duce interferon, this possibility seems unlikely. Evidence supporting such speculation was obtained by Guggenheim *et al.* (*9*) who showed that chick (nucleated) red cells, unlike other chick cells, were not stimulated by Sendai virus to produce interferon. Preformed interferon released by injection of endotoxin into mice is characterized by a peak at 2 hours and a rapid fall in titer. The finding of a late peak of plasmodium-induced interferon and its persistence in the serum appears to preclude release of existing interferon as a result of injection. The potential role of interferon in plasmodial infection has been emphasized also by findings (*10*) that various interferon inducers exert a protective effect in mice against infection with *P. berghei*. In view of the observation that interferon inhibits other nonviral intracellular microorganisms such as *Chlamydia* (*11*) and *Toxoplasma* (*12*), interferon apparently possesses a wider and more complex spectrum of activity than is now understood. Investigations of the relation between interferon and *Plasmodium* may not only reveal the role of interferon in malarial infection, but may further lead to the understanding of the action of interferon.

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Epinephrine: Effect on Uptake of Iodine by Dispersed Cells of Calf Thyroid Gland

Abstract. *In isolated thyroid cells* l-*epinephrine (0.1 and 10.0 micrograms per milliliter), norepinephrine and isoproterenol consistently stimulated the accumulation and organic binding of iodine. The effect was partially inhibited by phentolamine, but not propranolol, and hence may be mediated by alpha receptors. Theophylline did not mimic or enhance the epinephrine effect, suggesting that the latter may not result from activation of adenyl cyclase.*

3′,5′-Cyclic adenosine monophosphate may be a mediator of the action of several hormones, notably vasopressin, adrenocorticotrophic hormone, adrenomedullary hormones (*1*), and parathyroid hormone (*2*). In thyroid homogenates (*3*) and isolated thyroid cells (*4*), thyrotropin (TSH) has been shown to stimulate the activity of adenyl cyclase, an enzyme that catalyzes cyclic adenosine monophosphate (AMP) synthesis. The dibutyryl ester of cyclic AMP, like TSH, increases the incorporation of labeled orthophosphate into the phospholipids of thyroid slices (*5*) and theophylline, an inhibitor of cyclic AMP hydrolysis, enhances the stimulatory effect of TSH on thyroid iodine metabolism (*6*). Several reports indicate that dispersed thyroid cells retain both the essential features of hormonal synthesis and the response to TSH which are characteristic of the intact gland (*7, 8*). Therefore, we have investigated the effect of *l*-epinephrine, a known stimulator of adenyl cyclase activity in other tissues (*1, 9*), and of related sympathetic amines, on the uptake and metabolism of iodine by dispersed cells of calf thyroid gland.

Fresh calf thyroids were collected and stored on ice during transport to the laboratory. Within 1 hour the glands were trimmed of fat and fascia, minced, and then treated with trypsin for 4 hours (*7*). The material collected between the 2nd and 4th hour of trypsin treatment was centrifuged and washed twice with 50 volumes of Earle's solution. This material was then suspended in Earle's solution supplemented with 2 μc of ^{131}I and 3 μg of ^{127}I as sodium iodide per milliliter. The suspension was allowed to stand for several minutes, during which time the aggregated cells and debris sedimented. The suspended cells, now dispersed in 50 to 100 volumes of Earle's solution (18 to 20 × 10^6 cell/ml), were separated by decantation. Portions (2 ml) were added to incubation vessels in which various substrates had been added in a volume of 0.5 ml. Incubations were carried out in duplicate for 45 minutes in a Dubnoff shaker at 37°C with either a mixture of 95 percent O_2 and 5 percent CO_2 or 100 percent O_2 as gas phase. In each experiment two or three vessels containing cells which had been boiled for 5 to 7 minutes were incubated under the same conditions. After incubation, measured portions were centrifuged, the supernatant was discarded, and the ^{131}I content of the sedimented cells was measured in a well-type scintillation counter. Accumulated ^{131}I was related

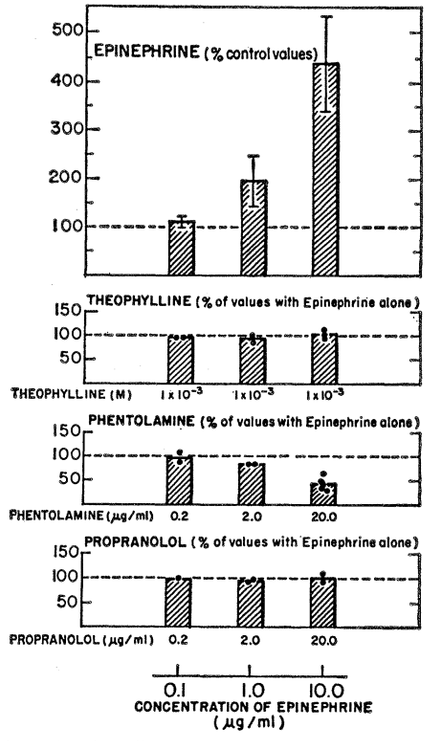

Fig. 1. The effect of epinephrine, theophylline, and adrenergic blocking agents on the uptake of ^{127}I by isolated thyroid cells. In this and Figs. 2 and 3, results are from incubations carried out in Earle's solution for 45 minutes at 37°C, with 100 percent O_2 as gas phase. Values shown in the upper panel represent the mean ± S.D. of results obtained in 12 experiments. The stimulation of iodine accumulation induced by epinephrine was consistently associated with an increase in the proportion of accumulated iodine incorporated into organic forms and an increase in organic iodine present as thyroxine. In the three lower panels, each point represents the results of a single experiment in which duplicate or triplicate vessels were incubated.

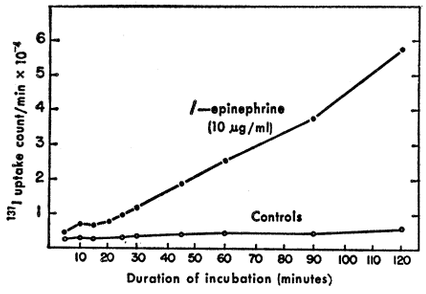

Fig. 2. Time course of the effect of *l*-epinephrine on the ^{131}I uptake by isolated thyroid cells. Each point represents the results obtained in a single measurement. The results shown are typical of those obtained in three experiments.

to the ^{131}I content of a measured portion of the uncentrifuged incubation mixture, and the absolute quantity of ^{127}I accumulated by the cells was calculated from the known specific activity of the incubation mixture. Inorganic and organically-bound ^{131}I in the cells was separated by filter paper electrophoresis (*10*) or by ascending paper chromatography in a butanol-ethanol-ammonia solvent system (5:1:2) after addition of 0.2 ml of calf serum containing methimazole (0.02*M*). In many experiments, cells suspended in the calf mixture of serum and methimazole were digested overnight with Pronase and were then subjected to paper chromatography for analysis of individual iodinated amino acids.

In control vessels, from 150 to 200 ng of ^{127}I were accumulated by the cells during the period of incubation. The percentage of accumulated ^{131}I which was incorporated into organic forms in control vessels varied widely in different experiments, but was consistent with each experiment. In cells which had been boiled, both the total accumulation of ^{127}I and the percentage incorporation into organic forms were greatly decreased. Epinephrine, in concentrations ranging between 0.1 and 10.0 μg/ml, consistently enhanced total iodine accumulation (Fig. 1). Furthermore, the stimulatory effect of epinephrine was accompanied by a dose-related increase, often striking, in both the percent of accumulated ^{131}I which had been incorporated into organic forms and the percent of organic ^{131}I present as thyroxine. As a result, the nanograms of ^{127}I incorporated into iodinated amino acids, and particularly into thyroxine, were greatly increased. At 10 μg/ml the effect of epinephrine on iodine accumulation was evident within 5 minutes (Fig. 2). Theophylline ($10^{-3}M$) had no significant effect on iodine accumulation when added alone and did not enhance the stimulatory effect of epinephrine (Fig. 1). The effect of epinephrine was partially inhibited by phentolamine, but not by propranolol (Fig. 1). Isoproterenol appeared to be slightly more effective than epinephrine, while norepinephrine was less so (Fig. 3).

The stimulatory effects of epinephrine were most marked when incubations were carried out in a gas phase of 100 percent O_2, which resulted in a final *p*H within the incubation mixture of 8.2 to 8.3. When incubations were carried out in a gas phase of 95 percent O_2 and 5 percent CO_2, ^{127}I accumulation was decreased by approximately 50 percent and a smaller stimulatory response to epinephrine was evident. The reason for this difference is unknown.

Our experiments indicate that epinephrine and related sympathetic amines stimulate both the accumulation of iodine and its incorporation into organic forms in dispersed thyroid cells. Extensive studies in vivo and in vitro have provided contradictory results about the influence of catecholamines on the thyroidal iodine metabolism (*11*). In isolated thyroid cells, however, a marked and highly reproducible effect of catecholamines is evident, possibly owing to the absence in this system of circulatory or other factors that may modify or mask the response in the whole animal. By conventional criteria, the effect on the thyroid cell would appear to be mediated by alpha-receptors, since it was inhibited by phentolamine, but not by propranolol (*12*).

In that TSH enhances iodine accumulation by the intact thyroid and by dispersed thyroid cells and also stimulates adenyl cyclase activity in the thyroid in vitro, the response to epinephrine may indicate that TSH exerts its effect on iodine accumulation through an action on the synthesis of cyclic AMP. However, in contrast to the situation in other tissues, in which the effects of epinephrine are increased by theophylline and in which theophylline itself mimics the effects of epinephrine (*12, 13*), theophylline in the dispersed thyroid cell preparation had no significant effect on iodine accumulation, either in the presence or absence of epinephrine. This can not be ascribed to a general ineffectiveness of theophylline in this system, since under similar experimental conditions we have found that theophylline greatly increases the concentration of pyridine nucleotides and of ATP in isolated thyroid cells (*14*). Zor, Lowe, and Field have found theophylline to be without effect on glucose oxidation and phospholipid synthesis in thyroid slices (*15*), although both functions have been shown to be stimulated by cyclic AMP itself or its dibutyryl ester (*5*). Hence, in contradistinction with other tissues (*16*), in the thyroid, the response to theophylline may not be an infallible criterion for reactions moderated by cyclic AMP.

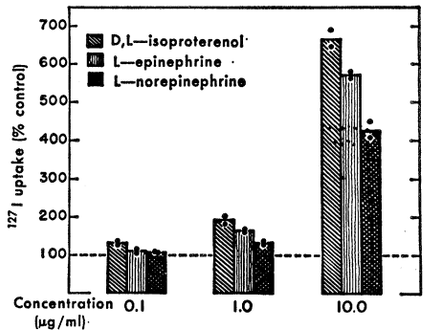

Fig. 3. The comparative effects of several sympathetic amines on the uptake of ^{127}I by isolated thyroid cells. Each point represents the results obtained in a single experiment in which duplicate vessels were incubated.

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