DISCUSSION

In 1947, TSH was unequivocally found in serum by Albert.[4] After that the presence of human serum TSH has been reported by many research workers, such as D'Angelo,[14] Gilliland,[19] and Adams.[2] The sensitivity of these assay techniques was in the order of 0.10 mU, 0.15 mU and 0.10 mU respectively. The method of McKenzie did not take the factor of animal variation into account; the mean precision index of the method is 0.24. The method used in our experi--ment followed that of McKenzie's[32] as modified by Good and Stenhouse.[22] Linearity of the dose-response curve was demonstrated over the range of 0.00625 to 0.80 ImU. The sensitivity and precision obtained are greater than those previously reported.

In order to obtain higher degree of sensitivity and increased precision, Good and Stenhouse[22] modified McKenzie's method[32] by the use of designed balance for residual effect to treatment, the factors of animal and day varia--tion, and, also residual effect could be removed in the statistical analysis. Only four treatments were used as the estimation of two unknown samples, although theoretically it was possible to use an unrestricted number of treat--ments. The test materials had to be injected twice on successive days but this was countered by the fact that control samples were not required. There--fore, the number of sample to be counted was not greater than that of the conventional McKenzie assay. This modified method has improvement over the original McKenzie's method by lowering the index of precision (λ) to 0.188.

In order to facilitate the steps of procedure, the following modifica--tions were adapted for our method:-

(1) Instead of using ^{131}I, the counting could be more conveniently done with ^{125}I which needs no correction for decay during the period of counting.

(2) The blood specimens were obtained by bleeding tail vein instead of retro-orbital venous sinus, which allowed more profuse bleeding and gave ade--quate amount of blood. By this way, increasing number of blood samples could be obtained.

(3) Since we have been using well counter counting each sample for 10 minutes, it becomes difficult when the number of sample is increasingly large. We have then adapted the existing equipment into autowell which takes care of the measurement automatically and continuously.

(4) The calculation was done with assistance of desk-computer, regressions calculated and graphs drawn.

For the methodology which is statistically treated in a similar fashion, our method yields the linearity of dose-response of 0.05 to 0.80 ImU (Table 4 p.24) and the precision indices (Λ) of 0.173 to 0.627 which agree favorably with those of McKenzie's and Good and Stenhouse's. In the assay of serum TSH, we do have some spurious results with precision indices greater than one, the error of which might be contributed from the use of different strains of mice. Some of the mice might be stressed from seasonal changes and too frequent bleedings. These factors were removed in subsequent studies in which the radio--active samples were subjected to long enough counting in the gamma autowell.

Similar to **in vitro** type of tests, the bioassay as we have done does not involve administration of radioisotopes into human bodies, thus obviating radiation exposure to the patients. The method is very helpful particularly in assessing the actual condition of treated patients when other methods are equivocal, as can be seen in the data of results presented in Table 7 to Table 18.

As determined from each dose-response curve, the sensitivity of this method is from 0.05 to 0.40 ImU which is quite adequate for detecting such small amount of circulating TSH in various conditions.

The normal values of TSH in serum as reported in the literatures[8,16,19,32,30] are 0.01 to 0.4 ImU/ml as seen in Table 19. Our normal values lie within a range from 0.056 to 0.328 ImU/ml. The mean value is 0.185 ImU/ml which compares favorably to those quoted elsewhere.

Table 19 - LEVEL OF TSH IN NORMAL SERUM

Assayists	Method	mean values of normal TSH ImU/ml
1. Bates, R.B. 1963	Bioassay	0.01-0.1
2. DiGeorge, D'Angelo and Paschkis 1957	Bioassay	0.40
3. Gilliland and Strudwick 1956	Bioassay	0.165
4. McKenzie 1958	Bioassay	0.2
5. Th. Lemarchand-Beraud and A. Vannotti 1965	Radioimmuno assay	0.36
6. The present series 1972	Bioassay	0.185

The values of serum TSH in hypothyroid are definitely higher than those of normal except one case showing 0.152 ImU/ml. This exceptional case shows no definite hypothyroid symptoms and the serum thyroxine is 2.5 ug%. The high value of TSH in hypothyroid conforms to the usual finding that whenever circulating thyroid hormones are low, the pituitary-thyroid axis feedback mechanism operates and the secretion of TSH from the anterior pituitary will increase. It might happen sometimes in hyperthyroid patients treated with ^{131}I that the symptoms of hypothyroid occurs later and the serum thyroxine decreases slowly, the TSH assay value might be decisive in withholding any additional treatment dose, if high TSH is found in the serum.

As the activity of the thyroid gland in hyperthyroidism is autonomous of anterior pituitary influence, the serum TSH will be present in normal or negligible amount. Our five cases give value not different from the normal.

Although the method such developed in our laboratory gained some sensitivity and precision, it is tedious and consumes much time and efforts. However in some situation whereby some cases may be problematic, it may be then very helpful to clarify the condition of patient. But if the demand increases, the method of determination of serum TSH level may very well be switched to radioimmunoassay which again may present some inherent problems of its own.

However, one of the means to save time and efforts might be that one could arrange so that the experiment could be double, triple, etc. with 2 or 3 groups of workers and mice, or alternatively a limited number of serum specimens could be estimated from a single dose-response curve done in the same assay.