and certain other substances (Y.L., I. Gigli, B.F. and E.C.F., in preparation), as well as further amino acid sequence studies will allow a more precise structural and functional comparison with C1t.

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1 Binette, P., Binette, M. & Calkins, E. *Biochem. J.* **143**, 253–254 (1974).
2 Skinner, M., Cohen, A. S., Shirahama, T. & Cathcart, E. S. *J. lab. clin. Med.* **84**, 604–614 (1974).
3 Pinteric, L., Assimeh, S. N., Kells, D. I. C. & Painter, R. H. *J. Immun.* **117**, 79–83 (1976).
4 Skinner, M., Benson, M. D. & Cohen, A. S. *Arthritis Rheum.* **19**, 822 (1976).
5 Kaplan, M. H. & Volanakis, J. E. *J. Immun.* **112**, 2135–2147 (1974).
6 Siegel, J., Rent, R. & Gewurz, H. *J. exp. Med.* **140**, 631–645 (1974).
7 Osmand, A. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 739–743 (1977).
8 Pisano, J. J. & Bronzert, T. J. *J. biol. Chem.* **244**, 5597–5607 (1969).
9 Summer, M. R., Smythers, G. W. & Oroszlan, S. *Analyt. Biochem.* **53**, 624–628 (1973).
10 Smithies, O. *et al. Biochemistry* **10**, 4912–4921 (1971).

An anterior pituitary factor stimulates thymidine incorporation in isolated thymocytes

A POSSIBLE tropic role of pituitary on thymus has been suggested by several studies. Hypophysectomy causes an atrophy of the gland[1–4] and thymus is poorly developed in Snellbagg genetically dwarf mice with hypopituitary function[5]. A diminution of thymus size and a reduction in immunological responses to T cell-dependent antigens is also seen after administration of anti-hypophyseal serum[6]. The pituitary may contain several active principles affecting directly or indirectly the thymus functions. Of these, growth hormone has been shown to have a promoting effect on metabolic activities of the thymus and on immune responses involving the participation of thymus-derived T cells[4,7,8]. Thyrotropin, via the thyroid hormones, also exercises a thymotropic action[9]. ACTH, via corticosteroids, causes the transient diminution of the gland[10]. We report here the presence in pituitaries of a previously unknown small molecular weight peptide(s) which stimulates markedly the incorporation of tritiated thymidine into DNA in isolated thymocytes.

Figure 1 shows that the addition of the anterior pituitary extract (APE) to the isolated thymocytes resulted in a notable stimulation of the incorporation of ^{3}H-thymidine into cold trichloroacetic acid (TCA)-precipitable pellet. A stimulation with APE was consistently obtained but the extent varied from preparation to preparation of APE and thymocytes. Thymocytes from young rodents gave a better response than those from aged animals. Table 1 shows the order of variation in stimulation by APE of ^{3}H-thymidine incorporation in thymocytes obtained from six different animals. Variance analysis of the data indicates that between-animal variations are significantly more marked than between tube variations (variance ratio $F = 29.9$, 8.5 times more than required for significance at 1% level). These variations probably arise due to different internal environments to which the thymocytes are exposed before the animals were killed. The effect of APE is highly

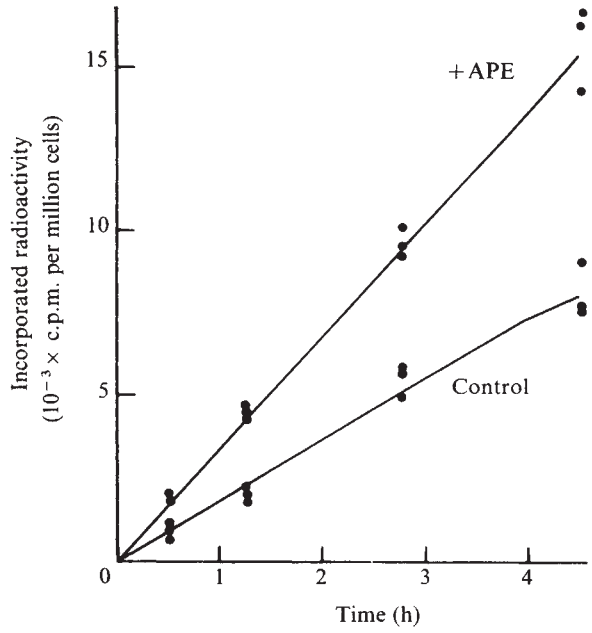

Fig. 1 Effect of anterior pituitary extract (APE) on ^{3}H-thymidine incorporation in isolated thymocytes. APE was the 2000*g* supernatant of rat anterior pituitary homogenates in GS medium (glucose salt medium, containing 120 mM NaCl, 5 mM KCl, 5 mM Na_2HPO_4, 1 mM $MgSO_4$, 0.8 mM $CaCl_2$, 5.5 mM glucose and 5 mM Tris-HCl buffer, *p*H 7.2). Rat thymocytes (15×10^6 ml^{-1}), were incubated in GS medium at 37 °C with ^{3}H-thymidine (0.5 μCi ml^{-1}, 2 μM) ± APE (added to a final concentration of 0.25 anterior pituitary equivalents per ml of final incubation medium). At different time intervals, the incorporation reaction was stopped by chilling and by adding excess (0.5 mM) of non-radioactive thymidine to the incubation tubes. Cells were centrifuged (600*g* for 10 min), washed with fresh GS medium, followed by two washings with cold 10% trichloroacetic acid and two with methanol. The pellet was dried and dissolved in 0.1 ml Soluene, transferred to the scintillation vials with a toluene-based scintillation mixture and the incorporated radioactivity measured in a Packard liquid scintillation spectrometer.

significant ($F = 1163.5$) and is much more than the animal-to-animal variations (variance ratio 159.2 times higher than that required for 1% significance level).

The incorporated radioactivity was sensitive to DNase but not to RNase treatment. Viability of cells was measured at each time point by Trypan blue exclusion and no significant difference was noted in control and APE added tubes.

To identify the pituitary hormones that may be responsible for this action of APE, rat growth hormones (NIAMD-rat GHB1), luteinising hormone (NIH-LH-S 17), follicle-stimulating hormone (NIH-FSH-S 9), thyrotropin (NIH-TSH-B 4), prolactin (NIH-P-B 3) and ACTH (pork ACTH, Chemical Lab. Frideriksberg, Denmark) were tested singly and in combination. Two hormone combinations were prepared: in mixture A the hormones were present at their approximate physiological level in pituitary and in mixture B, at five times higher concentration. These hormones caused a marginal increase in thymidine incorportion, but the effect was not comparable with that obtained with APE (Table 2). The active principle in the extract thus seems to be different from these hormones.

The factor was dialysable and heat stable and was distinct from LH and TSH releasing hormones. The activity was not present in hypothalamic extracts or in posterior pituitary and cerebral cortex extracts. Cyclic nucleotides and inorganic ions did not give parallel stimulation of this metabolic activity in isolated thymocytes. The stimulatory effect was not due to the possible supply of essential

Table 1 Effect of anterior pituitary extract (APE) on ^{3}H-thymidine incorporation in thymocytes from six young rats

Rat	Body weight (g) and sex	Cell density in assay medium (million per ml)	Incorporated radioactivity c.p.m. per million cells ± s.e.m. Control	+ APE	% Of control
1	94.5 (F)	4.40	2652±133 (3)	7086±174 (4)	266.5
2	77.6 (F)	4.52	1832± 55 (4)	4483±210 (4)	244.7
3	74.3 (M)	5.12	2189± 28 (4)	5451±169 (4)	249.0
4	88.7 (F)	3.88	2964± 80 (4)	6553± 84 (4)	221.1
5	69.1 (F)	4.80	2824± 64 (4)	6041±295 (4)	213.9
6	44.1 (M)	1.76	2184± 42 (4)	6696±195 (4)	306.6

Thymocytes prepared separately from six young rats were incubated at 37 °C for 2 h ± APE (0.8 anterior pituitary equivalent per ml) in GS medium containing 1 μCi ml^{-1} ^{3}H-thymidine (1 μM). Values in parenthesis are the number of replicates for each preparation of cells.

nutrients by the APE, and was also obtained when experiments were performed in tissue culture media RPMI and Medium 199.

The stimulatory activity of the APE on thymocytes was sensitive to hog intestinal peptidase (from Sigma, containing general proteolytic and aminopeptidase activity) but not to DNase, RNase and trypsin. The factor may therefore be a peptide. Its molecular weight (MW) is 500, and the isoelectric *p*H 7.9. Purified preparations of the factor do not show significant ultraviolet absorption above 230 nm. These properties indicate that this factor is distinct from other factors of pituitary origin described in recent years by other investigators. Fibroblast growth factor from bovine pituitaries[12] has a MW of 13,300. Chondrocyte mitogenic factor[13] is a heat-labile entity and was detected as a contaminant in anterior pituitary hormone preparations. The factor influencing the ovarian cells in culture[14] is a non-dialysable protein. Lipotropins[15] are big polypeptides (6,000 and 11,400 molecular weight). A hypophysial factor influencing the steroid metabolism in rat hepatoma cell lines[16] is a high molecular weight entity and in contrast to present case, is present only in female pituitaries. Pituitary endorphins[17] are polypeptides of 3,000–3,500 MW.

The action of APE on uptake and incorporation of thymidine seems to be tissue specific. Thymocytes show maximal stimulation but lymphoid cells derived from spleen and lymph nodes are also stimulated, though to a lesser extent. APE did not have stimulatory effect on thymidine incorporation in tissue slices from liver, kidney, heart and diaphragm.

The biological role of this factor(s) is not fully known. It may influence the proliferation of cells within thymus. Mouse embryonic thymus cultures in media containing this factor show higher cellularity than parallel control cultures.

Thymus is an organ characterised by a high rate of cellular proliferation, which is independent of the antigenic stimulus[11], and not much is known on the factors regulating the metabolic activities and the rate of cell proliferation in this tissue. The size of this gland shrinks with hypophysectomy[1–4]. The gland is also atrophied in states of hypopituitary function[5]. It is thus conceivable that pituitary exercises an effect on lymphoid cell proliferation in thymus. The presence of a factor exerting a marked influence on DNA synthesis in thymocytes is thus of interest.

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1 Smith, P. E. *Anat. Rec.* **47**, 119–129 (1930).
2 Atwell, J. W. *Proc. Soc. exp. biol. Med.* **29**, 1259–60 (1932).
3 Feldman, J. D. *Anat. Rec.* **110**, 17–40 (1951).
4 Talwar, G. P. *et al. Rec. Prog. Hor. Res.* **31**, 141–174 (1975).
5 Baroni, C. *Experientia* **23**, 282–283 (1967).
6 Pierpaoli, W. & Sorkin, E. *Nature* **215**, 834–837 (1967).
7 Li, C. H. & Evans, H. M. *Recent Prog. Horm. Res.* 3, 3–44 (1948).
8 Pandian, M. R. & Talwar, G. P. *J. exp. Med.* **134**, 1095–1113 (1971).
9 Pierpaoli, W., Fabris, N. & Sorkin, E. in *Hormones and the Immune Response, Ciba Foundation Study Group*, (ed Wolstenholme, G. E. W. & Night, J.), 126–153 (J. & A. Churchill, London, 1970).
10 Daugherty, T. F. *Physiol. Rev.* **32**, 379–401 (1952).
11 Metcalf, D. in *Thymus in Immunobiology* (eds Good, R. A. & Gabrielsen, A. E.), 150–182 (Hoeber Medical Division, Horper and Row Publishers, London, 1964).
12 Gospodarowicz, D. *J. biol. Chem.* **250**, 2515–2520 (1975).
13 Corvol, M. T., Malemud, C. J. & Sokoloff, L. *Endocrinology* **90**, 262–271 (1972).
14 Nishikawa, K., Armelin, H. A. & Sato, G., *Proc. natn. Acad. Sci. U.S.A.* **72**, 483–487 (1975).
15 Li, C. H. *Arch. Biol. Med. exp. (Chile)* **5**, 55–61 (1968).
16 Gustafsson, J. A., Larsson, A., Skett, P. & Stenberg, A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4986–4990 (1975).
17 Gentleman, S., Ross, M., Loweny, L. I., Cox, B. M. & Goldstein, A. in *Opiates and Endogenous Opioid Peptides* (ed Kosterlitz, H. W.), 27–34 (Elsevier/North Holland, Amsterdam, 1976).

Table 2 Effect of anterior pituitary hormones and anterior pituitary extract on the incorporation of thymidine into isolated thymocytes

Addition	Incorporated radioactivity c.p.m. per million cells Mean ± s.e.m.	% Of control
None	429±31 (4)	100.0±7.2
Hormone mixture (A)	536±42 (6)	124.9±9.8
Hormone mixture (B)	584±57 (5)	136.1±13.3
Anterior pituitary extract (APE)	1567±95 (5)	365.3±22.1

Five million rat thymocytes in 1 ml GS medium, were incubated with tritiated thymidine (0.5 μCi ml^{-1}, 2 μM) with or without hormones/APE at 37 °C for 2 h in a Dubnoff metabolic shaker. Hormone mixture (A) contained at final concentration, 25 μg of GH, 20 μg each of TSH, FSH, LH and prolactins and 10 μg of ACTH per ml of incubation medium. Mixture (B) had five times the concentration of these hormones. APE was added at one rat anterior pituitary equivlaent per ml of final incubation medium. Figures in parenthesis give the number of replicates in each case.

Propranolol increases binding of thyrotropin to thyroid membranes

β-Adrenergic antagonists such as propranolol possess two pharmacologically relevant properties—β-adrenergic blockade is observed at relatively low concentrations, whilst at higher concentrations they are effective membrane stabilising agents[1,2]. The latter property which, unlike β-adrenergic antagonism is non-stereospecific, is also described as local anaesthetic or quinidine like activity and it possibly accounts at least in part, for the anti-arrhythmic properties of propranolol—local anaesthetics and quinidine also showing anti-arrhythmic activity[3–5]. A wide variety of effects have been associated with local anaesthetic activity[1], but as yet there is no unifying explanation of the mechanism of action. We have investigated the effect of propranolol, acting in its capacity as a local anaesthetic, on a hormone receptor adenylate cyclase system which might be expected to be sensitive to membrane perturbation by local anaesthetics, and reported inhibition of thyro-