the salpinx were 8.6 mrad/g of tissue, 5.3 mrad/g of tissue and 5.3 mrad/g of tissue, respectively.

3. At the present time, radiation dose to the gonads due to diagnostic uses of radioisotopes in gynecological field makes less contribution to the genetically significant dose than medical exposure of X-ray and even in the case of ^{32}P, the estimated dose is not considerably higher than the recommendatory value of the ICRP for woman to be able to conceive.

Symposium V. Radioimmunoassay

(Chairman) M. Fukase, Univ. of Kyoto

Prenciples and Problems of Radioimmunoassay

K. Shizume

Department of Endocrinology, Toranomon Hospital, Tokyo

M. Irie

Third Department of Internal Medicina, University of Tokyo, Faculty of Medicine, Tokyo

Radioimmunoassay is an ingenious method for microassay of the substances which have antigenicity. This method is based on the high degree of specificity of antigen-antibody reaction and sensitivity of the measurement of radioactive substances and now is mainly used for the assay of peptide hormones.

The general principles of radioimmunoassay are as follows. Labeled hormone binds to its specific antibody to form a labeled antigen-antibody complex. Unlabeled hormone in plasma or other solutions competes with labeled hormone for antibody and thereby inhibit the binding of labeled hormone. Consequently the ratio of antibody-bound (B) to free (F) labeled hormone, denoted B/F, is diminished as the concentration of unlabeled hormone is increased. The concentration of hormone in an unknown sample is obtained by comparing the inhibition observed with that produced by standard solutions containing known amounts of added hormone ("standard hormone"). This is done by plotting B/F versus hormone concentration in known standards and, from the curve so obtained, finding the hormone conecntration that corresponds to the B/F ratio observed in the unknown sample.

For this method the following conditions are required.

1) Hormone to be assayed is obtainable in pure form and antibody for it available.

2) It is possible to radio-label the hormone and antigenicity is not altered by labeling.

3) Standard hormone and hormone in the sample behave similarly in antigen-antibody reaction.

4) Substances other than the hormone to be assayed has no influence on the antigen-antibody reaction.

As methodological problem, it is necessary to eliminate the radioactivity of other substances such as damaged hormone and inorganic iodine, and to make the separation of B and F complete.

This method is based on the immunological property within the chemical structure of the hormone. Therefore in case when immunological property and biological property are based on different portions in the chemical structure, disagreement can exsist between the value by radioimmunoassay and bioassay.

However such disagreement are rather exceptional and in most cases the value ob-

tained by this method agrees well with clinical observations and the agreement between the values by two assays has been reported by several investigations. This method has good recovery and reproducibility. By this method it is possible to assay various kinds of hormones in the plasma using 0.1 ml of the sample without extraction, and quite a number of samples can be assayed within a rather short period. Because of these advantages this method will be used more widely in the future.

Radioimmunoassay of Insulin

K. ONOE and K. KOBASHI

Second Internal Med., School of Med., Tokushima Univ., Tokushima

1) Studies on a differential immunoassay for endogenous and exogenous insulin using bonito insulin as exogenous insulin.

We had succeeded in a differential radioimmunoassay for endogenous and exogenous insulin. This assay is based on the Yalow & Berson's and our observations that bonito and mammalian insulin react differently with antipork insulin-binding antibodies in antisera from guinea pigs. Plasma endogenous dog insulin level was measured with antipork insulin serum, which reacts poorly with bonito insulin. Exogenous bonito insulin in dog plasma was measured with anti bonito insulin serum, which reacts poorly with endogenous mammalian insulin.

Intravenous injection of bonito insulin (0.2 U/kg), which induced hypoglycemia, inhibited completely endogenous dog insulin secretion which was stimulated after i.v. administration of xylitol (0.4 g/kg), glucagon (0.05 mg/kg), glucose (0.4 g/kg) nad tolbutamide (40 mg/kg) to dogs but a pretty amount of insulin secretion by xylitol (0.4 g/kg) was observed, when 0.1 μ/kg bonito insulin was injected and that inhibition of endogenous insulin secretion by exogenous bonito insulin was eliminated by the infusion of phentolamine (6 μg/kg/min.) and insulin secretion stimulated by xylitol was again appeared.

It was therefore concluded that inhibition of endogenous insulin secretion which occured on insulin hypoglycemia, might be arised from mediation of α-receptor of epinephrine.

2) Combined immunoassay of insulin and human growth hormone.

Table 1 shows the condition of our estimation of both hormones simultaneous using ^{131}I and ^{125}I tracers.

This method was very convenient and simple when differential countings of ^{131}I and ^{125}I might be accurately estimated.

3) Solid phase radioimmunoassay of insulin in antibody-coated polystyrene tubes.

Polystyrene tubes were purchased from R. M. Jones, Australia.

The conditions of our solid phase radioimmunoassay of insulin were showed on Tables 2, 3. The method is simple and suitable for automation.

PH (4.0~8.2) for coating of antibody, pH (6.8~8.2) for incubation, and the temperature (4~20°C) for antibody coating, incubation were suitable for this assay.

Blood insulin without insulin antibodies was estimated by method A.

Method B was deviced for solid phase radioimmunoassay of plasma insulin containing insulin antibodies.

After previous addition of samples contained insulin antibody in antibody coaed tubes, the tubes was washed with 1.0 ml of buffer solution (pH 8.2). And then ^{131}I-insulin was added in this assay system.

Insulin antibodies of sample plasma would be washed out by buffer solution. Blood insulin containing insulin antibodies might be estimated by this new method B although it was not succeeded by other radioimmunoassay of insulin.