extraction now gives the same number of counts as did the precipitate.

The error which appears as a result of differences between the counts in the *iso*butanol and in the precipitate is reflected in the specific activities, as indicated in the accompanying table.

The question now arises as to the nature of the contaminant, and preliminary experiments indicate that the adenosine polyphosphates are co-precipitated as, in fact, has been noted by Lehninger[2]. It has also been established that the degree of co-precipitation is dependent upon the amount of carrier phosphorus which has been added.

The preliminary results indicate that the long-accepted method for determination of the radioactivity of inorganic phosphate is subject to considerable error and, therefore, that the conclusions that have been drawn in the past on the turnover rate, etc., of an organophosphate are also subject to error. Experiments are now in progress to determine the magnitude of this error. It has also been established that the method described by Ennor and Stocken[1] is suitable for the determination of both phosphorus-31 and -32, and that it is free from error. A full report of the work will be submitted for publication elsewhere.

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May 18.

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A Particulate Impurity Found in Solutions of Radioactive Phosphorus

Experiments involving the use of radioactive phosphorus (phosphorus-32) incorporated into nucleoproteins have indicated that samples of nominally carrier-free radioactive orthophosphate contained, at various times during 1950, an appreciable proportion of activity associated with particulate matter. Recent samples of phosphorus-32 have, however, been free from this impurity. This impurity is of serious consequence when measurements of the radioactivity of proteins labelled with phosphorus-32 are to be made, as the impurity is carried down on protein precipitates, where it remains in spite of repeated washing.

The nature of this impurity has been investigated and a method for removing it from solutions of radioactive phosphorus has been devised. The uptake of the impurity by protein (crystalline horse serum albumin) precipitated from a carrier-containing solution by trichloracetic acid was unaffected by the amount of protein used or the amount of carrier present over a wide range. The protein precipitate (see table) was separated by centrifugation from the supernatant fluid, which was then neutralized, more protein was added and the precipitation repeated. The second precipitate was found to contain less than one-thousandth of the radioactivity found in the first, whereas both precipitates contained essentially the same amount of phosphorus. The degree of contamination of the first precipitate by carrier-free phosphorus-32 is indicated by the ratio of its specific activity to that of the supernatant fluid (final column of table), which in the absence of impurity cannot be greater than 1. This experiment has been repeated using a tissue homogenate instead of horse serum albumin, with similar results. A high activity persisted in the first precipitate after ten washings in phosphate-containing trichloracetic acid, whereas the second precipitate gave only background counts after six washings.

The impurity extracted has the same decay-rate as phosphorus-32 and was thought to be radioactive orthophosphate adsorbed on to, or combined with, some extraneous particulate matter suspended in the carrier-free solution. An attempt was made to remove the impurity by filtration of carrier-containing material through a 0·25-μ 'Gradocol' membrane. This proved as effective as protein precipitation in removing the impurity, only background counts being obtained on a washed precipitate from filtered material, an aliquot of which before filtration had given a highly active washed precipitate. The impurity may also be removed by extracting the orthophosphate in *iso*butyl alcohol as a phosphomolybdate complex, when the impurity remains in the aqueous phase; after alkaline hydrolysis the impurity behaves like orthophosphate and is found in the alcoholic phase.

In experiments involving nucleoproteins in which nucleic acid turnover is measured by means of phosphorus-32, Schmidt and Thannhauser's method of fractionating the nucleic acids is frequently used. In this fractionation the radioactivity due to the impurity is removed from the protein during alkaline hydrolysis and appears as radioactive orthophosphate in the ribose nucleotides fraction, where it will give rise to gross errors. If reliable results are to be obtained from experiments involving proteins labelled with phosphorus-32, it is essential to ensure that the tracer solution is free from this impurity. Filtration through a 0·25-μ 'Gradocol' membrane after addition of carrier provides a reliable and simple method of doing this, while at the same time sterilizing the solution.

	Total counts per min.	Percentage activity in precipitate	Phosphorus (μgm.)	Specific activity (counts per min. per μgm. phosphorus)	Ratio of spec. activities of precipitate to supernatant
Supernatant 1	195,000	—	858	228	—
Supernatant 2	198,000	—	900	222	—
Precipitate 1	10,800	5·5	3·9	2,700	11·8
Precipitate 2	7·8	0·004	4·5	1·74	0·008

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May 9.