in the latter were much lower (about 10%) than in the adult and further reduction may be particularly difficult. Small amounts of snake NGF injected together with the mouse antiserum partially prevented the destructive effects of the antibody and larger amounts produced a characteristic hypertrophy. Since there is no measurable cross-reactivity between this antibody and antigen[9] (and the amounts used preclude an effect based on a direct interaction between the two, see data in footnote to table 2) the snake NGF presumably replaces and supplements endogenous NGF removed by the antiserum. Our results are therefore consistent with and lend some support to the view that the antiserum normally acts by neutralizing circulating NGF. However, this theory does require a correlation between the potencies in vivo and in vitro of different batches and types of antisera. Such a correlation is claimed by some workers[16] but disputed by others[3,11]. While this discrepancy cannot exclude the postulated mechanism, which remains the most likely possibility, it is conceivable that a previously unconsidered effect is involved. Thus, NGF and its antibody may affect different target cells or the antiserum may promote a cell-mediated cytotoxic effect. Whatever the mechanism, it is however now clear that complement fixation is not involved.

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Uptake of tolbutamide by islets of Langerhans and other tissues

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Summary. 3-H-tolbutamide was distributed in a volume exceeding the space occupied by 14-C-sucrose in islets as well as in liver, kidney, muscle, and fat. In contrast to previous reports, the findings suggest that tolbutamide is not restricted to the extracellular space of islets.

Tolbutamide stimulates insulin release from the islet cells by a mechanism still unknown. It has been suggested that the sulfonylurea triggers the secretory response of the islet cell by altering the conformation of the membrane[1]. The insulin releasing activity of a tolbutamide derivative covalently linked to dextran[2] seemed to support this hypothesis. Recently the conclusions from this result have been questioned, however, as instability of the complex might well account for its insulin releasing potency[3]. Another argument for an extracellular action of tolbutamide was the finding that the agent was restricted to the extracellular space of islets[4] that had been marked by 14-C-sucrose. This finding was surprising for 2 reasons: Firstly, the sulfonylurea glibenclamide fairly exceeded the sucrose space[5] of islets. Secondly, tolbutamide is readily metabolized by the liver, indicating that it was taken up by the liver cell. Thus in the present study the uptake of tolbutamide in excess of the sucrose space into islets was re-investigated and compared to liver, kidney, muscle, and fat.

Materials and methods. 50–100 islets obtained from albino mice by collagenase digestion[6] of the pancreas were incubated for 30 min at 37 °C in Krebs-Ringer buffer containing 0.4% albumine, 0.28 mM H-3 tolbutamide (1.6 µCi/ml) (Hoechst AG, Frankfurt) and 1 mM sucrose (0.6 µCi/ml) (Amersham-Buchler, Braunschweig). In parallel experiments slices of liver, kidney, muscle, and fat from the same mouse were incubated. At the end of the incubation period, the tissue slices were freed from adhering buffer on filter paper and dissolved in TS-1 tissue solubilizer (Zinsser, Frankfurt). The islets were collected on pieces of aluminium foil, and the buffer was carefully removed with the aid of a micropipette. The dissolved samples were added to Instagel scintillator (Packard) and counted in an Intertechnique liquid scintillation counter. The channel ratio was used for the quench correction and the calculation of the H-3 and C-14 fractions. The amount of tolbutamide

Uptake of tolbutamide in excess to the sucrose space

Liver	0.22±0.03
Muscle	0.11±0.01
Kidney	0.24±0.04
Fat	0.25±0.02
Islets	0.10±0.03

Values are given as nmole/µl sucrose space. The results represent means ± SEM of 8 experiments. The incubation media contained 0.28 nmoles/µl tolbutamide.

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