irregular in shape with the membranes widely separate and the contents poorly defined. Again various stages in their development were found, as is shown in Figures 3 and 4.

Protrusions resembling that shown in Figure 4a where the outer margin was ruptured and granular material seen passing into the cytoplasm, were frequently observed. In such cases the inner of the two elements of the nuclear envelope was always reconstituted across the surface of the nucleus. It is probable that the fully developed vesicle shown as Stage (VI) in Figure 3 represents a section through a protrusion of the type shown as Stage (V). It is unlikely that final separation of the protrusions from the parent nuclear envelope was necessary before release of the contents could take place. Release seemed to begin immediately the integrity of the inner element of the nuclear envelope was restored. Such completely separated structures were only rarely observed and then never free in the cytoplasm.

The material included in the protrusions frequently resembled nucleoplasmic constituents, particularly the chromatin. Neither ribonuclease nor pepsin had a marked effect upon the appearance of the contents of the protrusions, apart from occasionally reducing the contrast of the matrix material.

Discussion. It is difficult to visualize the significance of either the development of small vesicle structures from the outer element of the nuclear envelope or the development of protrusions from both the elements of the nuclear envelope except in terms of nucleo-cytoplasmic transfer.

The development of irregularities in the outer element of the nuclear envelope of cells of both the liver and kidney could conceivably be related to a need to increase the surface area of the perinuclear space-cytoplasmic interface in certain metabolic states. The development of small vesicles from many of the irregularities however, would suggest the transport of small molecules or other electron transparent components from the perinuclear space to the cytoplasm. Such material, presumably, cannot traverse the nuclear membrane by diffusion. The nuclear envelope and endoplasmic reticulum are known to be structurally related. The observed relationship between the points of origin of the small vesicles and lamellae of the endoplasmic reticulum suggests that the nuclear envelope is able to contribute material to the endoplasmic reticulum. Vesicles budded from the nuclear envelope could perform a combined function of carrying small molecules as well as contributing to the mass of the endoplasmic reticulum by fusing with it.

The function of the large membrane protrusions is more difficult to explain, partly because of their restricted occurrence in the kidney and partly because of their relative infrequence. The presence of included material of nuclear origin and its apparent release would suggest that they are also connected with some mechanism of nucleocytoplasmic transfer. It has never been fully established to what extent nucleopores can cope with the transfer of particles from nucleus to cytoplasm. It is conceivable that the development of large nuclear membrane protrusions might represent a means of transferring large particles, in bulk, in certain cell types. SCHARRER and WURZELMANN[1] suggested such an explanation for the development of similar structures from the nuclei of oocytes of the South African Lungfish.

It is interesting to note, here, the observations of LÖWENSTEIN[6] concerning the properties of the nuclear envelope and the necessity of at least one of the membrane components remaining intact for the maintenance of nuclear function. Particulate material could be transferred directly from the nucleus to the cytoplasm by means of protrusion development, without disturbing the structural integrity of the nuclear envelope, only one of the membrane elements being disturbed at any one time.

SCHARRER and WURZELMANN[1] and KILARSKI and JASINSKI[5] correlated the development of large vesicle structures from the nuclear envelope of cells of *Protopterus* and *Perca fluviatilis* with altered cellular activity. In the case of the gas gland cells of *Perca* the production of vesicle structures was associated with increased protein synthetic activity. We were able to demonstrate an increase in the frequency of both the small vesicles and large protrusions with thyroid hormone treatment, which is known to stimulate protein synthesis[7,8].

Although it is difficult to ascribe any definite functional significance to the development of both small vesicles and large membrane protrusions from the nuclear envelope their occurrence appears to be related to increased protein synthetic activity and to nucleocytoplasmic transfer mechanisms.

Zusammenfassung. Erstmals werden bei Säugetieren Vesikulationen der äusseren Lamelle der Kernmembran bei Nieren- und Leberzellen von Mäusen gefunden und diese mit der Proteinsynthese in Verbindung gebracht.

SUSAN E. NICHOLLS[9] and A. J. MATTY

Department of Biological Sciences, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (England), 9 May 1974.

[6] W. R. LÖWENSTEIN, Protoplasmatologia *5*, 26 (1964).
[7] S. E. NICHOLLS and A. J. MATTY, J. Endocr. *48*, 8 (1970).
[8] S. E. NICHOLLS and A. J. MATTY, unpublished data.
[9] Present address: Biochemistry Research Unit, University of Keele, Staffordshire, England.

Nicotine and Placental Iron Transport

Several reports during the past few years have suggested that there may be a causal relation between maternal smoking and certain adverse pharmacologic responses in gravid women and human fetuses. Compared with non-smokers, women who smoke tend to have smaller babies and a greater incidence of premature delivery, abortion and stillbirth[1-4]. BECKER et al.[5,6] have obtained data in rats which demonstrate that nicotine is effective in producing retardation of fetal weight. Since nicotine can readily traverse the placental barrier[7] it may exert a

[1] P. UNDERWOOD, L. L. HESTER, T. LAFFITTE and K. V. GREGG, Am. J. Obstet. Gynec. *91*, 270 (1965).
[2] C. S. RUSSELL, R. TAYLOR and R. N. MADDISON, J. Obstet. Gynaec. Br. Commonw. *73*, 742 (1966).
[3] D. RUSH and E. H. KASS, Am. J. Epidem. *96*, 183 (1972).
[4] H. GOLDSTEIN, Nature, Lond. *245*, 277 (1973).
[5] J. E. KING and R. F. BECKER, Am. J. Obstet. Gynec. *95*, 508 (1966).
[6] R. F. BECKER, C. R. D. LITTLE and J. E. KING, Am. J. Obstet. Gynec. *100*, 957 (1968).
[7] H. D. MOSIER and R. A. JANSONS, Teratology *6*, 303 (1972).

Table I. Effect of nicotine on maternal weight-gain, and fetal and placental weights

Treatment	No. of gravid rats	No. of nidations [a]	Maternal weight-gain [b] (g) [a]	Fetal weight [c] (mg) [a]	Placental weight [c] (mg) [a]
Control	4	9.0 ± 2.0	48 ± 3	312 ± 9	324 ± 8
Nicotine					
1 mg/kg/day	5	8.8 ± 1.1	34 ± 4 [d]	309 ± 7	305 ± 10 [d]
2 mg/kg/day	5	10.2 ± 1.6	22 ± 4 [d]	292 ± 6 [d]	268 ± 1 [d]
6 mg/kg/day	7	12.4 ± 1.0	17 ± 7 [d]	250 ± 4 [d]	202 ± 6 [d]

[a] Mean ± SEM. [b] Weight-gain between gestation day 9 and 16. [c] Gestation day 16. [d] Statistically different from control value at $p < 0.01$.

direct effect on fetal tissues. However, the alkaloid may also influence fetal development indirectly via the placenta. Although TANAKA[8] has shown that respiration of the human placenta is reduced by smoking during pregnancy, there is no information concerning the affect of nicotine on this tissue.

Because of the important role of the placenta in maintenance of a normal pregnancy, the present investigation was undertaken to determine the affect of chronic ingestion of nicotine on placental growth and function. Sprague Dawley rats were used and the sperm positive date indicated by the suppliers (Holtzman Co., Madison, Wis.) was designated day 1 of gestation. Animals were distributed randomly into 4 groups, housed individually and fed Purina rat chow and water ad libitum. Control animals were injected s.c., twice daily from gestation day 9 through 15 with 0.9% saline. Each of the 3 remaining groups was injected according to the same regimen with either 0.5, 1 or 3 mg of nicotine per kg body weight. The low and perhaps the intermediate dosage is within the physiological level of nicotine consumed by heavy smokers[9]. Serum containing ^{59}Fe-labeled transferrin was prepared and administered to each maternal rat under anesthesia according to the method of GARRETT et al.[10]. Each animal was allowed to recover from the ether anesthesia for 30 min, was again anesthetized, laparotomized and the uterine horns excised. The total number of nidation sites was determined and each fetus and chorioallantoic placenta was weighed and counted for radioactivity in a NaI (Tl) crystal scintillation counter. Statistical analysis of the data was obtained using an IBM 360/50 computer and the Statistical Analysis System[11]. Level of significance was determined using Student's *t*-test.

Maternal rats receiving nicotine became anorexic and the rate of weight gain was less than that of control animals. As shown by data in Table I, interference with maternal weight-gain and conceptus growth correlated positively with increasing dosage of the alkaloid. Rats treated with nicotine at 1, 2 and 6 mg/kg/day gained, respectively, 71%, 46% and 35% of the weight gained by corresponding controls. The ratio of the conceptus weight and the maternal weight-gain was 0.0013 for controls, 0.0018, 0.0019 and 0.0027 for rats receiving nicotine at 1, 2 and 6 mg/kg/day. As shown by this ratio, the alkaloid exerted a more pronounced influence on the maternal system than on the fetal-placental unit per se. However, within the fetal-placental unit, growth of the placenta was affected more by nicotine than was growth of the fetus. The ratio of fetal weight to placental weight was 0.96 in controls and 1.01, 1.09 and 1.24 in rats receiving nicotine at 1, 2 and 6 mg/kg/day, respectively. Fetal death occurred in approximately 60% of the rats receiving nicotine at each dosage level but in only 25% of the control rats, and the incidence of resorptions increased by approximately 10% in drug treated rats. Results obtained in the present study relating to the effect of nicotine on maternal

[8] M. TANAKA, J. Jap. Obstet. Gynec. Soc. *14*, 45 (1967).

[9] W. A. WOLFF, M. A. HAWKINS and W. E. GILES, J. Pharmac. exp. Ther. *95*, 145 (1949): 0.2 to 0.4 mg of nicotine absorbed per pack of cigarettes smoked; also, private communication, Dr. H. B. KOSTENBAUDER: 0.54 mg of nicotine absorbed per pack of cigarettes smoked; nicotine absorption, however, varies from person to person and is related to puff volume, inhalation of smoke, etc.

[10] R. J. B. GARRETT, N. E. GARRETT and J. W. ARCHDEACON, Life Sci. *11*, 1 (1972).

[11] A. J. BARR and J. H. GOODNIGHT, *A User's Guide to the Statistical Analysis System* (Student Supply Stores, North Carolina State University, Raleigh, N.C., 1972). Data analysis conducted by Mr. R. GURAL, College of Pharmacy, University of Kentucky.

Table II. Effect of nicotine on maternal-fetal transport of ^{59}Fe on the 16th day of gestation

Treatment	No. of gravid rats	No. of fetal-placental units	^{59}Fe CPM × 10^3 [a]	
			Fetus	Fetal-placental unit
Control	5	44	3.3 ± 0.1	7.2 ± 0.3
Nicotine:				
1 mg/kg/day	4	23	3.0 ± 0.3	7.5 ± 0.7
2 mg/kg/day	6	61	1.8 ± 0.3 ($p < 0.005$)	4.5 ± 0.8 ($p < 0.025$)
6 mg/kg/day	6	63	1.6 ± 0.3 ($p < 0.001$)	4.4 ± 0.7 ($p < 0.01$)

[a] Mean ± SEM.

weight-gain, fetal weight and fetal resorptions are similar to but not as pronounced as the finding of HUDSON and TIMIRAS[12]. These investigators administered nicotine at 2, 6 and 10 mg/kg/day to rats from gestation Day 0 to Day 21 or from Day 0 to Day 7 only. They found that the percentage of resorptions increased and that body weight of the offspring decreased when increasing amounts of nicotine were administered. The authors suggested that nicotine affects processes operative during the first week of gestation and that these effects may be manifested in developmental disturbances in later growth stages. Results of the present study, however, demonstrate that fetal development is also affected even when nicotine administration is initiated after the first third of gestation.

In the rat a transport system for iron develops in the placenta[13] and on the 16th day of gestation iron can be transmitted to the fetus by this system[10]. Since accumulation of iron from maternal transferrin by the placenta is a process which is not dependent upon the presence of a normal fetus[13], ^{59}Fe uptake can be used as one index of placental function. As shown by data in Table II iron nuclide was effectively accumulated by the placenta of control animals and was transported to the fetus on the 16th day of gestation. Nicotine at 1 mg/kg/day did not significantly influence uptake and transport of ^{59}Fe by the placenta. In rats receiving nicotine at 2 and 6 mg/kg/day placental uptake of iron was depressed significantly. Approximately 40% less iron was present in the fetal-placental unit of rats receiving nicotine. Furthermore, on a weight basis, nuclide transport by the palcenta was reduced 22 to 34%.

Results of the present study support the findings of other investigators that nicotine ingestion adversely affects fetal weight-gain. In addition, however, results from the study show that nicotine adversely affects placental growth and at least one aspect of placental function, namely, accumulation and transport of iron. Investigations on the effect of nicotine on other biochemical and morphological parameters of placental development, metabolism and transport may provide insight into how and to what extent cigarette smoking may affect gravid women and their fetuses.

Zusammenfassung. Nikotin in 1, 2 und 6 mg/kg täglichen Dosen in Ratten vom 9. bis zum 15. Tage der Trächtigkeit s.c. injiziert verursachte herabgesetzte Gewichtszunahme der Mutter sowie geringeres Wachstum des Fötus und der Plazenta. Bei einer täglichen Dosis von 2 und 6 mg/kg Nikotin war die ^{59}Fe-Anreicherung in der Plazenta beträchtlich vermindert.

R. J. B. GARRETT

College of Pharmacy, University of Kentucky, Lexington (Kentucky 40506, USA), 13 November 1974.

[12] D. B. HUDSON and P. S. TIMIRAS, Biol. Reprod. *7*, 247 (1972).
[13] M. M. MANSOUR, A. R. SCHULERT and S. R. GLASSER, Am. J. Physiol. *222*, 1628 (1972).

Etude par immunofluorescence de l'apparition de protéines contractiles, myosine et actine, dans des Myoblastes d'Amphibiens en différenciation in vitro

Immunofluorescence Studies on the Appearance of Myosin and Actin During in vitro Differentiation of Amphibian Myoblasts

Le but de notre travail a été d'étudier un aspect de la différenciation morphologique in vitro des myoblastes d'Urodèle et plus particulièrement d'effectuer une étude comparative de l'apparition et l'évolution de protéines myofibrillaires, myosine et actine, à l'aide de la technique d'immunofluorescence qui permet de déceler avec précision la présence d'une quantité minime de ces protéines.

Matériel et méthodes. – Préparation du matériel embryonnaire et immunofluorescence. Des cultures de cellules prélevées sur la paroi dorsale de jeunes neurulas (St. 13) de *Pleurodeles waltlii* sont effectuées selon une technique déjà décrite[1]. La technique d'immunofluorescence indirecte utilisée est essentiellement la même que celle déjà publiée[2,3].

Préparation des antigènes et antisérums. Les détails de la préparation des antigènes, leur identification, le contrôle de leur pureté, l'absorption des antisérums, etc... sont décrits dans de précédentes publications[2–4].

Contrôles. Cellules: 1. Transférées seulement dans le tampon salin; 2. traitées au SwAR/FITC (Sevac) seulement; 3. traitées avec du sérum anti-myosine (anti-actine) seulement; 4. où le sérum normal de lapin est utilisé à la place de l'antisérum antimyosine (anti-actine). Tous ces contrôles ont donné des résultats négatifs.

Résultats. A 18 °C, 3 à 4 jours sont nécessaires aux cellules pour s'attacher et s'étaler sur le support. A ce stade, les myoblastes généralement fusiformes ne présentent encore aucune autre différenciation morphologique. A partir de ce moment, il n'y a plus de mitose dans cette catégorie cellulaire. Ce n'est qu'après le 5e jour de culture que la striation myofibrillaire commence à être visible en microscopie à contraste de phase, dans la zone paranucléaire. Au cours des jours qui suivent, les myofibrilles progressent vers les extrémités de la cellule, dont la plage cytoplasmique s'accroît. La différenciation morphologique de la cellule musculaire est complète au 10e jour de culture environ[1].

Il est à noter que la cytodifférenciation des myoblastes d'Urodèles se déroule normalement in vitro bien que ces cellules restent souvent complètement isolées[5]. La fusion des myoblastes n'est pas nécessaire. Ceci est contraire à ce qui se passe pour la différenciation de cellules isolées de muscle squelettique d'embryons d'Oiseau ou de Mammifère, qui nécessite une fusion de myoblastes en un syncytium se transformant ensuite en fibre musculaire typique avec l'apparition de myofibrilles caractéristiques.

Nous avons eu recours à une technique immunochimique pour détecter les premiers signes d'apparition des protéines

[1] A. M. DUPRAT, Annls. Embryol. Morph. *3*, 411 (1970).
[2] A. ROMANOVSKÝ et J. MÁCHA, Folia biol. *17*, 387 (1971).
[3] A. ROMANOVSKÝ, D. HURYCHOVÁ et J. MÁCHA, Folia biol. *19*, *43* (1973).
[4] A. SMIDOVÁ, D. HURYCHOVÁ, A. ROMANOVSKÝ et J. MÁCHA, sous presse.
[5] A. M. DUPRAT et C. MATHIEU, C. r. Acad. Sci., Paris *277*, 1705 (1973).