The step served to eliminate the spurious counts which were due to traces of radioactivated metals in the samples and interfering with $[^{32}P]PO_4^{3-}$ measurements. The $[^{32}P]PO_4^{3-}$ was separated from the contaminating radionuclides by a modification of a previous method (Sabbioni *et al.*, 1970) based on selective absorption of phosphate on acid aluminum oxide.

The DNA digests were diluted in 5 ml of 7 M HNO_3 and passed through a 0.7 × 3.0 cm column of acid aluminum oxide previously equilibrated with the same acid. Under these conditions, acid aluminum oxide retained quantitatively PO_4^{3-}, As^{III}, W^{VI}, and partially Br^{-I}, Ge^{IV}, Mo^{VI}, Sb^{III-V}, Sn^{IV}, and Ta^{V} among the 49 ions originally tested (Sabbioni *et al.*, 1970). Except for ^{122}Sb and ^{124}Sb which were discharged in minute quantities from neutron activated silica vials, none of the adsorbable elements were likely to contaminate nucleic acid samples. When the nucleotides were irradiated in quartz tubes, 0.5 g of carrier $SbCl_3$ was used for reducing to negligible level the acid aluminum oxide absorption of the Sb radionuclides.

The column was washed with the aid of 20 ml of 7 M HNO_3 and the $[^{32}P]PO_4^{3-}$ was specifically eluted by a 15-ml aliquot of 6 M HF. The effluent was quantitatively collected into commercial counting bottles for liquid scintillation spectrometry.

Radioactivity Measurements. The ^{32}P present in the acid aluminum oxide eluate was estimated by measuring the Cerenkov radiation due to the emission of its β^- particles in acid solution. The determination was performed in a liquid scintillation counter (Mark 1 from Nuclear-Chicago

TABLE I: Recovery of $[^{32}P]PO_4^{3-}$ in Redissolved DNA Digest Obtained after Mineralization in Glass and Teflon Vessels.[a]

Mineralization Method	Redissolving Medium	Yield of ^{32}P for Glass Series		Yield of ^{32}P for Teflon Series	
		Measured Cpm	% Recov	Measured Cpm	% Recov
H_2SO_4–$HClO_4$	0.5 M H_2SO_4–$HClO_4$	46,820	94	47,500	95
		40,920	82	48,510	97
		46,560	93	47,520	95
		44,970	90		
		42,500	85		
		47,300	95		
$Mg(NO_3)_2$–C_2H_5OH	0.5 M HCl	44,890	90	47,000	94
		42,000	84	47,500	95
		45,510	91	45,520	91
HNO_3–HCOOH–H_2O_2	1 M HNO_3	49,500	99	49,010	98
		47,400	95	49,500	99
		43,430	87	49,600	99
	7 M HNO_3			50,000	100
				49,510	99
				48,020	96
				48,490	97
	0.5 M HCl			49,000	98
				49,500	99
				49,020	98

[a] The nucleic acid samples were traced with the constant aliquot of neutron activated DNA corresponding to 5×10^4 cpm. DNA was irradiated in Ispra 1 reactor for 5 min and let decay for 15 days.

TABLE II: Precision of Determination of Neutron Activation Analysis for Nucleotide Phosphorus.

μg of DNA Phosphorus in Each Assay	No. of Assays	$\bar{X}$[a]	σ[b]	σ_m[c]	$\bar{X}_{(p)}$[d]	$\sigma_{(p)}$[e]	$\sigma_{m(p)}$[f]
350	8	336	7.2	2.6	96.6	3.7	1.04
300	5	292	5.2	2.4			

[a] $\bar{X} = \Sigma X_i/n$ = mean value of data. [b] $\sigma = (\Sigma(\bar{X} - X_i)^2/(n-1))^{1/2}$ = standard deviation of data. [c] $\sigma_m = \sigma/(n)^{1/2}$ = standard error of data. [d] $\bar{X}_{(p)}$ = mean value of per cent recovery from the 13 assays. [e] $\sigma_{(p)}$ = standard deviation of per cent recoveries. [f] $\sigma_{m(p)}$ = standard error of per cent recoveries.

Corp.) which was calibrated for optimal detection of the Cerenkov effect produced by a pure ^{32}P source in 6 M HF. Interferences by other radionuclides might be noticed from either changes in the channel ratios or half-life of the radiation. The efficiency of the radioactivity counting was 0.47 (Girardi *et al.*, 1969).

The quantity of phosphorus in a sample was calculated from the ratio between its ^{32}P counting rate and that of the known amount of $(NH_4)_2HPO_4$ which had been simultaneously irradiated and further processed as a paired standard.

Analytical Parameters. The fractional recovery of nucleotide phosphorus from the overall method was determined as follows. DNA samples of 3.2–3.8 mg were irradiated for 5 min and the radioactivity of the produced $[^{32}P]$DNA was large enough to allow direct measurement of DNA phosphorus in an nondestructive manner after 15-days decay, when the contribution of contaminating radionuclides was negligible. The $[^{32}P]$DNA was then mineralized and the $[^{32}P]PO_4^{3-}$ isolated on acid aluminum oxide from the digested material was counted. The ratio between the amount of phosphorus found as $[^{32}P]PO_4^{3-}$ at the end of the radiochemical separation and that detected as $[^{32}P]$DNA soon after irradiation corresponded to the effective yield of the nucleotide phosphorus in the overall neutron activation analysis procedure. As shown in Table II, the mean per cent recovery of DNA phosphorus throughout the stepwise procedure was 96.6 with a standard deviation of 3.7. The single measurements were well reproducible and might be routinely corrected for the 3.4 negative bias noted for the average per cent yield.

The method was tested for accuracy (deviation of ^{32}P measured from the true value) and precision (reproducibility of repeated determinations) at low levels of nucleotide phosphorus.

An aqueous sample of calf thymus DNA was dialyzed to equilibrium against 10 mM Tris-acetate (pH 8.0) and its concentration was determined by combining the standardized mineralization of nucleic acid with a colorimetric analysis for phosphate (Morrison, 1964).

From 6 independent measurements, an average concentration of 4.70 ± 0.08 mM DNA phosphate was found and the mother solution was used to prepare 4 reference specimens containing exactly 1 μg of DNA phosphorus/ml. A series of DNA dilutions was derived from each reference solution and aliquots corresponding to a range from 10^{-1} to 10^{-4} μg of total phosphorus were withdrawn for neutron activation analysis.

The results are shown in Table III. For all the orders of magnitude under investigation, the average amount of estimated DNA phosphorus approached very well the quantity actually present, the difference between such values being usually smaller than the standard deviation of the measurements. The findings prove that the method was without systematic errors and fully accurate for sample as small as 10^{-4} μg of nucleotide phosphorus.

TABLE III: Accuracy and Precision of Neutron Activation Analysis for Nucleotide Phosphorus at Decreasing Concentration of DNA.

DNA Phosphorus (μg)[d]	No. of Determinations	$\bar{X}$[a]	σ[b]	σ_m[c]
1	4	1.004	0.036	0.018
10^{-1}	4	0.998×10^{-1}	0.058×10^{-1}	0.004×10^{-1}
10^{-2}	4	0.968×10^{-2}	0.021×10^{-2}	0.011×10^{-2}
10^{-3}	4	1.005×10^{-3}	0.070×10^{-3}	0.035×10^{-3}
10^{-4}	4	0.969×10^{-4}	0.080×10^{-4}	0.040×10^{-4}

[a] $\bar{X} = \Sigma X_i/n$ = mean value. [b] $\sigma = (\Sigma(\bar{X} - X_i)^2/(n-1))^{1/2}$ = standard deviation. [c] $\sigma_m = \sigma/(n)^{1/2}$ = standard error. [d] Theoretical value.

Discussion

The described micromethod for nucleotide phosphorus retains the reliability and the great sensitivity inherent to neutron activation analysis, and involves a minimum of chemical manipulations. The mineralization of DNA was required for changing all the possible ^{32}P species, produced by the irradiation, into a single form, namely $[^{32}P]PO_4^{3-}$, which could be readily separated from other radioactivable metals with the aid of an inorganic exchanger. These steps may also be avoided when the level of interfering radionuclides generated by neutron activation is insignificant as in the case of carefully prepared samples containing more than 100 μg of total phosphorus and irradiated for 1 or 2 min.

The presence of chlorine and sulfur in materials subjected to neutron activation analysis for phosphorus must be prevented since both elements yield ^{32}P by neutron capture of ^{35}Cl and ^{32}S, with simultaneous emission of an α particle and a proton, respectively

$$^{35}Cl + n \dashrightarrow {}^{32}P + \alpha$$

$$^{32}S + n \dashrightarrow {}^{32}P + p$$

These reactions occur only with high-energy neutrons and can be minimized by exposing the specimens to a well thermalized neutron flux. In the reactor positions used for irradiation, as much as 12×10^4 μg of chlorine or 7×10^3 μg of sulfur was needed to obtain the radioactivity induced in 1 μg of phosphorus (Bartolini, 1969). The given instructions for eliminating chlorine and sulfur contaminants from nucleic acids were fully adequate. However additional provisions may be necessary when salts and buffers containing Cl and S had been used in large quantities.

The procedure can be easily adopted by biologists albeit it requires neutron activation of phosphorus in a nuclear reactor, all other operations being performable in an ordinary laboratory. Moreover, the measurements of ^{32}P by counting its Cerenkov effect takes advantage of a commercial liquid scintillation spectrometer. Neutron activation analysis of phosphorus is at least 200 and 10,000 times more sensitive than the photometric assay of inorganic and organic phosphate, respectively (Morrison, 1964). It has been employed here measuring "cold" nucleic acids with the same resolution of the radioisotope tracer techniques which are accessible only to biological compounds labeled by incorporation of radioactive chemical precursors. The method is so sensitive that it may be properly coupled with a phosphomonoesterase reaction for measuring specifically total and terminal phosphates in polynucleotides, thus providing an end-group analysis for determination of chain length and molecular weight of nucleic acids. Such an application is the matter of a paper presently under preparation, and it represents the extention of an experimental approach first proposed for oligonucleotides (Rushizky and Miller, 1967).

A noteworthy outcome of the technicalities developed in this research is the establishment of a dependable procedure for mineralizing as low aliquots of nucleotide phosphorus as those detectable by the chemical tests for free phosphate.

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Isoaccepting Transfer Ribonucleic Acids in Specialized Mammalian Tissues*

B. J. Ortwerth

ABSTRACT: The transfer ribonucleic acids of two specialized mammalian tissues, lens and muscle, were isolated and compared by cochromatography on reversed-phase Freon columns. A comparison of each isoaccepting tRNA species showed several significant differences between these two tissues. Quantitative differences of two- to threefold were seen in the amount of at least one isoaccepting tRNA species with aspartyl-, isoleucyl-, leucyl-, and lysyl-tRNA. Alanyl-tRNA from muscle and methionyl- and tyrosyl-tRNA from lens contained one species that was almost completely absent in the other tissue. Artifacts due to isolation, aminoacylation, chromatographic conditions, and ribonuclease activity were eliminated. These data suggest that specialized mammalian tissues have unique populations of tRNA which may function to promote the rapid synthesis of the proteins peculiar to that tissue.

Several different hypotheses have been advanced which propose that protein synthesis could be controlled at the translational level by tRNA (Sueoka and Kano-Sueoka, 1970). Supportive evidence is usually obtained by comparing the tRNA of two organisms or tissues to determine what significant differences exist. These can be either differences in the amount of one isoaccepting tRNA species or differences in the number of isoaccepting species present. In mammalian tissues differences of these types have been reported when comparisons were made between erythrocytes and reticulocytes (Lee and Ingram, 1967), normal and leukemic lymphocytes (Gallo and Pestka, 1970), normal liver and hepatoma (Baliga *et al.*, 1969), normal and hormone-treated cells (Busby and Hele, 1970; Jackson *et al.*, 1970), normal and regenerating liver (Agarwal *et al.*, 1970), and different lines of cells in tissue culture (Taylor *et al.*, 1968). Comparisons have also been made between various normal tissues and also between tissues from different species (Taylor *et al.*, 1967; Holland *et al.*, 1967). In most instances, little or no differences were seen, however, a recent report by Hatfield and Portugal (1970) has shown that major differences do exist between the seryl-tRNAs of brain and liver tissues. We would like to report here the results of a study of the tRNA populations of two specialized mammalian tissues, lens and muscle. These tissues were selected because they have different specialized protein-synthetic functions and different embryological origins.

Materials and Methods

Isolation of tRNA from Lens Tissue. Frozen bovine lenses (500 g) were homogenized in a high-speed Waring blender with 3 vol of an extraction buffer containing 0.15 M KCl–0.001 M Tris-HCl buffer (pH 7.5)–0.01 M $MgCl_2$–0.001 M Na_2EDTA–0.02% polyvinyl sulfate. This homogenate was stirred with an equal volume of water-saturated phenol at 45° for 1 hr. This mixture was centrifuged at 40,000*g*, for 15 min, and the aqueous layer was removed. The nucleic acids were precipitated from the aqueous layer by the addition of 2.5 vol of 95% ethanol. After storing overnight at −15° the RNA was collected by centrifugation and dissolved in a solution containing 0.05 M NaCl–0.01 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.001 M Na_2EDTA. This sample was applied to a 2.0 × 40 cm DEAE-cellulose column which had been previously equilibrated with the sample buffer. The column was washed with 250 ml of sample buffer, followed by 250 ml of sample buffer containing 0.30 M NaCl. The tRNA was then eluted with sample buffer containing 0.70 M NaCl. The peak of 260-nm-absorbing material was pooled, and 2.5 vol of ethanol was added. The precipitated tRNA was collected by centrifugation, dissolved in water to a concentration of 40 A_{260} units/ml, and stored at −15°.

Isolation of Aminoacyl-tRNA Synthetases from Lens Tissue. Bovine eyes were obtained from the local slaughterhouse soon after the death of the animal and transported to the laboratory in an ice bucket. The lenses were rapidly removed and the nucleus of each lens was removed by punching out the center with a no. 8 cork borer. The remainder of the lens was homogenized with a Teflon in glass homogenizer in a solution containing 0.03 M KCl–0.01 M Tris-HCl buffer (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–0.001 M Na_2EDTA (1.0 ml of buffer/g of lens tissue). This homogenate was centrifuged at 30,000*g* for 20 min followed by another centrifugation at 160,000*g* for 75 min. Glycerol was added to the resulting supernatant to make a 10% solution (v/v). This mixture was applied to a 2.5 × 80 cm reverse-flow Sephadex G-75 column which had been previously equilibrated with 0.01 M KCl–0.05 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–10% glycerol. The column was eluted with equilibrating buffer and the absorbance

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