that exceeded the sucrose space was calculated as nM per µl sucrose space.

Results and discussion. Tolbutamide was taken up into the tissue samples employed as it clearly exceeded the sucrose space. Due to the small quantitiy of the material, the absolute uptake of tolbutamide into islets was rather small but safely detectable: the ratio of H-3 to C-14 was significantly increased, indicating that H-3 tolbutamide had exceeded the sucrose space. Calculated as nmole/µl sucrose space (table) the uptake into islets and the other tissues ranged within the same order of magnitude, and the differences might simply be due to different ratios of intracellular to extracellular space.

In view of our results, the agent does not seem to be restricted to the extracellular space, and an intracellular locus of action may thus be discussed. However, the concept of a membrane action of tolbutamide is not ruled out by these findings. We still favour this concept of an isothiocyano-sulfonylurea that probably binds covalently to the beta-cell and is thus unlikely to penetrate the membrane, exerted a full sulfonylurea-like activity[7,8].

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Biological activities and receptor affinities of some natural and synthetic oestrogens and their D-homo analogues

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Summary. The activities of a series of D-homo- and D-homo-Δ^{16}-oestrogens on cornification of the vaginal epithelium of ovariectomized rats were tested and compared with their affinities in vitro for the rat uterine cytosol receptor. The effectiveness of the substances in both tests decreased in the order normal > D-homo > D-homo-Δ^{16}.

Like other steroid hormones, oestrogens are bound to specific cytoplasmic receptors following passage of the cell membranes in target organs[2]. These oestrogen-receptor complexes are then modified in such a way that they can enter the cell nucleus[3] where they are bound to a basic non-histone protein[4]. Such binding is evidently the prerequisite for a number of biochemical and molecular biological changes which can then be observed in the oestrogen-sensitive cell[5]. In addition to these effects which occur within the first 6 h after oestrogen administration, for example in the uterus, anatomical changes ensue after 20–48 h. Thus the uterotrophic effect of oestrogens in rats and mice and cornification of the vaginal mucosa in castrate rats have been known and utilized for bioassay purposes for many years.

We tested a number of oestrogens (table) of the normal, D-homo and D-homo-Δ^{16} series in rats in vivo (cornification of the vaginal mucosa) and in vitro (affinity for the uterine cytosol receptor) and compared the results of the two. For vaginal studies, ovariectomized rats were treated for 5 days with various concentrations of the steroids[6], administered daily in SSV[7] by the s.c. route. The vaginas were flushed with saline solution 24 h after each injection, and the flushings were utilized for cytological analysis. Predominance of cornified cells in the smear was regarded as evidence of mucosal cornification, and this occurrence was classified as an 'oestrous event'. The total number of oestrous events was then recorded as a percentage of all practically possible such events. For receptor studies, uteri from 20-day-old rats were homogenized in 10 mM Tris-HCl buffer (pH 8.0) containing 1.5 mM EDTA and then centrifuged at 105,000 × g, 4 °C, for 1 h. The cytosol was then incubated for 18 h at 4 °C either with 1.33×10^{-7} M [2,4,6,7(n)-^{3}H]-oestradiol (87 Ci/mmole, Amersham) alone or combined with various concentrations of the test substances. Free ^{3}H-oestradiol was separated from bound with dextran/charcoal; radioactivity was measured following addition of 10 ml Insta-Gel (Packard) in a liquid scintillation spectrometer (Packard). The maximal specific binding of ^{3}H-oestradiol, recorded as 100%, was generally ca. 60 fmoles/mg protein.

The results of the in vivo experiments, as exemplified by 17α-ethynyloestradiol, oestradiol and oestrone, are shown in figure A. The analogues of these substances gave similar dose-response curves. The median effective oestrogen doses (ED_{50}) were determined from the dose-response curves; they are listed in the table. Enlargement of the 5-carbon D ring by a methylene group reduced the activity, and introduction of a C_{16}-C_{17}-double bond lowered it still further. The relative activities of the members of the 17α-ethynyl-oestradiol series were greater than those of the oestradiol

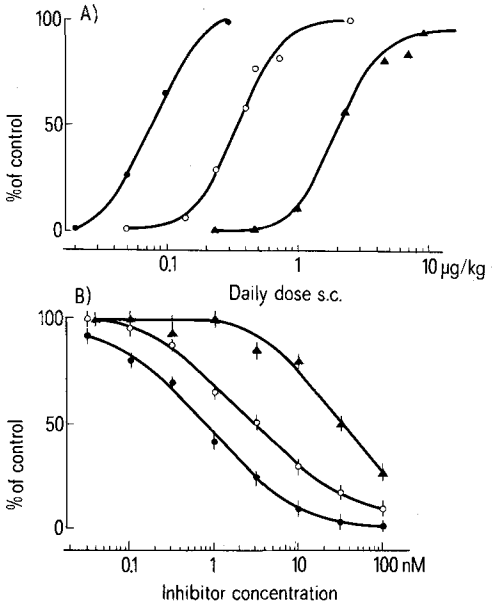
	Vaginal cornification ED_{50} (µg/kg/day) s.c.	Uterine cytosol receptor IC_{50} (nM)
17β-Oestradiol	0.4	3.0
D-homo-Oestradiol	18	23
D-homo-Δ^{16}-Oestradiol	130	50
Oestrone	2	30
D-homo-Oestrone	70	40
D-homo-Δ^{16}-Oestrone	150	100
17α-Ethynyloestradiol	0.075	0.8
D-homo-17aα-Ethynyloestradiol	15	13
D-homo-Δ^{16}-17aα-Ethynyloestradiol	100	33
17α-Oestradiol	85	30
Oestriol	150	10

series, which in their turn were greater than those of the oestrone series. The relative potency of 17α-oestradiol as compared with 17β-oestradiol was 0.4%.

Figure B shows the results of the in vitro tests with the same substances. As in the in vivo tests, activity declines from the normal to the D-homo substances, and further from these to the D-homo-Δ^{16} series. The median inhibitory concentrations (IC_{50}) are presented against the median effective in vivo doses (ED_{50}) in the table. Statistical analysis of the values of both tests gave a correlation coefficient of $r = 0.85$ ($p < 0.01$).

The oestriol results, not included in the foregoing analysis, show that this compound behaves differently from the others in both tests. Despite a good affinity for the receptor ($IC_{50} = 10$ nM), it has only a weak effect on the vaginal mucosa. This qualitative difference from oestradiol was also observed by Anderson et al.[8], who attributed the low biological activity to a comparatively short period of stay of the oestriol-receptor complex in the nucleus.

The reduction of oestrogenic activity resulting from enlargement of the D rings is scarcely surprising in view of the fact that, in addition to 2- and 16α-hydroxylation, production of a 6-membered D ring is a normal process in the metabolic inactivation of oestradiol in both rabbits and humans[9].



A Number of oestrous events as per cent of all possible such events in a group of ovariectomized rats (n = 10) as a function of daily s.c. administered dose of test compound.
B Competition of test substances with ^{3}H-oestradiol for the uterine cytosol receptor expressed as per cent inhibition of oestradiol binding (n = 5). Results are mean values±SEM. Symbols: ●—● 17α-ethynyloestradiol, ○—○ oestradiol, ▲—▲ oestrone. Note the differing abscissas.

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Effect of borax on testis of Indian desert gerbil, *Meriones hurriane* Jerdon

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Summary. Borax was injected at a dose level of 250 mg/kg b.wt for 16 days (total dose 4 g/kg b.wt) s.c. to active adult male gerbils. Borax caused several degenerative changes in the testes, of which giant cell formation, pyknosis and exfoliation are prominent. The increased activity of phosphatases was also noticed.

Several metallic salts have been said to evoke alterations in the testis, of which the compounds of lithium, molybdenum, thallium manganese, lead, cadmium seems to be the most effective[2-4]. Zinc and selinium salts have been reported to be antagonistic to these salts[5-7]. Borax, a compound of sodium, Boron and Oxygen ($Na_2B_4O_7$), has been reported to be effective on the female reproductive system in some ayurvedic literature. The aim of present study is to evaluate its effect on the testis.

Active adult male gerbils of approximately equal b.wt were collected locally and maintained under suitable laboratory conditions for a few days and were sorted out into 2 groups of 6 each. The animals of the 1st group were given 16 daily s.c. injections of borax in water at a dose level of 250 mg/kg b.wt (total dose 4 g/kg b.wt). The 2nd group received the same volume of saline in same way and served as controls. The animals were sacrificed 24 h after the last injection. 1 testis was immediately processed for histological studies. Biochemical estimations for enzymes listed in the table were carried out using standard techniques.

Result and discussion. None of the animals showed sign of morbidity or mortality. There was no variation in the b.wt and the testicular weights in both the groups. The testis of the control animals exhibited normal spermatogenesis leading to the formation of motile spermatozoa. Testis of the treated animals showed degenerative changes in the seminiferous tubules. The reduction in the diameter of seminiferous tubules was noticed.