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Use of I^{125}-Labeling in Radioimmunoassays.* (31027)

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Since the classical work of Yalow and Berson(1), radioimmunoassays for insulin and human growth hormone (HGH) have become almost routine procedures in many laboratories. Iodination of these protein hormones with NaI^{131} by the method of Greenwood, Hunter and Glover(2) has yielded labeled hormones of high specific activity with minimal loss in immunologic competence. The use of I^{131} with a half-life of 8 days has the disadvantage of necessitating frequent iodinations of these hormones to provide an adequate number of disintegrations per minute for their use in the chromatoelectrophoretic method of radioimmunoassay. To obviate this problem, an isotope of longer half-life is desirable. Therefore, I^{125} with a half-life of 56 days has been extensively evaluated in our laboratory and is now being used exclusively for radioimmunoassays using the double antibody technique(3,4). While specific activities of I^{125}-labeled hormones must be less than I^{131}-labeled hormones to prevent damage to these proteins(5), preparations with specific activities of 50-100 millicuries per milligram have proven to be very acceptable. When

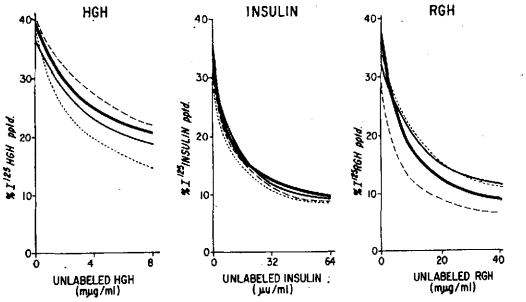

FIG. 1. Radioimmunoassay standard curves using I^{125}-labeled hormones. The stability of I^{125}-labeled hormones is demonstrated by the similarity of individual standard curves using the same iodinated preparations over a 4-month period in the HGH, insulin, and RGH radioimmunoassays. ——— 1st month, ——— 2nd month, — — — 3rd month, -------- 4th month.

frozen in aliquots containing 30% bovine serum albumin in 0.07 *M* barbital buffer (pH 8.6), I^{125}-HGH, I^{125}-insulin, and I^{125}-rat growth hormone (RGH) have been found to be stable over a period of 4 months (Fig. 1). In the double antibody radioimmunoassay technique, the total radioactivity in each assay tube as well as the bound fraction is counted. For this method, therefore, labeled hormones of lower specific activity are quite satisfactory, in contrast to the needs of the

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chromatoelectrophoretic method in which only an aliquot of the radioactivity in each tube is counted.

Summary. I^{125}-labeled hormones have been satisfactorily used in the double antibody radioimmunoassay technique for insulin, human growth hormone and rat growth hormone. The longer half-life isotope offers the important advantage of decreasing the need for frequent iodinations of these hormones as is necessary with I^{131}.

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Calcium and Potassium Uptake from Sodium Free Media by Frog Stomach Muscle.* (31028)

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When longitudinal strips of frog stomach muscle are immersed overnight in phosphate Ringer solutions containing 6 mEq potassium per liter and in which sodium is completely replaced by lithium, they lose the greater part of their potassium and virtually all their sodium. The sodium and potassium ions lost from the fibers are replaced by lithium. On reimmersion, at room temperature, in Ringer solutions containing 106 mEq sodium and 10 mEq potassium per liter the muscles readily accumulate potassium ions, but, when sodium is replaced by lithium in the reimmersion fluid no net uptake of potassium occurs. Partial replacement of lithium by sodium in the reimmersion fluid results in net potassium uptake in amounts roughly proportional to the external sodium concentration(1). Thus it appears that sodium plays an essential role in the uptake of potassium by this tissue.

These results are compatible with a direct stimulatory effect of external sodium on potassium uptake by frog stomach muscle(1,2). However, they do not exclude the possibility that the effects observed are due to other causes. One alternative explanation is suggested by the work of Judah and his associates(3,4). These workers found that rat liver slices immersed in low sodium media take up calcium. This uptake of calcium has marked effects on cellular metabolism, the turnover of ATP and of phosphoprotein being greatly reduced. These effects are readily reversed by sodium. Somewhat earlier, Cosmos and Harris showed that, in frog skeletal muscle exposed to low sodium media, there is an enhanced uptake of calcium by the fibers(5).

On the basis of Judah's observations, the failure of isolated strips of frog stomach muscle to accumulate potassium in the absence of sodium ions(1) might be explained as follows. Since all the immersion fluids contained the normal amount of calcium (1.8 mEq per liter) it is possible that, during overnight soaking in sodium free Ringer solutions, the muscles so treated took up relatively large amounts of calcium. During reimmersion in sodium free media the internal calcium concentration would be expected to remain high, with a consequent disruption of ATP production and

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