Biochem. J. (1968) **109**, 325
Printed in Great Britain

The Mechanism of Thyrotrophin Action in Relation to Lipid Metabolism in Thyroid Tissue

By T. W. SCOTT, S. C. MILLS AND N. FREINKEL
Thorndike Memorial Laboratory and Second and Fourth (Harvard) Medical Services, Boston City Hospital and Department of Medicine, Harvard Medical School, Boston, Mass. 02118, *U.S.A., and the Commonwealth Scientific and Industrial Research Organization, Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia, and the Department of Medicine, Northwestern University, Chicago, Ill.* 60611, *U.S.A.*

(*Received* 26 *March* 1968)

1. The effects of thyrotrophin *in vitro* on the incorporation of [^{14}C]-glucose, -glycerol, -palmitate and -oleate into the lipids of thyroid tissue were examined. 2. Thyrotrophin increased the incorporation of these ^{14}C-labelled precursors into phosphatidylinositol specifically. 3. Thyrotrophin also increased the proportion of ^{14}C radioactivity from labelled glucose, glycerol, palmitate and oleate incorporated into the 1,2-diglycerides. 4. The addition of thyrotrophin to thyroid slices for 10min., after 2hr. of prelabelling with [^{14}C]glycerol, also increased the proportion of ^{14}C radioactivity incorporated into the 1,2-diglyceride fraction. 5. After incubation of thyroid tissue with [1-^{14}C]palmitate, thyrotrophin caused a two- to three-fold increase in the specific radioactivity of palmitate isolated from phosphatidylinositol and 1,2-diglycerides. In contrast, the specific radioactivity of palmitate isolated from the choline and ethanolamine phosphoglycerides, 1,3-diglycerides and triglycerides was not increased by thyrotrophin.

A variety of endocrine glands, when stimulated *in vitro*, exhibit characteristic increases in phospholipid metabolism. This has been demonstrated in thyroid (Freinkel, 1957; Scott, Jay & Freinkel, 1966), adrenal (Hokin, Hokin, Benfey & Hokin, 1958), anterior pituitary (Hokin, Saffran, Schally & Zimmerman, 1958), pancreas (Hokin & Hokin, 1956) and other non-endocrine tissues, e.g. polymorphonuclear leucocytes (Karnovsky, 1962), rat superior cervical ganglia (Larrabee, Klingman & Leicht, 1963) and avian salt gland (Hokin & Hokin, 1964). Further, this stimulation appears to be specific for the acidic phospholipids, particularly phosphatidylinositol. In an attempt to obtain further information on the interrelationships between phosphatidylinositol and other lipids, thyroid tissue was incubated with ^{14}C-labelled precursors, namely glucose, glycerol and free fatty acids, and the pattern of labelling was studied, both in the presence and absence of TSH.* The results indicate that the selective stimulation of phospholipid metabolism by TSH is also probably associated with a change in the equilibrium between the diglyceride and triglyceride fractions. A preliminary report of part of these findings has already been published (Freinkel & Scott, 1964).

* Abbreviation: TSH, thyrotrophin.

MATERIALS AND METHODS

Incubation of thyroid tissue. Dog and sheep thyroid glands were quickly removed from mature animals, sliced and incubated in Krebs–Ringer bicarbonate buffer containing 1mM-phosphate buffer, pH7·4, in an atmosphere of O_2+CO_2 (95:5), by using general techniques described by Freinkel (1957) and Scott *et al.* (1966). The TSH [obtained from the National Institute of Health, Bethesda, Md., U.S.A. (Bovine NIH-TSH-B_2; Ovine NIH-TSH-S_2)] was dissolved in Krebs–Ringer bicarbonate buffer containing 0·2% gelatin and used at a final concentration of 0·1 U.S.P. unit/ml. of medium. Timed exposure of the tissue to TSH (i.e. acute effects) was carried out as described by Scott *et al.* (1966). Where the tissue was incubated with ^{14}C-labelled fatty acid, the Krebs–Ringer bicarbonate buffer containing albumin–fatty acid complex was prepared by using the procedures for preparation of complex B described by Kessler, Demeny & Sobotka (1967).

Lipid extraction and fractionation. After incubation, the tissue was immediately frozen by using liquid N_2 or solid CO_2. The frozen material was homogenized in 10ml. of chloroform–methanol (2:1, v/v) and filtered through fine glass wool. The filtrate was washed once with water (2·0ml.) and thrice more with theoretical 'Folch' upper-phase Ca^{2+} (Folch, Lees & Sloane-Stanley, 1957). Neutral lipids were separated from phospholipids by silicic acid (Mallinkrodt) column (10cm.×2cm.) chromatography; neutral lipids were eluted with chloroform (50–60ml.) and phospholipids with methanol (50–60ml.). Samples of each

fraction were assayed for radioactivity as described by Scott *et al.* (1966) with an automatic liquid-scintillation counter (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.).

Fractionation of neutral lipids. Individual neutral lipids were initially separated on thin-layer plates (silica gel G, 250μ thickness; E. Merck A.-G., Darmstadt, Germany) developed with the solvent system light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (90:10:1, by vol.). However, a more effective resolution between the 1,2- and 1,3-diglycerides was obtained by using larger plates (40cm.× 20cm.) and these were developed in two solvent systems run in the same direction [solvent I: di-isopropyl ether–acetic acid (45:2, v/v) for 45min.; solvent II: light petroleum (b.p. 60–80°)–diethyl ether–acetic acid (90:10:1, by vol.) for 90min.]. Individual neutral lipids were localized by radioautography and aspirated from the plate by using the device of Goldrick & Hirsch (1963). The gel, containing the radioactive glyceride, was either counted directly in scintillation vials (Scott *et al.* 1966) or the lipid was eluted by shaking the silica gel with ethanol–chloroform–water (10:3:2, by vol.) for subsequent fatty acid or glycerol analysis.

Fractionation of phospholipids. After incubation of the tissue with [^{14}C]glucose or [^{14}C]glycerol, the individual phospholipids were separated chromatographically after mild alkaline degradation (Dawson, Hemington & Davenport, 1962). The resulting water-soluble glyceryl-phosphoryl derivatives were localized by radioautography and radioactivity was determined as described by Scott *et al.* (1966). After incubation of the tissue with [^{14}C]palmitic acid or [^{14}C]oleic acid, the individual phosphoglycerides were separated into lipid classes by thin-layer chromatography. Silica gel G plates (500μ thickness) were developed in chloroform–methanol–water–acetic acid (65:50:4:1, by vol.) and the lipid fractions were located by radioautography. The gel containing the phospholipid was either aspirated directly into counting vials for radioactive assay (Scott *et al.* 1966) or, alternatively, the lipid was eluted by shaking the gel with ethanol–chloroform–water (10:3:2, by vol.) for determination of the specific radioactivity of the component fatty acids.

Gas–liquid chromatography. The various lipid classes (separated by thin-layer chromatography) were saponified and fatty acids extracted by using the procedures described by Scott, Setchell & Bassett (1967). Methyl esters of the fatty acids were prepared by the method of Schlenk & Gellerman (1960) and were separated on a 5ft. column (×¼in. internal diam.) of diethylene glycol succinate (17%, w/w) on 80–100-mesh Chromosorb W with a gas chromatograph (Aerograph 200 series; Wilkins Instrument and Research Inc., Walnut Creek, Calif., U.S.A.). The chromatograph was fitted with a stream splitter, which allowed approx. 50% of the effluent gas containing the ^{14}C-labelled fatty acid to pass directly into the flame detector, while the residual gas was diverted away and the radioactive methyl ester was collected into anthracene as described by Karmen, Giuffrida & Bowman (1962). Methyl [1-^{14}C]palmitate of known specific radioactivity was used to calculate the specific radioactivity of the isolated fatty acids.

Other analytical techniques. Phosphorus was determined by the method of Fiske & Subbarow (1925). Glycerol was determined in lipid extracts as described by Hanahan & Olley (1958).

Radioactive chemicals. [U-^{14}C]Glucose and [1,3-^{14}C]-glycerol were obtained from the New England Nuclear Corp., Boston, Mass., U.S.A. [1-^{14}C]Palmitate and [1-^{14}C]-oleate were obtained from The Radiochemical Centre, Amersham, Bucks.

Statistical analyses. The binomial distribution with $P=0{\cdot}5$ was used to assess whether TSH had an effect in a significant proportion of the experiments. Probability values were obtained from the Tables of *Binomial Probability Distribution*, National Bureau of Standards Applied Mathematic Series 6 (1950).

Table 1. *Effect of TSH on the incorporation of* ^{14}C *from* [U-^{14}C]*glucose into the phospholipids of dog thyroid slice*

Thyroid slices (100–200mg.) were incubated in Krebs–Ringer bicarbonate buffer containing glucose (5μC, 2mg./ml.) at 38° in an atmosphere of O_2+CO_2 (95:5). TSH (0·1 U.S.P. unit/ml.) or Krebs–Ringer bicarbonate buffer containing gelatin was added to each vessel at the commencement of incubation. Phospholipid ^{14}C radioactivity is that present in the glycerol part of the molecule. In all Tables radioactivity is expressed as ^{14}C counts/min./g. of tissue, based on initial wet weight. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

		TSH absent					TSH present				
Expt. no.	Incubation time (hr.)	Phospholipid ^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) PC	PE	PI	PS	Phospholipid ^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) PC	PE	PI	PS
1	3	11301	65·2	18·6	10·2	6·0	16090	68·6	14·9	12·8	3·7
2	3	9217	58·4	26·8	9·4	5·4	21447	64·6	17·7	13·7	4·0
3	3	8520	68·6	12·8	12·3	6·3	12641	60·9	13·2	20·6	5·3
4	3	8277	66·0	15·1	15·6	3·3	12845	58·5	14·6	18·8	8·1
5	2	5411	71·0	9·4	16·7	2·9	5857	66·3	7·5	23·7	2·5
6	2	5987	63·0	15·4	19·6	2·0	5392	53·1	11·8	33·0	2·1

Table 2. *Effect of TSH on the incorporation of* ^{14}C *from* [U-^{14}C]*glucose into the neutral lipids of dog thyroid tissue*

Details of tissue incubations are described in Table 1. Abbreviations: MG, monoglycerides; DG, diglycerides (1,2+1,3 forms); TG, triglycerides. Residual radioactivity [i.e. 100−(TG+DG+MG)] represents ^{14}C in cholesterol, cholesterol esters and free fatty acids, unless the components are mentioned. In Expts. 2 and 4 oleic acid (1 μmole/ml.) was added.

		TSH absent					TSH present				
Expt. no.	Incubation time (hr.)	Neutral-lipid ^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) MG	DG	TG	TG/DG ratio	Neutral-lipid ^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) MG	DG	TG	TG/DG ratio
1	3	4325	5·1	15·7	72·5	4·6	6918	3·5	16·3	72·5	4·4
2	3	24729	0·7	4·7	92·7	19·7	24219	0·8	6·6	91·8	13·9
3	3	4242	2·1	14·1	81·3	5·8	5283	3·1	17·8	77·2	4·3
4	3	9335	0·5	7·1	91·6	12·9	11584	0·6	8·5	90·6	10·7
5	2	3877	0·8	11·6	84·9	7·3	3926	0·8	13·8	80·3	5·8
6	2	2177	2·3	24·2	61·8	2·6	1865	3·1	31·9	55·2	1·7

Table 3. *Effect of TSH on the incorporation of* [1,3-^{14}C]*glycerol into the lipids of dog thyroid slices*

Thyroid slices were incubated for 2hr. in Krebs–Ringer bicarbonate buffer containing glycerol (5 μc, 46 μg./ml. of medium). TSH (0·1 U.S.P. unit/ml.) was added at the commencement of incubation. Residual radioactivity is present in other phospholipids. Abbreviations are as defined in Tables 1 and 2.

	TSH absent					TSH present				
	^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) PC	PE	PI	PS	^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) PC	PE	PI	PS
(*A*) Phospholipids	70615	66·5	11·0	13·0	4·0	70663	43·6	8·1	39·7	3·7
	^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) MG	DG	TG	TG/DG ratio	^{14}C radioactivity (counts/min./g.)	Distribution of ^{14}C (%) MG	DG	TG	TG/DG ratio
(*B*) Neutral lipids	10758	5·2	28·2	66·1	2·34	7485	5·2	48·0	46·3	0·96

RESULTS

Effect of TSH on the incorporation of ^{14}C *from* [U-^{14}C]*glucose into the lipids of thyroid slices.* Absolute quantities of individual phospholipids and neutral lipid moieties in the thyroid of the sheep (Freinkel, 1957, 1958, 1964) and dog (Scott *et al.* 1966) have been previously reported. The incorporation of ^{14}C from [U-^{14}C]glucose into the glycerol portion of the total phospholipids was usually increased by the addition of TSH (Table 1, $P=0·07$). However, the most striking change occurred in the distribution of ^{14}C radioactivity in the individual phospholipids, where TSH invariably increased the proportion of the label in phosphatidylinositol. To offset this, there were corresponding decreases in the proportions of radioactivity in the other phospholipids, but this was not confined to any particular component. Phosphatidylcholine always contained most of the ^{14}C incorporated from [^{14}C]glucose (Table 1).

The addition of TSH increased the incorporation of ^{14}C from [U-^{14}C]glucose into the total neutral lipid in only three out of six experiments. However, the hormone invariably increased the proportion of radioactivity in the diglyceride fraction (Table 2, $P=0·02$). The triglyceride/diglyceride ratio was decreased by adding TSH (Table 2, $P=0·01$). In Expts. 2 and 4 (Table 2) oleic acid (1 μmole/ml.) was added and this substantially increased the incorporation of ^{14}C from [^{14}C]glucose into the neutral lipids, particularly triglyceride, but the effect of TSH in lowering the triglyceride/diglyceride ratio was still observed (Table 2). TSH had no consistent effect on the proportion of radioactivity in the monoglyceride fraction.

Effect of TSH on the incorporation of [1,3-^{14}C]*glycerol into lipids of thyroid slices.* After incubation

Table 4. *Effect of 'acute' exposure to TSH on the distribution of* [^{14}C]*glycerol incorporated into the neutral lipids of thyroid slices*

Thyroid slices (dog 100–150mg., sheep 400–500mg.) were incubated for 2hr. in Krebs–Ringer bicarbonate buffer containing glycerol (5μc, 46μg./ml. of medium). After 2hr. TSH (0·1 U.S.P. unit/ml.) or Krebs–Ringer bicarbonate buffer containing gelatin was added and the tissues were incubated for a further 10min., except in Expt. 9 where the vessels were incubated for a further 60min. Individual neutral lipids were separated by thin-layer chromatography. Residual radioactivity [100−(DG+TG)] was present in the other neutral glycerides. Abbreviations are as defined in Table 2.

		TSH absent			TSH present		
		Distribution of ^{14}C (%)			Distribution of ^{14}C (%)		
Species	Expt. no.	DG	TG	TG/DG ratio	DG	TG	TG/DG ratio
Dog	1	15·8	81·6	5·2	31·5	64·3	2·1
	2	29·2	65·3	2·2	37·2	56·2	1·5
	3	43·2	50·1	1·2	46·4	46·5	1·0
	4	30·7	61·6	2·0	41·9	49·8	1·2
	5	33·7	58·6	1·7	40·6	42·1	1·0

		Distribution of ^{14}C (%)					Distribution of ^{14}C (%)				
		1,2-DG	1,3-DG	TG	TG/1,2-DG ratio	TG/1,3-DG ratio	1,2-DG	1,3-DG	TG	TG/1,2-DG ratio	TG/1,3-DG ratio
Sheep	6	23·3	4·3	64·1	2·8	14·9	28·6	5·0	57·8	2·0	11·6
	7	15·1	5·7	73·5	4·9	12·9	16·3	5·2	72·3	4·4	13·9
	8	17·1	3·3	73·3	4·3	22·2	17·2	3·2	73·2	4·2	22·9
	9	7·6	2·6	85·4	11·2	32·8	13·9	3·0	78·0	5·6	26·0

of dog thyroid slices with [^{14}C]glycerol, the proportions of ^{14}C radioactivity in the individual phospholipids were basically similar to those observed with [U-^{14}C]glucose, with phosphatidylcholine accounting for most of the radioactivity (Table 3). Addition of TSH substantially increased the proportion of ^{14}C in phosphatidylinositol, without affecting the amount of [^{14}C]glycerol incorporated into the total phosphoglycerides (Table 3). Moreover, TSH also dramatically increased the proportion of ^{14}C radioactivity in the diglyceride fraction, with a corresponding decrease in the triglycerides. Thus the hormone decreased the triglyceride/diglyceride ratio in a manner analogous to that observed when thyroid tissue was incubated with [U-^{14}C]glucose. This effect of the hormone was in no way associated with labelling in the fatty acids, because saponification of the glycerides revealed that less than 0·5% of the [^{14}C]glycerol incorporated was in the fatty acid fraction.

Effects of 'acute' exposure to TSH on the distribution of [^{14}C]*glycerol incorporated into thyroidal neutral lipids.* After 2hr. incubation with [1,3-$^{14}C_2$]-glycerol, a brief exposure of the tissue to TSH resulted in an increase in the proportion of ^{14}C radioactivity in the diglyceride fraction and a decrease in the triglycerides; this led to a decrease in the triglyceride/diglyceride ratio (Table 4). Further analysis with sheep thyroid tissue suggests that the increase in the proportion of radioactivity was principally in the 1,2-diglyceride fraction, because no consistent increase was observed in the 1,3-diglyceride fraction (Table 4). The ratio of the proportion of ^{14}C in the triglycerides and 1,2-diglycerides was lowered by TSH (Table 4, $P=0·03$), whereas the ratio of the proportion in the triglycerides and 1,3-diglycerides was not consistently decreased. Addition of TSH (for 60min. after prelabelling with [^{14}C]glycerol) increased the specific radioactivity of the glycerol in the 1,2-diglycerides (Expt. 9, Table 4; control 594 versus treated 1176counts/min./μg. of glycerol), whereas there was a slight decrease in the specific radioactivity of the glycerol in the 1,3-diglycerides (control 286 versus treated 238counts/min./μg. of glycerol) and triglycerides (control 656 versus treated 473counts/min./μg. of glycerol).

Effect of TSH on the incorporation of ^{14}C*-labelled fatty acids into the lipids of sheep thyroid slices.* During incubation of sheep thyroid slices in Krebs–Ringer bicarbonate buffer (without glucose) containing albumin–free fatty acid complexes, TSH increased the incorporation of both [1-^{14}C]-palmitate and [1-^{14}C]oleate into the total phospholipids (Table 5, $P=0·05$); this finding confirmed the observation by Freinkel (1964). Further fractionation of the phospholipids by thin-layer chromatography revealed that there was at least a twofold increase in the proportion of radioactivity in phosphatidylinositol, with corresponding decreases

Table 5. *Effect of TSH on the incorporation of* [1-^{14}C]*oleic acid and* [1-^{14}C]*palmitic acid into the phospholipids of sheep thyroid slices*

Thyroid slices (400–500mg.) were incubated for 3hr. in Krebs–Ringer bicarbonate buffer containing a complex of ^{14}C-labelled fatty acid with bovine serum albumin (Armour fraction V) (24mg./ml.). TSH (0·1 U.S.P. unit/ml.) was added at the commencement of incubation. Individual phospholipids were separated into classes by thin-layer chromatography.

		TSH absent					TSH present				
			Distribution of ^{14}C (%)					Distribution of ^{14}C (%)			
Fatty acid	Expt. no.	Total ^{14}C radioactivity (counts/min./g.)	Choline phospho-glycerides	Phospha-tidyl-inositol	Ethanol-amine phospho-glycerides	Other phospho-lipids	Total ^{14}C radioactivity (counts/min./g.)	Choline phospho-glycerides	Phospha-tidyl-inositol	Ethanol-amine phospho-glycerides	Other phospho-lipids
Oleic acid (0·52μmole/ml.)	1	591000	62·5	6·9	22·2	8·4	685000	51·0	19·3	22·8	6·9
Oleic acid (0·92μmole/ml.)	2	300000	56·6	9·4	13·6	20·4	444000	55·8	15·9	10·3	18·0
Palmitic acid (0·86μmole/ml.)	3	784000	70·7	7·2	3·7	18·4	1336000	64·9	13·0	5·8	16·3
Palmitic acid (0·86μmole/ml.)	4	2409000	68·3	7·0	9·0	15·7	2748000	57·8	13·8	10·0	18·4

Table 6. *Effect of TSH on the incorporation of* [1-^{14}C]*oleic acid and* [1-^{14}C]*palmitic acid into the neutral lipids of sheep thyroid slices*

Thyroid tissue was incubated as described in Table 5. Individual neutral lipids were separated by thin-layer chromatography. Abbreviations: FFA, free fatty acids: others as defined in Table 2.

		TSH absent							TSH present						
			Distribution of ^{14}C (%)							Distribution of ^{14}C (%)					
Fatty acid	Expt. no.	Total ^{14}C radioactivity (counts/min./g.)	FFA	MG	DG		TG	TG/DG ratio	Total ^{14}C radioactivity (counts/min./g.)	FFA	MG	DG		TG	TG/DG ratio
					1,2-DG	1,3-DG						1,2-DG	1,3-DG		
Oleic acid	1	949000	66·4	0·1	2·7		29·5	10·9	1043000	45·8	0·9	5·7		46·8	8·2
Oleic acid	2	1106000	72·3	0·2	1·8	0·9	24·0	8·9	1086000	73·1	0·2	2·7	0·9	22·2	6·2
Palmitic acid	3	1652000	64·4	0·5	5·8	1·5	26·1	3·6	1800000	69·2	0·1	6·8	0·9	21·9	2·8
Palmitic acid	4	2841000	59·4	3·5	5·1	2·6	29·4	3·8	2942000	60·0	3·0	6·4	2·3	28·3	3·3

in the proportion of the radioactivity present in the other phosphoglyceride fractions, particularly the choline phospholipids. However, in both the control and hormone-treated tissues the choline phosphoglyceride fraction accounted for more than 50% of the ^{14}C radioactivity incorporated (Table 5).

TSH had very little effect on the incorporation of either [^{14}C]oleate or [^{14}C]palmitate into the total neutral lipids (Table 6, $P > 0{\cdot}1$). On the other hand, further analysis revealed that the proportion of ^{14}C radioactivity in the diglyceride fraction, in particular the 1,2-diglyceride form, was consistently increased by the hormone (Table 6, $P = 0{\cdot}05$). In Expt. 1 (Table 6) the proportion of ^{14}C radioactivity in the triglyceride fraction from the treated slices was abnormally high (perhaps owing to contamination from the free fatty acid fraction), but, despite this, the ratio of the proportion of ^{14}C in the triglyceride and diglyceride was still decreased by hormonal treatment. Most of the ^{14}C radioactivity in the neutral lipid fraction was present as free fatty acids, but its relative proportion was not consistently affected by hormonal supplementation (Table 6). The proportion of ^{14}C radioactivity in the monoglyceride fraction, which only accounted for less than 1% of the total, was not consistently affected by TSH.

Effect of TSH on the specific radioactivity of palmitic acid isolated from sheep thyroidal lipids. TSH had no effect on the specific radioactivity of the palmitic acid in the choline or ethanolamine phosphoglycerides, but it did cause a threefold increase in the specific radioactivity of palmitic acid isolated from the phosphatidylinositol fraction (Table 7). Similarly, in the neutral glycerides, the hormone caused a threefold increase in the specific radioactivity of the palmitic acid in the 1,2-diglyceride fraction, but there was a decrease in its specific radioactivity in the 1,3-diglyceride and triglyceride fractions (Table 7). TSH increased the specific radioactivity of the palmitic acid isolated from the free fatty acid fraction, but the proportional increase was substantially smaller than that observed in the 1,2-diglycerides and phosphatidylinositol. In the control thyroid slices, the specific radioactivity of the palmitic acid in the 1,2-diglyceride fraction was slightly less than that observed in the phospholipids, but was much higher than the specific radioactivity of palmitic acid isolated from the triglycerides (Table 7). In the hormone-treated tissue, the specific radioactivity of palmitic acid in the 1,2-diglyceride was considerably greater than that isolated from the choline or ethanolamine phosphoglycerides, but was still below the specific radioactivity of palmitic acid in phosphatidylinositol.

Table 7. *Effect of TSH on the specific radioactivity of the palmitic acid fraction isolated from thyroidal lipids after incubation with* [1-^{14}C]*palmitic acid*

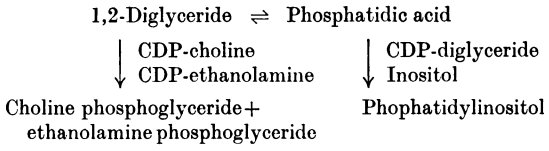
After incubation of thyroid slices (as described in Table 5), the individual lipids were separated by thin-layer chromatography. The lipids were eluted from the gel and saponified to isolate the fatty acid fraction. Individual fatty acid methyl esters were separated by gas–liquid chromatography and specific radioactivities obtained as described in the text.

	Specific ^{14}C radioactivity (counts/min./μg. of acid)	
	TSH absent	TSH present
(*A*) Phospholipids		
Choline phosphoglycerides	650	698
Ethanolamine phosphoglycerides	523	508
Phosphatidylinositol	738	2150
(*B*) Neutral lipids		
Free fatty acids	2610	3060
1,2-Diglycerides	474	1540
1,3-Diglycerides	673	418
Triglycerides	148	88

DISCUSSION

The present results clearly demonstrate that TSH increases the incorporation of [^{14}C]-glucose, -glycerol, -oleate and -palmitate into phosphatidylinositol selectively in thyroid slices. This specific response is similar to that reported for the effect of TSH on the incorporation of [^{32}P]orthophosphate into thyroidal phospholipids (Freinkel, 1957; Scott *et al.* 1966), or [^{14}C]inositol into thyroidal monophosphoinositide (Freinkel, 1960). Moreover, the hormonal stimulation of phosphatidylinositol metabolism is similar to that observed in many endocrine (Hokin & Hokin, 1963*a*) and non-endocrine tissues (Karnovsky, 1962) when functional activation is induced. Despite the recent speculations on the possible physiological functions of phosphatidylinositol (see Scott *et al.* 1966; Hokin & Huebner, 1967), it still remains an enigma as to why the turnover of that particular phosphatide is selectively stimulated.

However, perhaps of equal interest is the capacity of TSH to increase the proportion of ^{14}C radioactivity from labelled glucose, glycerol or fatty acids incorporated into the diglycerides, particularly the 1,2-diglyceride form. This stimulation tends to create a new dynamic equilibrium between the diglyceride and triglyceride fractions, and the net result is probably an increase in the turnover of diglycerides, which are active precursors for phospholipid biosynthesis (Kennedy, 1961). Further, it is puzzling why the specific radioactivity of the palmitic acid isolated from the choline and ethanol-



Scheme 1.

amine phosphoglyceride fraction is not increased by TSH (after incubation with [^{14}C]palmitate), when there is a threefold increase in the specific radioactivity of palmitic acid in the diglyceride fraction. From the accepted sequences of phosphoglyceride synthesis (Scheme 1) one would expect TSH to increase the specific radioactivity of palmitic acid in the choline and ethanolamine phospholipids. On the other hand, the increase in specific radioactivity of palmitic acid isolated from phosphatidylinositol is of the same order of magnitude as that observed in the 1,2-diglycerides. This may imply that phosphatidic acid is a common precursor for both diglyceride and phosphatidylinositol. Alternatively, it may also suggest that the 1,2-diglyceride is converted into phosphatidic acid [the reaction is catalysed by diglyceride kinase, which is present in many tissues, including erythrocyte membranes (Hokin & Hokin, 1963*b*) and pancreas (Prottey & Hawthorne, 1967)], which is then converted into phosphatidylinositol via the CDP-diglyceride intermediate. However, it is not feasible at this stage to eliminate completely the possibility that the increased diglyceride labelling could result from the hydrolysis of phosphatidylinositol by the action of phosphoinositide inositol phosphohydrolyase, as this pathway does exist in thyroid tissue (T. W. Scott, R. M. C. Dawson & N. Freinkel, unpublished work). Since the specific radioactivity of palmitic acid isolated from phosphatidylinositol is above that of the palmitic acid in the 1,2-diglyceride fraction, such a suggestion is not unreasonable. However extreme caution must be exercised in interpreting these results, because the direct acylation of lysophosphoglycerides (Lands, 1960; Keenan & Hokin, 1964) could also increase the specific radioactivity of the fatty acid isolated.

Irrespective of whether the increase in the proportion of labelling in the 1,2-diglyceride fraction is a result of increased synthesis or breakdown of glycerides, the question still remains why there is not an increase in the choline and ethanolamine phosphoglycerides. One possibility is that the specific radioactivity of palmitic acid isolated from the total choline and ethanolamine phosphoglycerides may not be representative of its specific radioactivity in an ethanolamine or choline phospholipid with a specific fatty acid composition. In fact, Rowe (1960) and Collins (1963) have shown that kephalin and lecithin fractions with different fatty acid composition turn over at different rates. Alternatively, there may be a fraction of the 1,2-diglycerides (not in equilibrium with the remaining diglyceride pool) that is located specifically in a lipoprotein membrane that is directly associated with the rapid cytostructural changes induced by TSH (Wetzel, Spicer & Wollman, 1965; Seljelid, 1967). These workers have shown that administration of TSH into rats causes a specific migration of lysosomes towards the apex of the follicular cell. Further, rapid membrane activity, with the formation of pseudopodia-like processes, occurs along the apical surface of the cell (Wetzel *et al.* 1965; Seljelid, 1967). It is tempting to speculate that the increased proportion of labelling in the phosphatidylinositol and 1,2-diglyceride fractions that is observed within as little as 10 min. after the introduction of TSH may be confined to such immediate cytostructural realignments in selected regions.

This work was supported in part by Research Grants A-1571 and AM-10699 and Training Grants A-5060 and AM-5071 from the U.S. Public Health Service. The authors are grateful to Dr R. M. C. Dawson for helpful criticisms of the manuscript.

Requests for reprints should be addressed to: T. W. Scott, C.S.I.R.O., Division of Animal Physiology, The Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia.

REFERENCES

Collins, F. D. (1963). *Biochem. J.* **88**, 319.
Dawson, R. M. C., Nemington, N. & Davenport, J. B. (1962), *Biochem. J.* **84**, 497.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Freinkel, N. (1957). *Endocrinology*, **61**, 448.
Freinkel, N. (1958). *Biochem. J.* **68**, 327.
Freinkel, N. (1960). *Endocrinology*, **66**, 851.
Freinkel, N. (1964). In *Metabolism and Physiological Significance of Lipids*, p. 455. Ed. by Dawson, R. M. C. & Rhodes, D. N. London: John Wiley and Sons (Inc.) Ltd,
Freinkel, N. & Scott, T. W. (1964). *Nature, Lond.*, **204**, 1313.
Goldrick, B. & Hirsch, J. (1963). *J. Lipid Res.* **4**, 483.
Hanahan, D. J. & Olley, J. N. (1958). *J. biol. Chem.* **231**, 813.
Hokin, L. E. & Hokin, M. R. (1956). *J. Physiol.* **132**, 442.
Hokin, L. E. & Hokin, M. R. (1963*a*). *Annu. Rev. Biochem.* **32**, 553.
Hokin, L. E. & Hokin, M. R. (1963*b*). *Biochim. biophys. Acta*, **67**, 470.
Hokin, L. E. & Huebner, D. (1967). *J. Cell Biol.* **33**, 521.
Hokin, M. R., Benfey, B. G. & Hokin, L. E. (1958). *J. biol. Chem.* **233**, 814.

Hokin, M. R. & Hokin, L. E. (1964). In *Metabolism and Physiological Significance of Lipids*, p. 423. Ed. by Dawson, R. M. C. & Rhodes, D. N. London: John Wiley and Sons (Inc.) Ltd.

Hokin, M. R., Hokin, L. E., Saffran, M., Schally, A. V. & Zimmerman, B. U. (1958). *J. biol. Chem.* **233**, 811.

Karmen, A., Giuffrida, L. & Bowman, R. L. (1962). *J. Lipid Res.* **3**, 44.

Karnovsky, M. L. (1962). *Physiol. Rev.* **42**, 143.

Keenan, R. W. & Hokin, L. E. (1964). *J. biol. Chem.* **239**, 2123.

Kennedy, E. P. (1961). *Fed. Proc.* **20**, 934.

Kessler, J. I., Demeny, M. & Sobotka, H. (1967). *J. Lipid Res.* **8**, 185.

Lands, W. E. M. (1960). *J. biol. Chem.* **235**, 2233.

Larrabee, M. G., Klingman, J. D. & Leicht, W. S. (1963). *J. Neurochem.* **10**, 338.

Prottey, C. & Hawthorne, J. N. (1967). *Biochem. J.* **105**, 379.

Rowe, C. F. (1960). *Biochem. J.* **73**, 438.

Schlenk, H. & Gellerman, J. L. (1960). *Analyt. Chem.* **32**, 456.

Scott, T. W., Jay, S. & Freinkel, N. (1966). *Endocrinology*, **79**, 591.

Scott, T. W., Setchell, B. P. & Bassett, J. M. (1967). *Biochem. J.* **104**, 1040.

Seljelid, R. (1967). *J. Ultrastruct. Res.* **18**, 479.

Wetzel, B. K., Spicer, S. S. & Wollman, S. H. (1965). *J. Cell Biol.* **25**, 593.