Triton X-100-toluene (1:2) scintillation solution. The radioactivity of the mixture was denoted by A_1. Then 0.5 ml of 50 mM EDTA plus 0.2 ml of 50% trichloracetic acid were added to the rest of each sample. This mixture was centrifuged and 1 ml of supernatant was added to 10 ml of the scintillation solution mentioned above. The radioactivity of that mixture was denoted by A_2. The radioactivity was measured with the liquid scintillation counter SL-4200 (Intertechnique). The data are presented as mean ± SE. The statistical significance of the difference between means was assessed by Student's t-test; p-value less than 0.05 was considered significant.

A_2 is a specific radioactivity, i.e. the radioactivity of 1 ml of supernatant of the mixture with EDTA and TCA (the total volume of this mixture is 1.4 ml); it does not differ from the specific radioactivity of the remainder, as we have found, therefore the total radioactivity A of each sample was calculated according to the formula: $A = A_1 + 1.4\,A_2$.

Results and discussion. The ratio A/A_1 (denoted by the parameter R) was chosen as a semiquantitative characteristic feature for cell membrane affinity to Ca^{2+}, because A_1 is the radioactivity of Ca^{2+} extracted under mild conditions (and seems to be proportional to the easily exchangeable Ca^{2+} of the cell surface). The data obtained are specified in tables 1 and 2.

Rat erythrocytes. The erythrocytes of SHR incorporated more ^{45}Ca in the control solution (without ACh and eserine) than the erythrocytes of NWR ($p < 0.005$). This was the result of (at least) an easier isotopic exchange, since this excess of labelling coincides with a smaller value of the parameter R ($p < 0.05$). ACh significantly inhibited the labelling of erythrocytes of SHR and NWR ($p < 0.001$ and $p < 0.002$, respectively) but the difference between these groups is not significant. The parameter R was not considerably altered. Eserine had insignificant influence on the ^{45}Ca-binding to erythrocytes of SHR and NWR; the parameter R was unchanged for SHR but was reduced significantly for NWR ($p < 0.02$). Thus a considerable variation in the parameter R under eserine influence between SHR and NWR was found ($p < 0.05$). It appears reasonable to assume that ^{45}Ca-ions are exchanged with membrane calcium, because the swelling of erythrocytes affected by ACh or eserine was not accompanied by any increase of label incorporation into the cells.

Human erythrocytes. No significant difference, either in ^{45}Ca-binding or in the parameter R between HP ($n = 8$) and NP ($n = 8$) were observed for the control incubation medium. Earlier it was found that HP-erythrocytes have less affinity to Ca^{2+} than the NP-erythrocytes[6]. The different conditions of Ca^{2+}-extraction from erythrocytes may be an explanation of the divergence between the old and new data. ACh reduced the label incorporation into erythrocytes for NP and HP ($p < 0.001$) but there was no difference in this property between these 2 groups. The parameter R was increased only for HP ($p < 0.05$) but the difference between the groups remained insignificant. Eserine reduced the ^{45}Ca-binding to erythrocytes of HP ($p < 0.005$) and did not significantly influence that of NP, but the difference between the groups became significant. The parameter R was decreased for HP and was increased insignificantly for NP; moreover, the difference in this property between these groups was significant ($p < 0.05$).

Thus, we have established that ACh and eserine (the inhibitor of acetylcholinesterase) influence Ca^{2+}-binding to erythrocytes of rats and humans. The variation of the parameter R is not always parallel to that of the incorporation of ^{45}Ca into erythrocytes. This may indicate that not only cell membrane affinity to Ca^{2+} but, in some cases, also the easily exchangeable Ca-pool of cell surface is altered under the action of ACh and eserine. Differences in Ca^{2+}-binding to SHR and HP erythrocytes, on one hand, and erythrocytes of the respective control groups, on the other, were found under eserine influence. It may well be that there is some interrelation between the difference in the influence of eserine on Ca^{2+}-exchange within the 2 rat groups and the different contents of erythrocyte membrane phosphoinositides of SHR and NWR reported earlier[2], because the close interrelation between mammalian erythrocyte membrane acetylcholinesterase and phosphoinositides has been established[7].

1 Reprint requests should be addressed to P.V.G.

2 G.M. Boriskina, P.V. Gulak and Yu.V. Postnov, Experientia *34*, 744 (1978).
3 R.H. Michell, L.M. Jones and S.S. Jaffarji, Biochem. Soc. Trans. *5*, 77 (1977).
4 W.H. Huestis and H.M. McConnel, Biochem. biophys. Res. Commun. *57*, 726 (1974).
5 W.H. Huestis, J. supramolec. Struct. *4*, 355 (1976).
6 Yu.V. Postnov, S.N. Orlov, A. Shevchenko and A.M. Adler, Pflügers Arch. *371*, 263 (1977).
7 M.G. Low and J.B. Finean, FEBS Lett. *82*, 143 (1977).

Effect of theophylline and triiodothyronine on some early estrogenic responses in the rat uterus[1]

J. Steinsapir, S. León, P. Bustón, M. Alburquerque, A.M. Rojas, A. Fernández, R. Pacheco and A. Tchernitchin

Department of Physiology and Biophysics and Laboratory of Experimental Endocrinology, Department of Experimental Morphology, University of Chile Medical School, Santiago Norte, Casilla 6524, Correo 7, Santiago (Chile), 2 January 1979

Summary. Theophylline increases and triodothyronine decreases uterine edema induced by physiological doses of estradiol-17β. Both of them decrease estrogen-induced uterine eosinophilia and the number of blood eosinophils, suggesting an explanation for the results in the uterus.

Thyroid hormones are known to have antiestrogenic properties[2]. Hyperthyroidism is associated with prolonged diestrus and hypothyroidism with a prolonged estrus cycle[2,3]. There is no agreement as to the mechanism whereby the thyroid influences uterine sensitivity to estrogens[4,5]. Evidence has been published for the mediation of various known metabolic effects of estrogens on the uterus by cyclic AMP[6,7]. Theophylline, when it is administered concurrently with cyclic AMP, also increases the activities of various key glycogenolytic and hexose monophosphate shunt enzymes, as well as uterine glycogen synthesis[6]. It has been proposed that uterine eosinophils mediate estrogen induced uterine edema[8], and it was suggested that this effect could be dependent on the number of eosinophils in the blood[9].

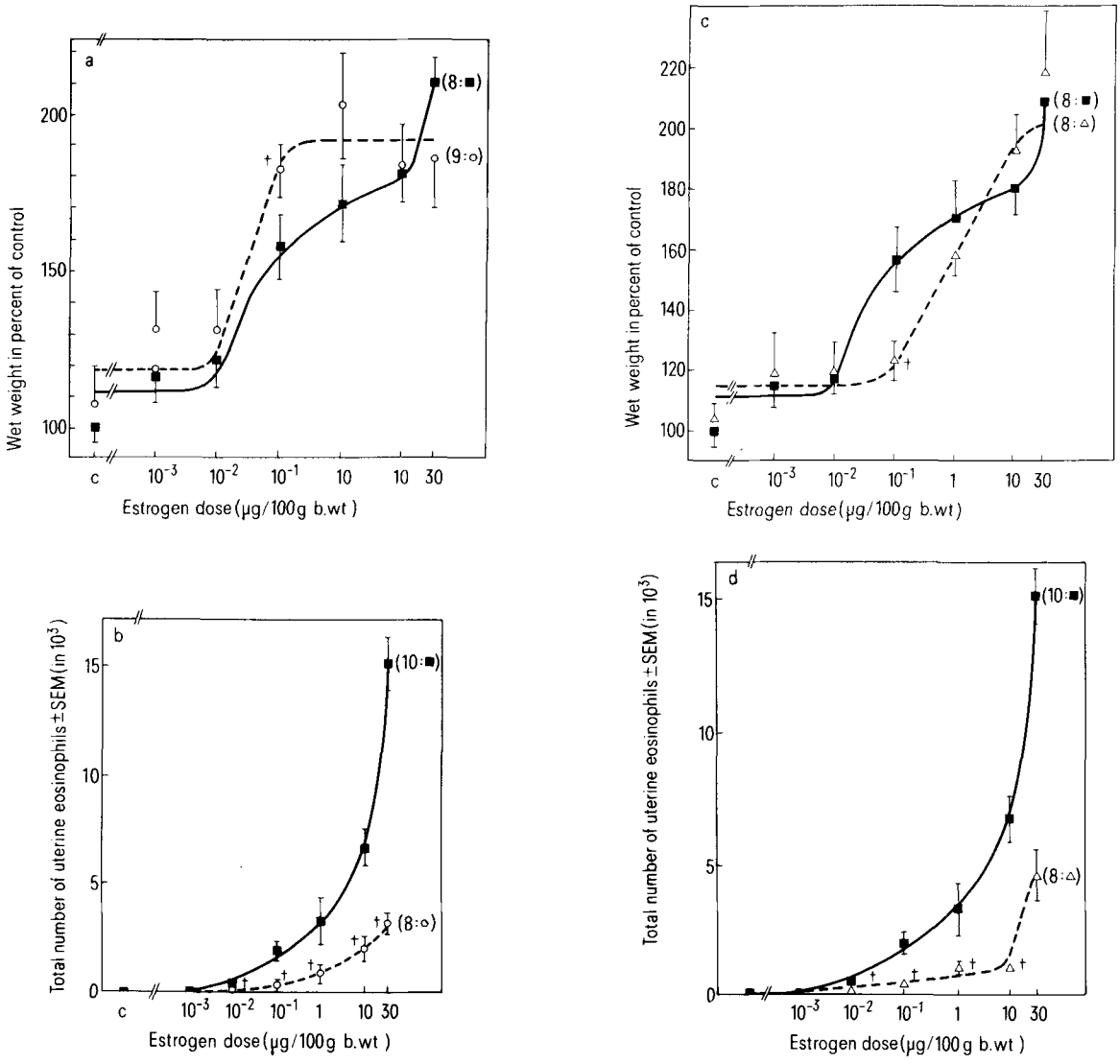
Therefore, any condition changing the number of blood eosinophils could modify this parameter of estrogen stimulation.

The aim of the present work was to study the effects of triiodothyronine and theophylline on estrogen induced uterine edema and eosinophilia, and correlate these effects with blood eosinophil levels in the female immature rat.

Material and methods. Effect of theophylline and triodothyronine on estrogen-induced uterine edema and eosinophilia. Immature female Sprague-Dawley rats of 50 g b.wt were injected with doses of estradiol-17 β in 5% ethanol-saline ranging from 0.001 to 30 µg per 100 g of b.wt (SIGMA) with or without 10 mg/100 g b.wt of theophylline (Knoll A.G. Ludwigshafen a.Rh., FRG) in saline, simultaneously with the estrogen injection. Thyroid hormone-treated animals were injected with 20 µg/100 g b.wt triiodothyronine (SIGMA) in 0.01 N NaOH in a pH 7.4 phosphate buffer, and 1 h after with the same estrogen doses as those used in theophylline-treated rats.

All injections were in the jugular vein under ether anaesthesia. The controls were similarly injected with equal amounts of the vehicle. 8–10 animals were used for each experimental group. The animals were killed 6 h after estrogen injection, and the uteri were excised. - The right uterine horn was used to measure the uterine wet weight and the left uterine horn was used to measure the total number of uterine eosinophils[9].

Effect of theophylline, triiodothyronine and/or estradiol on the number of eosinophil leukocytes in the blood. Immature female Sprague-Dawley rats, 50 g b.wt, were used in these experiments. Experimentals were injected with 30 µg/100 g b.wt estradiol in 5% ethanol-saline, triiodothyronine 20 µg/100 g b.wt in NaOH 0.1 N in a pH 7.4



Effect of theophylline (a and b) and triiodothyronine (c and d) on the estrogen-induced uterine wet weight increase and eosinophilia. Estradiol (■----■) and estradiol + theophylline (○---○) were given 6 h before the animals were sacrificed (a and b). Estradiol (■---■) and estradiol + triiodothyronine (△---△) were also compared 6 h after estrogen injection (c and d). - The uterine wet weight was expressed as percentage of the controls (c: ■, injected with the vehicle). Uterine wet weight of control animals was 19.31 ± 1.00 mg (mean ± SEM). Uterine eosinophilia was expressed as the total number of eosinophils in the uterus ± SEM (in 10^3). Number of animals per group in brackets (except in figures b and d, where n = 17 in all groups with 30 µg/100 g b.wt estradiol).

+ $p < 0.05$, as compared with the same estrogen dose, without theophylline (a, b) or without triiodothyronine (c, d). (Student's unpaired t-test).

Effect of triiodothyronine, estradiol 17β and/or theophylline on the number of eosinophil leukocytes in the blood of the female immature rat

Experimental condition	Number of eosinophils per mm^3 of blood ± SEM Before injection	6 h after injection	p*
Controls (injected with the vehicle)	22.47 ± 4.75 (100)	23.77 ± 3.57 (114)	n.s.
Triiodothyronine	52.01 ± 7.71 (100)	21.12 ± 3.98 (39)[a]	< 0.0025
Estradiol − 17β	50.14 ± 6.11 (100)	18.86 ± 2.45 (38)[a]	< 0.0005
Theophylline	32.50 ± 3.16 (100)	12.49 ± 1.48 (39)[a]	< 0.0005
Triiodothyronine + theophylline	58.29 ± 7.68 (100)	11.54 ± 3.23 (20)[a,c]	< 0.0005
Estradiol 17β + theophylline	50.27 ± 8.09 (100)	15.06 ± 4.38 (26)[a,b]	< 0.0025
Triiodothyronine + estradiol − 17β	58.26 ± 6.64 (100)	19.04 ± 4.17 (32)[a]	< 0.0005

The animals were i.v. injected with 10 mg/100 g b.wt theophylline and/or 30 µg/100 g b.wt estradiol, 20 µg/100 g b.wt triiodothyronine or the vehicle. Blood samples were taken from the tail before injection and 6 h after injection. Data are also given in percentages, taking the values of control animals as 100% (in brackets). * For comparison with values before injection. - 8 animals were used in each group, except theophylline + estradiol (n = 9). - Absolute values represent means ± SEM. - Student's paired t-test was used for comparison of absolute values before and after injection. - Kruskal-Wallis test[11] was used to compare average ranks of percentage values (in brackets) 6 h after injection. Superscripts indicate significant differences between percentage values 6 h after injection as follows: [a] $p < 0.0005$, with respect to controls; [b] $p < 0.0005$, with respect to estradiol or theophylline; [c] $p < 0.0005$, with respect to triiodothyronine or theophylline.

phosphate buffer and/or 10 mg/100 g b.wt theophylline in saline.

Controls were similary injected with equal amounts of the vehicle. All injections were in the jugular vein under ether anaesthesia. 8 animals were used per experimental group. Blood samples were taken from the tail before the injection and 6 h after the injection. EDTA was added to the blood samples, and they were stained with 1% eosin in acetone. The eosinophils were counted in a Neubauer chamber[10]. Since the levels of blood eosinophils/mm^3 before injections were different, the results are also given in percentage terms[11].

Results. Effects of theophylline and triiodothyronine on estrogen induced uterine edema and eosinophilia. Theophylline and triiodothyronine have opposite effects on estrogen-induced uterine edema. Theophylline increases uterine edema ($p < 0.05$) and triiodothyronine decreases uterine edema ($p < 0.05$) induced by physiological doses of estradiol-17β (0.1 µg/100 g b.wt) (figures a and c). Theophylline alone or triiodothyronine alone do not modify uterine wet weight.

Estradiol-17β induces uterine eosinophilia at doses ranging from 10^{-2} to 30 µg/100 g b.wt. Theophylline or triiodothyronine, when they are administered in the presence of estradiol, significantly decrease estrogen-induced uterine eosinophilia at 10^{-2}, 10^{-1}, 1, 10 and 30 µg/100 g b.wt estradiol-17β (figures b and d) ($p < 0.05$).

Theophylline, estradiol and triiodothyronine induce a decrease in the number of eosinophil leukocytes in the blood (table). Theophylline and estradiol or theophylline and triiodothyronine administered together induce a statistically significant greater percentage decrease than each one administered alone ($p < 0.01$) (table).

Discussion. Estrogen binding by uterine eosinophils is significantly decreased by triiodothyronine, in vitro[12]. Since binding of estrogen to specific eosinophil receptors should be necessary for early physiological action in the uterus, a decreased estrogen uptake by uterine eosinophils from triiodothyronine-treated rats could also result in impaired uterine function. Here we show that triiodothyronine induces a decrease in the number of uterine eosinophils and a decrease in the estrogen-induced uterine edema.

In this study, there is no correlation between the increase in uterine edema induced by estradiol-17β and the dose-dependent decrease of uterine eosinophilia, in the presence of theophylline and estradiol. This phenomenon could be explained by an increase in estrogen binding by each eosinophil[13], by theophylline. Another possible explanation is that theophylline could also induce an increase in the synthesis of endogenous estrogens by the immature ovary.

The decrease in the number of uterine eosinophils in the presence of estradiol and theophylline or triiodothyronine could be explained by the decrease in the number of blood eosinophils. The much greater decrease in the number of blood eosinophils observed when thyroid hormone and theophylline are administered together suggests a potentiation of its action on blood eosinophil levels. Thyroid hormone-induced blood eosinopenia could be mediated by cyclic AMP. Further studies are necessary to elucidate the mechanisms of interaction between thyroid hormones, theophylline and estrogens in the rat uterus.

1 Acknowledgments. - We wish to thank Dr G. Pineda, Salvador Hospital, Santiago, for the gift of L-triiodothyronine (Sigma). This work was supported by grant B 012-783, Servicio de Desarrollo Científico y Creación Artística, University of Chile.

2 W.M. Van Horn, Endocrinology *17*, 152 (1933).
3 J. Gilman and C.J. Gilbert, Obstet. Gynec. Br. Emp. *60*, 445 (1953).
4 M. Maqsood, Biol. Rev. *27*, 281 (1952).
5 M.F. Ruh, T.S. Ruh and H.M. Klitgaard, Proc. Soc. exp. Biol. Med. *134*, 947 (1970).
6 R.L. Singhal and R.T. Lafreniere, J. Pharmac. exp. Ther. *180*, 86 (1972).
7 R.T. Lafreniere and R.L. Singhal, Steroids *17*, 323 (1971).
8 A. Tchernitchin, Steroids *19*, 575 (1972).
9 A. Tchernitchin, J. Roorijck, X. Tchernitchin, J. Vandenhende and P. Galand, Nature *248*, 142 (1974).
10 J.V. Dacie and S.M. Lewis, in: Hematología Práctica, Chapter III, p. 66. Toray Press, Barcelona 1965.
11 E.L. Lehmann, in: Non-Parametrics: Statistical Methods Based on Ranks, p. 204. Holden-Day Inc., San Francisco 1975.
12 A. Tchernitchin, Biol. Reprod. *7*, 124 (1972).
13 A. Tchernitchin, J. Steinsapir, C. Unda, M. Mena, A. Rodríguez, M. Hernández, N. Soto, X. Tchernitchin, L. Bravo and M. De la Lastra, IRCS Med. Sci. *5*, 456 (1977).