

nearly bridged over in the treated animals than in the controls. A more objective means of estimating the degree of healing of fractures would be desirable. However, the observations indicated that in the majority of cases the drug exerted a positive effect even though the fractures with which we were dealing normally progress to rapid healing. Four determinations of serum inorganic phosphate and calcium, made at regular intervals during the course of 12 daily injections of 0.1 g of glycerophosphate, showed no consistent differences from the results obtained with untreated animals.

Sodium beta-glycerophosphate, when given by intravenous injection over a long time or in large doses, is not toxic. The liver, intestine, urinary bladder, kidney, heart and aorta of a rabbit which received 55 daily injections of 0.1 g of the drug were normal grossly and microscopically. Similar results were obtained with 3 rabbits following 6 daily intravenous injections of 1.0 g of the substance. No symptoms were produced following 26 daily subcutaneous injections of 0.1 g of sodium beta-glycerophosphate into a rabbit and no pathological findings were noted at autopsy. A 17 kg dog received 10 daily intravenous injections of 10 g of the substance in 40 cc of solution. No ill effects were produced and when the animal was sacrificed, 2 months after the last injection, and no pathological alterations of any organ were observed. Large doses (12 to 24 cc of a solution containing 0.25 g of the substance per cc) are fatal in 2-5 days when given daily to rabbits by intraperitoneal injection.

Since sodium beta-glycerophosphate is innocuous in a wide range of dosage it should be safe to administer it to the human in order to determine its effect on those fractures which heal at a rate slower than usual.

13503

Activity of Certain Isomers of Thyroxin.*

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Investigations on compounds closely related structurally to thyroxin have been limited. An isomer of thyroxin, β - β -di (3,5-diiodo-

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4-hydroxyphenyl) phenylalanine, has been synthesized by Harington and McCartney,¹ and found to be inactive. Thyronine has been found inactive,^{2, 3} and diiodothyronine has been shown to have slight activity.^{3, 4} Recently Nieman and coworkers^{5, 6} have synthesized two isomers of thyroxin differing from thyroxin only in the position of the hydroxyl group and iodine atoms in the second benzene ring.[†] These experiments were undertaken to determine the effect of these compounds on the basal metabolic rate of the rat.

The first isomer studied was dl-3,5-diiodo-4-(2'-4'-diiodo-3'-hydroxyphenoxy)-phenylalanine (II) which can be more conveniently termed "meta" thyroxin on the basis of the position of the hydroxyl group, ordinary thyroxin (I) being the para compound. As this compound was only available in limited quantity, studies on possible toxic effects and preliminary studies on the effect on the basal metabolic rate were made at the same time. The methods used for determination of the B.M.R. were given in an earlier publication.⁷ The meta thyroxin was dissolved in a minimum amount of sodium hydroxide, and injected subcutaneously at a level of 5 mg per kg of body weight, using a rat whose B.M.R. had previously been standardized. Since the B.M.R. determinations indicated that this level was ineffective, subsequent doses of 30, 60, 120, and 180 mg per kg of body weight were given over a period of 6 days, all without any toxic effect or without any definite effect on the B.M.R. To test larger amounts, doses of 250 and 500 mg per kg of body weight were given to 2 rats whose B.M.R. had been previously standardized. These doses had little or no effect as the B.M.R. remained at a normal level of about 1000 calories per square meter per 24 hrs. In contrast to the lack of effect of the meta thyroxin, synthetic dl-thyroxin in doses of 1 mg per kg resulted in an increase of 25-35% in the B.M.R.

Preliminary studies with "ortho" thyroxin, dl-3,5-diiodo-4-

¹ Harington, C. R., and McCartney, W., *J. Chem. Soc.*, 1929, 892-7.

² Gaddum, J. H., *J. Physiol.*, 1930, **68**, 383.

³ Thompson, W. O., Thompson, P. K., Taylor, S. G., III, Nadler, S. B., and Dickie, L. F. N., *Endocrinology*, 1935, **19**, 14.

⁴ Rapport, D., and Canzanelli, A., *Am. J. Physiol.*, 1933, **103**, 279.

⁵ Niemann, C., and Redemann, C. E., *J. Am. Chem. Soc.*, 1941, **63**, 1549.

⁶ Niemann, C., and Mead, J. F., *J. Am. Chem. Soc.*, 1941, **63**, 2685.

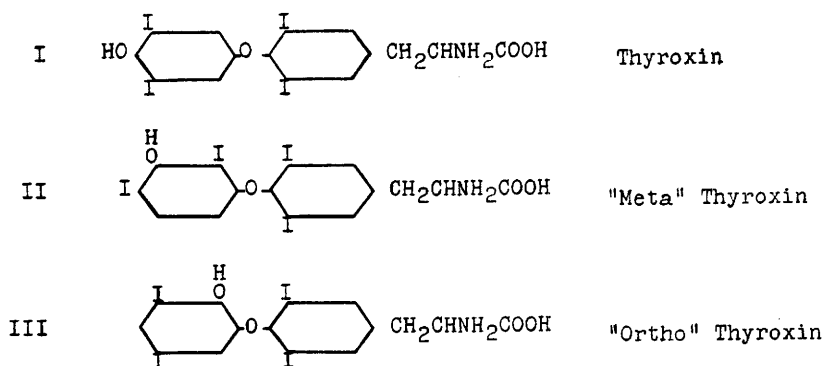
[†] These compounds have been made available to us by Dr. Carl Niemann, of the California Institute of Technology, with whom this was a coöperative research project.

⁷ Boyer, P. D., Evans, R. J., and Phillips, P. H., *J. Pharm. and Exp. Therap.*, 1941, **73**, 176.

TABLE I.
Effect of "Ortho" Thyroxin* on B.M.R. of Thyroidectomized Rat.

Days after injection	Calories per sq m per 24 hrs		
	Rat No. 3 25 mg/kg	Rat No. 4 25 mg/kg	Rat No. 5 50 mg/kg
Thyroidectomized			
B.M.R.	620	880	740
4	950	1100	940
11	740	1030	790
18	640	930	690
25	650	900	690

*dl-3,5-diiodo-4-(3',5'-diiodo-2'-hydroxyphenoxy)-phenylalanine.



Formulae

(3'-5'-diiodo-2'-hydroxyphenoxy)-phenylalanine (III), indicated that this compound had some biological activity. Accordingly, studies were made using thyroidectomized rats. A test was not considered successful unless the thyroidectomized animal had a stable lowered B.M.R. both before and after showing a response to the test compound. The results showed that the ortho thyroxin possessed some activity in moderately large doses, as indicated in Table I. The responses were of the same magnitude as those given by synthetic dl-thyroxin in doses of 0.5-1.0 mg per kg of body weight. Thus the "ortho" thyroxin had an activity approximately 1/25-1/50 that of thyroxin. This compound is the only isomer of thyroxin which has been shown to have some biological activity. The activity of the ortho compound and lack of activity of the meta compound support the hypothesis advanced by Niemann^{5, 6} that thyroxin-like activity is dependent upon the formation of a quinoid structure.

Summary. Two closely related isomers of thyroxin have been tested biologically. Meta thyroxin had little if any effect upon the

B.M.R. of the rat in doses up to 500 mg per kg of body weight, while ortho thyroxin was found to have an activity of 1/25 to 1/50 of that of thyroxin.

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Lysine Requirement of the Chick.

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In a continuation of studies on the quantitative amino acid requirements of the young chick for optimum rate of growth, evidence has been obtained on the level of lysine needed in the diet. The diet used was the same as that reported previously,¹ except for differences in the proteins and amino acids added. Calculations of amino acid levels in the diets are based upon the amino acid composition of proteins as summarized by Calvery.² Such calculations are, of course, subject to revision in the event that any of the cited values should be greatly altered in the future. The fact that lysine is required by the chick has been previously indicated.³

The initial experiment was conducted with zein since this protein contains no appreciable amount of lysine. After some failures in preliminary trials, the manifold deficiencies of zein were corrected by addition of pure amino acids and satisfactory rates of growth of

TABLE I.
Growth-promoting Effects of Lysine Additions to the Zein Chick Diet.

l-(+)-lysine added, %	Avg gain per chick, g	Gain per day, %
0.0	- 6.2	-1.7
0.4	+ 1.0	+0.2
0.8	+33.1	+6.4
Positive control diet	+40.7	+6.9

Each group contained 5 chicks with an average starting weight of 57 g. The experiment lasted 7 days. The positive control diet contained an equal weight of casein in place of zein. No methionine, threonine, tryptophane or lysine was added.

¹ Almquist, H. J., and Mecchi, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **48**, 526.

² Calvery, H. O., *Chemistry of the Amino Acids and Proteins* (edited by C. L. A. Schmidt), Charles C. Thomas, publisher, Baltimore, Maryland, first ed., p. 217.

³ Buckner, G. D., Peter, A. M., Wilkins, R. H., and Hooper, J. J., *Ky. Agr. Exp. Sta. Bul.*, 1919, **220**.