each chamber, a steel-ball-type flow meter is inserted at a point before the air is heated and humidified.

Results and discussion. The design and arrangement of the chambers is such that they can be cleaned and sterilized by UV treatment prior to exposure, and tissue or organ cultures installed in a quite convenient manner. In addition, the set-up is easy to dismantle so that cultures can be viewed or treated almost immediately after exposure. Although the system has been in operation for only a short time, it has already proved reliable. Work is now progressing, and in a future publication we hope to present quantitative data on the reaction of cells in monolayer and organ culture to mainstream cigarette smoke under such conditions[10].

Zusammenfassung. Eine Apparatur zur Berauchung von Zell- und Organkulturen unter kontrollierten Bedingungen (Anzahl und Dauer der Puffs, Temperatur, Luftfeuchtigkeit) wird beschrieben. Das System basiert auf der Verwendung der Filtrona-Berauchungsmaschine (Cigarette Components Ltd.); die zusätzlichen Elemente können leicht aus laboreigenem Material zusammengestellt werden. Frischrauch und wasserdampfgesättigte Gasmischungen (z.B. Luft/CO_2) können in der Berauchungskammer in variablen Proportionen gemischt werden.

P. DAVIES and G. S. KISTLER

Department of Anatomy, Division of Electron Microscopy, University of Zürich, Gloriastrasse 19, CH-8006 Zürich (Switzerland), 29 October 1973.

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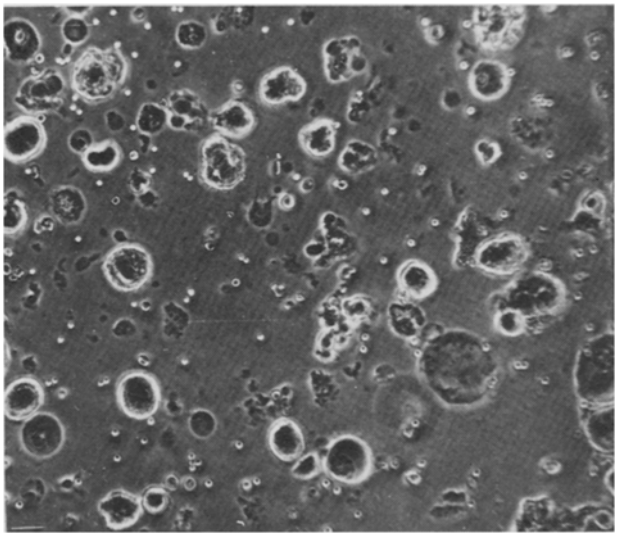
Free Cell Suspensions from Rat Placental Tissue: Metabolism of Pregnenolone-4-^{14}C and Progesterone-4-^{14}C

In this laboratory we have shown the disappearance of 17α-hydroxylase and 17–20 lyase activity after homogenization of the placental tissue. In vitro conversion of labelled C_{21} precursors to androgens occurs only in undamaged placental tissue[1]. These studies were carried out with tissue slices or small cubes of cut tissue. In such experiments, several methodological problems are encountered: broken cells, difficulties in getting homogenous tissue replicates, and gradients of penetration into the tissue of precursors. In work with adrenal[2] and ovarian[3] tissue, these problems are partially overcome by preparing free cell suspensions. We believe that a method of preparing free cells from placenta will make it possible to isolate the hormonal active cells from this morphologically and functionally complicated organ, and will facilitate studies on the regulation of steroid secretion.

We shall describe here a technique of preparing free cells from rat placental tissue. Our results indicate that suspensions of dissociated placental cells can be used to study the metabolism of labelled steroids.



Cell suspension prepared from rat placenta by treatment with trypsin (1:200, Biomed, Warsaw), collagenase (W.S.S., Warsaw) and DNAse (2,000 U/mg, B.Z.S.S., Warsaw). The cells are seen in phase contrast. ×250.

Preparation of placental cell suspensions. Wistar rats were used in these studies. Proestrous females were placed with males; the day that sperm were found in the vaginal smear was designated as day 1 of pregnancy. The animals were decapitated on day 15. The placentas, quartered and washed with Krebs-Ringer bicarbonate buffer, and then chopped and transferred (1.0 g of tissue) into a 25 ml Erlenmeyer flask containing 10.0 ml of 0.25% solution of trypsin, 0.25% collagenase and 0.013% DNAse in medium of pH 7.2–7.3 containing per 100 ml NaCl 0.8 g, KCl 0.02 g, Na_2HPO_4 0.115 g, KH_2PO_4 0.02 g and $MgCl_26H_2O$ 0.01 g. DNAse was added to digest DNA released from damaged cells[4] which otherwise would form a slimy material entrapping the liberated cells. The flask was gassed with 95% O_2–5% CO_2 and incubated at 37 °C for 15 min with shaking. At the end of the incubation period, the fluid containing freed cells was transferred with a siliconized pasteur pipette to a cold siliconized 50 ml centrifuge tube. To the residual 10 ml of fresh digestion medium was added and the process was repeated 3 times. The cell suspensions were combined and centrifuged at $100 \times g$ for 10 min at 4 °C. After centrifugation, the supernatant was removed and the pellet was washed twice with 10 ml portions of Krebs-Ringer bicarbonate buffer. Cell viability was tested by mixing the cell suspension with an equal volume of 0.2% methylene blue. Only the nulcei of living cells take up this stain. The number of cells was estimated by using a Bürker's counting chamber. A total yield of $5.0–6.0 \times 10^6$ cells was obtained from 1.0 g of placental tissue.

Incubation. The pellet was resuspended in Krebs-Ringer bicarbonate medium (pH 7.4) containing 0.2% glucose and 0.2 μCi of pregnenolone-4-^{14}C (S.A. 24 mCi/m*M*) or progesterone-4-^{14}C (S.A. 60 mCi/m*M*, Radiochemical Centre Amersham, England). The suspension

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was adjusted to contain 2.5–3.0 $\times 10^6$ cells in a volume of 2 ml in the incubation vial. The vials were incubated for 1 h in a shaking water bath at 37 °C under 95% O_2–5% CO_2. The samples were frozen immediately after incubation and stored in a freezer until assayed for steroids.

Extraction, purification and identification of steroids were carried out exactly by a procedure described by REMBIESA et al.[1]. Radioactive counting was performed in a SL-30 Liquid Scintillation Spectrometer (Intertechnique, Paris). Radioactivity obtained was corrected for quenching by external standard method.

Results and comments. The pattern of steroids synthesized from pregnenolone-4-^{14}C and progesterone-4-^{14}C of placental cells is shown in the Table. The rat placental cells produce androgens from C_{21} steroids. 17-hydroxyprogesterone and 4-androstane-3,17-dione were obtained as the metabolites of pregnenolone and progesterone, and 3α-hydroxy-5α-pregnan-20-one and 3α,17-dihydroxy-5α-pregnan-20-one were isolated as the products of the precursors reduction. From pregnenolone, progesterone was also isolated.

Percentage conversion of radioactive precursors to various metabolites in free cell suspensions from rat placental tissue

Metabolites	Precursor	
	Pregnenolone	Progesterone
Unknown metabolite	9.7	8.3
3α,17-Dihydroxy-5α-pregnan-20-one	11.0	14.8
17-Hydroxyprogesterone	2.4	2.6
4-Androstene-3,17-dione	4.5	9.5
3α-Hydroxy-5α-androstan-17-one	—	14.5
3α-Hydroxy-5α-pregnan-20-one	22.5	33.4
Pregnenolone	45.3	—
Progesterone	4.2	14.4
5α-Pregnane-3,20-dione	1.0	2.7

The percentage conversion of labelled precursors was calculated by eluting radioactive peaks present on chromatograms (average of 4 incubations). For other details of incubation see text, and of extraction, purification and identification see the paper[1].

The comparison of the results presented here with those reported earlier[1] indicate that free cell suspensions from rat placenta reveal the pattern of steroidogenesis similar to that of placental quarters but different from that of homogenates. Placental homogenates convert progesterone only to reduced metabolites[1,5].

Our preliminary results revealed that gradient centrifugation of the free cell suspensions results in several distinct fractions. We believe that using these fractions in studies on steroid synthesis will enable us to obtain better insight into the role of tropic hormones on placental steroidogenesis.

Zusammenfassung. Methode zur Gewinnung einer Suspension isolierter Plazentazellen der Ratte mittels dreier Enzyme (Trypsin, Kollagenase, Streptodornase). Ähnlich wie bei Placentagewebeschnitten[1], wurde die Steroidenzymaktivität festgestellt.

IRENA DZIADKOWIEC and R. REMBIESA

Institute of Pharmacology, Polish Academy of Sciences, 52 Ojcowska Street P–31-344 Kracow (Poland), 20 September 1973.

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A New Spectrophotometric Determination of Nitrazepam

It is known that compounds having active methylenic groups give rise with the quinones to the CRAVEN reaction[1–4]. We have taken interest in the action of mononuclear and binuclear quinones on several substances of toxicological interest having an active methylenic group, e.g. barbituric acid, brucine, strychnine, diazepam and nitrazepam, in order to establish either the nature of the reaction products or the analytical employment of such reactions. The reaction between barbituric acid and chloranil has been reported in a previous note[5]. We have now examined the reaction between nitrazepam and several quinones in dimethylformamide (DMF) solution in presence of ammonia, and we have found that 1,2-naphtoquinone gives the more sensitive chromatic reaction. The stoichiometry of this reaction has been established spectrophotometrically, according to the method of continuous variations of JOB[6]. On the basis of the results obtained, we can affirm that nitrazepam and 1,2-naphtoquinone give rise in solution to a molecular adduct with molar ratio 1:1 between the components. The optical density of this orange DMF solution is a linear function of nitrazepam concentration in the range $0.125 \times 10^{-4} \div 2.5 \times 10^{-4}$ moles/l. The suitability of this method of inquiry has been verified by adding variable and known amounts of nitrazepam (in the above range) to a series of known samples of the biological liquids blood-serum, saliva and urine. In the blood-serum, the quantity of nitrazepam evaluated was 95 ± 1.5% of the present amount; in the saliva the 96 ± 2% and in the urine the 94 ± 3%. Such results are better than those reported with other methods[7,8], and therefore this chromatic reaction

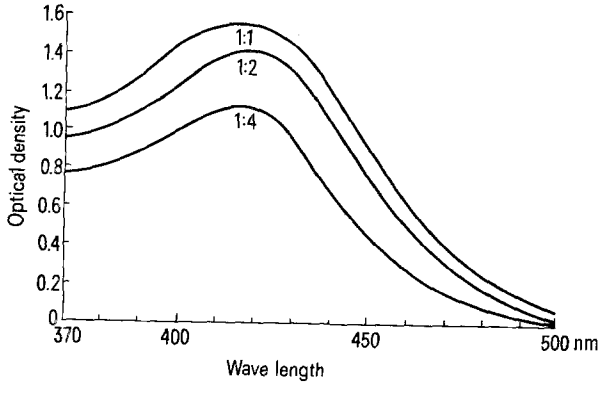

Fig. 1. Absorption spectra of ammoniacal DMF solutions containing nitrazepam and 1,2-naphtoquinone in molar ratios 1:1, 1:2, 1:4.

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