Previous work by other investigators and our work would seem to indicate that nuclei, in general, contain the following:

1. Nucleohistones or nucleoprotamines extractable with water or NaCl solutions.

The mammalian spermatozoa so far studied differ from other nucleated cells in containing no substances soluble in water or NaCl. These substances may be present in an altered form with different solubility characteristics.

2. Proteins extractable with alkali and precipitating when the solution is made acid.

The pH 6 fraction from thymus nuclei (Mayer and Gulick) and the pH 6 and 4.5 fractions from boar spermatozoa are protein fractions of this nature. This type of protein can be included only provisionally as a constituent of nuclei in general.

3. A highly insoluble residual material containing proteins and nucleic acids.

Green's residue from ram spermatozoa, the tryptophane-containing protein of Mirsky and Pollister, the "residual chromosome" of Mirsky and Ris, the "chromosomin" of Stedman and Stedman, and our residual material from boar spermatozoa all seem to belong in this category. They are all highly insoluble and require drastic treatment to bring even a part into solution.

A highly significant characteristic of the residue obtained by Green from ram spermatozoa and of our residue from boar spermatozoa is the high arginine content. The absence of lysine from these residues is also of interest.

Davidson and Lawrie recently reported (*1*) the results of amino acid analysis by paper chromatography of histone and residual material from calf thymus, rat liver, and fowl erythrocyte nuclei. These authors noted the absence of lysine in the residual material from all three of these sources. The quantity of arginine present was not reported.

The presence of more than one type of protein in each of these insoluble residues must be considered. The proteins with high arginine content in some of these residues may be, as suggested, altered forms of nucleohistones. On the other hand, the proteins of high arginine content in these residues may represent an entirely different type of protein.

References

1. Davidson, J. N. and Lawrie, R. W. *Biochem. J.*, 1948, **43**, XXIX.
2. Green, W. W. *Anat. Rec.*, 1940, **76**, 455.
3. Mathews, A. *Z. physiol. Chem.*, 1897, **23**, 399.
4. Mayer, Dennis T. and Gulick, Addison. *J. biol. Chem.*, 1942, **146**, 433.
5. Mirsky, A. E. and Pollister, A. W. *Proc. nat. Acad. Sci., Wash.*, 1942, **28**, 344.
6. ———. *J. gen. Physiol.*, 1946, **30**, 117.
7. Mirsky, A. E. and Ris, Hans. *J. gen. Physiol.*, 1947, **31**, 7.
8. Schmiedeberg, O. (Ed.). *Die histochemischen und physiologischen arbeiten von Friedrich Miescher.* Leipzig: von F. C. W. Vogel, 1897.
9. Stedman, E. and Stedman, E. *Nature*, Lond., 1943, **152**, 267.
10. ———. *Sympos. quant. Biol.*, 1947, **12**, 224.
11. ———. *Sympos. Soc. exp. Biol.*, 1947, **1**, 232.

A Possible Standard for Radioiodine

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Recently a radioactive thallium isotope has been suggested as a standard for comparison with I^{131} (*1*). It seems likely, however, that Cl^{36} will be more satisfactory for the purpose. Its maximum β-energy, 0.66 mev, differs very little from the corresponding value for I^{131}, 0.60 mev. Radioiodine has a second limit of minor importance at about half this value (*3*, *4*).

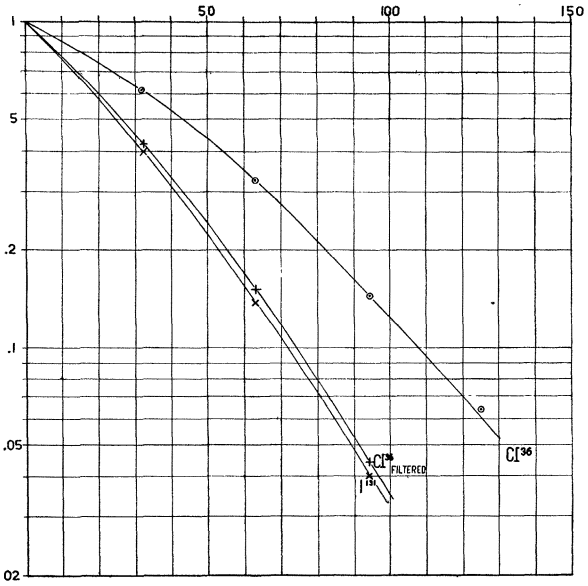

Fig. 1. Absorption of the beta rays of Cl^{36} and I^{131} in aluminum. The curve +++ indicates beta particles already filtered through 125 mg/cm². It is the continuation of the top curve.

The difference between the β energies is, however, sufficient to make the absorption curves quite dissimilar (Fig. 1).[1] As both lines curve downward in the normal way, it is possible to select two parts, one for each line, which have the same direction. Thus the absorption of Cl^{36} β particles, which have passed through 125 mg/cm² of aluminum, coincides almost exactly with the absorption of the unfiltered β radiation of I^{131}.[2] This suggests the use of a preparation containing Cl^{36} covered with 125 mg/cm² of aluminum as a standard for I^{131}. As only weak gamma rays are emitted by Cl^{36} (*2*) there is no ob-

[1] The absorption of the β radiation of Cl^{36} was determined in connection with measurements performed for Mr. C. B. Heyn. The Cl^{36} had been allotted to him for physiological investigations by the Atomic Energy Commission. The radioiodine had been furnished by the Isotope Branch of the Atomic Energy Research Establishment, Harwell.

[2] Radiochlorine had been purified from radiophosphorus and radiosulfur. Further purification caused no change in the absorption curve. The absorption of radioiodine beta rays was checked by comparison with a second preparation, obtained a few weeks earlier.

jection to the reduction of the intensity caused by this filtering.

A Cl^{36} sample, prepared as described, was compared with a radioiodine sample, using different counters which happened to be readily available in our institute. The results are seen in Table 1.

TABLE 1

Counter type	Window thickness mg/cm²	Ratio of cpm with standard and with radioiodine
Tracerlab	2.73 (mica)	1.08
Beta counter	1.5 (mica)	1.06
X ray counter	4.3 (mica)	1.06
Philips Eindhoven	about 80 (steel tube)	1.41

It is seen that the agreement with mica window counters is excellent, and even with a steel tube counter, a type of instrument which will never be chosen for standardizing radioiodine, the agreement is not worse than the average result obtained in different laboratories (*1*). This means that, with our standard, radioiodine samples can be counted under different counting arrangements without corrections.

The standard should not be used at a distance much less than half the diameter of the counter's sensitive surface. Results are best between this distance and twice its value; if larger variations are allowed results tend to be less satisfactory and deviations of about 25% may occur.

References

1. FEITELBERG, SERGEI. *Science,* 1949, **109,** 456.
2. JOHNSTON, F. and WILLARD, J. E. *Phys. Rev.,* 1949, **75,** 528.
3. METZGER, F. and DEUTSCH, M. *Phys. Rev.,* 1948, **74,** 1640.
4. ZAFFARANO, D. J., MITCHELL, A. C. G., and KERN, B. D. *Phys. Rev.,* 1949, **75,** 1632.

The Inhibition of the Cholinesterase Activity of Human Blood Plasma by Neutral Phosphate Esters. II: Studies with Hexa 1-C^{14}-Ethyl Tetrapolyphosphate[1]

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Compounds containing the grouping P-O-R (R = alkyl or aryl) are potent inhibitors of human plasma cholinesterase if they also include a sterically strained configuration around the phosphorus atom (as in tri *o*-cresyl phosphate), or if that atom participates in an acid anhydride linkage (as in the case of the tetraalykl pryophosphates) (*2*). The reaction between enzyme and inhibitor results in the rapid inactivation not only of the enzyme, but also of the inhibitor. Inactivation of the inhibitor is observed only on reaction with active esterase, and inactivation of the enzyme does not take place if the pyrophosphate linkages of compounds like tetraethyl pyrophosphate have been hydrolyzed by even brief contact of the inhibitor with water. The inhibition of plasma cholinesterase activity by such compounds is not reversed on removal of the inhibitor by hydrolysis and prolonged dialysis.

These observations strongly suggest that the interaction of plasma cholinesterase with such phosphorus compounds results in the formation of rather stable compounds involving the active groupings of the enzyme.

TABLE 1

Specific activity of HETP	7.02×10^2 cpm/mM
Activity added as HETP to all preparations	5.74×10^3 cpm/mg protein
Activity recovered in protein precipitated after dialysis:	
From active enzyme + intact HETP	8.05 cpm/mg protein
From active enzyme + hydrolyzed HETP	1.43 cpm/mg protein
From denatured enzyme + intact HETP	1.03 cpm/mg protein

In order to further test this hypothesis, hexaethyl tetrapolyphosphate (HETP) containing P^{32} or C^{14} as tracer atom has been prepared. An earlier test employing P^{32} tagged material (*2*) merely demonstrated that less than 1.0% of the HETP added was fixed on the enzyme, suggesting that the anticholinesterase activity of this material might reside in a minor component obtained in the course of preparation; possibly this component could be tetraethyl pyrophosphate (TEPP), the most active member of this group detected so far. The purpose of the present note is to report results obtained under more favorable conditions involving the use of C^{14}-labeled HETP.

The preparation of the inhibitor from P_2O_5 and $P(O)(OC_2H_5)_3$ was carried out as described previously (*2*), except that C^{14}-containing triethyl phosphate, prepared by reacting $CH_3C^{14}H_2I$ with silver phosphate, was employed.[2] The preparation obtained had a specific activity of 3 mc/mM of hexaethyl tetraphosphate. The human plasma esterase preparation employed[3] had a cholinesterase activity of 5.24 M CO_2 evolved/hr/g under standard conditions (0.8 M acetyl choline bromide) (*1*); this preparation is approximately ten times as pure as the fraction IV-6-3 previously employed.

The plan of the experiments, based upon these methods may be seen from Table 1. The following are additional details of preparation: a slight excess of inhibitor, or its equivalent, was added to the enzyme; HETP was hydrolyzed by contact with water (1% solution) for 72

[1] Supported by a grant from the Division of Research Grants and Fellowships, U. S. Public Health Service.

[2] This preparation was carried out for this laboratory by Tracerlab, Inc., Boston, Massachusetts.

[3] This material was made available through the kindness of Dr. Surgenor, Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts.