5. King, E. J., *Micro-Analysis,* 1956, Grune and Stratton, N. Y.

6. Anigstein, L., Whitney, D. M., Rennels, E. G., *Tex. Rep. on Biol. and Med.,* 1958, v16, 303.

7. Anigstein, L., Eklund, G. W., Whitney, D., *ibid.,* 1957, v15, 205.

Received September 17, 1959. P.S.E.B.M., 1959, v102.

Release of Inorganic Iodine from Thyroid Gland in Initial Phase of TSH Action. (25391)

SHIGENOBU NAGATAKI, KAZUO SHIZUME, KUNIO MATSUDA AND JUN ISHII
(Introduced by R. L. Swank)
Prof. Okinaka's Medical Clinic, University of Tokyo Medical School, Hongo, Tokyo, Japan

It has been well established that TSH accelerates release of thyroid hormone from the thyroid gland and increases uptake of inorganic iodine by the thyroid gland(1,2,3,4). While the acceleration of the hormone release takes place within several minutes after injection of TSH, there is a latent period of several hours before the thyroid gland starts to increase its uptake of inorganic iodine(1,5,6,8) and few studies have been reported on the behavior of the thyroid gland in handling inorganic iodine shortly after injection of TSH. Our observation that TSH releases the inorganic iodine from the thyroid in the early phase of its effect prompted this report.

Materials and methods. Seventeen male dogs weighing about 10 kg and maintained on a low iodine diet for 2 weeks were utilized. In 13 dogs, 3 to 7 days before the experiments, 0.5 to 2 mc of carrier free I^{131} were injected intramuscularly, while in the other 4 dogs, a similar amount of I^{131} was injected 20 hours before the experiments. Under morphine anesthesia (100 mg), a midline incision was made in the neck to dissect out the thyroid gland and its vessels. The thyroid venous blood samples were collected from polyethylene catheters inserted into the inferior thyroid veins. A catheter was placed in the femoral artery to collect arterial blood samples and to measure arterial blood pressure. During the experimental period, about 10,000 units of heparin in saline were infused intravenously. Blood transfusion was used if necessary, to maintain constant blood pressure.

Method. In 14 dogs, after control period of 30 minutes to 2 hours, one USP unit of TSH in 10 ml saline was injected intravenously, and the effect of TSH observed for 2 to 10 hours thereafter. In 2 dogs, observation was started 8 hours after administration of TSH to determine duration of its action. At intervals of 15 to 30 minutes during control and experimental periods, blood samples were collected from catheters in the thyroid veins and femoral artery, and blood pressure at each time was recorded. Blood flow of the thyroid vein was estimated by volume of blood collected during each interval. Measurement of PBI^{131}: Samples from thyroid veins and femoral artery were centrifuged to separate 1 ml of plasma. Radioactivity of the plasma, measured in well type scintillation counter for 5 minutes, represented total plasma radioactive iodine. Five ml of 20% trichloroacetic acid was added in the same tube to precipitate the protein bound iodine, and the precipitate was washed twice with 5 ml of 10% trichloroacetic acid. The activity of the precipitate, redissolved with 0.5 ml of 2 N sodium hydroxide, which was measured in a well type scintillation counter for 5 minutes, represented the PBI^{131} concentration in 1 ml of plasma. Concentration of inorganic iodine was expressed by the difference between the activity of total and PBI^{131}. Radiopaperchromatographic technic: 3 to 5 ml of plasma was acidified to pH 2-3 with 5 N HCl, extracted 3 times with 0.1 N HCl saturated butanol after addition of carriers, neutralized with 4 N NH_4OH and evaporated to dryness in a 50°C water bath. The residue was dissolved in 0.2 ml of butanol and placed on Toyo filter

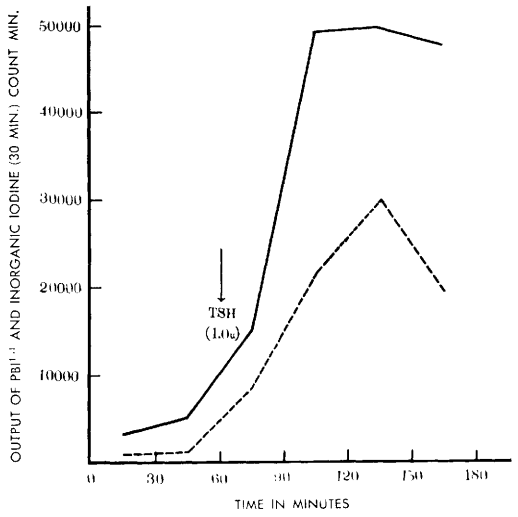

FIG. 1. Output of PBI^{131} and inorganic iodine in dog administered I^{131} 4 days before experiment. ——— PBI, - - - - - - inorganic iodine.

paper No. 50 in tertiary amyl alcohol-2 N ammonia or butanol acetic acid ascending system for 16 to 36 hours. The paperchromatogram was then cut into 0.5 cm horizontal strips for radioactivity measurement in a well type scintillation counter. Estimation of the output of PBI^{131} and inorganic iodine from the thyroid was made by the following formula: output of PBI^{131} (inorganic iodine) = (concentration of PBI^{131} (inorganic iodine) in thyroid venous plasma − concentration of PBI^{131} (inorganic iodine) in the femoral arterial plasma) × plasma flow of thyroid vein.

Results. Following a latent period of 15 minutes after one USP unit of TSH infusion, both PBI^{131} and inorganic iodine concentration of thyroid venous blood rose sharply, and the increased concentration continued for about 10 hours, while concentration of femoral arterial blood remained at control level. Fig. 1 shows a representative experiment in which I^{131} was injected 3 to 7 days previously, and Fig. 2 shows results in one experiment when I^{131} was injected 20 hours before the experiment. In the second experiment (Fig. 2) the thyroid gland was accumulating inorganic iodine before injection of TSH and started to release iodide shortly after injection.

Fig. 3 depicts one of 2 dogs in which observation was started 8 hours after injection of TSH to determine duration of the release of inorganic iodine by TSH administration. About 10 hours after injection of TSH, release of inorganic iodine terminated and the thyroid gland started to accumulate iodide. A similar result was obtained in one other dog. In one experiment release of inorganic iodine was observed even when the dose of TSH was 50 USP milliunits. No change of blood pressure or thyroid venous blood flow was observed after TSH infusion.

The radiopaperchromatogram of the thyroid venous blood and femoral arterial blood before and after TSH revealed thyroxine, triiodothyronine, and inorganic iodine, but spots of iodotyrosine were not recognized. The ratio of the output of triiodothyronine to thyroxine showed a tendency to increase after TSH infusion.

Discussion. In our experiments, injection of 1 USP unit of TSH was followed shortly by release of both protein bound and inorganic iodine from the thyroid gland. The possibility that the increased output of inorganic iodine

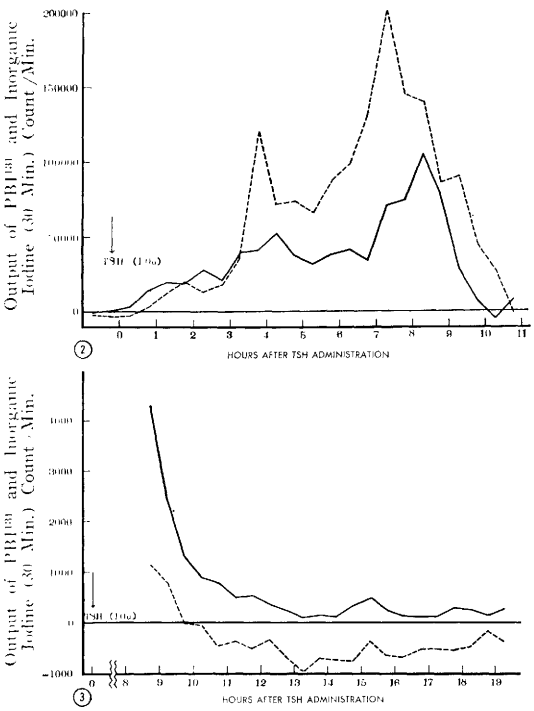

FIG. 2. Output of PBI^{131} and inorganic iodine in dog administered I^{131} 20 hr before experiment. ——— PBI, - - - - - - inorganic iodine.

FIG. 3. Output of PBI^{131} and inorganic iodine in dog administered I^{131} 3 days before experiment. ——— PBI, - - - - - - inorganic iodine.

was due to destruction of protein bound iodine before protein precipitation was most unlikely as thyroxine derivatives are known to be stable in the blood and the procedure was performed within 30 minutes after collection of samples. In one experiment the blood was collected directly into trichloroacetic acid without altering the results. The possibility that the increase of radioactivity in the unprecipitated fraction was due to free thyroxine, triiodothyronine, or iodotyrosine was excluded since most of the radioactivity was located in the inorganic iodine zone on the radiopaperchromatogram of this unprecipitated fraction. The observation that 50 USP milliunits of TSH released both inorganic and protein bound iodine indicated that this release was not caused by unphysiological dose of TSH.

Whether the release of inorganic iodine is due to deiodination of some products by the increase in thyroglobulin proteolysis, or due to change of permeability of cell membrane is still to be determined.

This phenomenon may explain the slowing of iodide disappearance in the plasma which occurs shortly after TSH administration in man(9) and also the increased urinary output of iodine within 24 hours after injection of TSH in man(7,8).

Summary. Measurement of inorganic I^{131} and PBI^{131} in thyroid venous blood obtained by catheterization was performed in 17 dogs to study the early effect of TSH. Following a latent period of about 15 minutes, output of both inorganic I^{131} and PBI^{131} started to increase sharply. The increased output continued for about 10 hours, and thereafter the thyroid gland started to accumulate inorganic iodine. The inorganic I^{131} fraction, represented in the trichloroacetic acid supernatant fraction, was confirmed by radiopaperchromatography. TSH apparently releases inorganic iodine, in addition to PBI, from the thyroid in the early phase of its action.

We express our thanks to Prof. Okinaka and the medical staff of our department for their advice. The well type scintillation counter was purchased by grant from Rockefeller Fn.

1. Stanley, M. M., Astwood, E. B., *Endocrinology,* 1949, v44, 49.
2. Goldsmith, R. E., Stanbury, J. B., Brownell, G. L., *J. Clin. Endocrinol.,* 1951, v11, 1079.
3. Keating, F. R., Jr., Rawson, R. W., Peacock, W., Evans, R. D., *Endocrinology,* 1945, v36, 137.
4. Wolff, J., *ibid.,* 1951, v48, 284.
5. Vander Laan, W. P., Greer, M. A., *ibid.,* 1950, v47, 36.
6. Halmi, N. S., Spirtos, B. N., Bogdanove, E. M., Lipner, H. J., *ibid.,* 1953, v52, 19.
7. Becker, D. V., Rall, J. E., Peacock, W., Rawson, R. W., *J. Clin. Invest.,* 1953, v32, 149.
8. Einhorn, J., Larsson, L., *J. Clin. Endocrinol. and Metab.,* 1959, v19, 28.
9. Deiss, W. P., O'Shaughnessy, Wynn, J. O., *ibid.,* 1959, v38, 334.

Received September 28, 1959. P.S.E.B.M., 1959, v102.

Potentiation of Lathyrogenic Effect of Beta Aminopropionitrile in Turkey Poults.* (25392)

D. N. ROY, S. H. LIPTON, F. M. STRONG AND H. R. BIRD
Depts. of Biochemistry and Poultry Husbandry, University of Wisconsin, Madison

Cyanoacetic acid (CAA) has been isolated from urine of rats and rabbits as a final product of metabolism of beta-aminopropionitrile (BAPN)(1,2). It is assumed that this conversion of BAPN to CAA is possible under the influence of the enzyme monoamine oxidase (MO). It has been shown that 1-isonicotinyl-2-isopropyl hydrazine (IIH) or iproniazid inhibits monoamine oxidase activity of rat liver *in vitro*(3), and the mode of action of IIH on mitochondrial MO of mammalian liver has been investigated(4). The inhibitory ef-

* Published with approval of Director of Wisconsin Agri. Exp. Station, Coll. of Agri., Madison. Supported in part by Research Committee of Graduate School from funds supplied by Wisconsin Alumni Research Fn., Madison, and by grant from Nat. Inst. Health, U.S.P.H.S.