Although this is an attractive hypothesis it is not substantiated by the present study, since there was no correlation between this ratio and the development of cirrhosis. Nevertheless, it is possible that animals developing cirrhosis have a relative inability to absorb or utilize choline, which defect might not be reflected in the ratio of food intake to weight gain.

Although differences in incidence of cirrhosis occurring in Experiments 1 and 2 suggest the influence of environmental factors, nonetheless the relative frequency of cirrhosis in the various strains remained constant. In each series the incidence of cirrhosis was greatest in the Wistars, less in the Sherman, and considerably less in the Long-Evans.

The present experiments suggest that conditions other than food intake or growth rate played a determining role in the varied susceptibility of different strains of rats to cirrhosis. Presumably these conditions are constitutional in nature. The mechanism responsible for these changes is not evident.

Conclusion. The present study reveals differences in strain susceptibility to dietary cirrhosis in the rat. Although environmental factors, such as food intake and growth rate may influence susceptibility to cirrhosis, they do not fully account for the changes observed. It is inferred that inherited traits in some unknown fashion exert an important effect on the susceptibility of the rat to dietary cirrhosis.

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In vitro Incubation of Rat and Rabbit Thyroid with L-Tyrosine-C^{14}, Sodium Iodide-I^{131}, and L-Mono- and Diiodotyrosine-C^{14}-I^{131}.* (30834)

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Reports of the fate of tyrosine-C^{14} added to the media bathing surviving thyroid tissue have been possibly conflicting. Dobyns found that some C^{14} was incorporated into iodothyronine but not iodotyrosine in rabbit thyroid slices(1), but Dillard *et al* recently found that none was converted into thyronine or its analogs by beef thyroid slices or rabbit thyroid homogenate(2). The present communication provides further data bearing on this problem and extends the observations to the fate of labelled iodide, tyrosine, and iodotyrosine in surviving rat and rabbit tissues. The results of these experiments provide further insight into the mechanisms of thyroxine biosynthesis and show that species differences exist in the conversion of the labelled substrates under these conditions.

Methods. The rabbits were adult New Zealand albinos; the rats were adults of the Long-Evans or Sprague-Dawley strains (Table I). Purina Rabbit Chow and Staley's Rockland Rat Diet were fed respectively. Food and tap water were given *ad lib*. The animals were killed by ether, blow on the head, or .22 caliber gunshot through the spine and heart. The thyroids were quickly dissected out and kept on cooled glass. Excess surrounding tissue was quickly trimmed away. In some experiments the glands were minced with a razor or scissors into fragments about 1.0 mm thick, weighed and placed in incubation medium. Homogenization prior to in-

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TABLE I. Summary of Results. See Fig. 1 for definition of terms.

Substrate	Animals			Tissue preparation	Hours incubated	Sample analyzed	Hydrolysis method*	Labelled products	
	Species	No.	Sex					Non-hydrolyzed	Hydrolyzed
Tyrosine-C^{14}	Rabbit	2	♂	minced	3	Tissue & Media	P,P-P,NaOH	TY	TY
"	"	1	♀	"	16	Tissue	P	O, TY	O, TY, Unknown 1
						Media	P	O, TY	O, TY, Unknown 1
"	"	2	♂	"	24	Tissue	P	O, Unknowns 2,3	O, TY, Unknown 1
		1	♀			Media	P	O, TY, Unknowns 1,2,3	O, TY, Unknowns 1,2,3
"	Rat†	2	♂	homogenate	1	Tissue & Media	P	TY	TY
"	" ‡	4	♂	minced	24	Tissue	P	O, TY	O, Unknown 1
						Media	P	O, TY, Unknown 2	O, Unknowns 1,2
Iodide-I^{131}	Rabbit	2	♂	"	2	Tissue & Media	P, P-P	O, I, Unknown 8	O, I, MIT, DIT, Unknown 7
"	"	1	♂	"	6	Tissue	P	O, I, Unknown 9	I, MIT, DIT, Unknown 6
						Media	P	O, I	I
"	"	1	♂	"	14	Tissue	P	O, I, Unknown 9	O, I, MIT, DIT, Unknown 4
						Media	P	O, I	I
"	"	1	♀	"	16	Tissue	P	O, I	I, MIT, DIT
						Media	P	O, I	I
"	"	1	♂	"	22	Tissue	P-P	O	I, MIT, DIT
						Media	P-P	O, I	not analyzed
"	"	2	♂	"	24	Tissue	P	O, I	O, I, MIT, DIT, Unknown 2
		1	♀			Media	P	O, I	I
"	Rat‡	2	♂	"	2	Tissue & Media	P, P-P	O, I, Unknown 8	O, I, MIT, Unknown 5
"	"	1	♂	"	6	Tissue	P	O, I, Unknowns 1,9	O, I, MIT, DIT, T_4, Unknown 3
						Media	P	O, I, Unknown 1	I
"	"	1	♂	"	14	Tissue	P	O, I, Unknown 9	O, I, DIT, T_4, Unknown 4
						Media	P	O, I	I, MIT
"	"	4	♂	"	20	Tissue	P	O, I, Unknown 9	I, MIT, DIT, Unknown 9
						Media	P	O, I	I, Unknown 9
MIT-I^{131}-C^{14}	Rabbit	2	♂	"	2	Tissue & Media	P, P-P	I, MIT-I^{131}-C^{14}	I, MIT-I^{131}-C^{14}
"	Rat‡	2	♂	"	2	Tissue & Media	P, P-P	I, MIT-I^{131}-C^{14}	O-I^{131}, MIT-I^{131}-C^{14}, Unknown 5-C^{14}
DIT-I^{131}-C^{14}	Rabbit	2	♂	"	2	Tissue & Media	P, P-P	O-I^{131}, I, DIT-I^{131}	O-I^{131}, I, DIT-I^{131}-C^{14}
"	Rat‡	2	♂	"	2	Tissue & Media	P, P-P	O-I^{131}, I, DIT-I^{131}	O-I^{131}, I, DIT-I^{131}-C^{14}

* P = Pangestin, P-P = Pangestin-papain. † Long-Evans strain. ‡ Sprague-Dawley strain.

cubation was done in a glass homogenizer chilled in ice.

The buffer used in these experiments was the Krebs-Henseleit Buffer, type A, of the following composition: 4 parts 1.15% KCl, 83 parts 0.9% NaCl, 1 part 2.11% KH_2PO_4, 1 part 3.82% $MgSO_4 \cdot 7H_2O$, 3 parts 1.3% $NaHCO_3$, 18 parts sodium phosphate buffer (110 parts 0.1 M $Na_2HPO_4 \cdot 2H_2O$ and 15 parts M $NaH_2PO_4 \cdot H_2O$), and 5 parts 4.6% glucose.

Five μc of L-tyrosine-HCl uniformly labelled with C^{14}, specific activity 11.8 mc/mM, was added to each reaction vessel. The solution was dried with gentle warmth (37°C) and air stream and stored dry until used.

Doubly labelled tyrosine-I^{131}-C^{14} was prepared by I^{131} labelling of tyrosine-C^{14} by a modification of the method of Roche *et al* (3). To 2.4 ml of carrier-free sodium iodide-I^{131} solution the following were added: 1.0 ml 0.1 M KI, 0.1 ml N HCl, 2.0 ml ethyl ether and 0.2 ml 0.6% H_2O_2. This was shaken occasionally in a glass-stoppered vessel at room temperature. Seven hours later the aqueous phase was removed and discarded and to the ether-iodine phase were added 1.2 ml 5% NH_4OH containing 1.1×10^{-5} gram moles L-tyrosine and 15 μc of L-tyrosine-UL-C^{14}. The mixture was shaken to decolorize ether, shaken overnight and dried in an air stream. The residue was taken up in methanol-ammonia (9:1) and chromatographed on paper in n-butanol-2 N acetic acid. The spots were eluted with methanol-ammonia, rechromatographed and finally eluted with 0.15 N NaOH. This solution was neutralized with N HCl and frozen until used.

Incubations were carried out in a Dubnoff incubator under 95% O_2 and 5% CO_2. After incubation enzymatic hydrolysis was begun in the buffer medium or tissue homogenate by adjusting the pH to 8.0 and adding Pangestin† 5 to 10 mg per 100 mg of tissue. This mixture was usually allowed to incubate at 37°C overnight with occasional stirring. In some experiments papain hydrolysis followed. The pH was brought to 5 with 0.4 M citric acid, and 0.5 ml 0.2 M L-cysteine HCl and 20 mg papain were added. This mixture was incubated 2 hours. After centrifugation of samples 10 to 20 μl of supernates and stable identifying compounds were added to Whatman I paper strips and chromatographed in a descending direction in both n-butanol-2 N acetic acid (1:1) and n-butanol-2 N NH_4OH (1:1) systems. After spraying to identify the stable carriers, radiation intensity on the strips was measured automatically with a Nuclear-Chicago Actigraph II utilizing a a thin-window gas flow detector, a 0.125 in. window slit, a Nuclear-Chicago ratemeter, and a Texas Instrument rectilinear recorder set at a chart speed of 1 in. per min. The ratemeter count rate and time constant settings were chosen to eliminate spurious background excursions in the tracings. In the experiments with doubly labelled compounds, recordings of radioactivity were repeated at intervals up to 18 months after the experiment to determine the relative amount of C^{14} present after decay of the I^{131}. The various experiments are listed in Table I.

Results. A. Incubation with L-tyrosine-C^{14}. Rabbit thyroid did not alter the chemical form of the labelled compound after 3 hours incubation and only free tyrosine-C^{14} appeared on the chromatograms. At 16 and 24 hours, there was incorporation of some of the label into material, presumably protein, which remained at the origin, and into 3 different unknown substances on the chromatograms. No iodotyrosine or iodothyronine was formed. Rat thyroid showed a similar pattern after 24-hour incubation with the exception that only 2 of the unknowns seen in the rabbit were present.

B. Incubation with inorganic iodide-I^{131}. Rabbit thyroid incubated 2 hours had incorporated significant amounts of I^{131} into iodotyrosine and several unknown substances. However, no thyroxine (T_4) or 3,5,3′-triiodothyronine (T_3) was present even after 24-hour incubation. Rat thyroid, on the other hand, had labelled thyroxine after 6 hours incubation; a number of unknown compounds also appeared.

C. Incubation with L-iodotyrosine-I^{131}-C^{14}. There was no formation of labelled thyronine or iodothyronine by either rat or rabbit. Both the MIT-I^{131}-C^{14} and DIT-I^{131}-C^{14} were es-

† Dried pancreas, Difco Laboratories.

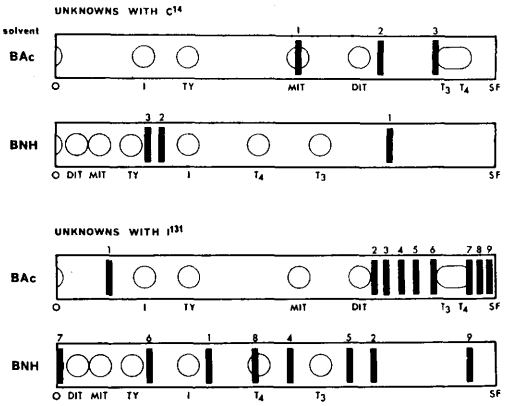

FIG. 1. Chromatogram loci. Open areas correspond to known compounds and solid bars indicate unknown compounds. Abbreviations: BAc = butanol-acetic acid solvent system; BNH = butanol-ammonia solvent system; O = origin; SF = solvent front; TY = tyrosine; I = iodide; MIT = 3-monoiodotyrosine; DIT = 3,5-diiodotyrosine; T_3 = 3,5,3'-triiodothyronine; T_4 = thyroxine. The unknowns are numbered separately for C^{14} and I^{131}. The I^{131} unknown number 3 was not located on the BNH chromatograms.

sentially 100% pure at incubation as shown by control chromatograms. Moderate deiodination occurred in all the incubations. In the case of MIT-I^{131}-C^{14}, the rat at 2 hours had converted the labelled material into a doubly labelled unknown. The fact that there was C^{14} activity in hydrolyzed tissue and not in non-hydrolyzed tissue may have been the result of a variance in the size of the aliquot or may indicate that the non-hydrolyzed material had bound the DIT to a non-soluble structure. Only the supernate of centrifuged samples was applied to chromatograms.

Table I lists the experiments and gives the results of each. Fig. 1 identifies the location of known and unknown compounds on the chromatograms.

Discussion. These results support the conclusion of Dillard *et al* that thyroid tissue does not synthesize thyronine from free tyrosine. Dobyns' findings were not confirmed. None of the 3 unknowns produced in the C^{14}-tyrosine experiments match the Rf values of thyronine for the two solvent systems used. In addition there was no incorporation of tyrosine into iodothyronine or iodination to form MIT or DIT. The double labelling experiments with iodotyrosines also showed no synthesis of iodothyronines utilizing the label.

On the other hand iodide in the media is incorporated into iodotyrosines of the media and in the case of the rat, iodothyronine. It seems most evident, therefore, that the steps of synthesis of iodothyronines are carried out with amino acids fixed in peptide chains. The nature of the last steps in thyroxine synthesis remains obscure.

Further studies of the *in vitro* system with iodide-I^{131} may reveal experimental conditions which favor iodothyronine formation in rabbit thyroid tissue. At present the cause of this difference between the two species studied is unknown.

Summary. Incubation of surviving rat and rabbit thyroid tissue in media containing labelled L-tyrosine, MIT or DIT failed to result in incorporation of the label into iodothyronines. When iodide-I^{131} was present in the media, the label was incorporated into iodothyronine by rat but not by rabbit thyroid tissue. The cause of this species difference is not known.

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