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Effect of Thyrotrophin on *in vivo* Thyroid Uptake of Labelled Tyrosine, Arginine and Iodine in the Chick.* (30172)

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Injection of thyrotropic hormone into normal guinea pigs has been shown to increase the *in vitro* incorporation of labelled amino acids into thyroid protein. An *in vivo* study was reported at the same time from this laboratory showing an increased thyroid uptake of C^{14} tyrosine in TSH-treated chicks (3).

Thyrotropic hormone has been shown to increase *in vitro* incorporation of labelled leucine, valine, tyrosine, arginine, aspartic acid, phenylalanine, and proline into protein of thyroid slices(1). It was also demonstrated that the incorporation of labelled leucine into protein is depressed in surviving thyroid slices from hypophysectomized rats and enhanced in thyroid tissue from TSH-treated rats. A part of the carbon skeleton of the thyronines has been recently shown to come from tyrosine H^3(4).

To gain additional information regarding the effect of TSH on *in vivo* incorporation of amino acids into thyroid protein, the present time-response study was done relating the *in vivo* effect of thyrotrophin on tyrosine and arginine uptakes by the thyroid gland.

Methods and materials. Newly hatched cockerel chicks were used because of their high sensitivity to thyrotrophin(5). A Remington iodine deficient diet (Nutritional Biochemicals Corp.) was fed *ad lib* prior to study and daily requirement for iodine was satisfied by addition of 0.01 μg/ml of NaI to the drinking water.

I. *Thyroid uptake of NaI^{125} in response to TSH.* Thirty chicks 2 days old received subcutaneous injections of TSH (thytropar-Armour) 0.8 I.U. once a day for 2 days. This dose level of TSH was used since preliminary experiments indicated that thyroid concentration of I^{125} was not significantly elevated by doses in excess of 0.8 I.U. In addition, it was determined that the maximum TSH effect on I^{125} uptake occurred between 24 and 48 hours after a single TSH injection and that uptakes were not significantly altered by more than 2 doses on consecutive days.

Twenty-four hours after the last TSH injection these animals and an equal number of untreated control chicks were injected I.P. with 0.25 μc of I^{125} as sodium iodide.

Five animals from each of the 2 groups were sacrificed using ether at 1, 2, 4, 8, 24 and 48 hours after the isotope injection.

The thyroid glands were rapidly excised and weighed on a microbalance. Thyroid radioactivity of I^{125} injected animals was determined by placing the glands in plastic test tubes and counting in a crystal scintillation detector.

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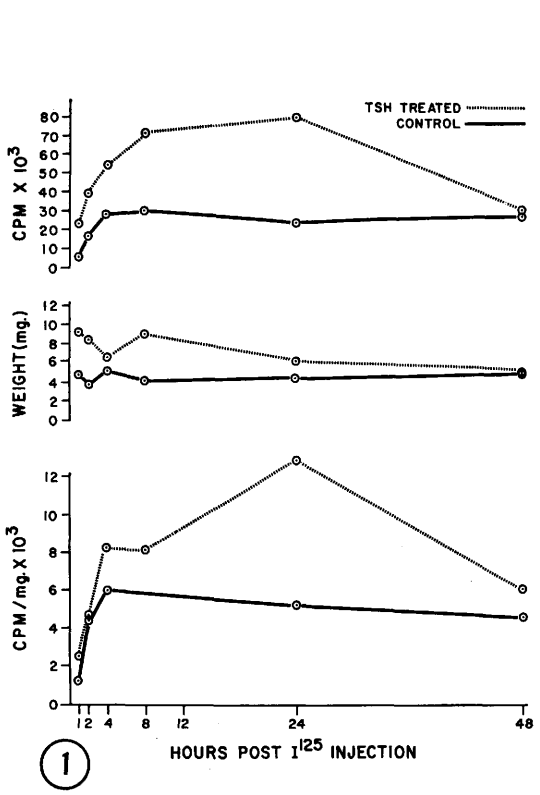

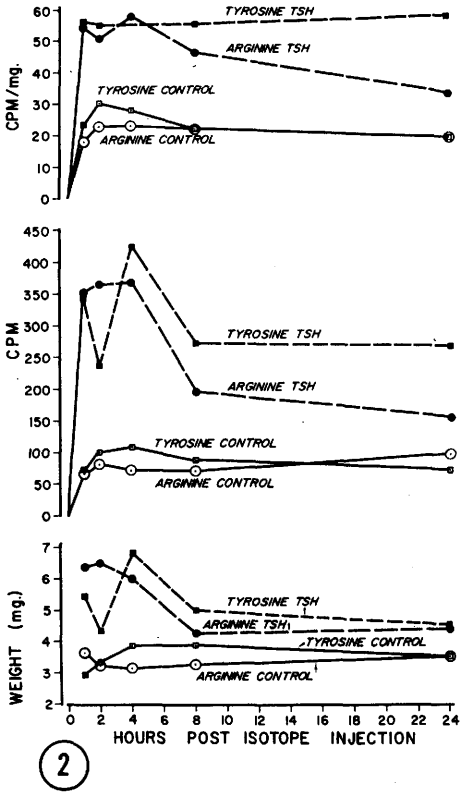

FIG. 1. Effect of thyrotrophin treatment on thyroid I^{125} uptake.
FIG. 2. Effect of thyrotrophin on thyroid uptake of C^{14} tyrosine and C^{14} arginine.

II. *Thyroid uptake of C^{14} tyrosine and C^{14} arginine in response to TSH.* An experiment identical to that described above was subsequently done to compare the effect of TSH pre-stimulation of the thyroid gland on uptake of C^{14} tyrosine and C^{14} arginine. Chicks were sacrificed at 1, 2, 4, 8 and 24 hours after intraperitoneal administration of 2 μc of the isotope. Thyroid glands in this experiment were homogenized with one ml of 10% (w/v) trichloroacetic acid solution. Prior to centrifugation, an 0.4 aliquot was removed and prepared for liquid scintillation counting (6). The remaining sample was centrifuged and the precipitate washed 3 times with one ml of 10% trichloroacetic acid. The radioactivities of the pooled washes and precipitate were then measured. Radioisotope determinations were made with a counting error of less than one percent.

Results. As shown in Fig. 1, the thyroid uptake of I^{125} in the thyrotrophin-treated chicks was significantly elevated at 8 hours ($P = 0.02$) reaching a maximum of nearly 3 times that of the control by 24 hours ($P = 0.01$). This uptake response of the TSH stimulated glands was followed by a rapid release of labelled iodine so that no significant difference in amount of radioactivity between TSH treated and control was present after 48 hours ($P = 0.15$). The effect of TSH was evident whether the results of thyroid uptakes are expressed as total activity or activity per mg of thyroid.

In a second study (Fig. 2), a similar 3-fold increase in thyroid C^{14} tyrosine and C^{14} arginine uptake was observed after 2 hours in the group pre-treated with thyrotrophin when compared with the control group ($P = 0.001$). This increased uptake of the thyroxine precursor, tyrosine, and of arginine was significantly different from the controls from one hour after isotope administration. The increased uptake was evident regardless of the means used to express the results and was not due only to increased thyroid weights

observed in the TSH-treated group.

A correlation coefficient of 0.65 was found between the TSH stimulated iodine and tyrosine groups. The TSH treated tyrosine and arginine groups had a correlation coefficient of 0.84.

Discussion. The increased thyroid uptake of iodine, tyrosine, and arginine in the thyrotrophin stimulated chicks was expected since these components would be required for the increased synthesis of thyroid protein and hormones. Since it has been shown that thyroglobulin is high in arginine content, this amino acid was chosen for this study (7). The initially equal uptake values of the TSH-treated group were followed by a more rapid loss of radioactive arginine than labelled tyrosine. This may be the result of an accelerated turnover of incorporated arginine or an increased reutilization of tyrosine.

In vitro studies have shown an increased uptake of various amino acids by thyroid slices excised from animals pre-treated with thyrotrophin(1,2). Thyroid slices removed from hypophysectomized rats showed a depression of amino acid uptake. These latter studies, and the present report, have not distinguished between the altered synthesis of protein as related to intracellular components *per se* or extracellular thyroglobulin. An increased thyroid uptake of alpha-aminoisobutyric acid, a nonutilizable amino acid, was observed after the addition of thyrotrophin to the reaction medium(8). It is interesting to note that the addition of thyrotrophin to the reaction medium of the earlier *in vitro* investigations did not result in an increased uptake of amino acids(1).

Using tritium-labelled leucine and autoradiographic techniques, radioactivity has been found in both the epithelial cells and extracellular colloid in normal animals(9). Evidence has been presented, using similar techniques, that intracellular colloid droplets become conspicuous within 8 to 12 minutes after thyrotrophin treatment compared with only a few droplets in the control animals(10).

A good correlation was found between the TSH stimulated uptake of tyrosine and arginine (0.84) while there was poor correlation between tyrosine and iodine (0.65). An explanation for this discrepancy may be that tyrosine and iodine are incorporated into thyroid tissue differently. Tyrosine will eventually become part of the sedentary or exportable protein of the thyroid gland through the process of protein synthesis(11). The iodine will be incorporated into tyrosine which pre-exists in peptide linkage(12,13). Since these processes are quite different, it would appear that the rates of tyrosine and iodine incorporation might be different. From these results, it is suggested that TSH is controlling the rate of protein synthesis in the thyroid gland in addition to thyroid hormone production.

Summary. The effect of TSH on the iodine, tyrosine, and arginine uptake by the thyroid gland was studied. The results of TSH stimulation revealed greater than a two-fold increase in uptake of all materials studied. A correlation between the TSH stimulated uptakes of tyrosine and arginine was observed; however, there was poor correlation between the tyrosine and I^{125} uptakes. This study has demonstrated a definite *in vivo* increase in the amount of radioactive tyrosine and arginine in the TSH stimulated thyroid gland. These results support the hypothesis that TSH may be controlling the rate of protein synthesis in the thyroid gland, in addition to its effect on hormone production.

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Effect of Pneumococcus SIII Polysaccharide on Viscosity and Heat Transfer of Human Blood. (30173)

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The problems related to the Anomalous Viscosity of Blood are well described by Bayliss as one of a compilation of papers on blood and other body fluids showing non-Newtonian rheological properties(1).

It is recognized that blood cells having varying degrees of aggregation might easily be disturbed by shear forces as blood is passed through a capillary tube under pressure of gravity forces, and thus affect the rheological properties of blood. Therefore, I tried several approaches to minimize these shear forces with the hope of developing a new technique for measuring the viscosity of blood. Included in these methods was a procedure to measure by a photo cell and record the movement of an 8 mm round aluminum disc balanced with a Du Nuoy tensiometer as the sample was oscillated on a vertical axis at varying speeds. The results were unsatisfactory. Finally it was decided to measure the time required for the transfer of heat from a water bath at 42° to a mercury thermometer through a 5 ml sample in a test tube and to determine the time required for the sample temperature to rise from 35° to 40°.

The importance of viscosity as affecting heat transfer in Newtonian liquid systems is well accepted. Less evidence exists as to the relationship between viscosity of non-Newtonian systems and heat transfer.

This paper contains data comparing the time required for samples to pass through an Ostwald viscosimeter at 36° and the time for the temperature of the sample (5 ml) to rise from 35° to 40° when the sample was placed in a water bath at 42°. Samples of oxalated (0.2%) human blood containing varying amounts of pneumococcus SIII polysaccharide were examined. Also samples of oxalated human plasma with different concentrations of SIII* were tested, as were varying concentrations, by weight, of glycerol in water. Glycerol solutions were used since this system has been extensively used in viscosity measurements.

Methods. It was recognized that heat transfer as measured in these experiments depended on (1) the temperature gradient between the water bath and the sample, (2) heat transfer from the water bath reservoir through a film of water on the external surface of the sample tube, (3) through the glass wall of the tube, (4) through a film of the sample on the inner surface of the sample tube, (5) agitation of the sample by external forces or convection currents, (6) heat transfer through a film of sample on the thermometer and (7) through the thermometer wall and the mercury in the thermometer. The variables related to the nature of the sample are listed as 4, 5 and 6 above. By attention to certain details, an effort was made to minimize variation in factors, 1, 2, 3 and 7. These experimental details are listed:

1. All glassware was rinsed with ammonium hydroxide, water, ethanol, ethyl ether and dried between tests on each sample.

2. The water was circulated in a bath with a baffle by means of a pump.

3. In the work presented 5 measurements were made on each sample by the heat trans-

* Obtained from Dr. Michael Heidelberger.