Figure 1 shows that the milk-ejection activity and the neurophysin content of the incubation media following electrical stimulation are related to stimulation intensity, the 2 curves being S-shaped. In Figure 2, results from each individual incubation have been plotted according to its neurophysin (abscissa) and hormone (ordinate) content. The 2 regression lines ($x = -14.6 + 0.79y$, and: $y = 21.4 + 1.14x$) have been computed; their correlation coefficient is equal to 0.95. The fact that the intercept is different from zero is not uncommon when biological and immunoreactive potencies of substances are determined, and may also depend on the different sensitivities of the 2 assays.

Studies on the binding of the polypeptide hormones to neurophysins[3,5] have led to the suggestion that neurophysins are involved in the intracellular storage and transport of the hormones; their fate during the secretory process has however long remained obscure. The present study indicates that neurophysins and polypeptide hormones are released in a fixed ratio from isolated rat neurohypophyses over at least a 10-fold range of hormone output. UTTENTHAL et al.[14] have recently shown that isolated porcine neurohypophyses liberate lysine-vasopressin and neurophysin when exposed to solutions containing a high KCl concentration. These results suggest that the determination of plasma neurophysin levels could serve as a clinically useful index of the secretory activity of the neurohypophysis, since the assay of the very small quantities of polypeptide hormones in blood poses important technical problems. It remains to be shown however that the ratio is always fixed with different experimental conditions, and whether it reflects the ratio found in the neurosecretory granules[15].

Résumé. La stimulation électrique de neurohypophyses de rat in vitro provoque la libération conjointe d'hormones et de neurophysine. Cette observation suggère que la secrétion neurohormonale implique l'extrusion du contenu des granules neurosecrétoires.

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[15] This work was supported by grants from the Swiss National Science Foundation No. 5340.3 and from the F. Hoffmann-La Roche Foundation No. 117. The technical assistance of Miss R. E. BIANCHI is acknowledged.

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Subcellular Distribution of ^{32}P Labelled Phospholipids: Stimulation by Thytropar and Long Acting Thyroid Stimulator

Thytropar (TSH) elicits numerous biochemical responses in its target tissue-thyroid, both in vivo and in vitro[1,2]. Increased incorporation of ^{32}Pi into the phospholipids of thyroid is one of the earliest effects of TSH[3-7]. Long acting thyroid stimulator (LATS) present in the serum of patients suffereing with Graves' disease has also been shown to mimic the action of TSH on thyroidal phospholipid metabolism[8,9].

Subcellular distribution of ^{32}P-labelled phospholipids in control and TSH-stimulated thyroids has been studied by KOGL and VAN DEENEN[10] and KERKOF and TATA[11]. In the present studies subcellular fractionation and localisation of the radioactivity in the TSH and LATS stimulated thyroids have been attempted with a view to analyze the distributory pattern of the ^{32}P-labelled phospholipids in these organelles.

Materials and methods. Thytropar (TSH) was a generous gift of Armour Pharmaceutical Company, Chicago. LATS was gift of MRC, London. $Na_2H^{32}PO_4$ was the product of Isotope Division, BARC, India.

Bovine thyroids from freshly slaughtered animals were sliced (100–150 mg bits) and 5 g of bovine thyroid slices (pooled slices from at least 6 thyroids) were incubated in 150 ml *Tris*-Cl buffer (pH 7.4; 50 m*M*) containing NaCl 131 m*M*; KCl 5 m*M*, $MgSO_4$ 1.2 m*M*; $CaCl_2$ 0.8 m*M* and $Na_2H\ ^{32}PO_4$ – 1 mCi for 1 h at 37 °C in a metabolic shaker under 100% oxygen. TSH and LATS were used at a concentration of 100 mU/ml and 1.5 MRC milli units/ml respectively. After the incubation period, the slices were washed several times with 0.1 *M* KH_2PO_4 and normal saline. Subcellular fractions were prepared by the differential centrifugation of the thyroidal homogenate in 4 volumes of 0.25 *M* sucrose. The 1000 *g* sediment (10 min centrifugation) was discarded. The 10,000 *g* sediment obtained after 20 min centrifugation was taken as the mitochondrial fraction. The microsomal fraction was the 105,000 *g* sediment (1 h centrifugation). Lipids were extracted from the homogenate, subcellular fraction and a portion of the 105,000 *g* supernatant with 20 volumes of $CHCl_3$: CH_3OH (2:1 v/v) mixture and purified according to the method of FOLCH et al.[12]. The total lipid radioactivity was measured using 15.0 ml of the scintillator fluid (PPO-5 g and dimethyl POPOP-0.3 g in IL Toluene) and counted in Nuclear Chicago Liquid Scintillation Counter. Individual phospholipids were resolved on TLC and the phospholipid zones

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Table I. Subcellular distribution of ^{32}P into bovine thyroidal phospholipids – effect of TSH and LATS

Experiments	Specific radioactivity ^{32}P incorporation into phospholipids (counts/min/μg P)							
	I		II		III		IV	
	Conc.	TSH	Conc.	TSH	Conc.	TSH	Conc.	TSH
Homogenate	252	430	221	470	450	531	463	563
Mitochondria	130	259	161	249	146	213	197	216
Microsomes	244	456	244	489	570	665	551	604
Supernatant	169	372	224	407	242	340	345	471

5 g bovine thyroid slices (pooled slices from at least 6 different thyroids) were incubated in Erlenmeyer flasks in 150 ml of the buffer medium given in 'Methods' in the presence of 1 °ci $NaH_2{}^{32}PO_4$ for 1 h at 37 °C under 100% O_2 before subcellular fractionation and isolation. TSH concentration studied was 100 mU/ml and LATS was used at 1.5 MRC mU/ml.

Table II. Distribution of ^{32}Pi into phospholipids of subcellular components of bovine thyroids

Phospholipid fraction	Radioactivity in phospholipids (% of total activity spotted)											
	Homogenate				Mitochondria				Microsomes			
	Conc.	TSH	Conc.	LATS	Conc.	TSH	Conc.	LATS	Conc.	TSH	Conc.	LATS
PE	6.66	4.33	4.88	5.66	6.08	5.16	6.30	5.74	1.88	2.69	9.38	13.39
PI	45.50	64.67	44.25	41.87	37.29	63.66	37.13	15.18	62.02	71.59	57.36	51.07
PC	44.60	28.81	49.95	51.63	54.41	29.56	56.12	78.80	34.56	24.68	31.87	34.90
SP	3.18	2.37	0.89	0.83	2.20	1.67	0.43	0.26	1.52	1.01	1.36	0.82

Experimental details as in Table I. The results from a typical experiment are expressed as the percentage of the total activity spotted.

were scraped and counted by forming a homogenous suspension of these scrapings with thixotropic gel (40 g/l) in the above scintillator. Total lipid P was determined by the method of FISKE and SUBBAROW[13] and individual phospholipid P by BARTLETT procedure[14].

Results and discussion. The subcellular distribution of the TSH and LATS stimulated ^{32}Pi incorporation into the phospholipids are summarized in Table I. The microsomal fraction of the control slices showed the highest specific activity. Thyroidal stimulation by TSH and LATS augmented ^{32}Pi incorporation in all the subcellular components examined, but that in the microsomal component was prominent.

The individual phospholipid components of these subcellular fractions resolved on TLC reveals a differential distribution of ^{32}Pi in the phospholipids of subcellular organelles (Table II). Whereas all the subcellular components and the homogenate in TSH provoked thyroids have the highest counts in PI, the maximum radioactivity was present in PC in the control and LATS treated mitochondria and homogenate. However, the PI of the microsomes of these thyroids exhibited the highest radioactivity.

According to KERKOF and TATA[11], the rate of incorporation of ^{32}Pi into the mitochondrial phospholipids was 70–100% higher than that into the microsomal lipids over a 6-hour period. They separated the rough and the smooth microsomal fractions and their studies were performed for 2, 3, 4 and 6 h. The results of the present studies, where the bovine thyroids were incubated for 1 h, show the microsomal lipids to be highly labelled. Many of the enzymes involved in the biosynthesis of phospholipids have been localized in the microsomes[15]. The exchange of phospholipids between the liver mitochondria and microsomes in vitro has been established[16]. MCMURRAY and DAWSON[17], working with the isolated preparations of mitochondria and microsomes of rat liver, have established that the microsomes are the seat of ^{32}Pi incorportaion into phospholipids and that mitochondria fail to show this effect. In the light of these reports, it is not unreasonable to assume that our observations represent the earlier phase of this labelling, and those of KERKOF and TATA form the later phase when the ^{32}P-phospholipids have been transported to the other sites within the cell. They have shown that, at the end of 6 h incubation, 2-to 3-fold of the label accumulated in the mitochondria compared to the microsomes. TSH and LATS might be expected to enhance both the microsomal phospholipogenesis as well as transfer of these phospholipids to other intracellular sites. The high activity of PI in the microsomes of the control, TSH and LATS stimulated thyroids further lends support to this contention.

Zusammenfassung. An Schilddrüsenschnitten wurde der subzelluläre Einbau von Phosphor in Phospholipide und die Wirkung von Thyreotropin und von LATS untersucht, wobei die mikrosomale Fraktion die höchste Radioaktivität aufwies.

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