These contradictions can be explained on the grounds that endogenous CFU-S are evidently part of a population of stem cells in a state of active proliferation [9], whereas the transplantable CFU-S are perhaps mainly in the G_0 stage or in the long G_1 phase [10]. There is evidence that sublethal irradiation synchronizes cells in the S phase to some degree, and in that phase CFU-S are more resistant to irradiation than cells not in the cycles [9, 15]. It can be postulated that pertussis vaccine and *M. arthritidis* differ in their effects on the proliferative state of the CFU-S population, and this problem is the subject of a current study.

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ROLE OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN THE ACTION OF ESTRADIOL IN UTERINE TISSUE

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Changes in the cyclic nucleotide concentration in the cells of animal tissues under the influence of hormones as a rule take place on account of changes in activity of adenylate cyclase, associated with receptors for hormones in the outer membrane of cells which react to them [3, 14]. As regards another enzyme involved in cyclic nucleotide metabolism, namely phosphodiesterase (PDE), concentrated mainly in the cell cytosol, according to the generally accepted view its role can be reduced to removal of an excess of cyclic nucleotides formed as a result of activation of adenylate cyclase, the immediate target for the action of a hormone entering the cell.

The sole and unique exception to this rule is the photoreceptor cell of the retina (rod). In this cell the receptor of photons (rhodopsin) is linked with PDE and, as a result of illumination, the activity of this enzyme changes sharply [1, 5, 6]. The nature of the stimulus reaching the cell in this particular case is unusual (quanta of light). In addition, this stimulus does not arise from the internal medium, like a hormone, but from the external

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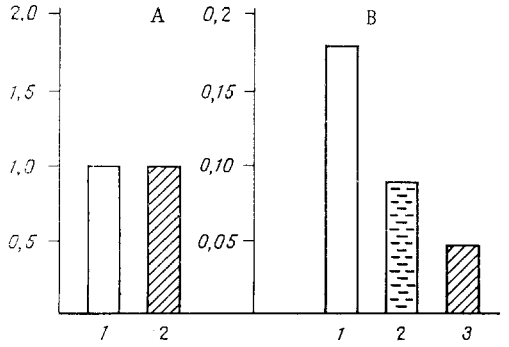

Fig. 1. Effect of estradiol on adenylate cyclase (A) and PDE (B) in uterine tissue preparations. Ordinate, enzyme activity: A) in picomoles; B) in conventional units (optical density at 660 nm). 1) Control, 2) estradiol concentration $2 \cdot 10^{-5}$ M, 3) 10^{-6} M.

medium. The photoreceptor cell is also characterized by a unique structure: The receptor component in it is intracellular in its localization. It can be postulated on the basis of all these facts that the association of rhodopsin with PDE in the photoreceptor represents a unique situation characteristic of this strictly specialized cell only.

To test this hypothesis and to shed light on the problem of whether such a situation may arise in other, ordinary cells reactive to hormones, the effect of estradiol was studied on enzymes of cyclic nucleotide metabolism in the tissues of the uterus. The uterus was chosen as test object because estradiol receptors in the uterine tissue are localized intracellularly [2]; meanwhile, under the influence of estrogens changes have been observed in the cyclic nucleotide concentrations in this tissue in experiments *in vivo* [7, 11], and on that basis a role has been postulated for these nucleotides as secondary mediators of estradiol action. It is not yet known whether one of the enzymes of cyclic nucleotide metabolism must be coupled with estrogen receptors.

EXPERIMENTAL METHOD

Experiments were carried out on preparations obtained from the uterus of sexually mature Wistar rats.

To determine adenylate cyclase [4] the coarse membrane fraction (residue obtained by centrifugation of the homogenate at 1000g for 10 min) was used. To prepare the homogenate, tissue of the uterus was homogenized (1:10) in buffer containing 10 mM Tris-HCl, pH 7.5; 0.5 mM EDTA, and 2 mM β-mercaptoethanol. The protein content in the sample was 50-100 μg and the incubation time was 10-20 min.

PDE was determined [8] in preparations obtained by the following scheme: The tissue homogenate (1:10) in the same buffer was centrifuged at 100,000g for 60 min, after which the supernatant was concentrated on Amicon membrane filters by means of an FMO-1 apparatus. The protein content in the sample was 50-100 μg and the substrate was cyclic AMP (1 mM). The 17-β-estradiol, obtained from Sorin Biomedica (Italy), was used in concentrations of 10^{-7}-10^{-5} M.

To study binding of the hormone by cytosol receptors, uterine tissue was homogenized (1:10) in the same buffer and the mixture centrifuged as described above (for determination of PDE). Binding was measured by the method in [10]; samples, each containing 200-400 μg protein, were incubated for 30 min with labeled (2,4,6,7-^{3}H)-estradiol (85 Ci/mmole) or with a mixture of labeled and unlabeled estradiol; specific binding was estimated by the usual method. Receptors were blocked with clomiphene (from Egyt, Hungary), which other workers also have used for this purpose [12]. Radioactivity was counted (for determining adenylate cyclase and hormone binding) on a "Rack Beta-1216" liquid scintillation counter (LKB, Sweden).

TABLE 1. Specificity of Effect of Estradiol on Uterine PDE (mean results of 5-7 experiments)

Source of enzyme	Estradiol concentration, M	Enzyme activity, optical density units at 660 nm	
		control	estradiol
Rat uterus	$5 \cdot 10^{-6}$	0,10	0,03
	$1 \cdot 10^{-6}$	0,12	0,06
	$1 \cdot 10^{-7}$	0,13	0,09
	$1 \cdot 10^{-7}$	0,29	0,18
Rat brain	$5 \cdot 10^{-6}$	0,58	0,58
Bovine heart*	$5 \cdot 10^{-6}$	0,26	0,26
Outer segments of rods of bovine retina	$1 \cdot 10^{-6}$	0,36	0,36
	$1 \cdot 10^{-7}$	0,36	0,38

*PDE preparation from bovine heart, from Sigma

TABLE 2. Effect of Estradiol ($1 \cdot 10^{-7}$ M) on PDE Activity from Uterine Tissue against the Background of Clomiphene (mean results 5-7 experiments)

PDE activity, optical density units at 660 nm			
control	+ estradiol	+ clomiphene	+ estradiol + clomiphene
0,14	0,09	0,14	0,13
0,14	0,10	0,14	0,14
0,24	0,18	0,24	0,24
0,25	0,17	0,25	0,25
0,29	0,18	0,28	0,27
0,17	0,09	0,16	0,15
0,14	0,07	0,13	0,13

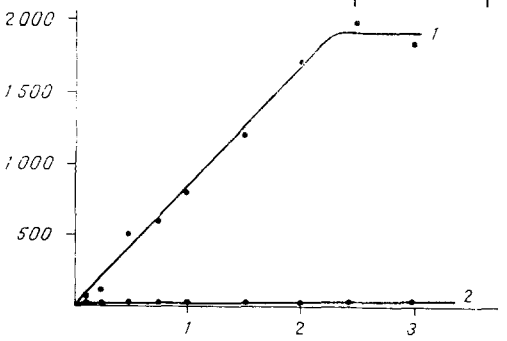

Fig. 2. Binding of estradiol by cytosol from uterine tissue. Abscissa, concentration of labeled estradiol (in $M \cdot 10^{-7}$); ordinate, specific binding (in counts per sample/min). Protein content in sample 300 μg. 1) Control, 2) in presence of clomiphene.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that estradiol, in concentrations of 10^{-6}-10^{-5} M, did not affect adenylate cyclase activity of the uterine tissue homogenate but significantly inhibited the PDE activity of preparations obtained from this tissue. The degree of inhibition was determined by the hormone concentration and depended also on the quality of the preparations used (their keeping time, the age of the rats, and so on). These data, which we obtained *in vitro* and which are evidence of inhibition of PDE in the presence of estradiol, are in good agreement with the results of investigation into accumulation of cyclic nucleotides in the uterine tissues following administration of the hormone *in vivo* [7, 11].

In the next series of experiments it was shown that the effect of estradiol on PDE is specific for uterine tissue. The hormone had no action on activity of the partially purified preparations obtained from the outer segments of the retinal rods [9], brain tissue [13], and a preparation of the enzyme from heart tissue (from Sigma, USA), nor did it affect Ca-dependent and Ca-independent forms of the enzyme isolated from tissues other than the uterus (Table 1).

The special reactivity of PDE from uterine tissue to estradiol could be explained by coupling of the enzyme in this case with the specific hormone receptor that is absent in other tissues. In that case blockade of the receptor ought correspondingly to abolish the effect of the hormone on PDE. The presence of specific binding of the hormone with cytosol receptors of uterine tissue and the possibility of complete blocking of this process in the presence of clomiphene were tested first (Fig. 2).

When the effect of estradiol was studied against the background of clomiphene, addition of which to the mixture itself had no effect on PDE activity, it was found that after blockade of the receptors the hormone did not affect the enzyme (Table 2). These results are thus direct proof of binding of PDE by the receptors.

It can be concluded from all the results described above that besides the unique photoreceptor cell, binding of the receptor component with PDE may also take place in ordinary cells responding to the hormone. A decisive role in the binding of one of the enzymes of cyclic nucleotide metabolism with the receptor is evidently played by the location of the receptor in the cell. If it is intracellular, it can bind with PDE, which in that case plays the key role in the regulation of the cyclic nucleotide content in response to reception of different adequate stimuli. Consequently, the functional role of PDE is much more varied than was hitherto considered. The common principles governing binding of receptors with enzymes of cyclic nucleotide metabolism in cells of widely different types (photoreceptor, uterine cell) must also be specially emphasized.

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