On hybridization of mouse and Chinese hamster cells resistant to ouabain with normal human cells the ouabain concentration was increased to 1 μM, which caused more rapid death of the human cells (on the 3rd day) without affecting viability of the hybrid cells. In turn, the more rapid death of the human cells ought to prevent metabolic cooperation between unfused human and rodent cells.

Altogether 15 colonies of presumptive human–Chinese hamster hybrid cells and one colony of mouse–human hybrid cells were isolated. All clones were cultured on HATG medium for not less than 3 months. At the first subcultures the hybrid cells differed in their morphology from the parental cells. By the 20th-25th subculture the external appearance of the hybrid cells began to resemble that of the hamster and mouse cells, i.e., the fusiform shape of the human cells was completely lost. Ten clones were subjected to karyologic analysis. Analysis confirmed the hybrid nature of all the clones analyzed. Karyotypes of the parental human IMG812 cells are shown in Fig. 1 and of the Chinese hamster MO1 cells in Fig. 2. IMG812 cells have the 46 XY karyotype. The modal number of chromosomes in MO1 cells is 36 (60% of cells). Two metaphase plates of the hybrid clone MOI8, produced by fusion of MO1 and IMG812 cells, are illustrated in Fig. 3. Elimination of both human chromosomes and Chinese hamster chromosomes was observed in the hybrid clone after 8 months of culture.

It can be concluded from the results as a whole that the method of hybridization of cells using PEG as agent inducing cell fusion is encouraging. The selective HATG medium with ouabain kills parental mouse, hamster, and human cells while allowing only hybrid cells to multiply.

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HORMONAL SPECIFICITY OF THE AFFINITY OF AN UNUSUAL RAT LIVER ESTROGEN-BINDING PROTEIN

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The unusual estrogen-binding protein (UEBP) of rat liver differs from other intra- and extracellular proteins specifically binding sex steroids primarily in the high lability of the complexes which it forms with estradiol (E_2) and the unique specificity of its affinity for ligands [3, 6]. UEBP has been found in intact sexually mature males and can be induced in females by injecting them with testosterone propionate [4]. The ability of this protein to interact both with estrogens and with androgens suggests that UEBP may play an important role in the regulation of reception and metabolism of sex steroids in liver cells and in the whole organism [3, 5]. To elucidate the concrete forms of participation of UEBP in these processes it was decided to study a wide range of hormonal compounds with respect to their ability to interact with UEBP.

EXPERIMENTAL METHOD

Sexually mature male Wistar rats or a mixed population weighing 150-250 g were used. The preparation of partially purified UEBP was obtained from liver cytosol by fractionation

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TABLE 1. RCA of Different Steroids and Their Analogs Assayed by 50% Inhibition of Binding of [^{3}H]-E_2 with the Unusual Estrogen-Binding Protein from Male Rat Liver

Compound	Number of determinations	RCA, %
1,3,5(10)-estratriene-3,17β-diol (stradiol-17β)		100
1,3,5(10)-estratrien-17β-ol	2	∿1.0
1,3,5(10)-estratrien-3-ol	3	∿1.0
1,3,5(10)-estratriene	2	<0.5
1,3,5(10)-estratriene-3,17β-diol-17 valerianate	3	5.3 ± 0.3
1,3,5(10)-estratriene-3,17β-diol-17 benzoate	2	∿1.6
1,3,5(10)-estratriene-3,17β-diol dipropionate	2	∿3.8
3-methoxy-17α-ethynyl-1,3,5(10)-estratrien-17β-ol (mestranol)	2	<0.5
1,3,5(10)-estratriene-3,17α-diol (epiestradiol)	3	0.8 ± 0.05
3-hydroxy-1,3,5(10)-estratrien-17-one (estrone)	4	12.6 ± 2.0
1,3,5(10)-estratriene-3,16α,17β-triol (estriol)	9	170 ± 37
3,16α-dihydroxy-1,3,5(10)-estratrien-17-one	3	32.0 ± 0.8
1,3,5(10)-estratriene-3,16α,17α-triol (17-epiestriol)	4	14.9 ± 1.3
1,3,5(10)-estratriene-3,16β,17β-triol (16-epiestriol)	3	17.5 ± 0.6
1,3,5(10)-estratriene-3,15α,16α,17β-tetrol (estetrol)	4	16.9 ± 1.8
17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol	4	32.0 ± 5.4
2-methyl-1,3,5(10)-estratriene-3,17β-diol	7	10.2 ± 1.1
2-methoxy-1,3,5(10)-estratriene-3,17β-diol	3	14.3 ± 0.8
11β-methoxy-17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol (moxestrol)	3	16.7 ± 1.3
Hexestrol (stilbene estrogen)	2	∿1.2
Dienestrol diacetate	2	<0.5
Clomiphene (triphenylethane antiestrogen)	2	<0.5
5α-androstane-3α,17β-diol	7	13.8 ± 1.2
17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone)	3	2.0 ± 0.5
17β-hydroxy-4-androsten-3-one (testosterone)	6	5.9 ± 0.6
17β-hydroxy-1,4-androstadien-3-one (Δ^1-testosterone)	7	11.3 ± 2.2
5α-androstane-3α,17α-diol	3	Δ0.5
17α-hydroxy-4-androstan-3-one (epitestosterone)	2	∿1.0
17α-hydroxy-5α-androstan-3-one	3	1.0 ± 0.07
17β-hydroxy-4-androsten-3-one-17 propionate	2	∿0.5
2α-methyl-17β-hydroxy-5α-androstan-3-one-17 propionate	2	∿1.0
3α-hydroxy-5α-androstan-17-one (androsterone)	4	<0.5
4-androstene-3,17-dione	3	<0.5
17β-hydroxy-4-estren-3-one (19-nortestosterone)	2	∿1.0
5β-androstane-3α,17β-diol	4	∿1.0
5β-androstane-3β,17β-diol	2	∿0.5
5α-androstane-3β,17β-diol	2	<0.5
2α-methyl-17β-hydroxy-5α-androstan-3-one	3	4.4 ± 0.5
2α,17β-dihydroxy-4-androsten-3-one	3	32.0 ± 3.9
2β-17β-dihydroxy-4-androsten-3-one	3	4.9 ± 0.8
6β,17β-dihydroxy-4-androsten-3-one	2	<0.5
5α-androstane-3α,6β,17β-triol	2	<0.5
17α-methyl-17β-hydroxy-1,4-androstadien-3-one (dianabol)	2	∿0.5
16α,17β-dihydroxy-4-androsten-3-one	3	∿0.5
3β-hydroxy-5β-androstan-17-one	2	<0.5
5β-androstane-3,17-dione	2	<0.5
3α-hydroxy-5β-androstan-17-one (etiocholanolone)	2	<0.5
5α-androstan-17-one	2	<0.5
17β-hydroxy-4-estren-3-one-17 phenylpropionate (phenobolyl)	2	<0.5
4-androstene-3,11,17-trione	2	<0.5
3α-hydroxy-5β-androstane-11,17-dione	2	∿0.5
3β-hydroxy-5-androsten-17-one (dehydroepiandrosterone)	3	∿0.5
4-pregnene-3,20-dione (progesterone)	2	<0.5

(continued)

TABLE 1. (continued)

Compound	Number of determinations	RCA
17α-hydroxy-4-pregnene-3,20-dione	2	<0.5
1,2-methylene-6-chloro-4,6-pregnadien-17α-ol-3,20-dione-17 acetate (cyproterone acetate)	2	<0.5
6-methyl-17α-hydroxy-4,6-pregnadiene-3,20-dione-17 acetate (megestrol acetate)	2	<0.5
11β-21-dihydroxy-4-pregnene-3,20-dione (corticosterone)	2	<0.5

with ammonium sulfate (50-75% saturation), gel-filtration on Ultrogel AcA-44 columns and ion-exchange chromatography on DEAE-Sephadex A50 as described previously [3]. Values of relative competitive activity (RCA) of the various ligands studied were determined with respect to 50% inhibition of binding of [2,4,6,7-^{3}H]-estradiol-17β (specific radioactivity 102 Ci/mmole, from Amersham Corporation, England) with UEBP. Free and protein-bound hormone were separated by adsorption on activated charcoal, covered with dextran, for 1 min [3]. Equilibrium association constants (K_a) of interaction of UEBP with [2,4,6,9-^{3}H]-estriol ([^{3}H]-E_2; specific radioactivity 109 Ci/mmole) and 5α-[1α,2α(n)-^{3}H]-androstane-3α,17β-diol (specific radioactivity 40 Ci/mmole, from Amersham Corporation, England) were determined by the method of equilibrium dialysis [3]. The radioactivity in the samples was measured on a liquid scintillation counter (Mark 2, Nuclear Chicago) with a counting efficiency of about 40%.

EXPERIMENTAL RESULTS

The results of determination of RCA values for different steroids and their analogs with respect to displacement of [^{3}H]-E_2 from complexes with UEBP are given in Table 1. It will be clear that all the estrane derivatives tested except 3- and 17-deoxy-E_2, 3,17-bis-deoxy-E_2-17α, and derivatives of E_2 conjugated at positions 3 and 17 possess the ability to interact actively with UEBP. The 16α-hydroxy group has a potentiating action on affinity for UEBP, whereas replacement of the 17β-hydroxyl by a keto group and also introduction of additional 15α-hydroxy, 2- and 11β-methoxy, 2-methyl, and 17α-ethyl groups and radicals into the E_2 molecules has a moderate inhibitory effect on steroid—protein interaction. Synthetic estrogens of the stilbene series and antiestrogen of the triphenylethane series do not have marked affinity for UEBP. The results suggest that the binding site of UEBP for estrogens contains two subcenters reacting with oxygen functions of the A and D rings of the estrogens. The subcenter for the D ring probably includes three loci capable of forming bonds with 17β-hydroxy, 16α-hydroxy, and 17-oxo groups of estrogens, and allosterically linked with one another.

Of the 30 androstane derivatives tested only seven compounds have the ability to interact sufficiently intensively with UEBP: testosterone, its 2α- and 2β-hydroxy derivatives, 5α-dihydrotestosterone and its 2α-methyl derivative, 5α-androstane-3α,17β-diol, and Δ^1-testosterone; their affinity for the protein, moreover, is much weaker than the affinity of E_2 and estriol. The affinity of androgens for the protein falls sharply with a change in orientation of the 17β-hydroxyl group to 17α, its substitution by a 17-keto group, and blocking by ester bonds. The 6β- and 16α-hydroxy derivatives of the androgens, their 5β-metabolites, 19-nortestosterone, and 5α-androstane-3β,17β-diol have no marked affinity for UEBP. Pregnene derivatives, including the antiandrogen cyproterone acetate, do not compete for binding sites of UEBP with [^{3}H]-E_2. The results suggest that the binding site of the UEBP molecule for androgens contains two subcenters which interact with oxygen functions of the A and D rings of androstane derivatives. The subcenter for the A ring perhaps contains three active loci, capable of forming bonds with 3α- and 2α-hydroxy groups and the 3-keto group of androgens. This subcenter does not impose excessively rigid limitations on the conformation of the A ring of steroids. The subcenter for the D ring of C_{19}-steroids is much more selective toward the structure of the ligands and, unlike the corresponding subcenter for estrogens, it probably contains only one locus, reacting with the 17β-hydroxy group of the androgens.

The ratio between the directly measured values of K_a for binding of [^{3}H]estriol and [^{3}H]-E_2 with UEBP (1.3×10^8 and 1.1×10^8 M^{-1}) and for binding of [^{3}H]-5α-androstane-3α,17β-

diol and [^{3}H]-E_2 with UEBP (7.6×10^6 and 5.5×10^7 M^{-1}) is in satisfactory agreement with the values of RCA. Together with data on the competitive character of inhibition of [^{3}H]-E_2 binding with unlabeled ligands this is evidence of very close coupling between the binding sites of estrogens and androgens in the UEBP molecule. The results of the investigation correspond most closely to two types of interconnection between these sites: 1) The subcenters of the binding sites for estrogens and androgens are different, but the binding sites themselves partly overlap; 2) the subcenters for the A ring of estrogens and androgens coincide whereas the subcenters for the D ring of these steroids are different.

Comparison of the values of RCA for interaction of estrogens and androgens and their natural metabolites with UEBP and with the receptors of classical target organs for these hormones [1, 2] shows that there is no direct correlation between the intensity of estrogenic or androgenic activity of the steroids and their affinity for UEBP. Meanwhile evidence is constantly accumulating that several of the sex steroids also possess other forms of biological activity (anabolic, lipotrophic, and so on), probably mediated through other types of receptors [1, 2]. It is quite possible that UEBP serves to distribute the flows of steroids with qualitatively different biological activity. Another possibility is that the function of UEBP is linked with regulation of metabolic conversions of sex steroids in cells of the liver — the central organ of steroid metabolism.

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MYELOPOIESIS IN THE EMBRYONIC HUMAN LIVER

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Interest in the *in vitro* study of hematopoiesis in human embryonic organs at different stages of their development has recently shown a marked increase, mainly as a result of attempts to use these organs for transplantation into patients with combined immunodeficient states and with hypoplastic and aplastic anemias [8, 9, 14].

However, there have been a few such investigations so far and the conclusions drawn are based on an insufficient quantity of factual data and are often contradictory [3, 4, 10, 11].

It was accordingly decided to study myelopoiesis in the most active human organ of embryonic hematopoiesis, namely the liver (from the 6th to the 28th week of fetal development), using a method of cloning hematopoietic cells in semisolid nutrient media.

EXPERIMENTAL METHOD

A suspension of embryonic liver cells from 113 human fetuses obtained after abortions and by minor caesarian section was used.

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