J. Indian Inst. Sci., 64 (C), Mar. 1983, Pp. 27–35

Effect of estradiol on the *in vitro* assembly of rat brain tubulin

R. RAVINDRA*
Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India.

Received on March 8, 1982; Revised on May 11, 1982.

Abstract

In order to examine if an interaction existed between estradiol and microtubules, tubulin from rat brain was isolated. Ability of this tubulin preparation to hydrolyse GTP and to assemble into microtubules *in vitro* was assessed. Tubulin-induced GTP hydrolysis was observed to occur as almost a single burst. It was found that estradiol inhibited this GTPase activity of tubulin in a dose-dependent manner and this inhibition could be overcome by a large excess of GTP. Estradiol also inhibited the rate as well as the extent of tubulin assembly *in vitro*, as assessed by turbidity changes and electron microscopy.

Key words: Estradiol, tubulin, GTP hydrolysis.

1. Introduction

Tubulin, the monomeric unit of microtubules, has a molecular weight of about 110,000 daltons and is composed of two non-identical sub-units alpha and beta[1]. Tubulins have remained very stable in evolution, histones apparently being the only class of proteins which have undergone less change since the origin of eukaryotes. Common antigenic determinants in microtubules from mammals, birds, reptiles, teleosts and diptera have been reported[2].

Earlier work strongly suggested that microtubules are involved in the inhibitory effect of estradiol on progesterone secretion by corpus luteum of pregnant hamster[3,4]. In

Abbreviations

EGTA : Ethylene glycol-bis (β-amino ethyl ether)-N, N′-tetraacetic acid.
GTP : Guanosine triphosphate.
MES : 2N-Morpholino-ethane sulfonic acid.
TCA : Trichloroacetic acid.

* Present address: Department of Biological Science, College of Letters and Science, University of Idaho, Moscow 83843, Idaho, U.S.A.

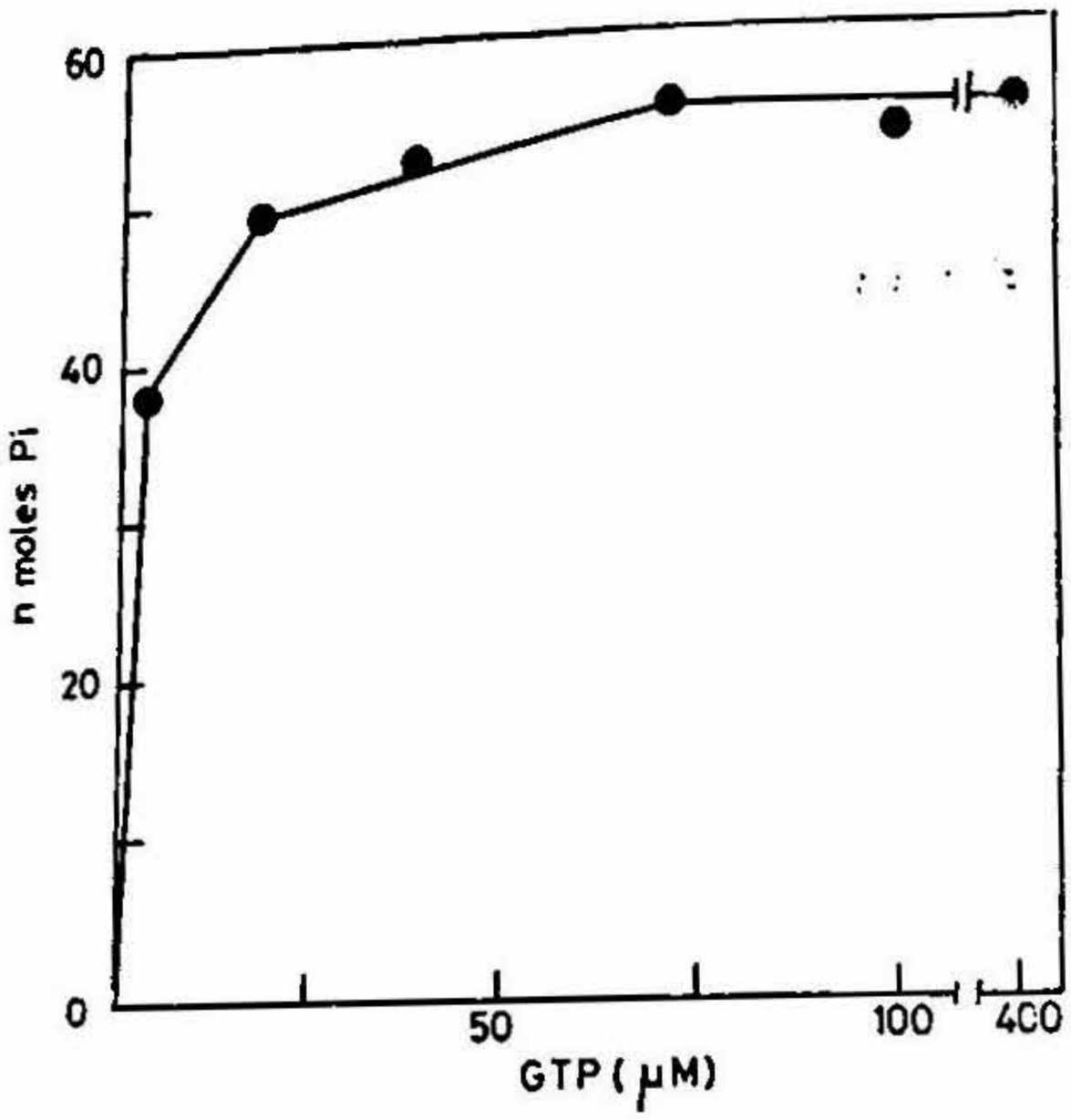

FIG. 1. P_i liberation as a function of GTP concentration. 500 nM of tubulin preparation was used. The reaction was conducted for 30′. Each point represents the mean of duplicate determinations. This experiment was repeated twice.

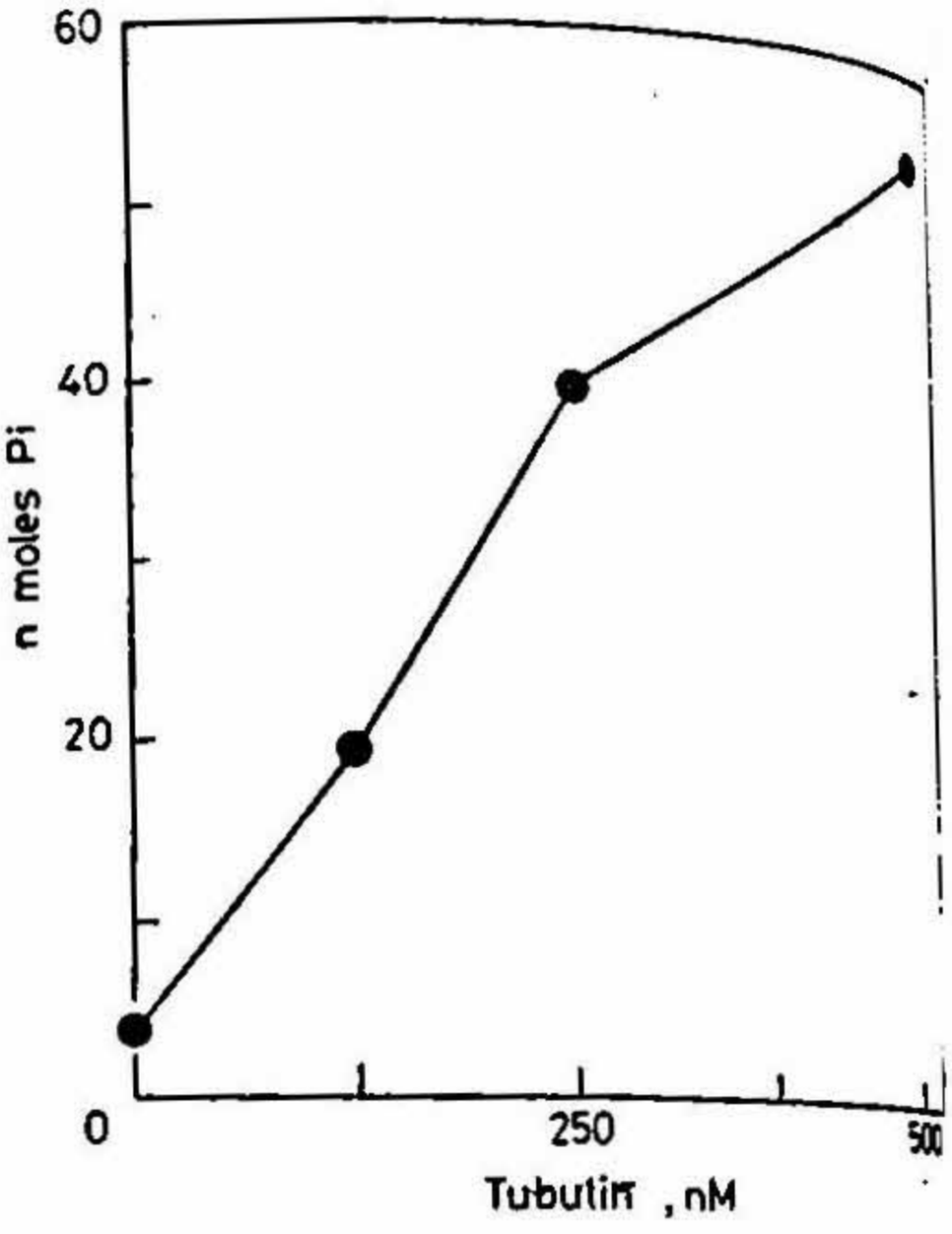

FIG. 2. GTP hydrolysis as a function of tubulin concentration. GTP 100 μM was used and the reaction was terminated at 30′. Each point represents mean of duplicate determinations. The experiment was repeated twice.

the present study, the effect of estradiol on tubulin assembly has been followed by examining its effect on GTP hydrolysis, turbidity measurements and changes observed in electron microscopy.

2. Materials and methods

2.1. *Tubulin*

Tubulin was isolated from rat brain using phosphocellulose chromatography as described earlier[5].

2.2. *Determination of GTP hydrolysis*

Tubulin-induced GTP hydrolysis was measured according to Jacobs *et al*[6]. Tubulin (12–25 μg/ml) was incubated at 30° C in MES buffer. Buffer, pH 6·8, containing 5 mM 2-N-morpholino ethane sulphonic acid, 1·0 mM EGTA (Sigma), 0·5 mM $MgCl_2$ and KCl 50 mM. The reaction was initiated by the addition of GTP, TCA final concentration, 10% (w/v) being used to terminate the reaction. The volume of the reaction mixture was 500 μl. After centrifuging at 3000 rpm, the supernatant was assayed for P_i according to the method of Fiske and Subbarow[7].

2.3. *Tubulin assembly* in vitro

Turbidimetric studies of the *in vitro* assembly of tubulin was performed according to the method of Gaskin *et al*[8]. 1·4–3·0 mg/ml of rat brain tubulin was incubated at 37° C in MES buffer, pH 6·8 (100 mM MES, 0·5 mM $MgCl_2$, 1·0 mM EGTA and KCl 50 mM). The reaction was initiated by the addition of GTP. Absorbance at 350 nm was monitored using SP 8–100 Pye unicam recording spectrophotometer with temperature-controlled chamber attached to it.

2.4. *Electron microscopy*

1·0 mg/ml of rat brain tubulin was allowed to assemble into microtubules as described above. For electron microscopy, samples were negatively stained with 6% (w/v) uranyl acetate, after drop-loading on to parllodion carbon grids as described by Kirschner *et al*[9]. The samples were visualised in Philips electron microscope.

3. Results

3.1. *GTP hydrolysis by tubulin preparation*

First of all, the parameters related to GTP hydrolysis were investigated. Liberation of inorganic phosphate as a consequence of GTP hydrolysis by tubulin preparation

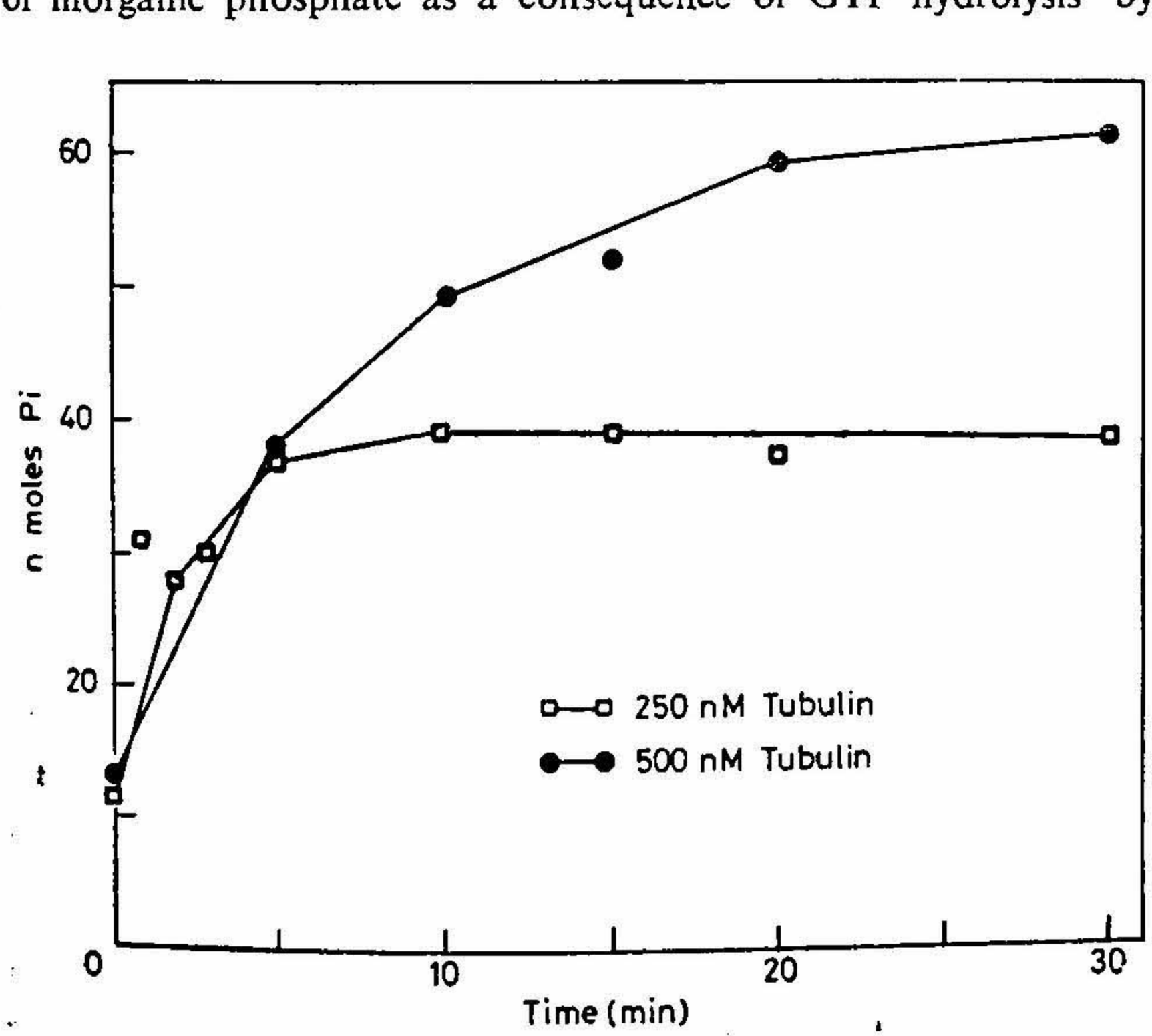

FIG. 3. Time course of GTP hydrolysis. 100 μM of GTP was used in all the tubes. Each point represents mean of duplicate determinations.

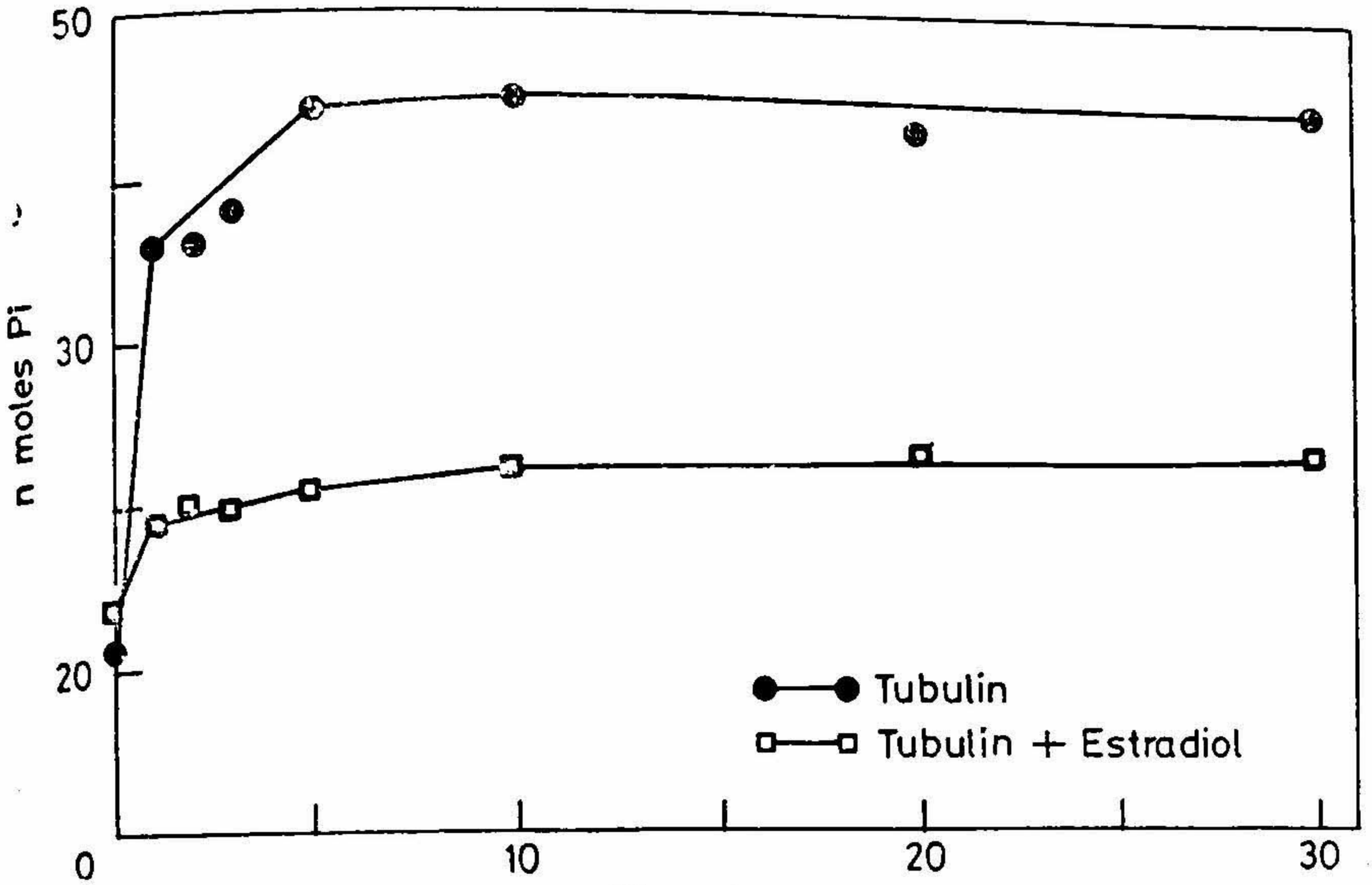

FIG. 5. Time course of inhibition of GTP hydrolysis by estradiol-17β. Each point represents mean of duplicate determinations. GTP—10μ0 M, Tubulin—250 nM, Estradiol—300 nM.

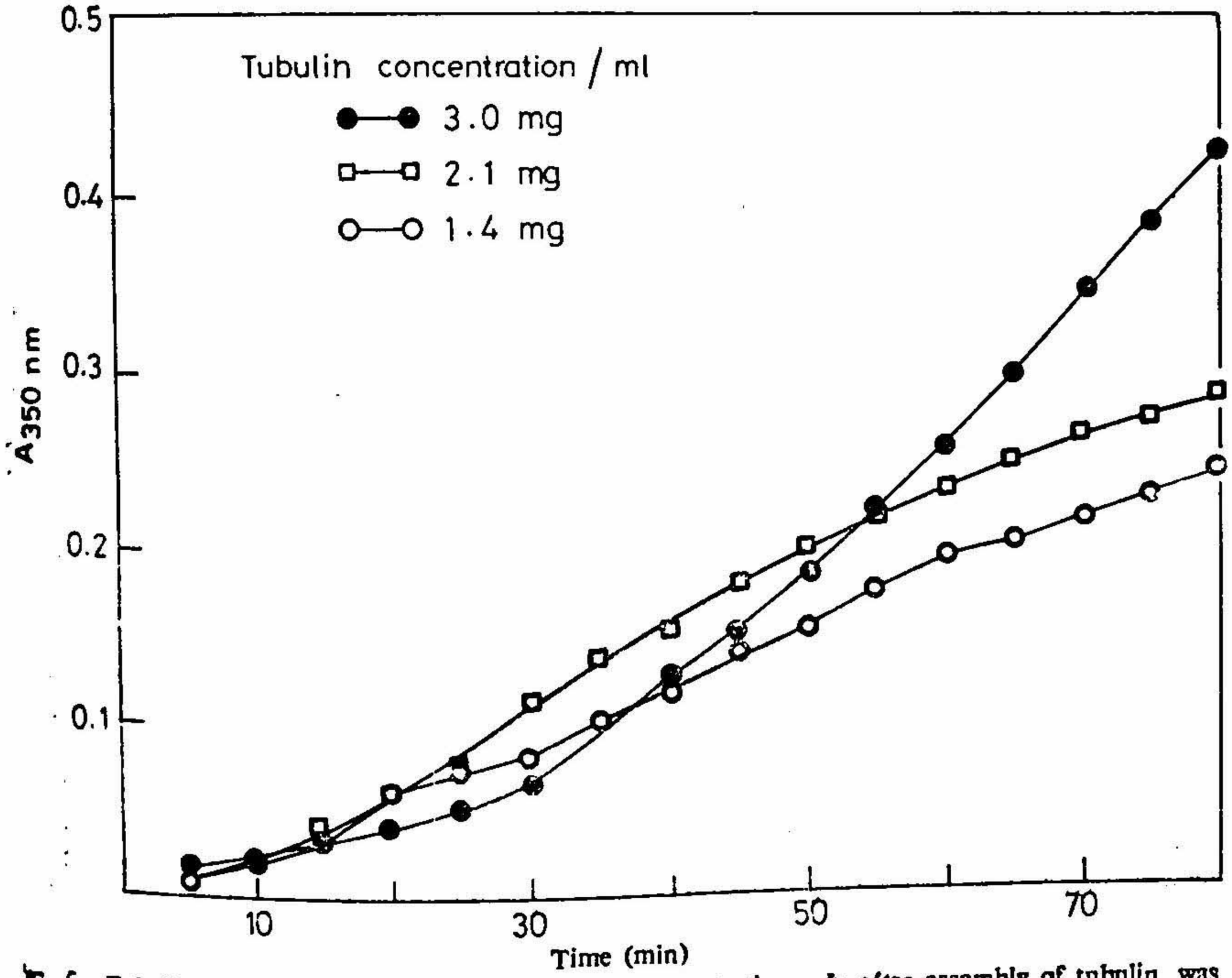

FIG. 6. Tubulin assembly as a function of tubulin concentration. *In vitro* assembly of tubulin was monitored in presence of 100 μM GTP.

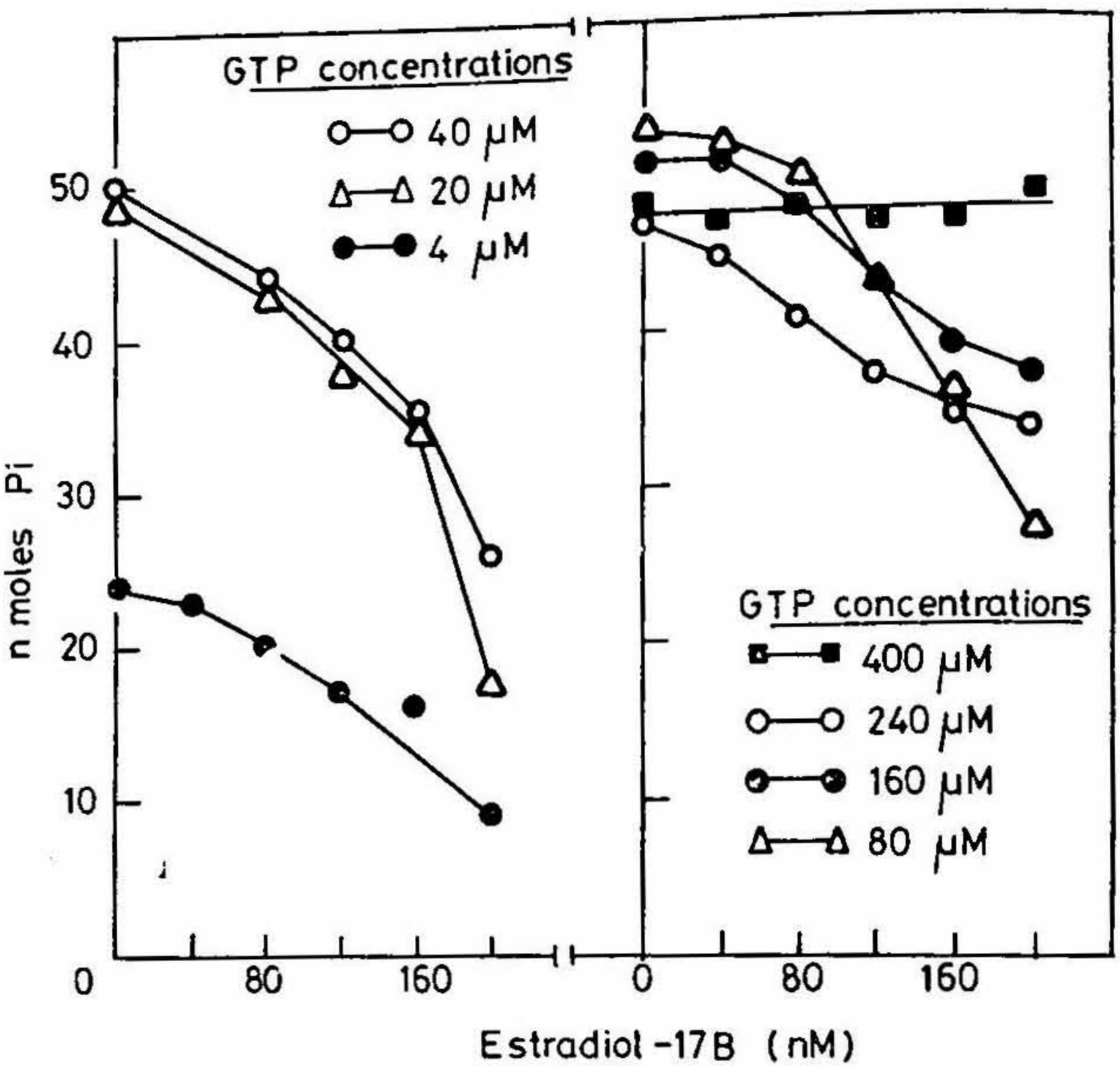

FIG. 4. Effect of estradiol-17β on GTP hydrolysis. GTPase assay was conducted for 15′ as described in materials and methods section. 250 nM tubulin was used in all the experiments. Each point represents mean of duplicate determinations.

indicated that maximum hydrolysis occurred at a GTP concentration of at least 50 μm (Fig. 1). Increasing the concentration of tubulin resulted in increased liberation of inorganic phosphate (Fig. 2). A time course study indicated that GTP hydrolysis occurred as a burst, reaching an apparent peak by 5′ in case where 250 nM tubulin was used. Increasing tubulin concentration to 500 nM resulted in slowing down the reaction rate to a certain extent, although about 60% of hydrolysis was observed in 5′ (Fig. 3).

3.2. *Effect of estradiol on GTP hydrolysis*

Dose response curves of the effect of estradiol on GTP hydrolysis was studied by fixing the tubulin concentration and varying GTP concentration. A range of GTP concentration (4–400 μM) was employed. It is evident from the results (Fig. 4) that the effect of estradiol is maximal at lower GTP concentrations, while higher amounts of GTP seem to override the inhibitory effect of estradiol. It was observed that maximal inhibition of GTP hydrolysis occurred at a concentration of 200 nM estradiol. A time course study revealed that estradiol effect is almost instantaneous, about 50% inhibition of GTPase activity being observed by 1′ (Fig. 5).

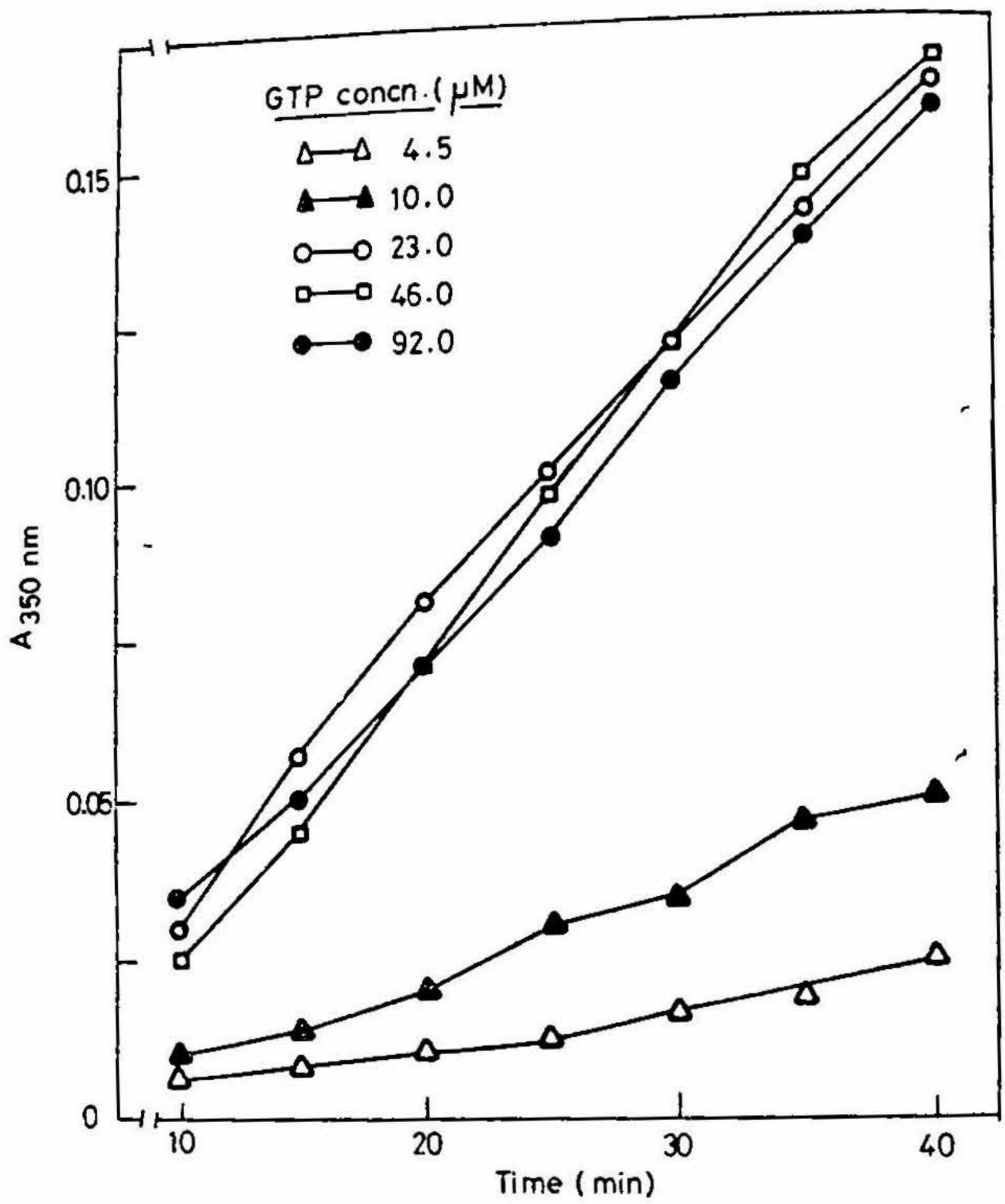

FIG. 7. Tubulin (1·4 mg/ml) assembly as a function of GTP concentration.

3.3. *Effect of estradiol on tubulin assembly*

The extent of tubulin polymerisation, measured by turbidity changes, showed a dependence on tubulin concentration (Fig. 6). Under conditions of fixed tubulin concentration and varying GTP concentration, tubulin assembly showed a dependence on GTP concentration and proceeded at almost the same rate beyond a concentration of 23 μM (Fig. 7). Estradiol inhibited tubulin polymerisation in a dose-dependent manner. Increasing concentrations of estradiol showed a progressive inhibition of the extent of tubulin assembly (Fig. 8). Electron microscopic analysis revealed that tubulin polymerisation occurred under the *in vitro* conditions described under Materials and methods. However, treatment with estradiol (50 μM) completely blocked the assembly of tubulin (Fig. 9).

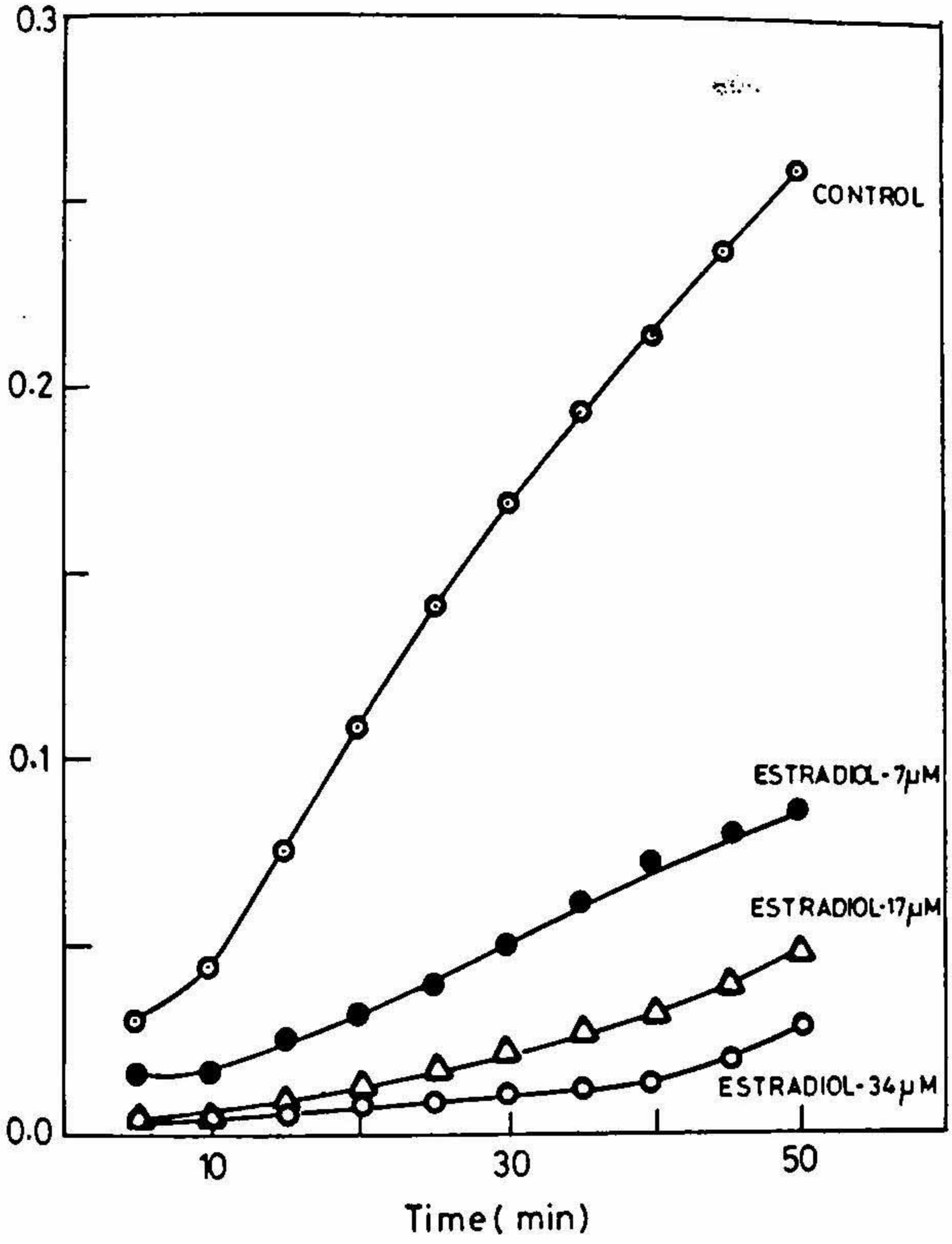

FIG. 8. Effect of estradiol-17β on tubulin assembly. Assembly was monitored spectrophotometrically at 350 nM. 25 μM GTP and 2·5 mg/ml tubulin were used in all these experiments.

4. Discussion

The inhibition of GTP hydrolysis by estradiol suggests a role for this steriod hormone in controlling tubulin assembly. At the same time, the fact that this inhibition could be overcome by excess GTP indicates that the estradiol effect may be reversible. The method of choice to study tubulin assembly is to measure turbidity at 350 nm and using this assay it is observed that estradiol affects the assembly of tubulin. These results were further confirmed by electron microscopic analysis.

Thus, estradiol appears to affect the function of the tubulin-microtubule system. Based on these results as well as those reported earlier[3,4] it can be suggested that

(A) (B

FIG. 9. Electron micrographs of tubulin (1 mg/ml) assembly. A– without estradiol (× 4800). B–in presence of 50 μM estradiol (× 4800).

estradiol could be inhibiting progesterone secretion by the corpora lutea by interfering with tubulin assembly. It is possible that estradiol causes accumulation of tubulin monomers in the luteal cell, resulting in a derangement of secretory processes.

It is essential to maintain the equilibrium between monomeric and polymeric form of tubulin in the cells, and regulatory mechanisms must exist to prevent all cytoplasmic tubulin from assembling into microtubules. It is tempting to speculate that estradiol may be one such molecule which has a role in regulating tubulin function *in vivo*

Acknowledgement

The author is grateful to Professor G. Padmanaban for helpful discussions.

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