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A Source of Error in Tracer Experiments with P^{32} [1]

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In dealing with radioactive solutions of high specific activity where total phosphorus is small, the amount of P^{32} adsorbed on laboratory glassware may become significant.

An experiment done in this laboratory to measure this adsorption and to determine the efficacy of silicone-coated glassware illustrates the magnitude of the problem.

A solution of P^{32} containing about 1×10^{-4} mg /ml of total phosphorus was drawn up once into each of four 2-ml pipettes, all new and identical except that two had been coated with silicone. Each of the pipettes was then washed by drawing up 2-ml amounts of distilled water. The wash water from each pipette was collected into two counting sample cups (1 ml each), evaporated, and the number of radioactive disintegrations determined in a Nucleometer.

Average values, in cpm/ml were:

	Coated	Uncoated
Wash 1	390	6,000
Wash 2	110	300
Wash 3	background	110

The count in the first wash of the uncoated pipette is high and variable, depending on both the P^{32} and P^{31} contents, and can cause serious error where it is not suspected.

To avoid this difficulty, we have adopted several techniques. Wherever possible all counting solutions contain a uniform 1 mg/ml of carrier phosphorus at pH 4.5. Where less carrier must be used, care is taken to pipette as rarely as possible and only in solutions containing as much carrier as the procedure permits. When conditions are extremely unfavorable, requiring exceedingly low carrier or high pH values, silicone coating of glassware is necessary.

The coating technique we used was modified from Johannson and Torek (*1*). A 2% solution of "DC 200" was prepared in pure grade CCl_4 (the Dow Company recommends chlorinated organic solvents). The glassware, preferably new, is scrupulously cleaned and heat-dried, completely immersed in the silicone solution, and carefully drained. It is then placed into an oven at 90° C for 2 hr, to evaporate the solvent. The temperature is then raised to 250° C for a period exceeding 4 hr but not longer than overnight. Detailed experiments on the lasting qualities of this type of coating have been done by the Dow Company. This treatment caused no detectable change in calibration.

There is another group of compounds which have been similarly used, called "silanes" (hydrochlorides of unpolymerized silicones). These may be vaporized onto a glass surface, where they react with the film of moisture to form a silicone layer. This process of hydrolysis and polymerization is somewhat difficult to control.

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Free Amino Acids and Peptides in Frog Embryos

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Relatively large quantities of free amino acids have been detected by two-dimensional paper chromatography in tissues of the mouse (*3, 4*), the human, and the frog (*Rana pipiens*). Each of the normal adult tissues examined appeared to have a distribution of free amino acids characteristic for that tissue. Therefore, it was apparent that the ability of these tissues to maintain the characteristic distributions of these constituents must have arisen at some time during embryonic or postembryonic development.

As the first step in the study of the free amino acids of frog tissues at various stages of development, examinations were made of alcohol extracts of material ranging from ovarian eggs to larvae, corresponding to stages 0, 1, 2, 8, 10, 12, 17, and 21 according to Shumway (*5*). In the last stage examined, there was still no intake of food from the environment. Exactly 260 mg of fresh weight of material was placed in ethyl alcohol, the final concentration of alcohol being 70–73%. The material was homogenized in a ground glass homogenizer, centrifuged, and the supernatant fluid decanted and evaporated to dryness. The residue was thoroughly extracted with 3 ml of distilled water. This was then dialyzed against 10 ml of distilled water in a rocking dialyzer (*2*). One half of the dialyzate was evaporated to dryness and placed on paper for chromatography (*1*), and the other half was chromatographed after hydrolysis with 6 N HCl.

In none of the stages studied were there detectable quantities of free amino acids in the extracts. Several ninhydrin-reactive materials were located at spots which disappeared on acid hydrolysis, and which did not correspond in position to any known amino acids. These were