saline was added to the initial homogenate, which was further processed through a Waring Blender to a fine consistency, placed in a Serval refrigerated centrifuge and spun for 30 min at 13,500 *g* (22,000 *g*), at 4 °C. The surface lipid material and the cellular residue were discarded. The remaining yellowish solution was filtered through a 0.45 micron millipore filter, lyophilized and stored at 0 °C until added to the experimental medium. The protein concentration of the extract was determined from LOWRY [12] protein analysis with human serum albumin (Cutter Laboratory, Berkeley, California) as protein standard. In these extracts the ratio of carbohydrate to protein was approximately 1:50 as determined by an anthrone test with dextrose as standard.

Results and discussion. In Figure A, changes in mitotic index, cell volume and cell number are shown for normal control cultures of human primary foreskin cells. It may be seen that the cell volume reaches a maximum at about 21 h after seeding. Following this cell volume maximum, one sees the initial increase in cell number, which begins to rise about 24 h after seeding. Also, at 24 h the mitotic index begins to rise dramatically, coming to a maximum halfway through the initial increase in cell number, and decreasing rapidly as this increase in cell number levels off to a plateau, having completed the first division cycle. This plateau occurs between 35–40 h after seedings, after which time a second cell division wave commences, although the effect is not as marked. This damping of the response is also shown by the mitotic index and cell volume curves. In all control growth experiments, we have found this highly predictable initial temporal surge of cell division. This 'pseudosynchronous' pattern can probably be attributed to our standard method of serial cultivation.

Cell cultures initiated with experimental medium containing skin extract and adrenalin exhibit a definite increase of 8 h in the lag period preceeding the first wave of cell division. This longer lag period is apparent in all three cell parameters studied as shown in Figure B. In comparison with the control cell growth parameters, the initial step increase in cell number has been eliminated, and the well defined peaks of mitotic index and cell volume have been reduced in amplitude and sharpness. These changes strongly indicate a decrease in the degree of 'pseudosynchrony' as compared to control cultures. Growth parameters of cell cultures initiated with adrenalin alone were not different fron control cultures. Also, cultures treated at various times (32–42 h after seeding) during active cell division with biochemical extracts obtained from leg muscle alone (1.0 mg protein/ml), leg muscle (1.5 mg protein/ml) plus adrenalin, or lung extract (1.5 mg protein/ml) plus adrenalin showed no difference from control cultures. Growth inhibition was seen with cultures treated only with skin extract (1.0 mg protein/ml) and this inhibition was slightly potentiated by the combination of skin extract with adrenalin. This data indicates that while adrenalin will potentiate the effect, the active component is contained in the skin extract.

Our skin extract can be considered patent, as mouse in vivo experiments indicate approximately a 16% depression in ear epidermis mitotic index as compared to control animals after i.p. injections of 1.0 mg protein/g of animal. Also, in preliminary experiments investigating possible cellular modes of action of skin extract, effects on rat liver mitochondrial respiration and oxidative phosphorylation in vitro were examined. No effects of extract were found, suggesting that the effect of mitotic inhibition is not via inhibition of cell respiration or oxidative phosphorylation.

Although our extract has definite effects on the mode of growth of human primary foreskin cells in tissue culture, further work in extract purification and mode of action is needed.

Zusammenfassung. Der Einfluss eines biochemischen, zellfreien Hautextraktes auf menschliche Vorhautzellen wurde in Kultur geprüft. Allgemeine Hemmung von Zellwachstum, Zellvermehrung, Zellvolumen und Mitoserate sprechen für aktiven Bestandteil des Extraktes.

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The Effect of Pretreatment with Rat Placental Homogenate on Subsequent Pregnancy in the Rat

There is increasing interest in the problem of intrauterine fetal malnutrition in human pregnancy as a result of accumulating evidence which suggests that severely affected infants may suffer physical and/or mental retardation in later life [1–6]. Its cause is not known.

The present paper describes an attempt to produce fetal growth retardation in pregnant rats using an immunologic method which involves repeated injection of homologous rat placental homogenate in Freund's adjuvant.

Materials and methods. Three groups of rats were investigated: 1. Rats injected with homologous placenta in Freund's adjuvant before pregnancy. The preparation of homogenate and injection was as described by OKUDA and GROLLMAN [7]. A regime of 8 bi-weekly injections was administered i.p. under ether anaesthesia to a group of 25 rats, after which the animals were mated. Rats weighed from 153 to 196 g at the start of the experiments.

2. Rats injected with homologous placenta in Freund's adjuvant during pregnancy. Twice weekly injections were given i.p. under ether anaesthesia during the 3 weeks of gestation.

3. Control rats. These were normal untreated rats which were not tampered with in any way before or during pregnancy.

Rats were mated, proteinuria determined and placenta and fetal tissues obtained by methods described elsewhere [8].

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Average litter size and weights of fetuses and placentas in normal pregnant rats and in rats injected with rat placental homogenates plus Freund's complete adjuvant

Type of pregnancy	Day of pregnancy	No. of liveborn fetuses	Fetal weight (g)	Placental wt. (g)	Fetal: placental weight (ratio)
Normal	21	11.14 ± 3.14 (59)[a]	3.9 ± 0.58 (22)	0.57 ± 0.08 (22)	6.9 ± 0.96 (22)
	20	12.00 (2)	2.2 (2)	0.54 (2)	4.08 (2)
Injected with rat placental homogenate before pregnancy	21	8.50 ± 4.00 (12) $P < 0.02$	3.6 ± 0.43 (12)	0.66 ± 0.05 (12) $P < 0.02$	5.7 ± 1.49 (12) $P < 0.01$
	20	8.00 (1)	1.8 (1)	0.78 (1)	2.3 (1)
Injected with rat placental homogenate during pregnancy	21	10.70 ± 2.52 (9)	3.6 ± 0.31 (9)	0.54 ± 0.05 (9)	6.8 ± 0.45 (9)

[a] Figures in parenthesis are the number of samples. All values are the mean ± standard deviation.

Results. Only 13 of the rats injected with the homogenate preparation before pregnancy were subsequently mated successfully; some rats never showed spermatozoa in vaginal smears despite repeated mating attempts, while others killed 21 days after a positive smear was obtained, showed no evidence of pregnancy then. These rats excreted up to 300 mg/100 ml protein in the urine on occasion; however, the amount of proteinuria fluctuated considerably in the same rat. Control rats generally showed lesser degrees of proteinuria than those treated.

As indicated in the Table, rats which had been injected with placental homogenate prior to pregnancy had a significantly smaller number of live-born fetuses per litter than did the controls ($P < 0.02$). The average weight of surviving fetuses was similar to that of controls at day 21 of gestation, although fetal size was abnormal in 2 cases at this time of pregnancy; fetuses weighed from 2.8 to 2.9 g in one rat, and in another, one live fetus weighing 2.5 g was observed with 2 resorptions. A third rat killed on day 20 of gestation showed abnormally small fetuses. The average placental weight was significantly greater than normal in these rats, while the fetal/placental weight ratio was significantly less than in the controls.

The Table shows that rats injected with placental homogenate in Freund's adjuvant during pregnancy only did not differ significantly from controls with respect to litter size and fetal and placental weight.

Discussion. The present attempt to induce intrauterine fetal malnutrition using the immunologic technique of repeated injection of rat placental homogenate in Freund's adjuvant, either before or during pregnancy, was not successful from the point of view that it did not consistently result in pregnancies with growth retarded fetuses.

It is interesting, nevertheless, to compare the effect of this treatment on other aspects of the pregnancy outcome which were observed in this study and by other investigators. In a few rats given 12 bi-weekly injections of placental homogenate and subsequently mated, OKUDA and GROLLMAN[7] observed bleeding from the vagina, necrotic fetuses and in one case, very small fetuses with normal-sized placentas. Using their technique, but a different strain of rats and a total of only 8 injections, we obtained normal-sized fetuses in most cases but the number of live-born fetuses per litter were less than in controls. The latter finding was due in part to fetal death in utero as indicated by the presence of incomplete fetal resorptions. These fetal deaths probably occurred in the latter half of pregnancy since in similar (but not identical) experiments of KETCHEL et al.[9] litter size was observed to be normal at day eleven of gestation.

As in the present study, LANGFORD et al.[10] observed smaller litters at term in placental homogenate treated rats but these authors did not report on fetal or placental size. The abnormal fetal/placental weight ratio observed in our experiments was due mainly to increased placental mass which probably resulted from the smaller litter size. According to studies in mice, placental size within a given strain is inversely related to litter size[11].

The cause of the reduced number of live-born fetuses per litter in the rats treated with placental homogenate in Freund's adjuvant before pregnancy is not clear. OKUDA and GROLLMAN found that this treatment resulted in rats (non-pregnant) showing kidney glomerular lesions similar to those observed in human toxemia of pregnancy. An increased incidence of intrauterine fetal malnutrition has been noted in the latter condition[2,12] and it may be due to an impairment of uteroplacental circulation for which evidence has been observed by a number of investigators[13–16]. It is possible that a disturbance in renal function in a species which normally has many fetuses per pregnancy may result in a reduction in litter size by causing metabolic disturbances which would ultimately adversely affect uteroplacental blood flow.

Résumé. L'effet d'un traitement immunologique sur la grossesse du rat a été étudié. Les animaux du groupe expérimental ont reçu 8 injections d'homogénat placentaire à raison de deux par mois, puis ont été accouplés. Après 21 jours de grossesse il y avait moins de fétus nés vivants que dans le groupe témoins non-traité; le poids des fétus en vie était en général normal mais on a noté une augmentation significative de la masse placentaire par rapport au groupe témoin.

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