curve of incubation medium was paralleled with the standard curve. Repeatability of immunoassayable ACTH in withinassay was 174±5.0 pg/tube (CV=2.9%).

A log dose-response relationship was observed between the amounts of stalk median eminence extracts (SME: NIAMDD) added to the incubation medium and its ACTH releasing activities. The sensitivity of this assay method was at least 0.1 SME of 10 mU of LVP and AVP.

Using this method, it was found that LVP, AVP, norepinephrine (100 ng/ml–200 ng/ml) and 5-hydroxytryptophane (1 μg/ml) had ACTH releasing activities but LH-RH, TRH, glucagon, dopamine, phentolamine propranolol, haloperidol, prostaglandin E_1 and indomethacin did not increase the release of ACTH.

Studies on a Radioimmunoprecipitation Method for the Determination of Insulin Antibody and the Binding Capacity of ^{125}Insulin to Insulin Antibody

N. MIZUNO, K. OKADA and S. BABA

The Second Department of Internal Medicine Kobe University School of Medicine

We have studied on the several basic investigation of a radioimmunoprecipitation method of insulin antibody in human serum. The most suitable condition in this method was obtained follows: 0.10 ml of ^{125}I-labeled insulin (0.02 ng/ml) was added to 0.05 ml of the 16-fold diluted human serum. This mixture was incubated for 72 hrs. at 4°C and to this, 0.20 ml of the 4-fold diluted anti-human IgG was added. Then, after the additional 48 hrs. incubation at 4°C, the total radioactivity (cpm) of the whole mixture was measured. Subsequently, the mixture was centrifuged at 3,000 rpm for 30 min, the supernatant was decanted and the precipitate was washed three times with phosphate-buffered saline, pH 7.5 to serve for the maeasurement of pure radioactivity in the precipitate. This method was superior in the specificity, reproducibility and sensitivity to those of the other method (PEG method).

Furthermore, in order to investigate the binding state of ^{125}I-insulin to insulin antibody, we performed the following procedure. 0.1 ml of ^{125}I labeled insulin (0.2 ng/ml) was added to 0.05 ml of human serum, and the mixture was incubated for 72 hrs. at 4°C. Then, ^{125}I-insulin bound to the antibody was separated by gel-filtration through sephadex G-50 column (0.9×25) cm. The ^{125}I-insulin-antibody complex solution obtained was fractionated by Pevikon electrophoresis and was precipitated by anti human γ-globlin. A peculior phenomenon, in which ^{125}I-insulin was liberated from ^{125}I-insulin antibody complex during either electrophoresis or immunoprecipitation, was observed. However it is obscure that this phenomenon was caused by either some structural change in insulin due to iodination or co-existence of human antibodies with weak affinity to insulin.