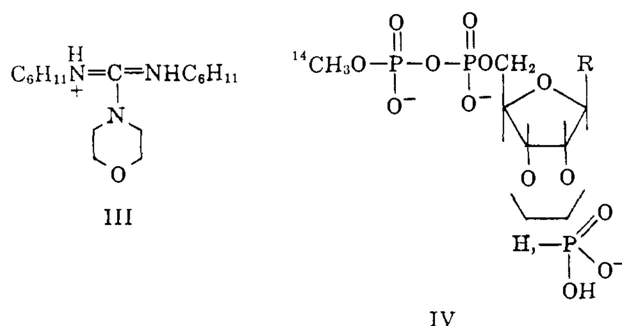

ture with water, removal of the resin and extraction with ether, the labelled nucleic acids were isolated by chromatography on a DEAE-cellulose column using a linear gradient of sodium chloride in 0.1 *M* tris-hydrochloride buffer. The labelled amino acid acceptor ribonucleic acid was eluted, as expected, at 0.4-0.45 molar salt concentration, the recovery after alcohol precipitation being about 80% of the material applied to the column. The specific activity of the product obtained in different labelling experiments was 30-50 c.p.m./optical density unit (260 mμ).

The labelled product (300 optical density units at 260 mμ) was hydrolyzed in 1 *N* potassium hydroxide for 25 hr. at room temperature and the hydrolysate after neutralization with sulfonic acid ion exchange resin was chromatographed for 3 days on a 25-cm. wide strip of Whatman paper 1 in isopropyl alcohol–ammonia–water (7–1–2) solvent mixture. Scanning of a portion of the strip in a radioactivity scanner showed that 80-85% of the total radioactivity was in a nucleotide travelling just ahead of guanosine-2′(or 3′),5′-diphosphate. The remainder of the radioactivity was present in a band travelling a little faster than the major radioactive band. After further purification by paper electrophoresis in pH 5 ammonium acetate buffer, the major radioactive product was identified as IV (R = guanine). The characterization was accomplished by absorption spectrum, specific activity, degradation with crude snake venom to C^{14}-methyl phosphate and guanosine-2′(3′),5′-diphosphate and, finally, degradation with bacterial alkaline phosphomonoesterase to a compound presumed to be P^1-guanosine-5′,P^2-C^{14}-methyl pyrophosphate. Although an insufficient amount of the minor radioactive product has been available, this has been tentatively identified, by similar techniques, as the adenine analog of IV. This result is in agreement with that reported previously[1] that a few amino acid acceptor ribonucleic acid chains end in adenosine-5 phosphate groups.

The extent of the reaction with the end groups has been in the range of 50% in the experiments carried out so far. The principles for subsequent degradations so as to recognize the sequences near the labelled terminus have been outlined previously.[1]

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Neutron Activation as a Method for Labelling the Phosphoros of Nucleotides

A CORRECTION

It was reported recently that neutron activation could be used to label the phosphorus of nucleotides,[1] the production of phosphorus-32 being proportional to the phosphorus content of the samples. There was no gross decomposition which could be detected by physical or biological means. For instance, 5′-adenosine triphosphate (ATP) irradiated in the water-cooled compartment of a graphite reactor at a neutron flux of 6.5×10^{11} neutrons/cm.^{2}/sec. for 62 hours, retained undiminished ability to induce luminescence in the luciferin-luciferase assay, which specifically requires the triphosphate.[2] The infrared and ultraviolet spectra of all samples were unaltered by the activation procedure.

While all the above findings could be duplicated, it was found on repetition of these experiments that there is extensive decomposition localized exclusively in the radioactive molecules. This decomposition, which is due presumably to recoil reactions, could be detected by the non-correspondence of ultraviolet absorbing spots and radioactivity after paper chromatography or paper electrophoresis. Since the "cold" molecules, making up the bulk of the irradiated nucleotides, were intact no gross decomposition could be found.

Repetitions of the chromatographic procedure coupled with strip counting reported in the original communication,[1] showed that only a small fraction of the radioactive phosphorus is associated with authentic nucleotides. Radioautography provided a more convenient method for obtaining the same results.

Purification of activated 5′-adenosine monophosphate by the successive use of Dowex formate[3] and Ecteola cellulose columns[4] and further analysis of the fractions containing radioactive ultraviolet absorbing materials indicated that not more than 1% of the phosphorus-32 was attached to adenosine, while there was no gross decomposition of the nucleotide.

The chromatography of "activated" ATP with or without treatment with hexokinase and glucose, followed by radioautography, showed that the distribution of radioactivity was not affected by this reaction, although ADP and glucose-6-phosphate were formed normally.[5]

It seems, therefore, that while neutron activation can label the phosphorus of nucleotides, it is not a useful method for labelling nucleotides.

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(1) H. G. Mautner, B. Donnelly, C. M. Lee and G. W. Leddicotte, *J. Am. Chem. Soc.*, **84**, 2021 (1962).

(2) B. L. Strehler and J. R. Totter in D. Glick, "Methods of Biochemical Analysis," Vol. I, Interscience Publishers, New York, N. Y., 1954, p. 345.

(3) G. R. Bartlett, *J. Biol. Chem.*, **234**, 459 (1959).

(4) G. M. Tener, H. G. Khorana, R. Markham and E. H. Pol, *J. Am. Chem. Soc.*, **80**, 6223 (1958).

(5) B. C. Pressman, C. Stroebel and P. D. Boyer "in Biochemical Preparations," Vol. 7, John Wiley & Sons, New York, N. Y., 1960, p. 18.

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