over 10 min with good stirring, keeping the temp below 20°. The cooling bath was removed and stirring was continued for 1 hr, after which time the evolution of $CO_2$ stopped. The $CHCl_3$ layer was washed with 20 ml of 5% HCl, then $H_2O$ and dried ($Na_2SO_4$) and the solvent evaporated. The oily product was crystallized from $CCl_4$–hexane, decolorizing with charcoal, to give 6.7 g (59%) of small white needles, mp 68–70°. Recrystallization from pet ether (bp 60–110°) gave an analytical sample: mp 72–74°; ir $\lambda_{max}^{CHCl_3}$ 3.0 (OH), 5.87 (C=O).

**o-Carbomethoxyphenyl *l*-Ephedrinecarbamate.**—A mixture of 4.95 g (0.03 mole) of *l*-ephedrine in 50 ml of $CHCl_3$ and 1.7 g (0.016 mole) of $Na_2CO_3$ in 20 ml of $H_2O$ was cooled to 5°. A soln of 6.66 g (0.031 mole) of *o*-carbomethoxyphenyl chloroformate in 20 ml of $CHCl_3$ was added over 10 min with good stirring, keeping the temp below 15°. The cooling bath was removed and the reaction mixture was stirred for 1.5 hr. The organic layer was washed with 20 ml of 5% HCl and 20 ml of $H_2O$ and dried ($Na_2SO_4$) and the solvent evaporated. The solid product was recrystallized from $CHCl_3$–hexane, decolorizing with charcoal, to give 7.66 g (74%) of fine long white needles, mp 95–96°.

**Bis(phenyl *dl*-*p*-hydroxy-α-methylphenethylcarbamate)piperazine Salt.**—A stirred mixture of 6.96 g (0.03 mole) of *dl*-*p*-hydroxyamphetamine·HBr, 3.4 g (0.032 mole) of $Na_2CO_3$, 30 ml of $H_2O$ and 60 ml of $CHCl_3$ was cooled in an ice bath. A soln of 4.85 g (0.031 mole) of phenyl chloroformate in 20 ml of $CHCl_3$ was added over 5 min. After stirring at room temp for 2 hr, the partially soluble free phenolic amine gradually reacted and dissolved in the $CHCl_3$ giving 2 clear layers. The $CHCl_3$ layer was washed with 2 × 50 ml of 5% HCl, dried ($Na_2SO_4$), and the solvent evaporated to give 8 g of a colorless oil. This was dissolved in 150 ml of $C_6H_6$ and 4 g of piperazine was added to the warm soln, followed by 80 ml of hexane. The resulting ppt was recrystallized from $C_6H_6$ giving 4.9 g (52%) of white crystalline product, mp 115–116°, that analyzed correctly for the bis salt.

**o-Nitrophenyl *dl*-α-Methylphenethylcarbamate.**—A soln of 13.9 g (0.1 mole) of o-nitrophenol in 30 ml of solvent A was added to a solution of 17 g (0.17 mole) of $COCl_2$ in 150 ml of solvent A at 0° with no noticeable rise in temperature. However, an exothermic reaction occurred during the gradual addition of 10.1 g (0.1 mole) of $Et_3N$ in 30 ml of solvent A and a solid formed. After stirring out of the cooling bath for 1 hr, 100 ml of $H_2O$ was added cautiously to dissolve the pptd salt. The organic phase was washed with $H_2O$, dried ($CaCl_2$), and evapd to about 50 ml of yellow soln containing the *o*-nitrophenyl chloroformate, from which the carbamate was prepared.

A mixture of 9.02 g (0.245 mole) of *dl*-amphetamine sulfate and 5.3 g (0.05 mole) of $Na_2CO_3$ in 70 ml of $H_2O$ with 50 ml of $CHCl_3$ was cooled to 0°. The *o*-nitrophenyl chloroformate solution was added with stirring and cooling over 10 min. The cooling bath removed and the mixture was stirred for 2 hr whereupon both layers became clear yellow at pH 7–7.5. The organic phase was washed with 50 ml of $H_2O$, two 50-ml portions of 5% HCl, $H_2O$ again and then dried ($Na_2SO_4$). Most of the solvent was evaporated and upon the addn of hexane a solid formed. Recrystallization from $CCl_4$ gave 12.3 g (84%) of crude product, mp 80–86°. Further recrystn from dry *i*-$Pr_2O$ gave analytically pure product, mp 89–90°, as very light yellow needles.

**o-Carbomethoxyphenyl [$^{14}$C]-*d*-α-Methylphenethylcarbamate (21).**—A mixture of 20 mg (0.055 μmole) of [$^{14}$C]-*d*-amphetamine sulfate (6.0 μCi/mg), 35 mg (0.33 μmole) of $Na_2CO_3$, 40 mg (0.2 μmole) of *o*-carbomethoxyphenyl chloroformate, 1 ml of $H_2O$, and 3 ml of $CHCl_3$ was shaken for 0.5 hr at room temperature. The mixture was dild with 10 ml of $CHCl_3$ and 4 ml of $H_2O$. The organic phase was washed with dil HCl and $H_2O$, dried ($Na_2SO_4$), and evaporated to an oil that crystallized. After trituration with pet ether the solid was recrystallized from $CCl_4$ and pet ether, eventually giving 30 mg (87%) of white needles with a specific activity of 3.5 μCi/mg. Tlc on an Eastman Kodak 6060 chromagram silica gel sheet using $C_6H_6$ developer indicated that the product was homogeneous with an $R_f$ 0.21 corresponding exactly to that of the unlabeled material. A radioscan of the strip showed a single peak corresponding to the visual spot, indicating radioactive homogeneity as well.

**o-Nitrophenyl [$^{14}$C]-*d*-α-methylphenethylcarbamate (22)** was prepd from 25 mg (0.068 μmole) of [$^{14}$C]-*d*-amphetamine sulfate (6.0 μCi/mg) by procedures similar to those in the preceding experiment and those given here for the prepn of the unlabeled product. A 30-mg yield of long, yellow-tinted needles was eventually obtained with a specific activity of 3.7 μCi/mg. Tlc on Eastman Kodak 6060 Chromagram silica gel sheet using $C_6H_6$ as the developer showed the product to be homogeneous with an $R_f$ 0.54 corresponding exactly to that of the unlabeled product. A radioscan indicated that the product was radiochemically pure and corresponded to the visual spot.

# Peripheral Inhibition of Thyroxine by Thiohydantoins Derived from Amino Acids[1a]

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A number of 3-phenyl-2-thiohydantoins with nonpolar substituents at the 5 position inhibited the peripheral effect of $T_4$ as measured by the liver GPD response. Like TU and PTU, they also increased the PBI in rats given exogenous $T_4$. These PTH derivatives of valine, leucine, norleucine, isoleucine, etc., were very weak goiterogens which had only little or no effect on thyroid weight or radioiodine uptake.

Barker, *et al.*[2a] originally showed that a given dose of $T_4$ did not restore the metabolic rate as well in rats made hypothyroid with TU as it did in surgically thyroidectomized rats. In a recent summary by de Escobar and del Rey[2b] the minimal daily $T_4$ requirement was found to be increased twofold in rats receiving TU, PTU, or MTU when a variety of biological endpoints was used (metabolic rate, liver GPD, goiter prevention, pituitary basophilia, normal plasma TSH, and suppression of thyroid $^{131}$I release). These thiouracils, in addition to preventing the biosynthesis of thyroid hormones, inhibited the peripheral action of $T_4$.[3,4]

This paper reports a study of the inhibition of the GPD response to exogenous $T_4$ by a series of 5-alkyl-3-phenyl-2-thiohydantoins derived from amino acids by the Edman reaction[5,6] in an attempt to establish the

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(1) (a) This study was aided by Grant No. 5-RO1-AM-09106 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, and Grant No. 5-RO1-CA-01852-17 from the National Cancer Institute, U. S. Public Health Service. (b) Abbreviations used are: GPD, mitochondrial α-glycerophosphate dehydrogenase (EC 1.1.99.5); MTU, 6-methyl-2-thiouracil; PBI, protein-bound iodine; PTH-AA, 5-alkyl-3-phenyl-2-thiohydantoin derived from amino acid; PTU, 6-*n*-propyl-2-thiouracil; $T_4$, 3,5,3′,5′-tetraiodo-L-thyronine, L-thyroxine; TSH, thyroid stimulating hormone; TU, 2-thiouracil; RAIU, radioiodine uptake.

(2) (a) S. Barker, C. Kiely, and H. Lipner, *Endocrinology*, **45**, 624 (1949); (b) C. Morreale de Escobar and F. Escobar del Rey, *Recent Progr. Horm. Res.*, **23**, 87 (1967).

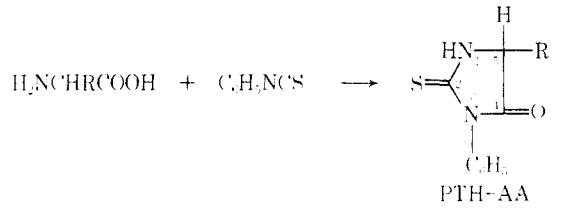
(3) W. Ruegamer, W. W. Westerfeld, and D. A. Richert, *Endocrinology*, **75**, 908 (1964).

(4) W. Hoffman, D. Richert, and W. W. Westerfeld, *ibid.*, **78**, 1189 (1966).

(5) P. Edman, *Acta Chem. Scand.*, **4**, 277 (1950).

(6) W. Westerfeld, R. Doisy, and D. Richert, *J. Nutr.*, **78**, 393 (1962).

molecular requirements for the peripheral inhibition of $T_3$. The effect of some of these PTH-AA's on the thyroid gland was also determined to see if there was a correlation between the structural requirements for the inhibition of the peripheral action of $T_3$ and the biosynthesis of the hormone.




## Methods and Materials

Groups of 6 to 9 male Sprague–Dawley rats weighing approx 150 g were fed a purified basal diet (6) ± 0.2% of the test compound and sacrificed on the 12th day. Some rats were simultaneously injected with 1.5 μg of $T_3$/100 g of body wt per day subcutaneously. Liver GPD was determined manometrically;[7] several of the active inhibitors (PTH-valine, PTH-leucine, PTU) were tested and found to have no effect on the GPD assay *in vitro*, and did not produce an inhibitor *in vivo* which interfered with the GPD determination in normal or hyperthyroid liver. RAIU values were determined by injecting rats subcutaneously with 0.2 μCi of $Na^{131}I$ (Oriodide from Abbott Radiopharmaceuticals without added carrier); 24 hr later the thyroid gland was weighed and counted in a Packard Auto-γ solid scintillation system. Chemical PBI determinations were performed by Bio-Science Laboratories on frozen sera pooled from several rats.

PTH-AA's were synthesized from 60 ml of phenyl isothiocyanate and 25 g of a DL-amino acid and the products were checked for identity and purity by comparison with available standards (Mann Research Laboratories). The melting point and mixture melting point were essentially the same as those reported by Edman[5] and Brautlecht.[8] The $R_f$ values on tlc agreed well with those reported by Stahl,[9] and a mixture of each compd with its own standard could not be sepd by tlc.

## Results

**Induction of Liver GPD by $T_3$.**—Table I shows the inhibition of the $T_3$-induced increase in liver GPD when rats were also given various PTH-AA's in the diet. Those compds with a nonpolar side chain in the 5 position were most potent; those with polar substituents had little or no activity. A comparison of those derivatives with increasing carbons in an unbranched, nonpolar side chain (PTH-α-aminobutyrate, -norvaline, -norleucine) shows increasing activity with chain length up to 4 C (PTH-norleucine); the 6-carbon side chain (PTH-α-aminooctanoate) was less active. PTH-glycine and alanine were also relatively inactive, but the results are not comparable because of poor food consumption and growth with these compds. Compds with branched 3 or 4 C, nonpolar side chains (PTH-valine, leucine) had good activity, but the isoleucine derivative was less active. Phenylisothiocyanate itself and monophenylthiourea were relatively toxic, but did not inhibit GPD induction by $T_3$; another side product formed in the synthesis of the PTH-AA's, 1,3-diphenylthiourea, was a moderate inhibitor of $T_3$ without inhibiting growth or food consumption appreciably. None of the PTH-AA's which showed good activity against the peripheral action of $T_3$ were contaminated by 1,3-diphenylthiourea.

When no $T_3$ was administered simultaneously with the drug, the latter could decrease the basal GPD level by blocking the biosynthesis of $T_4$ as well as by inhibiting its peripheral action. While many of these compounds did depress the basal GPD levels somewhat (Table I), only the proline derivative produced thyroidectomy levels of liver GPD. PTH-proline was also very effective in blocking exogenous $T_3$, but it was relatively toxic.

Some compds which inhibit the peripheral action of $T_3$ (*e.g.*, TU, PTU) also increase the PBI in rats fed a casein diet[20] and given exogenous $T_3$. Within this series of compds, some of the better peripheral inhibitors of $T_3$ (PTH-valine, -leucine, -isoleucine) also increased the PBI in rats given $T_3$ (133 to 158% of the 8.8 μg % found in control rats fed the basal diet and also injected with $T_3$) (Table I). However, the two phenomena were not always associated. PTH-proline inhibited the GPD induction by exogenous $T_3$, but did not cause an accumulation of exogenous $T_3$ in the plasma; monophenylthiourea increased the PBI accumulation in plasma, but did not inhibit the peripheral effect of $T_3$. When exogenous $T_3$ was not administered, many of the compds decreased the PBI, and the correlation coefficient between this effect and a decrease in basal GPD was 0.62; both effects were consistent with a mild goiterogenic action.

The 24-hr RAIU's (Table II) were unaffected by all compds tested except PTH-valine, and the effect of this compd was very weak by comparison with PTU. Several compds (PTH-valine, -leucine, -proline, -glycine, -norleucine) increased the wt of the thyroid gland slightly, but they were all very weak goiterogens by comparison with PTU. Unlike the inorganic thiocyanates, phenyl isothiocyanate was found to have no effect on the RAIU or wt of the thyroid gland.

## Discussion

The results of the present study showed that the PTH-AA's with a nonpolar side chain in the 5 position were the most effective in preventing the induction of liver GPD by exogenous $T_3$; the PTH-AA's with polar substituents were generally inactive. While these PTH-AA's are moderate inhibitors of $T_4$ peripherally, they are not very potent inhibitors of the thyroid gland; however, the same structure–activity relationship in this series is applicable to the inhibition of the peripheral action of $T_3$ as was previously developed for the inhibition of the biosynthesis of the thyroid hormone by other thioamides.

The most active thioamides which produce gross and histological hyperplasia of the rat thyroid gland are the

(7) D. Richert, J. Schenkman, and W. Westerfeld, *J. Nutr.*, **83**, 332 (1964).
(8) C. Brautlecht, *Am. Chem. J.*, **14**, 446 (1914).
(9) E. Stahl, "Thin-Layer Chromatography, A Laboratory Handbook," Academic Press, New York and London (1965).

TABLE I
THE EFFECTS OF PTH-AA'S ON THE LEVELS OF LIVER GPD AND SERUM PBI[a]

| Compd | Av weight gain +$T_4$ | Av weight gain No $T_4$ | Av food consumption ±$T_4$ | Liver GPD +$T_4$ | Liver GPD No $T_4$ | Serum PBI +$T_4$ | Serum PBI No $T_4$ | % inhibition of $T_4$[c] |
|---|---|---|---|---|---|---|---|---|
| PTH-proline | −7 | 17 | 74 | 36 ± 6* | 36 ± 6* | 84 | 65 | 100 |
| PTH-valine | 93 | 74 | 93 | 37 ± 4* | 61 ± 6* | 156 | 47 | 81 |
| PTH-leucine | 87 | 59 | 85 | 41 ± 3* | 59 ± 4* | 158 | 80 | 88 |
| PTH-norleucine | 52 | 32 | 70 | 46 ± 5* | 67 ± 16 | | | 97 |
| 1,3-diphenylthiourea | 130 | 80 | 101 | 53 ± 4* | 79 ± 7 | 122 | 71 | 53 |
| PTH-isoleucine | 74 | 93 | 109 | 57 ± 8 | 70 ± 8* | 133 | 67 | 50 |
| PTH-norvaline | 75 | 75 | 91 | 61 ± 4* | 72 ± 9 | | | 51 |
| PTH-α-aminobutyrate | 64 | 59 | 96 | 63 ± 10 | 73 ± 5* | 95 | 56 | 43 |
| PTH-tryptophan | 98 | 99 | 101 | 66 ± 6 | 89 ± 8 | | | 55 |
| PTH-2-methylalanine | 40 | 41 | 92 | 69 ± 10 | 50 ± 2* | 91 | 67 | 37 |
| PTH-phenylalanine | 95 | 87 | 95 | 70 ± 7 | 80 ± 9 | 83 | 72 | 45 |
| PTH-threonine | 87 | 74 | 93 | 71 ± 4* | 72 ± 8 | 131 | 58 | 38 |
| PTH-alanine | −46 | 0 | 69 | 73 ± 6 | 50 ± 9* | 94 | 46 | 41 |
| PTH-α-aminooctanoate | 81 | 76 | 102 | 76 ± 6 | 83 ± 5 | | | 32 |
| PTH-serine | 123 | 83 | 105 | 77 ± 6 | 55 ± 12* | 111 | 84 | 25 |
| PTH-cystine | 104 | 104 | 104 | 82 ± 6 | 100 ± 10 | | | 31 |
| PTH-methionine | 56 | 53 | 78 | 86 ± 7 | 72 ± 10 | 81 | 49 | 23 |
| PTH-glycine | −95 | −54 | 50 | 88 ± 9 | 70 ± 13 | | 49 | 23 |
| PTH-arginine | 56 | 55 | 102 | 97 ± 2 | 87 ± 9 | | 98 | 4 |
| PTH-tyrosine | 88 | 79 | 94 | 99 ± 5 | 67 ± 7* | 77 | 66 | 0 |
| PTH-asparagine | 104 | 100 | 92 | 100 ± 7 | 79 ± 12 | | 99 | 0 |
| PTH-aspartate | 94 | 117 | 91 | 105 ± 11 | 94 ± 10 | | 109 | 0 |
| Monophenylthiourea | −200 | −108 | 31 | 105 ± 12 | 73 ± 6 | 176 | 42 | 0 |
| PTH-glutamate | 94 | 87 | 92 | 113 ± 6 | 111 ± 8 | | 98 | 0 |
| Phenyl isothiocyanate | −27 | −2 | 66 | 127 ± 9 | 127 ± 9 | 101 | 62 | 0 |
| Basal diet (control) | 100 | 100 | 100 | 100 ± 7 | 100 ± 6 | 100 | 100 | |
| Absolute values[b] | 51 ± 3 | 72 ± 4 | 16.4 ± 0.4 | 132 ± 12 | 19 ± 1 | 8.8 ± 0.3 | 5.6 ± 0.2 | |

[a] Each compound was fed as 0.2% of the diet for 11 days, and the amount of drug administered was proportional to the food consumption as listed. All values are shown as a percent of the corresponding control (basal diet rats). For GPD values, the means are significantly different from the controls at $p = 0.02$ or less as indicated by *. [b] The units for the abs values on the basal diet are as follows: GPD = μl of $O_2$ consumed per 10 min per 150 mg of wet liver at 30°. PBI = μg of iodine per 100 ml of serum. Food consumption (g/rat per day) was not altered significantly by $T_4$. Weight gain = number of grams gained during 11 days of treatment. [c] Restoration of liver GPD from 132 to 19 by the consumption of 0.14 mmole of drug/day would be 100% inhibition of the $T_4$. All values were calculated as a per cent of this inhibition at this constant dosage as an approximation of relative activities.

TABLE II
THE EFFECTS OF SEVERAL PTH-AA'S ON THE WEIGHT AND RADIOIODINE UPTAKE OF THE THYROID GLAND

| Compd | % dose uptake (RAIU)[a] | mg of gland/100 g of body wt |
|---|---|---|
| PTH-valine | 4.3 ± 0.5* | 6.7 ± 0.5* |
| PTH-leucine | 7.2 ± 0.5 | 5.6 ± 0.3 |
| PTH-aspartate | 6.5 ± 0.4 | 5.0 ± 0.4 |
| PTH-methionine | 6.4 ± 0.8 | 5.1 ± 0.3 |
| PTH-norleucine | 9.4 ± 1.6 | 6.6 ± 0.7 |
| PTH-tyrosine | 6.9 ± 0.6 | 5.1 ± 0.3 |
| PTH-proline | 7.9 ± 0.7 | 5.7 ± 0.2* |
| PTH-glycine | 8.0 ± 0.4 | 8.3 ± 0.8* |
| Phenylisothiocyanate | 7.6 ± 0.7 | 5.1 ± 0.4 |
| 0.06% PTU | 0.5 ± 0.05* | 18.8 ± 0.8* |
| Basal diet (control) | 7.2 ± 0.4 | 4.7 ± 0.1 |

[a] Mean ± SE. The means are significantly different from the controls (basal diet) at $p = 0.02$ or less as indicated by *.

2-thiouracils.[10-13] Substitution with alkyl groups at the 5 or 6 position gives peak activity with the 6-propyl or 5-ethyl-2-thiouracil. Of the thiohydantoins tested[13-15] 5,5-dimethyl-2,4-dithiohydantoin[13] and 5-isobutyl-2-thiohydantoin[14] have good activity; in general, 2-thiohydantoin derivatives with a nonpolar substituent at the 5 position effectively depress the RAIU of the thyroid gland[15] while derivatives with polar substituents at the 5 position are ineffective. Similarly the 3-allyl-2-thiohydantoin derivatives containing nonpolar substituents at the 5 position decrease $Na^{131}I$ uptake, whereas those with polar substituents do not.[16]

The inhibition of $T_4$ biosynthesis can be separated from the inhibition of the peripheral effect of $T_4$. Methimazole is a very effective inhibitor of $T_4$ biosynthesis, but has no effect on the peripheral action of $T_4$. Several of the PTH-AA's are moderate inhibitors of $T_4$ peripherally, but have only little or no effect on the gland.

(10) E. Astwood, *Harvey Lect.*, **40**, 195 (1945).
(11) E. Astwood, A. Bissell, and A. Hughes, *Endocrinology*, **37**, 456 (1945).
(12) E. Astwood, J. Sullivan, A. Bissell, and R. Tyslowitz, *ibid.*, **32**, 210 (1943).
(13) J. Cheymol, P. Chabrier, and Y. Gay, *Arch. Int. Pharmacodyn.*, **88**, 343 (1951); **90**, 78 (1952).
(14) M. Jackman, M. Klenk, B. Fishburn, B. Tuller, and S. Archer, *J. Amer. Chem. Soc.*, **70**, 2884 (1948).
(15) R. Kilpatrick, D. Elmore, and D. Wood, *Brit. J. Pharm.*, **13**, 350 (1958).
(16) P. Langer, L. Drobnica, and J. Augustin, *Physiol. Bohemoslov.*, **13**, 450 (1964).