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# ISOLATION OF HYBRID HUMAN–RODENT SOMATIC CELL CLONES

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UDC 576.363/.364.082.623

KEY WORDS: hybrid cells; man; mouse; Chinese hamster.

Hybridization of mammalian somatic cells is widely used in the investigation of differentiation, of regulation of gene action, and chromosome mapping. The most popular method of obtaining hybrid cells is by fusion of cells of two mutant lines and growth of the cells on a selective medium [1]. Definite difficulties arise in the production of hybrid cells when normal nonmutant human fibroblasts are used as one of the partners.

In the investigation described below a selective system was worked out for obtaining hybrids by fusing normal human cells growing on a substrate with mutant Chinese hamster and mouse cells.

## EXPERIMENTAL METHOD

Mutant Chinese hamster cells (M) resistant to 30 μg/ml of 6-mercaptopurine, clone M01 isolated from M cells after culture on medium with ouabain (1 mM), normal human embryonic skin fibroblasts of strains F20 and IMG795, normal human embryonic muscle cells of strain IMG812, mutant LTK mouse cells resistant to 30 μg/ml of 5-bromodeoxyuridine, and clone L01, obtained after culturing LTK cells on medium with ouabain (1 mM) were used. The cells were cultured in Carrel flasks on Eagle's medium with the addition of 20% bovine serum without antibiotics; this medium will be described later as normal medium.

The components of selective medium HATG3 were hypoxanthine (from Chemapol, Czechoslovakia) $10^{-4}$ M, aminopterin (from Schuchardt, West Germany) $4 \cdot 10^{-7}$ M, thimidine (from Serva, West Germany) $1.6 \cdot 10^{-7}$ M, and glycine (from Reanal, Hungary) $3 \cdot 10^{-6}$ M.

Ouabain (from Serva, West Germany) was used in the concentrations indicated below. The experiments were conducted on plastic petri dishes (diameter 40 mm, Leningrad Factory) or glass dishes (diameter 60 and 100 mm, from Anumbra, Czechoslovakia). To maintain the pH of the medium between 7.2 and 7.4 the dishes were kept in airtight containers under an atmosphere containing 5% $CO_2$. To count the growing colonies the cells were stained with methylene blue. To count the number of growing colonies, nuclei were stained with azure-eosin. Films for karyologic analysis were prepared with cells harvested by shaking the Carrel flasks. The films were air-dried by the usual method and stained with 2% Giemsa stain by the G method, using trypsin or CsCl. At least 10 metaphase plates were analyzed in each clone.

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0007-4888/82/9411-1571$07.50  

TABLE 1. Effect of Ouabain on Survival Rate of Chinese Hamster Cells (M) and Human Cells (F20)

| Ouabain concentration, μM | Relative seeding efficiency of hamster cells, % of control | | Number of human cells per dish (in thousands) | |
|---|---|---|---|---|
| | experiment 1 | experiment 2 | experiment 1 | experiment 2 |
| 0 | 100 | 100 | 425 | 389 |
| 0,005 | 99,9 | — | 399 | 401 |
| 0,05 | 91,6 | — | 1,4 | 2.7 |
| 0,5 | 91,8 | — | 0 | 0 |
| 5 | 92,9 | 92,2 | — | — |
| 50 | 20,8 | 27,6 | — | — |
| 1000 | 0,00104 | 0,00115 | — | — |

TABLE 2. Results of Experiments on Selection of M × F20 Hybrid Cells on HATG Medium with Ouabain

| Cells | Number of cells seeded per dish | Mean number of colonies per dish | | |
|---|---|---|---|---|
| | | experiment 1 | experiment 2 | experiment 3 |
| Hamster–hamster | 50 000 | 0,2 | 0 | 0 |
| Human–human | 50 000 | 0 | 0 | 0 |
| Hamster–human | 50 000 | 15,3 | 1,5 | 29,6 |
| Hamster–human | 30 000 | — | 0,2 | 0 |

## EXPERIMENTAL RESULTS

Before the hybridization experiments began it was necessary to estimate the viability of the parental cells quantitatively on selective media.

Survival Rate of Cells on HATG Medium. Chinese hamster cells, deficient for hypoxanthine phosphoribosyltransferase, when seeded at up to 50,000 cells per dish did not grow on HATG medium. After mass seeding (up to 200,000 cells or more per dish), mass death of the cells also was observed. However, on the 12th day a few separate cells, normal in their external appearance, still remained. Nevertheless, changing the HATG medium for normal did not restore viability, which means that the lethal action of HATG medium for 12 days is irreversible. Human cells, seeded at the rate of 200,000-500,000 cells per dish, grew on HATG medium as rapidly as on normal medium (with no change in external appearance), at least for 20 days. Mouse cells, deficient in thymidine kinase, like hamster cells, died after mass seeding on HATG medium by the 8th-10th day of culture.

Effect of Ouabain on Viability of Cells. The data in Table 1 show that human cells were 1000 times more sensitive to the action of ouabain than hamster cells. A mass culture of mouse cells grew in the presence of 50 μM ouabain just as on normal medium. These results agree with data in the literature on differences in the sensitivity of human and rodent cells to ouabain [2]. The resistance of hybrids to ouabain is known to be intermediate between the sensitivity of one and resistance of the other partner. To cause death of human cells but allow survival of hybrid cells, ouabain was used in a concentration of 0.5 μM. The results encouraged the hope that the parental cells would die on selective HATG medium with ouabain but the hybrid cells would survive.

Effect of Polyethylene Glycol (PEG). Treatment with a 50% solution of PEG for 1 min reduced the survival rate of the hamster cells to 50%, and treatment for 2 min reduced it to 25% compared with the control. The number of hamster cells with two or more nuclei increased after treatment with PEG for 1 min to 10-18% compared with 1.5% in the control.

Cell Fusion. Hamster and human or mouse and human cells were seeded on Carrel flasks in the ratio of 1:1 and with about $1.5 \cdot 10^6$ cells of each type. After 24 h the cells were treated with 50% PEG solution for 1 min, quickly washed three times with Hanks' solution, and covered with normal medium. Next day the cells were reseeded on selective HATG medium with ouabain on petri dishes. The results of these experiments are given in Table 2. They show that virtually only the hybrid cells survived on selective medium. Hybridization of $LTK^-$ mouse cells with IMG795 human cells was much less effective. No colonies could be obtained on plastic petri dishes, and accordingly glass dishes were used. In three experiments only eight colonies were observed on 60 dishes.

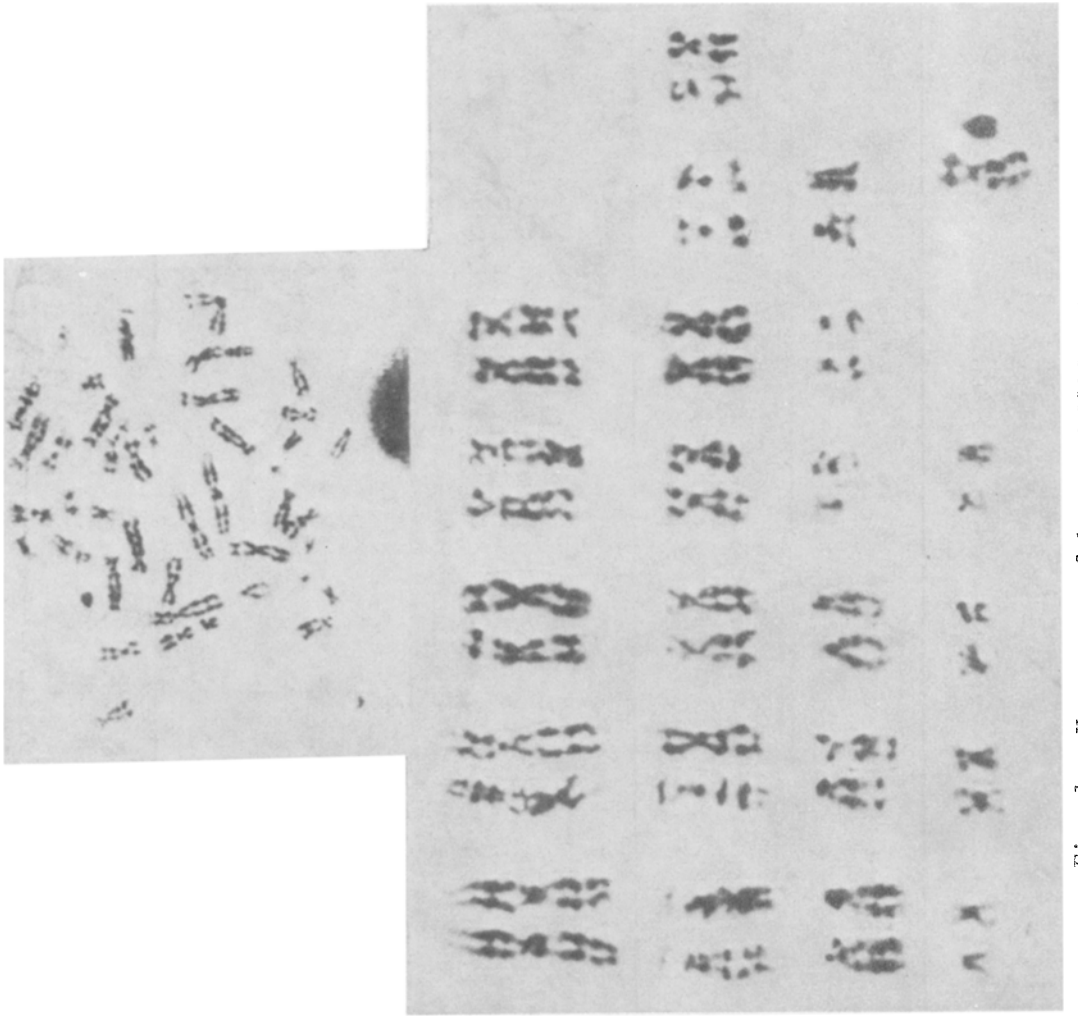

Fig. 1. Karyotype of human IMG812 cells.

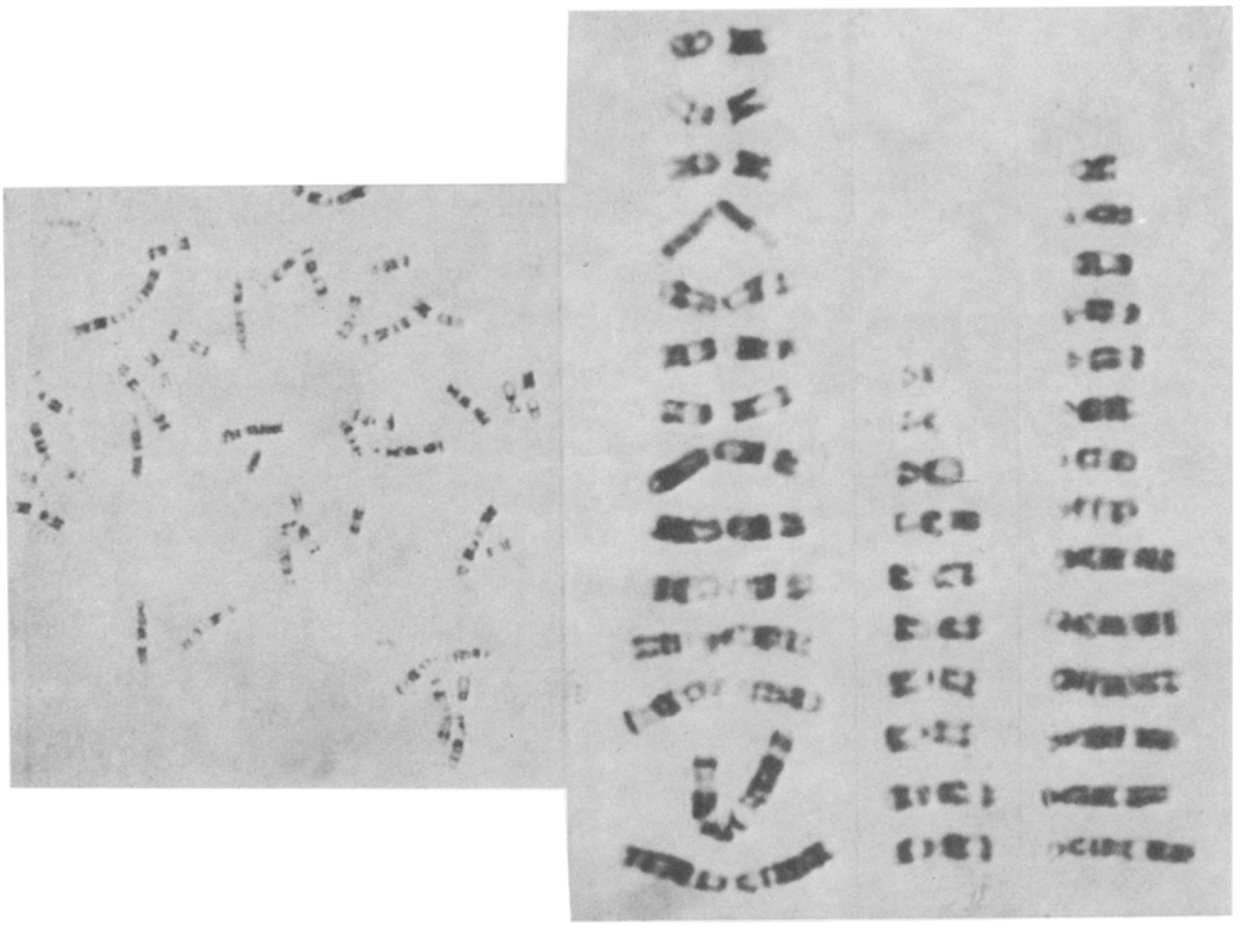

Fig. 2. Karyotype of Chinese hamster M01 cells.

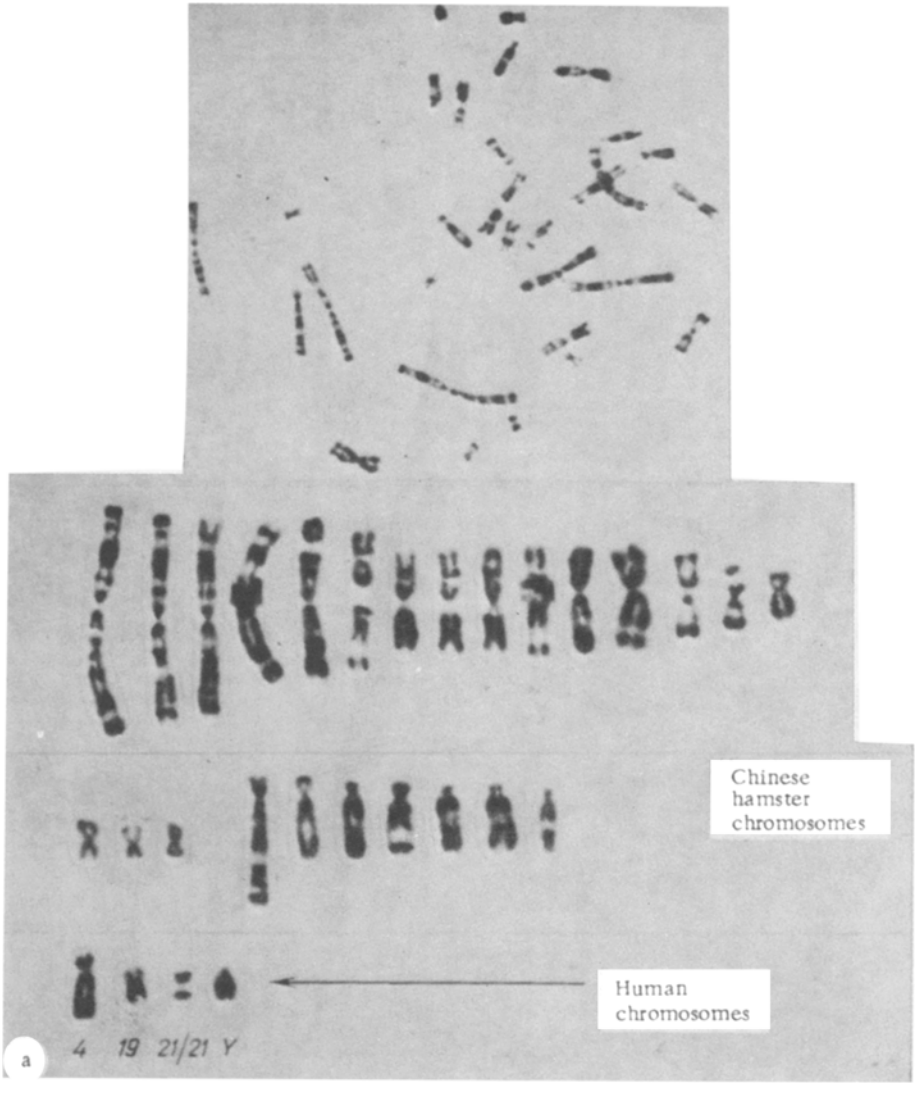


Fig. 3. Karyotypes of two metaphase plates (a and b) of hybrid MOI8 cells. [See next page for Fig. 3b.]

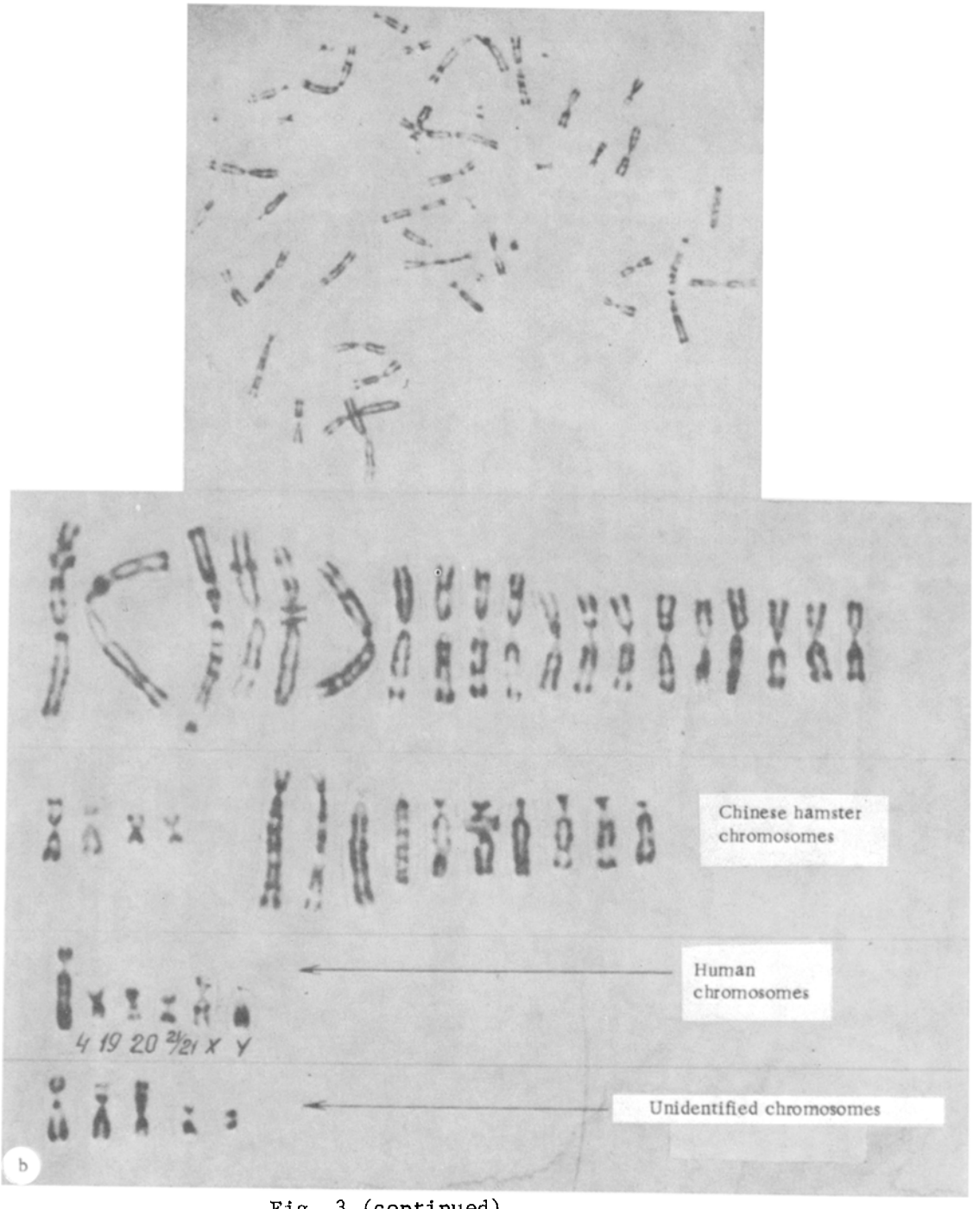


Fig. 3 (continued)

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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UDC 612.352.3:612.621.31

KEY WORDS: estrogens; androgens; binding; rat liver.


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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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 90-92, November, 1982. Original article submitted April 14, 1982.



0007-4888/82/9411-1577$07.50