JCI The Journal of Clinical Investigation

Stimulatory effects of induced phagocytosis on the function of isolated thyroid cells

K. Kowalski, … , S. Sato, G. Burke

J Clin Invest. 1972;51(11):2808-2819. https://doi.org/10.1172/JCI107103.

Research Article

Stimulation of endocytosis is a very early effect of thyrotropin on thyroid. However, the relationship of the endocytotic process to the many other thyrotropin effects on thyroid is not clearly defined. Since phagocytosis in isolated thyroid cells is a presumed model for in vivo endocytosis of colloid, we induced phagocytosis in isolated thyroid cells by incubating them at 37°C with 0.109-μ diameter polystyrene microbeads; phagocytosis was confirmed in each experiment by electron microscopy and/or spectrophotometric analysis of dioxane cell extracts. Cells incubated with 50-100-μ diameter polystyrene macrobeads (too large to ingest) served as controls. Microbead-induced phagocytosis in isolated thyroid cells was consistently accompanied by increases in: (*a*) cyclic 3′,5′-adenosine monophosphate-^{14}C formation from adenine-8-^{14}C (66%); (*b*) iodide-^{131}I trapping (40%); (*c*) protein and RNA synthesis (30%); (*d*) phospholipogenesis (50%); (*e*) α-aminoisobutyric acid-1-^{14}C uptake (15%). 50- to 100-μ diameter polystyrene macrobeads did not influence cell function in any of these experiments. Aminotriazole, $5 \times 10^{-3}_{M}$, a peroxidase inhibitor, blocked the stimulatory effect of microbead-induced phagocytosis on phospholipogenesis only. These studies indicate that in isolated thyroid cells the phagocytotic process, per se, may alter activity of the membrane-bound adenyl cyclase enzyme. The resultant increase in cyclic 3′,5′-adenosine monophosphate may be a triggering mechanism for (some) subsequent metabolic changes occuring during phagocytosis. Since these changes mimic those induced by thyrotropin, it is suggested that a […]

Stimulatory Effects of Induced Phagocytosis on the Function of Isolated Thyroid Cells

K. KOWALSKI, D. BABIARZ, S. SATO, and G. BURKE

From the Division of Endocrinology and Nuclear Medicine, Department of Medicine, Michael Reese Hospital and Medical Center, and the Department of Medicine, University of Chicago Pritzker School of Medicine, Chicago, Illinois 60616

ABSTRACT Stimulation of endocytosis is a very early effect of thyrotropin on thyroid. However, the relationship of the endocytotic process to the many other thyrotropin effects on thyroid is not clearly defined. Since phagocytosis in isolated thyroid cells is a presumed model for in vivo endocytosis of colloid, we induced phagocytosis in isolated thyroid cells by incubating them at 37°C with 0.109-μ diameter polystyrene microbeads; phagocytosis was confirmed in each experiment by electron microscopy and/or spectrophotometric analysis of dioxane cell extracts. Cells incubated with 50–100-μ diameter polystyrene macrobeads (too large to ingest) served as controls. Microbead-induced phagocytosis in isolated thyroid cells was consistently accompanied by increases in: (*a*) cyclic 3′,5′-adenosine monophosphate-^{14}C formation from adenine-8-^{14}C (66%); (*b*) iodide-^{131}I trapping (40%); (*c*) protein and RNA synthesis (30%); (*d*) phospholipogenesis (50%); (*e*) α-aminoisobutyric acid-1-^{14}C uptake (15%). 50- to 100-μ diameter polystyrene macrobeads did not influence cell function in any of these experiments. Aminotriazole, 5×10^{-3} M, a peroxidase inhibitor, blocked the stimulatory effect of microbead-induced phagocytosis on phospholipogenesis only. These studies indicate that in isolated thyroid cells the phagocytotic process, per se, may alter activity of the membrane-bound adenyl cyclase enzyme. The resultant increase in cyclic 3′,5′-adenosine monophosphate may be a triggering mechanism for (some) subsequent metabolic changes occurring during phagocytosis. Since these changes mimic those induced by thyrotropin, it is suggested that a variety of thyrotropin effects on thyroid may be secondary to stimulation of colloid resorption and hormone secretion.

Received for publication 6 January 1972 and in revised form 5 July 1972.

INTRODUCTION

Stolc and Tong (1) reported stimulatory effects of induced phagocytosis on iodinating activity in thyroid cells. We report here the results of studies with isolated bovine thyroid cells which indicate that induced phagocytosis has a variety of stimulatory effects on isolated cell function.

METHODS

The isolated bovine thyroid cell suspension was prepared by the method of Edmonds, Row, and Volpé (2) using an intermittent trypsinization technique. The isolated cells were suspended in a volume of Eagle's minimum essential medium (MEM)[1] with Hanks' salts and 15% fetal calf serum, sufficient to obtain a concentration of 1.0×10^7 to 2.0×10^7 cells/ml. Contamination by leukocytes was estimated to be ≤ 200 cells/ml, i.e., $\leq 0.001\%$.

Iodide trapping. ^{131}I uptake in thyroid cells was measured in the manner of Edmonds et al. (2) as previously reported (3).

The iodide-concentrating activity of the thyroid cells was expressed as the cell-to-medium ratio of ^{131}I (C/M ratio).

$$\text{C/M ratio} = \frac{\text{cpm of } ^{131}\text{I/ml packed cells}}{\text{cpm of } ^{131}\text{I/ml medium}} \text{ (references 2, 3).}$$

Measurement of adenyl cyclase. Adenyl cyclase was assayed in 50-μl isolated thyroid cells using the procedure of Humes, Rounbehler, and Kuehl (4) which measures incorporation of adenine-8-^{14}C into ATP-^{14}C and the subsequent formation therefrom of cyclic 3′,5′-adenosine monophosphate-^{14}C (cyclic AMP), with minor modifications (5).[2]

[1] *Abbreviations used in this paper:* AIB-1-^{14}C, α-aminoisobutyric acid; C/M ratio, cell-to-medium ratio; cyclic AMP, cyclic 3′,5′-adenosine monophosphate; DBcAMP, dibutyryl cyclic AMP; MEM, Eagle's minimum essential medium; TCA, trichloroacetic acid; TSH, thyrotropin.

[2] Although this procedure has been designated as an adenyl cyclase assay (4), we do not consider that we have *directly* determined adenyl cyclase activity. Nevertheless, a number of considerations (discussed in detail in [5]) suggest that the

TABLE I

Adenyl Cyclase Assay—Per Cent Recovery of Labeled Cyclic AMP

Tube No.	TCA supernate	Dowex 50 [H^+] chromatography	1st $BaSO_4$ supernate	2nd $BaSO_4$ supernate	Dry residue	Thin-layer chromatography
1	89	78	71	58	49	41
2	95	83	73	59	47	39
3	93	79	73	61	48	38
4	89	77	70	63	47	42
5	94	81	72	60	46	39
6	92	82	74	63	49	37

Six replicate 2-ml portions of Krebs-Ringer bicarbonate buffer containing 0.1% bovine serum albumin and 0.1 μCi cyclic AMP-^{3}H were fractionated as previously described (4, 5). The amount of radioactivity was determined after every operation and the recovery based on the radioactivity in the initial portion.

Recovery studies with cyclic AMP-^{3}H [see Table I, Humes et al. (4)] revealed that the over-all recovery exclusive of cellulose chromatography was 47.6±1.0% (Table I); in view of the high reproducibility of these findings, recovery standards were not routinely run.

Formation of cyclic AMP-^{14}C was calculated as previously described (5).

Measurement of protein and RNA synthesis. Leucine-^{14}C incorporation into protein in 0.2-ml isolated thyroid cells was measured by the method of Tong (6); RNA synthesis in 0.2-ml isolated thyroid cells was measured by the method of Wilson and Wright (7) using uridine-^{3}H. Total protein was determined by the method of Oyama and Eagle (8); RNA was estimated spectrophotometrically by the method of Fleck and Begg (9). Results are expressed as protein-^{14}C specific activity (counts/minute per milligram protein) and as RNA-^{3}H specific activity (counts/ minute RNA-^{3}H per milligram cell RNA).

Glucose oxidation. Conversion of glucose-1-^{14}C to $^{14}CO_2$ in 50 to 80-μl isolated thyroid cells ($\simeq 10^6$ cells) was measured by the method of Tong (10). A blank incubation (no cells) was carried out in each experiment. The $^{14}CO_2$ collected from this blank ranged from 300 to 500 cpm and was subtracted from each of the other $^{14}CO_2$ assays.

Phospholipogenesis. ^{32}P incorporation into cell phospholipids was based on the methods of Tong (6) and Oka and Field (11). 0.1-ml packed cells ($0.8–1.2 \times 10^6$ cells) were suspended in 3 ml Eagle's basal medium balanced with Earle's salts containing 5 μCi/ml orthophosphate-^{32}P and 1 mg/ml glucose. The suspension was saturated with 95% O_2–5% CO_2. The samples, in sealed sterile 80 × 16-mm polypropylene tubes, were incubated for 90 min in a horizontal position at 37°C in a Dubnoff metabolic shaker. The incubation was terminated by the addition of 3 ml ice-cold 20% trichloroacetic acid (TCA) and the mixture centrifuged for 5 min at 120 *g*. The precipitate was then washed twice with 3 ml of 10% TCA. Supernates from subsequent serial extractions of the precipitate[8] with 3 ml each ethanol, ethanol-ether [1:1] and ether were pooled together and radioactivity was determined using 0.5 ml of this mixture in 10 ml of a scintillation mixture containing 10% naphthalene, 0.7% 2,5-diphenyloxazole, 0.03% 1,4-bis[2-(5-phenyloxazolyl)]benzene, and 0.4% thixotropic gel in dioxane. Results are expressed as ^{32}P cpm/10^6 cells.

α-Aminoisobutyric acid transport. Measurement of α-aminoisobutyric acid-1-^{14}C (AIB-1-^{14}C) accumulation in 0.2-ml isolated thyroid cells was carried out using the procedure described by Tong (10) as reported elsewhere (12).

As was the case in iodide-^{131}I studies, the C/M values for AIB-1-^{14}C were not corrected for the error due to contamination of packed cells with noncellular material (see Tong [10]).

Induction of phagocytosis in isolated thyroid cells. The phagocytotic process was induced in 50- to 80-μl isolated thyroid cells by the addition, to the appropriate incubation medium, of 10–200 μl of a 10% suspension (prepared in glucose-enriched [10 mM] Earle's salt solution, pH 7.3) of polystyrene "microbeads," diameter 0.109 μ (Dow Chemical Co., Midland, Mich.). 50- to 100-μ diameter polystyrene "macrobeads" [*i.e.*, beads which are too large to ingest; also obtained from Dow Chemical Co.] were used as controls in each of the studies outlined above.

The method of Rodesch, Neve, and Dumont (13), with minor modifications (14), was employed to monitor cell phagocytosis of latex beads, in *each* of the experiments reported here. In the absence of cells, no sediment of latex particles was discerned in the glass tubes after the last washing and there was no evidence of polystyrene in the dioxane extracts of these tubes. Extracts of cells incubated with beads for up to 120 min at 0°C exhibited negligible polystyrene content; in all experiments, the 0°C uptake was subtracted from results of experimental tubes. Results are expressed as micrograms of polystyrene incorporated/10^6 cells.

The same procedure was repeated, in each instance, with 50- to 100-μ diameter polystyrene macrobeads.

Eagle's medium, Earle's salt solution, 2.5% trypsin solution, L-glutamine, and fetal calf serum were obtained from Grand Island Biological Co., Grand Island, N. Y.; bovine serum from Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.; and DNase crystals from Sigma Chemical Co., St. Louis, Mo. Thyrotropin (TSH; in the form of Thytropar) was purchased from Armour Pharmaceutical Co., Kankakee, Ill.; sodium-N_6-2′-*O*-dibutyryl cyclic AMP (DBcAMP) was obtained from Schwarz Mann BioResearch. Aminotriazole was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Actinomycin D and puromycin were purchased from Sigma Chemical Co. 1-Methyl-2-mercaptoimidazole (Tapazole) was a gift from Dr. J. M. McGuire, Eli

increase in cyclic AMP-^{14}C content of cells previously incubated with adenine-^{14}C is physiologically relevant and is a valid index of adenyl cyclase activity.

[8] Addition of tracer quantities of orthophosphate-^{32}P to the TCA precipitate did not influence ^{32}P counts in the ethanol-ether extract.

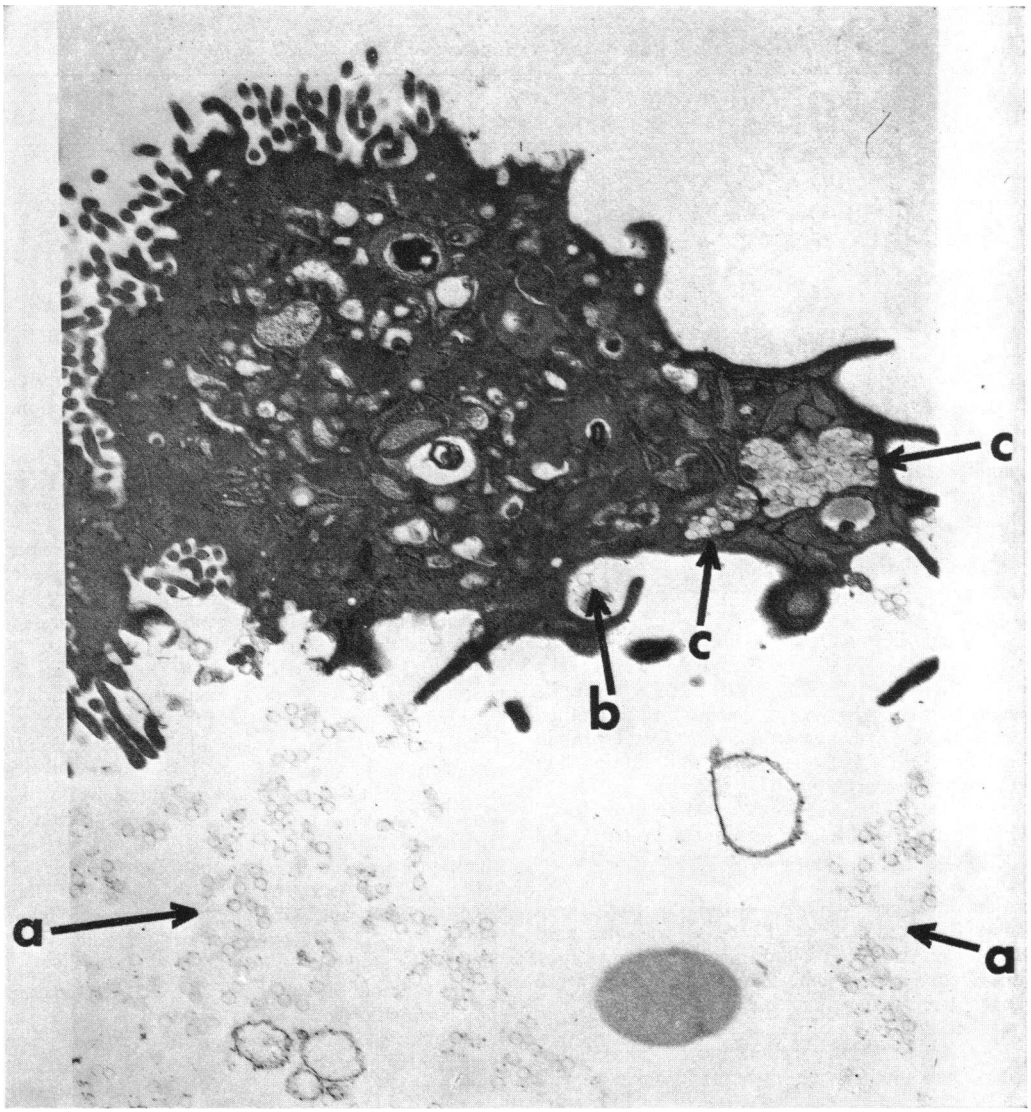

FIGURE 1 Electron micrograph of isolated thyroid follicular cell. As expected in these unwashed cell preparations, numerous latex microbeads are observed outside the cell (*a*). Pseudopods are branched out and enclose latex particles (*b*). Grouped intracellular latex particles are seen (*c*). Section obtained from cells incubated for 30 min at 37°C with 50 μl 10% microbead suspension (magnification × 15,200).

Lilly and Co., Indianapolis, Ind. AIB-1-^{14}C, SA 56 mCi/mM; L-leucine-U-^{14}C, 240 mCi/mM; uridine-5-^{3}H, 2.47 Ci/mM; glucose-1-^{14}C, 7.35 mCi/mM; and carrier-free orthophosphate-^{32}P were purchased from International Chemical and Nuclear Corporation, Irvine, Calif.

All studies were performed in duplicate or triplicate.

Statistical analysis of data was performed by paired *t* test (15) or, where appropriate, by Dunnet's test which permits comparison of several experimental groups with a single control (16). In some experiments, the significance of interaction between groups was established by factorial arrangement of treatments (17).

RESULTS

Phagocytosis. Biochemical studies of latex bead uptake by isolated bovine thyroid cells were essentially the same as those reported by Rodesch et al. (13) using horse thyroid cells. Thus, the dioxane extracts of isolated bovine thyroid cells[4] incubated with 0.109-μ diameter

[4] Since the cells were washed three times before dioxane extraction and the third washing contained no polystyrene, the dioxane extracts referred to here measure polystyrene *inside* the cell only (13, 14).

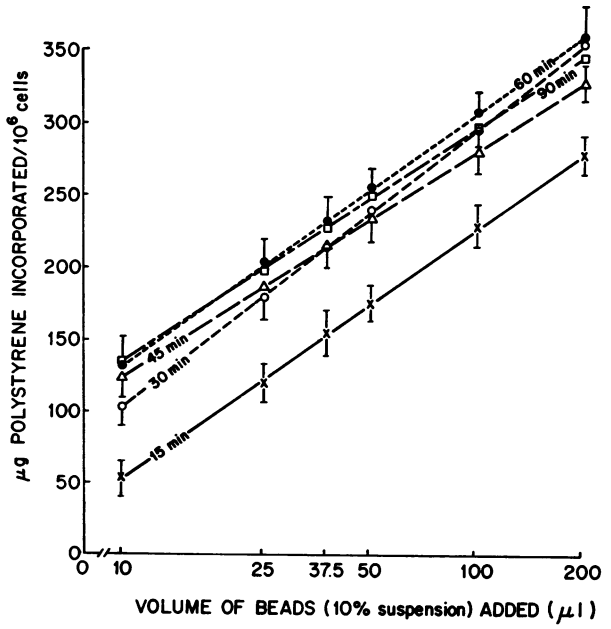

FIGURE 2 Polystyrene uptake by isolated thyroid cells incubated with latex microbeads. Results are mean (and when indicated) +, −, or ± SEM of three experiments, each experimental determination performed in duplicate.

(microbeads) latex beads had the characteristic absorption spectrum of polystyrene (18), whereas dioxane extracts of cells incubated with 50- to 100-μ diameter latex beads (macrobeads) contained no polystyrene.[5] The biochemical studies were confirmed by electron microscopic studies demonstrating the phagocytotic process (13, 14, Fig. 1). Prior washing of microbeads or macrobeads with Krebs-Ringer bicarbonate buffer, pH 7.4, did not influence the phagocytotic process or metabolic changes in the cells.

Significant cell uptake of latex microbeads ($\geq$ 50 μg polystyrene incorporated/10^6 cells) was evident within 15 min of incubation at 37°C (14). As can be seen from Fig. 2, no significant increase in cell uptake of microbeads occurred beyond 30 min of incubation. At each of the time periods studied, however, bead uptake was proportional to the volume of beads initially added to the cells.[6]

[5] Dioxane extracts of macrobeads alone had the same absorption spectrum as microbead-dioxane extracts.

[6] Since 50–80 μl of cell suspension ($\simeq 10^6$ cells) weighs ca60 mg, a 200 μg polystyrene uptake represents 0.3% (by weight) of cell mass; alternately, assuming bead density = 1.2 (g/ml), a 200 μg polystyrene/10^6 cells bead uptake represents a volume of ca0.15 μl, i.e., approximately 0.3% of total cell volume (ca50 μl). Correcting, in each instance, for extracellular space included in the packed cell volume (i.e., 49–60% of the volume of the packed cell mass [19]), the estimate rises to 0.6%. Accordingly, it seems reasonable to conclude that cell volume was not materially changed by incubation with the polystyrene microbeads.

Iodide trapping. In isolated thyroid cells, phagocytosis of latex beads (10 to 200 μl 10% microbead suspension added to incubation medium at 0 time) was consistently accompanied by a 10–40% increase in cell iodide-^{131}I trapping which was evident within 15 min of incubation and was directly related to the quantity of microbeads added to cells (Fig. 3). Prolonging the incubation period beyond 90 min or increasing the volume of added microbead suspension above 200 μl resulted in no further increase in ^{131}I C/M ratios.

In the presence of 50 μl of microbead suspension, polystyrene content of these cells varied from 176±15 to 235±16 μg polystyrene/10^6 cells during incubation periods ranging from 15 to 90 min. ^{131}I C/M ratios of cells incubated for 15–90 min with 10–200 μl 10% macrobead suspension were the same as those measured for cells incubated with ^{131}I in the absence of beads.

$NaClO_4$, 2 mM, abolished iodide trapping in isolated thyroid cells in the presence or absence of added latex microbeads. No microbead effect on ^{131}I C/M ratios was observed in the presence of dinitrophenol, 2×10^{-5} M.

During incubation periods of 60 min or less, TSH was without significant effect on cell iodide trapping. During 30- and 60-min incubations, the addition of TSH to the incubation medium significantly inhibited the stimulatory effect of latex microbeads on cell iodide trapping (Fig. 4*a*) Modest stimulatory TSH effects were observed at 90 min; combinations of maximally effective concentrations of TSH (20) and microbeads had an additive or near-additive effect on cell iodide trapping at 90 min and a synergistic effect at 180 min of incubation (Fig. 4*b*). Although not detailed here, results entirely analogous to those shown in Figs. 4*a* and 4*b* were obtained when DBcAMP and latex microbeads, in combination, were incubated with isolated thyroid cells. Heat-inactivated TSH [70°C, 1 hr], human growth hormone, and ACTH were without effect on cell polystyrene uptake

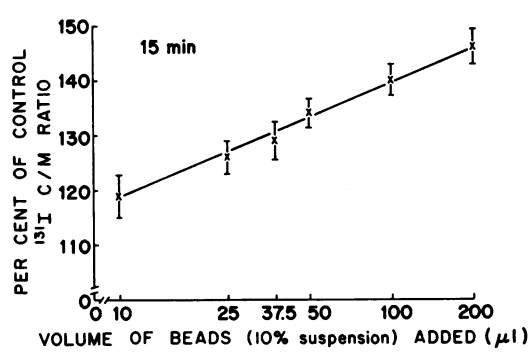

FIGURE 3 Effect of added latex microbeads on iodide trapping in isolated thyroid cells. Incubation time = 15 min; results are mean ±SEM of 10 experiments, each experimental determination performed in duplicate. Control ^{131}I C/M ratios in these experiments ranged from 6.3 to 7.9.

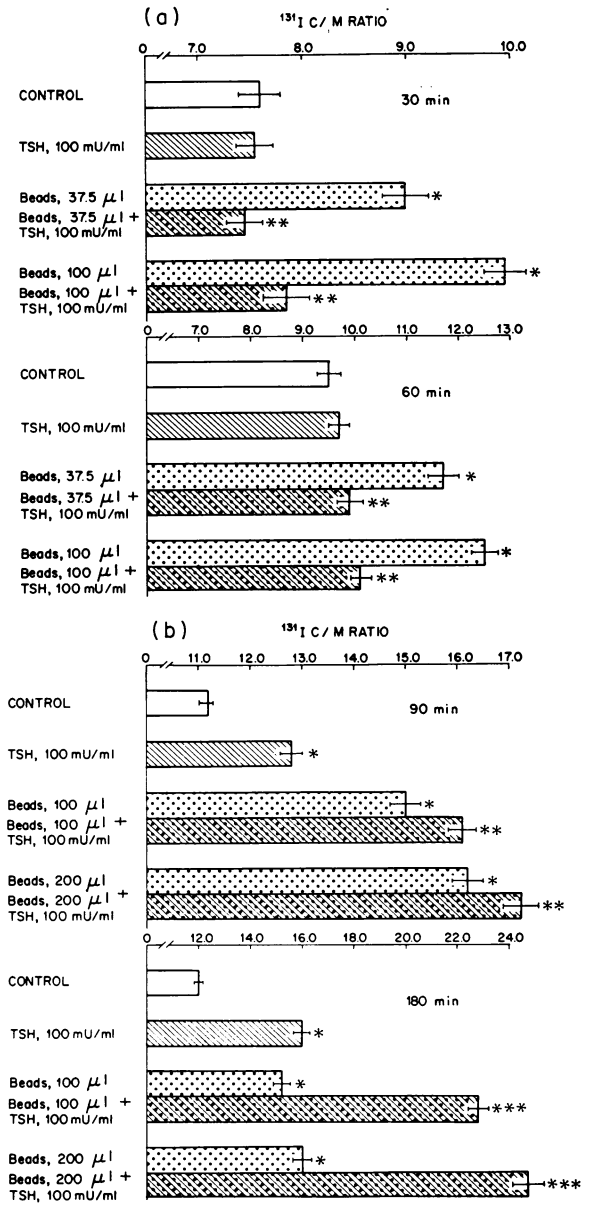

FIGURE 4(*a*) Effects of TSH and microbeads, combined, on iodide trapping in isolated bovine thyroid cells. Results are mean ±SEM of four experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.02$ to $P < 0.01$) greater than control; **, significantly ($P < 0.01$) less than beads alone. (*b*) Effects of TSH and microbeads, combined, on iodide trapping in isolated bovine thyroid cells. Results are mean ±SEM of same four experiments as detailed in Fig. 4*a*, each experimental determination performed in duplicate. *, significantly ($P < 0.02$ to $P < 0.01$) greater than control; **, significantly ($P < 0.05$) greater than beads alone; ***, significantly ($P < 0.01$) greater than (theoretical) additive effects of TSH and beads combined. The probability of significant interaction between TSH and beads during the 90- and 180-min incubations was also established by factorial arrangement of treatments (17).

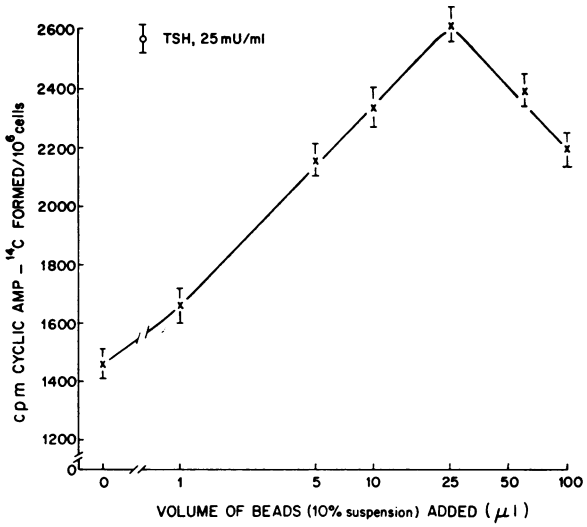

FIGURE 5 Effects of added latex microbeads on adenyl cyclase activity in isolated bovine thyroid cells. Incubation time = 10 min; results are mean ±SEM of four experiments, each experimental determination performed in duplicate. Response to 25 mU/ml TSH is included for comparative purposes.

and did not modify microbead effect on cell iodide trapping.[7] Cyclic 3′,5′-AMP and 5′-AMP were without effect on cell microbead uptake (14) and did not influence microbead effect on ^{131}I C/M ratios.

Adenyl cyclase activity. Microbead-induced phagocytosis in isolated thyroid cells was associated with a biphasic stimulatory effect on cyclic AMP-^{14}C formation reaching a maximum with 25 μl added microbead suspension and declining as larger volumes of microbead suspension were added (Fig. 5). In the presence of 25 and 50 μl of microbead suspension, polystyrene uptake by these cells during the 10 min adenyl cyclase incubation was measured at 106±11 μg/10^6 cells and 138±17 μg/10^6 cells, respectively. Cyclic AMP-^{14}C formation in cells incubated for 10 min with 1–100 μl 10% macrobead suspension was the same as that observed in cells incubated in the absence of beads.

When adenyl cyclase activity was measured in thyroid cells incubated with both TSH (25–100 mU/ml) and microbeads (1–100 μl), no additive effect on labeled cyclic AMP formation was observed; rather, the more potent TSH effect was "inhibited," the reduction in TSH effect becoming more marked as microbead volume was increased (Fig. 6). That the reduction in TSH effect was not due to TSH adsorption by the latex microbeads was shown in experiments where TSH (100 mU/ml) and microbeads (25 μl) were "preincubated" at 37°C for 10 min, centrifuged at 30,000 *g*, and the supernate (TSH) and sediment (microbeads) tested for effects

[7] Unpublished data.

on isolated cell adenyl cyclase activity. In three separate experiments, the supernate and sediment stimulated cell cyclase activity to the same extent as did TSH and microbeads, respectively, not previously combined in a "preincubation" (supernate, 64±9% increase; sediment, 55±12% increase vs. TSH, 25 mU/ml, 59±11% increase; 25 μl microbeads, 62±13% increase). The TSH effect on cyclic AMP-^{14}C formation was not modified by the addition of 1–200 μl [10% suspension] of macrobeads to the thyroid cell suspension.

Protein and RNA synthesis. During a 90 min incubation, microbead-induced phagocytosis in isolated thyroid cells was associated with a 10–30% increase in protein and RNA synthesis; although the increase appeared related to the quantity of microbeads employed, no true "dose-response" relationship could be established (Table II). Microbead effects on protein and RNA synthesis were additive with those of TSH and DBcAMP, even at maximally effective concentrations (Table III).

In the presence of 100 μl of microbead suspension, polystyrene content of these cells ranged from 267 to 288 μg/10^6 cells (90 min incubation). 50–200 μl of 10% macrobead suspension did not influence cell protein or RNA synthesis, basal or stimulated.

Although not detailed here, microbead-induced augmentation of protein and RNA synthesis was abolished

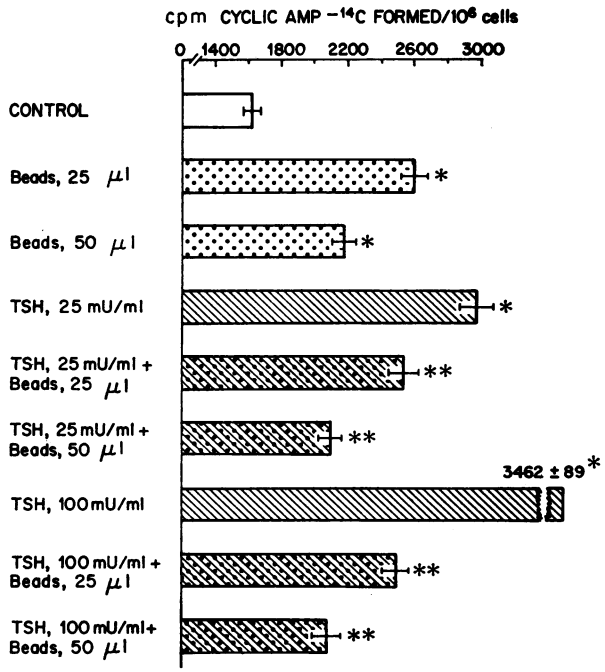

FIGURE 6 Effects of TSH and microbeads combined on adenyl cyclase activity in isolated bovine thyroid cells. Incubation time = 10 min; results are mean ±SEM of four experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.01$) greater than control; **, significantly ($P < 0.02$ to $P < 0.01$) less than TSH alone.

TABLE II

Latex Microbead Stimulation of Leucine-^{14}C and Uridine-^{3}H Incorporation into Protein and RNA, Respectively, by Thyroid Cells

Exp. No.	Additions	Protein-^{14}C SA*	Stimulation	Uridine-^{3}H SA*	Stimulation
		cpm/mg	%	*cpm/mg*	%
1	None	1008±36	—	1460±38	—
	TSH, 10 mU/ml	1321±39‡	31	1971±48‡	35
	Beads, 25 μl	1112±41‡	10	1635±52‡	12
	Beads, 37.5 μl	1175±38‡	17	1734±44‡	20
	Beads, 50 μl	1216±43‡	22	1813±50‡	24
	Beads, 100 μl	1314±37‡	31	1948±49‡	33
	Beads, 200 μl	1272±42‡	27	1823±51‡	25
2	None	1802±56	—	2422±63	—
	TSH, 10 mU/ml	2308±61‡	28	3147±65‡	30
	Beads, 25 μl	2003±44‡	11	2660±43‡	10
	Beads, 50 μl	2162±53‡	20	2954±48‡	22
	Beads, 100 μl	2306±57‡	28	3223±69‡	33
3	None	1937±45	—	2496±68	—
	DBcAMP, 3 mM	2578±43‡	33	3253±57‡	30
	Beads, 25 μl	2132±38‡	10	2751±49‡	10
	Beads, 50 μl	2342±41‡	21	2953±51‡	18
	Beads, 100 μl	2520±39‡	30	3204±46‡	28

* Mean±SD of triplicate determinations, 90 min incubation of 0.2-ml cells in 3 ml Earle's solution with 0.1 mM leucine-U-^{14}C and 0.1 mM uridine-5-^{3}H.
‡ Significantly ($P < 0.05$ to $P < 0.01$) greater than control.

TABLE III

Effects of Latex Microbeads, TSH, and DBcAMP, Separately and in Combination, on Leucine-^{14}C and Uridine-^{3}H Incorporation into Protein and RNA, Respectively, by Thyroid Cells

Exp. No.	Additions	Protein-^{14}C SA*	Stimulation	Interaction‡	Uridine-^{3}H SA*	Stimulation	Interaction‡
		cpm/mg	%	*P*	*cpm/mg*	%	*P*
1	None	2233±38	—		3084±62	—	
	TSH, 0.1 mU/ml	2409±35§	8		3381±59§	9	
	DBcAMP, 1 mM	2502±41§	12		3396±63§	10	
	Beads, 25 μl	2461±33§	10		3487±58§	13	
	TSH, 0.1 mU/ml + Beads, 25 μl	2658±39§	19	<0.01	3733±64§	21	<0.01
	DBcAMP, 1 mM + Beads, 25 μl	2705±36§	21	<0.01	3823±66§	24	<0.01
2	None	1170±31	—		1520±34	—	
	TSH, 10 mU/ml	1555±37§	33		1993±38§	31	
	TSH, 50 mU/ml	1523±39§	30		2021±40§	33	
	Beads, 50 μl	1406±33§	20		1902±43§	25	
	Beads, 100 μl	1558±41§	33		1996±41§	31	
	TSH, 10 mU/ml + Beads, 50 μl	1754±44§	50	<0.01	2359±48§	55	<0.01
	TSH, 10 mU/ml + Beads, 100 μl	1932±47§	65	<0.01	2494±43§	64	<0.01
3	None	2004±38	—		2862±43	—	
	DBcAMP, 3 mM	2666±43§	33		3719±51§	30	
	DBcAMP, 4.5 mM	2607±40§	30		3751±49§	31	
	Beads, 50 μl	2402±36§	20		3465±44§	21	
	Beads, 100 μl	2583±41§	29		3721±48§	30	
	DBcAMP, 3 mM + Beads, 50 μl	3108±57§	55	<0.01	4231±59§	48	<0.01
	DBcAMP, 3 mM + Beads, 100 μl	3209±53§	60	<0.01	4666±63§	63	<0.01

* Mean±SD of triplicate determinations, 90 min incubation of 0.2-ml cells in 3 ml Earle's solution with 0.1 mM leucine-U-^{14}C and 0.1 mM uridine-5-^{3}H.

‡ The probability of significant interaction between groups was established by factorial arrangement of treatments (17).

§ Significantly ($P < 0.05$ to $P < 0.01$) greater than control.

by puromycin (5×10^{-5} M) and actinomycin D (20 μg/ml), respectively.

Glucose-1-^{14}C oxidation. Although Tong (10) and Maayan and Ingbar (21) reported stimulation of glucose oxidation in isolated thyroid cells in response to TSH (10) and catecholamines (21), we were unable to demonstrate any stimulatory effect of TSH [in concentrations up to 500 mU/ml] in our system and only a 10–20% increase in $^{14}CO_2$ production in the presence of 10^{-5} M epinephrine (six separate experiments). In none of these cell preparations did the addition of latex microbeads (10–200 μl) augment glucose-1-^{14}C oxidation above control levels, although the cell polystyrene uptake (140–312 μg/10^6 cells) did not differ from that observed in other experiments.

^{32}P Incorporation into phospholipids. During a 90 min incubation, microbead-induced phagocytosis in isolated thyroid cells was associated with a (microbead) concentration-related increase in ^{32}P incorporation into phospholipids (Fig. 7 *a*); maximal stimulation of cell phospholipogenesis in the presence of latex microbeads (50±2% increase) exceeded that observed when TSH

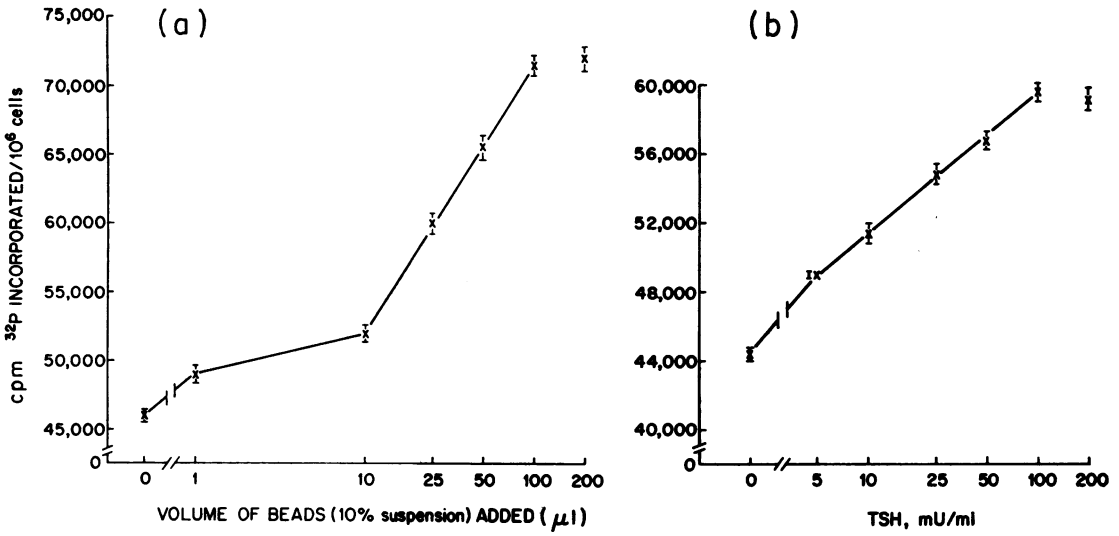

FIGURE 7 Effects of added microbeads (*a*) and TSH (*b*) on ^{32}P incorporation into phospholipids in isolated bovine thyroid cells. Incubation time = 90 min; results are mean ±SEM of three experiments, each experimental determination performed in duplicate.

was added to the incubation medium (36±2% increase) (Fig. 7*b*). Cell polystyrene uptake in these experiments ranged from 121±19 μg/10^6 cells (10 μl added microbead suspension) to 286±23 μg/10^6 cells (100 μl added microbead suspension). Macrobeads did not influence basal or TSH-stimulated cell phospholipogenesis.

When cells were incubated in the presence of maximally effective concentration of microbeads (100 μl) and minimally effective concentrations of TSH (1 mU/ml), cell phospholipogenesis was consistently reduced below that seen with microbeads alone ($P < 0.01$). Maximally effective concentrations of microbeads (100 μl) and TSH (100 mU/ml) combined had no additive effect on ^{32}P incorporation into cell phospholipids (Fig. 8). Heat-inactivated TSH (70°C, 1 hr) or human growth hormone (0.5 mg/ml) did not influence basal or microbead-induced cell phospholipogenesis.

AIB-1-^{14}C accumulation. When isolated thyroid cells were incubated for 3 hr with AIB-1-^{14}C, in the presence of latex microbeads, there was a 10–15% increase in AIB-1-^{14}C uptake over that seen when no beads were

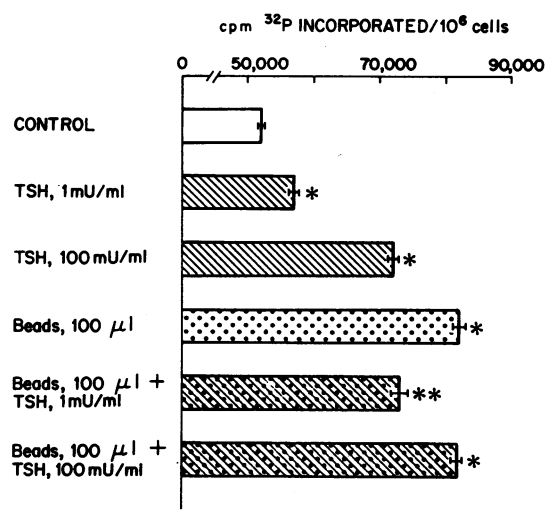

FIGURE 8 Effects of TSH and microbeads, combined, on ^{32}P incorporation into phospholipids in isolated bovine thyroid cells. Incubation time = 90 min; results are mean ±SEM of three experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.02$ to $P < 0.01$) greater than control; **, significantly ($P < 0.01$) less than beads alone.

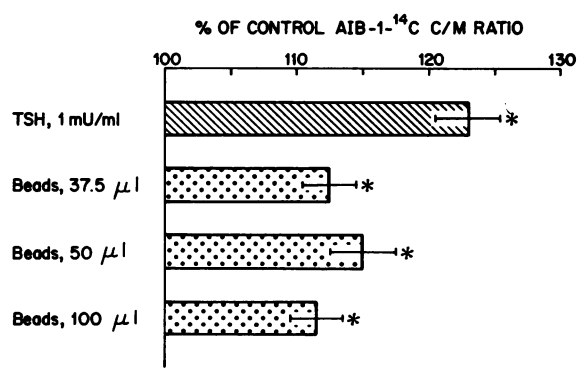

FIGURE 9 Effects of TSH and latex microbeads on AIB-1-^{14}C uptake in isolated bovine thyroid cells. Incubation time = 3 hr; results are mean ±SEM of six experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.05$ to $P < 0.01$) greater than control. Control AIB-1-^{14}C C/M ratios in these experiments ranged from 8.2 to 11.7.

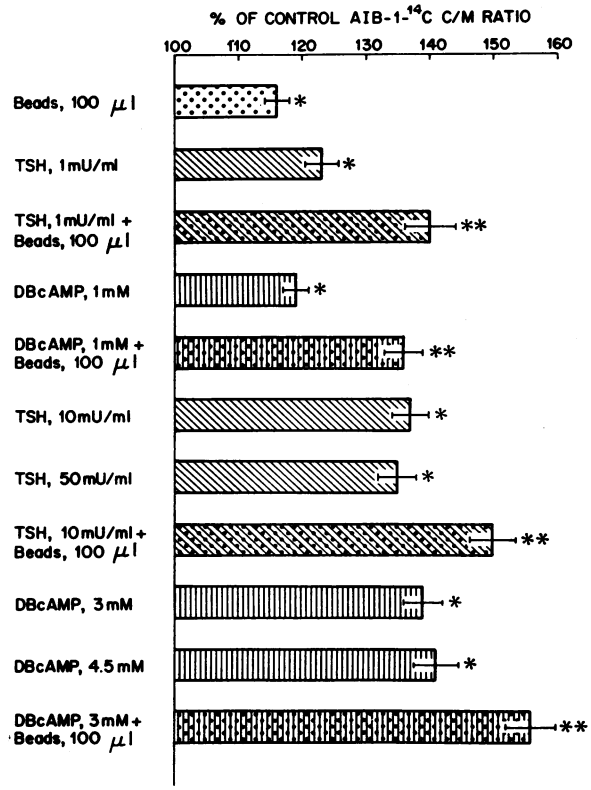

FIGURE 10 Effects of TSH or DBcAMP and microbeads, combined, on AIB-1-^{14}C uptake in isolated bovine thyroid cells. Incubation time = 3 hr; results are mean ±SEM of four experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.02$ to $P < 0.01$) greater than control; **, significantly ($P < 0.02$ to $P < 0.01$) greater than the same concentration of TSH, DBcAMP or beads alone. Control AIB-1-^{14}C C/M ratios in these experiments ranged from 6.7 to 10.3.

present. Although this modest increase in AIB-1-^{14}C C/M ratios was, in every instance (eight experiments), statistically significant ($P < 0.05$ to $P < 0.02$), the increase in AIB accumulation was not related to the volume of microbead suspension used (Fig. 9). Cell polystyrene uptake in these experiments ranged from 227±28 μg/10^6 cells (37.5 μl added microbeads) to 329±25 μg/10^6 cells (100 μl added microbeads). Addition of 50–200 μl of 10% macrobead suspension did not influence cell AIB accumulation.

Submaximal or maximally effective concentrations of TSH or DBcAMP were additive with microbeads on cell AIB-1-^{14}C uptake (Fig. 10).

No stimulatory effect of added microbeads was seen when boiled thyroid cells were used in any of the experimental systems studied here.

Stolc and Tong (1) suggested that the phagocytosis-induced increase in iodinating activity in thyroid cells and leukocytes was associated with augmented peroxidase activity. We therefore studied the effects of aminotriazole, a known inhibitor of thyroid peroxidase (22–24) on phagocytosis-induced changes in thyroid cell function. Aminotriazole, 10^{-3}–10^{-2} M, did not influence the stimulatory effects of TSH- or microbead-induced phagocytosis on cell iodide trapping, adenyl cyclase activation, protein/RNA synthesis, or AIB transport; however, aminotriazole, 5×10^{-3} M, did abolish the increase in ^{32}P incorporation into cell phospholipids induced by TSH or latex microbeads without altering basal phospholipogenesis (Fig. 11).

The effects of microbead-induced phagocytosis on thyroid cell function, alone and in combination with TSH or DBcAMP, are summarized in Table IV.

DISCUSSION

Stimulation of endocytosis is a very early effect of TSH on thyroid (25). However, the relationship of the endocytotic process to the many other TSH effects on thyroid is not clearly defined. Since phagocytosis in isolated thyroid cells is a presumed model for in vivo endocytosis of colloid (13, 14), the primary aims of this study were: (*a*) to determine whether induction of phagocytosis in thyroid cells effected any other alteration[s] in cell function; and, (*b*) to determine whether such responses involved mechanisms activated by TSH and/or mediated by cyclic AMP.

The results obtained indicate that while bead-induced phagocytosis is associated with a number of stimulatory effects on thyroid cell function, such stimulation varied in magnitude depending on the parameter studied and

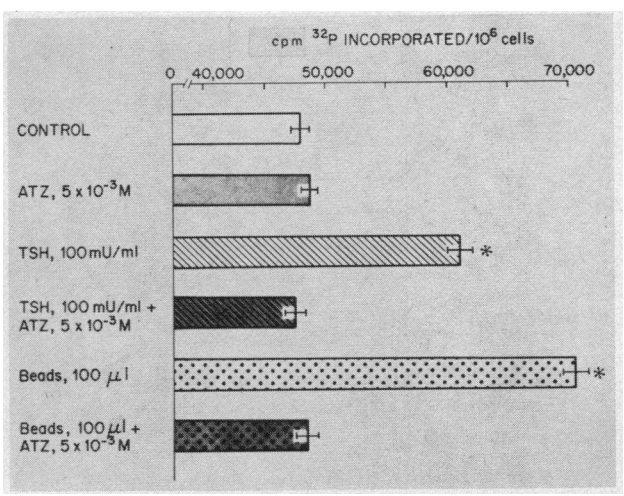

FIGURE 11 Effects of aminotriazole (ATZ) on TSH- and microbead-induced cell phospholipogenesis. Incubation time = 90 min; results are mean ±SEM of three experiments, each experimental determination performed in duplicate. *, significantly ($P < 0.01$) greater than control.

TABLE IV

Effects of Microbead-Induced Phagocytosis on Thyroid Cell Function

Cell function	Dose-related* stimulatory effect	Inhibition by aminotriazole	Combined effects of microbeads and TSH (or DBcAMP)
Iodide trapping	+‡	−§	TSH (or DBcAMP) inhibits more potent bead effect (<60 min); additive (90 min) or synergistic (180 min) at maximally effective concentrations
Adenyl cyclase	+ [Biphasic]	−	Microbeads inhibit more potent TSH effect
Protein/RNA synthesis	±	−	Microbeads and TSH (or DBcAMP) additive at maximally effective concentrations
Phospholipogenesis	+	+	Minimally effective TSH inhibits maximal microbead effect; nonadditive at maximally effective concentrations (of each)
AIB transport	−	−	Microbeads and TSH (or DBcAMP) additive at maximally effective concentrations

* i.e., microbead concentration-related.

‡ Present.

§ Absent.

was not uniformly related to the quantity of microbeads employed. In addition, significant qualitative differences between various aspects of cell function were readily demonstrable. Thus, the magnitude of bead-induced increase in cell iodide trapping differed sharply from the rather small effects on AIB uptake. A bead-concentration related effect on cell function was not clearly demonstrable in protein/RNA incorporation or AIB transport studies and the biphasic bead effect on cyclic AMP-^{14}C formation[8] or microbead inhibition of the more potent TSH effect on adenyl cyclase was not mirrored by any other bead-induced alteration in cell function. Although the bead- (and TSH-) induced increase in labeling of protein/RNA and phosphatides would suggest increased energy requirements, no effect on cell glucose oxidation was observed. While the inhibitory effects of aminotriazole on bead-induced cell phospholipogenesis suggest that this metabolic response to phagocytosis is dissociated from other alterations in cell function, labeling of lipids does not involve peroxidase (26) and we cannot at this time account for this observation.

Having cited the qualitative and quantitative differences between the various alterations in cell function consequent to induction of phagocytosis, it is important to note that these findings were not entirely unanticipated. Thus, earlier studies in our laboratory (5, 12) indicate that iodide and AIB transport in isolated thyroid cells are mediated by differing mechanisms. A number of recent reports (27–32) suggest a lack of direct coupling between thyroid hormone secretion and glucose oxidation. Studies with thyroid slices (33) and isolated thyroid cells (21, 34) indicate a similar lack of coupling between phospholipogenesis and many other parameters of thyroid function. Of perhaps even greater significance are results of several recent in vivo and in vitro studies (27, 28, 35–37) which suggest that adenyl cyclase activation and cyclic AMP formation may be dissociated from a variety of other TSH effects on thyroid, including those on endocytosis (27, 28).

Given these considerations, one cannot state with certainty whether the cell responses to induced phagocytosis reported herein involve mechanisms activated by TSH and/or mediated by cyclic AMP. (TSH *stimulates* cell microbead uptake at incubation times up to 120 min [14]; thus, any antagonism by TSH of bead effects on cell function is *not* a consequence of TSH inhibition of phagocytosis.) The finding that early ($\leq$ 60 min incubation) bead effects on cell iodide trapping were inhibited by ineffective concentrations of TSH (or DBcAMP) and demonstration of a synergistic effect during longer incubations (120 min) suggest utilization of [a] common pathway[s]. On the other hand, the additive effects observed with maximal concentrations of TSH (or DBcAMP) and microbeads, respectively, on cell protein/RNA synthesis and AIB transport suggest different mechanisms are involved. (Although TSH inhibited the effects of microbeads on iodide trapping in 30- and 60-min incubations, no microbead (or TSH) effect on protein/RNA synthesis or AIB uptake was observed during these shorter incubations, precluding examination of microbead-TSH interaction under these circumstances.)

The view is widely (albeit not universally [27, 28]) held that stimulation of the endocytotic process by TSH is a consequence of TSH activation of membrane adenyl cyclase (30). Thus, one would not have anticipated that

[8] Such a biphasic response is not obtained with TSH (5).

bead-induced phagocytosis would effect an increase in cell adenyl cyclase activity, i.e., increased formation of labeled cyclic AMP. These results, as well as the demonstration of a "competitive" interaction between beads and the more potent TSH on cell cyclase activity, raise the possibility that the "labilization" (30) of plasma membrane which occurs in association with the phagocytotic process might itself alter activity of the membrane-bound adenyl cyclase enzyme. The resultant increase in cyclic AMP may be a triggering mechanism for [some] subsequent metabolic changes during phagocytosis. Park, Good, Beck, and Davis (38) and Manganiello, Evans, Stossel, Mason, and Vaughan (39) recently reported that after incubation with polystyrene latex beads for 5 min, the cyclic AMP content of human peripheral blood leukocytes is increased severalfold. While each of these studies, as well as that reported herein, suggest that phagocytosis per se increases cyclic AMP levels, it should be noted that the nucleotide may well have a role in the control or regulation of this function (14, 40, 41).

In this regard, note should be made of recent findings in the laboratories of Williams and Wolff (42) and Nève, Willems, and Dumont (48) indicating a colchicine-sensitive role for microtubules in colloid endocytosis in the thyroid gland. The role of the microtubular-microfilamentous machinery of the cell in the in vitro phagocytotic process reported herein and the effects of colchicine on thyroid cell responses to induced phagocytosis are currently under study.

The reservations noted above notwithstanding, thyroid cell responses to phagocytosis observed herein generally mimic those induced by TSH. It is therefore suggested that a variety of TSH effects on thyroid may be secondary to stimulation of colloid resorption and hormone secretion.

ACKNOWLEDGMENTS

Mrs. H. Sanders, Department of Pathology, prepared the electron micrographs.

This study was supported by a grant (AM 11136) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

REFERENCES

1. Stolc, V., and W. Tong. 1971. Stimulatory effects of induced phagocytosis on iodinating activity in thyroid cells and leucocytes. Abstracts of the 53rd Meeting of The Endocrine Society. A134.
2. Edmonds, M. W., V. V. Row, and R. Volpé. 1970. Action of globulin and lymphocytes from peripheral blood of patients with Graves' disease on isolated bovine thyroid cells. *J. Clin. Endocrinol. Metab.* **31**: 480.
3. Burke, G., and K. Kowalski. 1971. Stimulatory effect of ammonium ion on iodide trapping in isolated thyroid cells. *Life Sci.* **10**: 361.
4. Humes, J. L., M. Rounbehler, and F. A. Kuehl, Jr. 1969. A new assay for measuring adenyl cyclase activity in intact cells. *Anal. Biochem.* **32**: 210.
5. Sato, S., M. Szabo, K. Kowalski, and G. Burke. 1972. Role of prostaglandin in thyrotropin action on thyroid. *Endocrinology.* **90**: 343.
6. Tong, W. 1967. TSH stimulation of ^{14}C-amino acid incorporation into protein by isolated bovine thyroid cells. *Endocrinology.* **80**: 1101.
7. Wilson, B. D., and R. L. Wright. 1970. Mechanism of TSH action: effects of dibutyryl cyclic AMP on RNA synthesis in isolated thyroid cells. *Biochem. Biophys. Res. Commun.* **41**: 217.
8. Oyama, V. I., and H. Eagle. 1956. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteau). *Proc. Soc. Exp. Biol. Med.* **91**: 305.
9. Fleck, A., and D. Begg. 1965. The estimation of ribonucleic acid using ultraviolet absorption measurements. *Biochim. Biophys. Acta.* **108**: 333.
10. Tong, W. 1964. *In vitro* effects of thyrotropin on certain metabolic activities of isolated thyroid cells. *Endocrinology.* **75**: 527.
11. Oka, H., and J. B. Field. 1966. Effects of ions on TSH stimulation of P^{32} incorporation into thyroid slice phospholipid. *Am. J. Physiol.* **211**: 1357.
12. Burke, G., K. Kowalski, and S-C. Yu. 1971. Effects of 3',5' cyclic nucleotides on membrane transport in isolated thyroid cells. *Acta Endocrinol.* **68**: 183.
13. Rodesch, F. R., P. Nève, and J. E. Dumont. 1970. Phagocytosis of latex beads by isolated thyroid cells. *Exp. Cell Res.* **60**: 354.
14. Kowalski, K., D. Babiarz, and G. Burke. 1972. Phagocytosis of latex beads by isolated thyroid cells: effects of thyrotropin, prostaglandin E_1, and dibutyryl cyclic AMP. *J. Lab. Clin. Med.* **79**: 258.
15. Dixon, W. J., and F. J. Massey, Jr. 1957. Introduction to Statistical Analysis. McGraw-Hill Book Company, New York. 124.
16. Dunnet, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Statist. Assoc.* **50**: 1096.
17. Snedecor, G. W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State University Press, Ames, Iowa. 5th edition. 333.
18. Roberts, J., and J. H. Quastel. 1963. Particle uptake by polymorphonuclear leucocytes and Ehrlich ascites-carcinoma cells. *Biochem. J.* **89**: 150.
19. Tong, W. 1964. Thyrotropin stimulation of thyroxine synthesis in isolated thyroid cells treated with perchlorate. *Endocrinology.* **75**: 968.
20. Burke, G., and K. Kowalski. 1971. Comparative effects of thyrotropin and long-acting thyroid stimulator on iodide trapping in isolated thyroid cells. *Acta Endocrinol.* **68**: 645.
21. Maayan, M. L., and S. H. Ingbar. 1970. Effects of epinephrine on iodine and intermediary metabolism in isolated thyroid cells. *Endocrinology.* **87**: 588.
22. Alexander, N. M. 1959. Antithyroid action of 3-amino-1,2,4-triazole. *J. Biol. Chem.* **234**: 148.
23. Alexander, N. M. 1959. Iodide peroxidase in rat thyroid and salivary glands and its inhibition by antithyroid compounds. *J. Biol. Chem.* **234**: 1530.
24. Strum, J. M., and M. J. Karnovsky. 1971. Aminotriazole goiter. Fine structure and localization of thyroid peroxidase activity. *Lab. Invest.* **24**: 1.
25. Field, J. B. 1968. Studies on the mechanism of action of thyroid-stimulating hormone. *Metab. (Clin. Exp.).* **17**: 226.

26. Freinkel, N. 1964. The intermediary metabolism of thyroid tissue. *In* The Thyroid Gland, Volume I. R. Pitt-Rivers and W. R. Trotter, editors. Butterworths, Washington. 131.
27. Burke, G. 1970. Effects of iodide on thyroid stimulation. *J. Clin. Endocrinol. Metab.* **30**: 76.
28. Dekker, A., and J. B. Field. 1970. Correlation of effects of thyrotropin, prostaglandins, and ions on glucose oxidation, cyclic-AMP, and colloid droplet formation in dog thyroid slices. *Metab. (Clin. Exp.)*. **19**: 453.
29. Pastan, I., and S. H. Wollman. 1967. Colloid droplet formation in dog thyroid *in vitro*. Induction by dibutyryl cyclic-AMP. *J. Cell Biol.* **35**: 262.
30. Onaya, T., D. H. Solomon, and W. D. Davidson. 1969. Effect of chlorpromazine on thyrotropin-stimulated endocytosis and glucose oxidation in canine thyroid slices. *Endocrinology*. **85**: 150.
31. Zor, U., I. P. Lowe, G. Bloom, and J. B. Field. 1968. The role of calcium (Ca^{++}) in TSH and dibutyryl 3',5' cyclic AMP stimulation of thyroid glucose oxidation and phospholipid synthesis. *Biochem. Biophys. Res. Commun.* **33**: 649.
32. Berens, S. C., J. A. Williams, and J. Wolff. 1971. Dissociation of thyrotropin-stimulated hormone secretion and glucose oxidation in thyroid glands by lithium and colchicine. *Biochim. Biophys. Acta.* **252**: 314.
33. Burke, G. 1971. Thyroid stimulators and thyroid stimulation. *Acta Endocrinol.* **66**: 558.
34. Scott, T. W., N. Freinkel, J. H. Klein, and M. Nitzan. 1970. Metabolism of phospholipids, neutral lipids and carbohydrates in dispersed porcine thyroid cells: comparative effects of pituitary thyrotropin and dibutyryl-3',5'-adenosine monophosphate on the turnover of individual phospholipids in isolated cells and slices from pig thyroid. *Endocrinology*. **87**: 854.
35. Zor, U., T. Kaneko, I. P. Lowe, G. Bloom, and J. B. Field. 1969. Effect of thyroid-stimulating hormone and prostaglandins on thyroid adenyl cyclase activation and cyclic adenosine 3',5'-monophosphate. *J. Biol. Chem.* **244**: 5189.
36. Burke, G. 1970. On the role of adenyl cyclase activation and endocytosis in thyroid slice metabolism. *Endocrinology*. **86**: 353.
37. Burke, G. 1970. Effects of prostaglandins on basal and stimulated thyroid function. *Am. J. Physiol.* **218**: 1445.
38. Park, B. H., R. A. Good, N. P. Beck, and B. B. Davis. 1971. Concentration of cyclic adenosine 3',5'-monophosphate in human leucocytes during phagocytosis. *Nat. New Biol.* **229**: 27.
39. Manganiello, V., W. H. Evans, T. P. Stossel, R. J. Mason, and M. Vaughan. 1971. The effect of polystyrene beads on cyclic 3',5'-adenosine monophosphate concentration in leukocytes. *J. Clin. Invest.* **50**: 2741.
40. Stossel, T. P., R. J. Mason, and M. Vaughan. 1971. A simple sensitive assay for the rate of phagocytosis. *Clin. Res.* **19**: 468. (Abstr.)
41. Bourne, H. R., R. I. Lehrer, M. J. Cline, and K. L. Melmon. 1971. Cyclic 3',5'-adenosine monophosphate in the human leukocyte: synthesis, degradation and effects on neutrophil candidacidal activity. *J. Clin. Invest.* **50**: 920.
42. Williams, J. A., and J. Wolff. 1970. Possible role of microtubules in thyroid secretion. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 1901.
43. Nève, P., C. Willems, and J. E. Dumont. 1970. Involvement of the microtubule-microfilament system in thyroid secretion. *Exp. Cell Res.* **63**: 457.