doses of cortisone acetate to immature rats maintained on a synthetic diet resulted in retarded growth, alopecia and death. These effects were largely counteracted by the administration of desiccated whole liver when fed at a level of 10% of the diet. The protective factor(s) in liver was distinct from any of the known B vitamins.

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Further Studies on Anti-Thyroxine Effects of Various Compounds.* (19237)

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The extension by Woolley(1) of the specific metabolic inhibitor principle into thyroxine analogues initiated an interest which has been taken up by other laboratories, reports of whose work have appeared during the past few years. Frieden and Winzler(2) expanded the variety of substituted ethers of 3,5-diiodo-4-hydroxybenzoic acid, demonstrating special activity in 4-benzyloxy-3,5-diiodobenzoic acid and 4-benzyloxy-3,5-diiodophenylalanine in preventing the thyroxine-induced acceleration of tadpole metamorphosis. Maclagan, Sheahan, and Wilkinson(3) noted interference with increased metabolic rates caused by thyroxine in mice when 4-benzyloxy-3,5-diiodobenzoic acid was injected, as well as the dimethyl acetal of 3,5-diiodoanisaldehyde. As was also pointed out by Barker *et al.*(4), the amounts of inhibitors required for mammalian experiments were greater than for amphibia. Cortell(5) was able to cause a considerable reversal of the thyroxine depression of thiouracil-induced hypertrophy of rat thyroid glands by the use of 2',6'-diiodothyronine. Barker and coworkers(4) presented the results of 3 different approaches to quantitative evaluation of thyroxine inhibition, two involving measurement of changes in metabolism and the third employing the depression of response to standard estrogen dosages produced by thyroxine. It was concluded that the thyroidectomized rat maintained at an approximately normal metabolic level by daily injections of 12 μg of DL-thyroxine† offered a highly sensitive preparation for evaluation of antithyroxine effects. This technic was used by Klitgaard and coworkers(6) in their study of iodinated phenoxyacetic acids, several of which were found to antagonize the energy metabolism control of the thyroid hormone, with greatest activity when iodine was in the 2, 4, and 6 positions in the phenyl ring.

The present investigation was concerned with a varied group of substances which either have been reported to show thyroxine inhibition or might be expected to do so.

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† DL-Thyroxine was made available by Dr. K. W. Thompson, Organon, Inc., Orange, N. J.

Experimental methods. The general procedure was that described by Klitgaard *et al.* (6), which should be consulted for details. The effectiveness of thyroxine antagonism was calculated as the percentage of return of the thyroxine-maintained metabolic rate of thyroidectomized rats produced by a daily dose of the compound being studied. These values were obtained by measuring the oxygen consumption 4 weeks or more after thyroidectomy and after the athyroid animals had been maintained on 12 μg of thyroxine per kg body weight per day. Metabolic rates were then determined one week and 2 weeks after commencing administration of the material, and the 2 values averaged unless there was a continuing downward trend, in which case the 2-week value was used, or the experiment continued another week. In order to be as certain as possible of the genuineness of the change, the animal's metabolic rates usually were determined for several weeks after cessation of drug administration, until plateauing had occurred. This value was averaged with the preliminary figure. With the exception of iodothiouracil, which was fed after thorough mixing with dry powdered diet, the substances being tested‡ were injected subcutaneously, in saline, propylene glycol (PG) or sesame oil solution, using 0.5 to 1.0 cc per kg body weight per day. Dosages of specific compounds are shown as number of times isomolar with the dose of 12 μg of thyroxine injected per kg per day to maintain normal metabolic rates in thyroidectomized rats.

Results and discussion. The results obtained are shown in Table I. Each set of data represent 4 animals, since the metabolism apparatus used was set up for this number. Control groups of rats, injected with saline, PG or sesame oil, showed variations from their basal oxygen consumption values of about ±10%. When these data were recalculated as though some potential inhibitor compound were being evaluated, "antagonisms" of —3 to —24% (*i.e.*, "thyroxine-like" action) and +6 to +21% were obtained, indicating that differences of less than 20% can probably be ignored as to be anticipated from spontaneous fluctuations.

Vit. A (synthetic crystalline material administered as the palmitate) revealed unmistakable effects at 300 times isomolar, equivalent to 4416 I.U. of vit. A per kg per day. The depression was somewhat higher at 500x, and considerably so at 1000x, indicating a competitive type of inhibition. The effects were always more marked after 2 weeks of administration than after one, and the metabolic rates invariably remained depressed for at least one week after injections were stopped, a phenomenon probably resulting from hepatic storage of the vitamin. β-Carotene was less effective than the oil solution of vit. A, suggesting that conversion to the biologically active form was required. The basis for these effects must lie outside of the ordinary functions of the vitamin, since the animals were fed a basal diet rich in vit. A; furthermore, the 100x dosage exerted no thyroxine inhibition, even though it furnished 1472 I.U. of vit. A per kg of body weight per day above the amount in the diet. Several reports have previously discussed the ameliorating effects of large quantities of vit. A upon clinical as well as experimental hyperthyroidism(7,8). In addition, the inadequate hepatic conversion of carotene in hypothyroidism has been well established(9), but it is very doubtful that these two processes can be related.

Folic acid was found ineffective, and no effects of vit. B_{12} could be demonstrated con-

‡ Thanks are due the following for furnishing compounds for testing: Dr. M. J. Schiffrin, Hoffmann-LaRoche, Inc., for Vit. A palmitate in oil and in saline, ribofuranoside of 5, 6-dimethylbenzimidazole (Ro 2-3950), 5,6-dimethylbenzimidazole, and N- [4-(4'-hydroxyphenoxy)-phenyl]-glycine (Ro-2-3137); Dr. Cecil R. Kemp, Flint, Eaton and Co., for folic acid and Vit. B_{12}; Dr. R. J. Winzler, University of Southern California, for 4-benzyloxy-3, 5-diiodophenylalanine; Dr. N. F. Maclagan, Westminster Medical School, London, for the 3,5-diiodoanisaldehyde dimethyl acetal and butyl-3, 5-diiodo-4- hydroxybenzoate; Dr. Domenick Papa, Schering Corp., for the rest of the series of 3,5-diiodo-4-hydroxyphenyl substituted compounds; Knoll A.-G. Chemische Fabriken, Ludwigshafen am Rein, Germany, for 3-fluoro-4-hydroxyphenylacetic acid ("Capacin"); Dr. S. C. Wang, State University of Iowa, for 2,4,6-triiodophenoxyacetic acid. Commercial sources were used for the remaining substances.

TABLE I. Effect of Various Compounds on Oxygen Consumption of Thyroxine-Maintained Thyroidectomized Rats.

Inhibitor: Compound	Dosage*	Oxygen consumption, cc O_2/100 g/hr: Pre-T	On T	T + inhib.	Inhib. of T, %
Saline only		71.8	103.7†	96.9	21.3
		69.8	97.4†	103.9	—23.6
Sesame oil only		70.9	101.1†	95.4	18.9
PG only		67.4	99.4†	97.5	6
		70.1	98.3†	99.2	—3.1
Vit. A palmitate in oil solution	100	70.9	100.7†	100	2.3
	100	68.2	90.9	94.6	—16.3
	300	68.2	102.2†	89.1	38.5
	500	70.9	100.7†	92.7	26.8
	500	68.2	102.2†	88.5	40.3
	500	65.8	97.1†	80.4	53.4
	1000	68.2	98.7†	75.8	75.1
Vit. A palmitate in saline emulsion	500	65.8	98.9†	92.5	19.3
	2000	65.8	98.9†	90.1	26.6
β-Carotene	500	68.6	102.1†	102	.3
	1000	68.6	102.1†	86.9	45.4
Folic acid	500	74.6	102	102.7	—2.6
Vit. B_{12}	1*	74.6	83.4	83.8	—4.5
	10	74.6	100.1†	98.2	7.5
	10	74.6	92.4†	86.9	30.9
	10	66.3	96.8†	88.9	25.9
Ribofuranoside of 5,6-dimethylbenzimidazole (R_o 2-3950)	500	69.3	100.1†	95.9	13.6
5,6-Dimethylbenzimidazole	500	66.3	95 †	91.8	11.1
3,5-Diiodotyrosine	500	71.5	91.2	98.5	—37.1
3,5-Dibromotyrosine	500	69.3	96.3†	93.5	10.4
3,5-Diiodo-2-hydroxybenzoic acid	500	74.6	98.9	91.8	29.2
" 4 " "	500	74.6	94.4†	88	32.3
4-Benzyloxy-3,5-diiodo-phenylalanine	100	68	94.3†	89.5	18.3
	500	68	94.3†	95.8	—5.7
3,5-Diiodoanisaldehyde dimethyl acetal	500	70.1	98.6†	100.7	—7.4
	2000	69.8	100.6	93	24.7
Butyl-3,5-diiodo-4-hydroxybenzoate	500	70.7	100.3†	88.7	39.2
	500	73.3	98 †	86.2	47.8
	2000	68.5	103.1†	89.5	39.3
2-(3,5-Diiodo-4-hydroxyphenyl)-benzoic acid	500	65.7	93.4	92.9	1.8
3,5-Diiodo-4-hydroxyphenylacetic acid	500	64.6	97.2	106.5	—28.5
α-Methyl-3,5-diiodo-4-hydroxycinnamic acid	500	70.7	102.3†	93.8	26.9
α-Methyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid	500	70.6	98.6†	81.6	60.7
α-Phenyl-β- " ("Priodax")	500	72.1	96.7	86.9	39.8
	500	66.7	97.5†	93.1	14.3
γ-(3,5-Diiodo-4-hydroxyphenyl)-butyric acid	500	70.7	103 †	92.4	32.8
ω-(3,5- " hexoic "	500	67.8	91.4†	84.1	30.9
ω-(3,5- " capric "	500	69.5	100 †	88.6	37.4
3-Fluoro-4-hydroxyphenylacetic ("Capacin")	500	67.6	96.1†	85	38.9
	2500	66.2	95.9†	88.6	24.6
5-Iodo-2-thiouracil	500	70.9	99.3†	86.8	44
	500	72.9	100.3†	92.4	28.8
	500	70	102.1†	91.7	32.4
	2000	70	102.1†	91.1	34.3
2,4,6-Triiodophenoxyacetic	100	70.8	107.3†	100.9	17.5
	100	73.3	95.7†	93.5	9.8
	300	70.8	108.6†	96.5	32
	300	73.3	95.7†	87.4	27.1
	500	73.3	101.7†	87.4	50.4
Cholesterol	500	70	103 †	80.2	69.1
N-[4-(4'-Hydroxyphenoxy)-phenyl]-glycine (R_o 2-3137)	500	72.9	98.7†	101.6	—11.2

* Doses expressed as molar ratio of inhibitor to thyroxine maintenance dose; latter was 12 μg/kg/d. in all instances except the one indicated, which was 6 μg/kg/d.

† These values are averages of metabolism values after withdrawal and before administration of inhibitor.

vincingly at the low molar ratios employed (1x to 10x). The larger of these two doses involved the use of 250 μg B_{12} per kg per day; larger quantities were not tried. Several laboratories have noted that the growth rates of young rats are adversely affected by inclusion of large amounts of desiccated thyroid substance in the food mixture, an interference which could be removed by the addition of liver to the diet (Ershoff)(10). At various times, the active substance has been reported as folic acid and vit. B_{12}. Although it now seems probable that some other substance may be concerned (Ershoff)(11), another factor could be the known increase in requirement for many substances needed for growth in hyperthyroid rats (Drill)(12). Since our animals were not in an active growth phase, such a complication should not enter. Two products of fragmentation of vit. B_{12}, the ribofuranoside of 5,6-dimethylbenzimidazole (Ro 2-3950) and 5,6-dimethylbenzimidazole, proved without effect at 500x, although the metabolic rates of the animals receiving the latter compound dropped 44% one week after injections had been stopped and rose only slowly over the next three weeks to the pre-injection level.

Because of its relationship to thyroxine, the 3,5-diiodo-4-hydroxyphenyl substitution has been placed in many compounds, a high proportion of which exhibit thyroxine antagonism. Outstanding in this regard are the previously reported 4-benzyloxy-3,5-diiodobenzoic acid(2-4) and N-(3,5-diiodo-4-hydroxybenzoyl)-3,5-diiodotyrosine(4), as well as α-methyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid, butyl-3,5-diiodo-4-hydroxybenzoate, ω-3,5-diiodo-4-hydroxyphenyl)-capric acid and γ-(3,5-diiodo-4-hydroxyphenyl)-butyric acid, results of which are shown in Table I. In contrast, several other compounds also containing this grouping showed little or no activity, including 3,5-diiodotyrosine and 4-benzyloxy-3,5-diiodophenylalanine, the latter reported highly effective in tadpoles by Frieden and Winzler(2). A marked species difference has previously been noted to exist between the sensitivity of tadpole and rat to both thyroxine-like and thyroxine-inhibiting analogues, the former reacting to much smaller amounts that the latter(4,13). This difference may be due partially to the different criteria used for evaluation, namely, metamorphosis in the tadpole and metabolic rate changes in the rat.

2 - (3,5-diiodo-4-hydroxyphenyl) - benzoic acid exhibited no thyroxine inhibition, nor did 3,5-diiodo-4-hydroxyphenylacetic, although the latter falls structurally between 3,5-diiodo-4-hydroxybenzoic and γ-(3,5-diiodo-4-hydroxyphenyl)-butyric acids, both of which exhibited about 33% inhibition. The dimethylacetal of 3,5-diiodoanisaldehyde was found much less effective than was indicated by the results of Maclagan, Sheahan, and Wilkinson (3), that inhibition of 55% of the thyroxine effect was caused by a molar ration of 900x. The butyl-3,5-diiodo-4-hydroxybenzoate exhibited a much more pronounced antagonism than the diiodoanisaldehyde, but appeared less than would be expected from the 82% decrease reported by Sheahan, Wilkinson, and Maclagan(14) for 460x and 56% for 22x. Its antagonism in the present series was non-competitive. The English workers used a single massive thyroxine dose in normal animals, which we have found a considerably less satisfactory technic than the maintenance of thyroidectomized, wherein changes are followed over a longer period of time.

"Capacin" (3-fluoro-4-hydroxyphenylacetic acid), the subject of a favorable report in the German clinical literature(15), was found to exert some non-competitive inhibition. In view of the toxicity of the fluoride ion, such an effect might be due to splitting of the organic fluoride. Cortell(5) found only thyroxine-like activity from fluoroiodothyronines, with F substituted for one or more of the iodines.

5-iodo-2-thiouracil administered orally, mixed in the diet because of its insolubility in the solvents employed, produced an appreciable anti-thyroxine action, which appeared to be non-competitive. 2,4,6-triiodophenoxyacetic acid was found to exhibit an increasing amount of antagonism to thyroxine as the dose was increased. The 50.4% inhibition obtained at 500x was, however, appreciably less than the marked 80% previously reported for this compound (Klitgaard *et al.*)(6).

Because of the well known alterations of

TABLE II. Summary of Most Active Thyroxine Antagonists as Studied in this Laboratory.

Compound	Dosage*	Inhibition, %
3-Iodophenoxyacetic acid	500	56
2,4-Diiodophenoxyacetic acid	500	46
2,6- " "	500	67
2,4,6-Triiodophenoxyacetic acid	500	66
Butyl-3,5-diiodo-4-hydroxybenzoate	500	44
4-Benzyloxy-3,5-diiodobenzoic acid	1000	66
α-Methyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid	500	61
N-(3,5-diiodo-4-hydroxybenzoyl)-3,5-diiodotyrosine	500	82
β-Carotene	1000	45
Vit. A	1000	75
Cholesterol	500	69

* Doses expressed as molar ratio of inhibitor to thyroxine maintenance dose of 12 μg DL-form/kg/d.

cholesterol metabolism in hypo- and hyperthyroidism, the 69% reversal of thyroxine effect produced in the single series thus far attempted is a striking observation. It amplifies the observation of Marx, Meserve, and Deuel(16) that addition of cholesterol to rats' diets containing desiccated thyroid would protect the animals against the deleterious effects of the large quantities of hormone. Further exploration is obviously required.

As a general summary of the thyroxine antagonism work of this laboratory to date, Table II shows those compounds which have exerted about 50% or greater interference by the metabolic technic of survey discussed in this paper. It can be seen that an inhibitor: thyroxine ratio of about 500x is required to produce such an effect, in contrast to ratios in the neighborhood of 10x and 20x, as reported by others(2,13). No clear response has ever been obtained at less than 300x, the very slight effects at 10x with vit. B_{12} (averaging 21% inhibition) being merely suggestive. It is easy to picture a blocking action of compounds containing the 3,5-diiodo-4-hydroxyphenyl grouping in accord with the general principles of specific metabolic inhibition, but no consistent pattern of response has yet emerged covering active and inactive positions in aliphatic or aromatic compounds. A theory of response to iodinated phenoxyacetic acids has been evolved(6), to some extent coordinating the activity of this structure with that of the diiodohydroxyphenyl radicle discussed above. The presence of vit. A and cholesterol in the list of active compounds indicates a variety of structure which may require a still more flexible concept of inhibitor action than any yet proposed. As recently discussed in a review(17), it is still impossible to attribute to thyroxine a specific function in any enzyme system or series of systems, so the locus of action of any thyroxine antagonist must also remain in doubt for the present.

Summary. (1) The study of compouds inhibiting the peripheral metabolic action of thyroxine in thyroidectomized rats has been extended to such substances as vit. A, vit. B_{12}, cholesterol and a series of aliphatics and aromatics carrying the 3,5-diiodo-4-hydroxyphenyl grouping. (2) The compounds found capable of producing approximately 50% inhibition were vit. A, β-carotene, cholesterol, butyl-3,5-diiodo-4-hydroxybenzoic acid, and α-methyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid. Several other substances were found to be less active than these. No compound tested was found clearly active as a thyroxine antagonist at an inhibitor:thyroxine ratio of less than 300.

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Relation of Sperm Morphology to Genotypically Controlled Variations in Fertility. (19238)

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Since the classic investigations of the relation between sperm morphology and impaired fertility made by Williams and Savage(1), Moench(2), and Lagerlof(3), studies of sperm cytology have provoked great interest and some controversy.

In the laboratory mouse a number of mutations have been found, which by their interaction affect the reproductive efficiency of the male in various degrees—from almost complete sterility to almost complete fertility, while their sisters of the same genotype are normal in fertility. The factors responsible for this impairment have been described in several papers from our laboratory(4). Here it need only be recalled that these mutations belong to a series of alleles at or near the T locus in Chromosome IX. Of these alleles 7 have been studied in some detail. They have been normally maintained in balanced lethal lines in which T/t^x is tailless and T/t^u is similarly tailless, whereas crosses between these lines produce compounds $t^u t^x$ distinguished by normal tails and impaired fertility in the males. Examination by Bryson(5) of spermatozoa from males of one of these gene combinations, $t^u t^1$, which was completely sterile, revealed that, although these sterile males copulated normally and produced a large number of spermatozoa, about a quarter of the sperm had abnormally shaped heads, which were less frequently found in fertile males. He also logically concluded that the normal looking spermatozoa of these sterile males were incapable of fertilization. Recently, spermatozoa have been examined from males of 2 other gene combinations, $t^0 t^3$, and $t^1 t^3$. These males, unlike $t^0 t^1$ males, are not quite sterile. These "quasi-sterile" males produce less than 20% the number of young sired by "normal" males. Attempts to correlate percentages of morphologically abnormal spermatozoa with the breeding efficiency of such males reveal that, while one may with some caution distinguish between normal fertile males and $t^0 t^3$ males whose fertility is impaired in various degrees, any attempt to set numerical boundaries to distinguish absolute sterility from quasi-sterility would be highly misleading.

The average percentage of abnormal sperm in fertile controls is 8.18 ± 0.98, in quasi-steriles 32.20 ± 1.14, and in steriles 43.16 ± 3.69. While the difference in the average percentages of abnormals, between quasi-steriles and steriles, is significant, there is an overlap in their ranges. There is the further evidence that there is no correlation between percentages of abnormal sperms and degree of sterility. All one could safely say is that a higher incidence of abnormal sperms indicates lowered fertility, and there is no justification for treating morphology of spermatozoa as the only diagnostic factor for impaired fertility. The source of the alterations in the sperm pathology was sought by the study of spermatogenesis in quasi-sterile and sterile males. No major cytological aberrations were found.

It has become apparent of late that several different anatomic components of the spermatozoon are significant in evaluating fertility. With improved staining technics, it is becoming clear that nuclear and acrosomal ab-