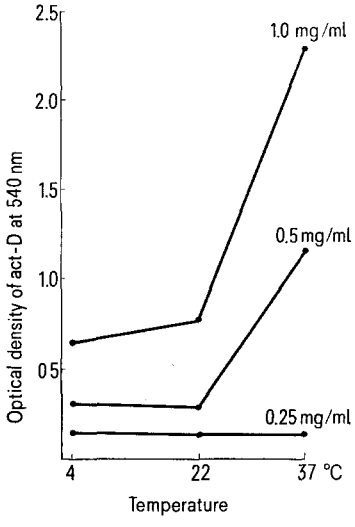
With an increase in temperature the drug came out of solution and the resulting turbidity was read at 540 nm on a Beckman Spectrophotometer with an attached Haake Constant Temperature Circulator. Readings were taken while the solutions were maintained at 4°, 21–22° (room temperature), and 37 °C.

The results of this study are summarized in the Figure. Optical density was partially due to the yellow-orange color of the drug. At 4 °C each concentration was clear and free from turbidity. It was obvious the drug was completely in solution and optical density was wholly concentration dependent at this temperature. At room temperature only the highest concentration of act-D (1.0 mg/ml) began to come out of solution and thus registered an increased turbidity. This insolubility was heightened 3-fold at 37 °C. Actinomycin D at the lower concentrations of 0.25 and 0.5 mg/ml stayed in solution at room temperature. At 37 °C, 0.5 mg/ml became insoluble with a 4-fold increase in turbidity; 0.25 mg/ml remained in solution.




The turbidity of actinomycin D at different temperatures. 3 concentrations of the drug in phosphate buffer (0.25, 0.5, 1.0 mg/ml) were maintained at 4°, 21–22° (room temperature), and 37 °C. Turbidity was measured, (as a change in optical denstiy), spectrophotometrically at 540 nm. Optical density was partially due to the color of the drug. The variation in optical density at 4 °C was wholly concentration dependent; the drug was completely in solution at this temperature.

The physico-chemical property of increased solubility with decreasing temperature is not unique to act-D but has been observed with other compounds[6]. By a freeze-thaw technique concentrations of act-D in aqueous medium can be realized as high as 0.5 mg/ml at room temperature. For rapid bioavailability, however, we suggest the administration of act-D at 0.25 mg/ml. Solutions containing 0.5 mg/ml are liable to precipitate, at body temperature, at the site of injection. This, then, eliminates the necessity of using foreign vehicles to dissolve act-D and thus the possibility of their effects[7].

*Résumé.* L'actinomycine D est solubilisée dans un milieu aqueux par une technique de congélation suivie de dégel, ce qui permet d'atteindre des concentrations de 1 mg/ml à 4 °C et de 0.5 mg/ml à la température du laboratoire. Pour obtenir une absorption rapide de l'actinomycine D les concentrations ne doivent pas dépasser 0.25 mg/ml.

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[7] Acknowledgments. This research was supported by California Tuberculosis and Respiratory Disease Association. We thank Mr. Crawford H. Brown for his helpful advice and offer our sincere appreciation to Dr. A. Heusner, Professor of Physiology at UCD for translating the summary from English to French language.

## Vesiculation of the Nuclear Envelope of the Liver and Kidney of the Mouse

The development of vesicle structures from the nuclear envelope has been described in various species of oocytes[1–4] and in the gas gland cells from *Perca fluviatilis*[5], the development of such structures from the nuclear envelope of a mammalian somatic cell has not been observed. We here report the development of small, single membrane-bounded vesicles from the outer element of the nuclear envelope of both the liver and kidney cells of the mouse and large nuclear envelope protrusions in proximal convoluted tubule cells of the kidney.

In cells of both liver and kidney proximal tubules the contour of the outer nuclear membrane was frequently irregular and puckered. Fingerlike projections and pockets were pinched-off to form small, electron transparent vesicles, 50–100 nm in diameter. A complete developmental series has been traced in both liver and kidney, as is shown in Figures 1 and 2. There was no evidence that the vesicle profiles represented sections of microtubules or lamellae derived from the nuclear envelope.

Although there was no apparent association between the position of the nucleolus within the nucleus or with mitochondria or Golgi apparatus with sites of small vesicle production, there was an association with the endoplasmic reticulum. Especially in the liver, rows of small vesicles occurred between the parent nuclear envelope and adjacent lamellae of endoplasmic reticulum. Such vesicles were often flattened, suggesting their conversion into elements of endoplasmic reticulum but no evidence was found of their fusion with lamellae.

Nuclear envelopes of some 15% of kidney cells were also observed with large local protrusions which were apparently associated with the production of double membrane bound vesicles containing material of apparently nuclear origin. These protrusions were almost always

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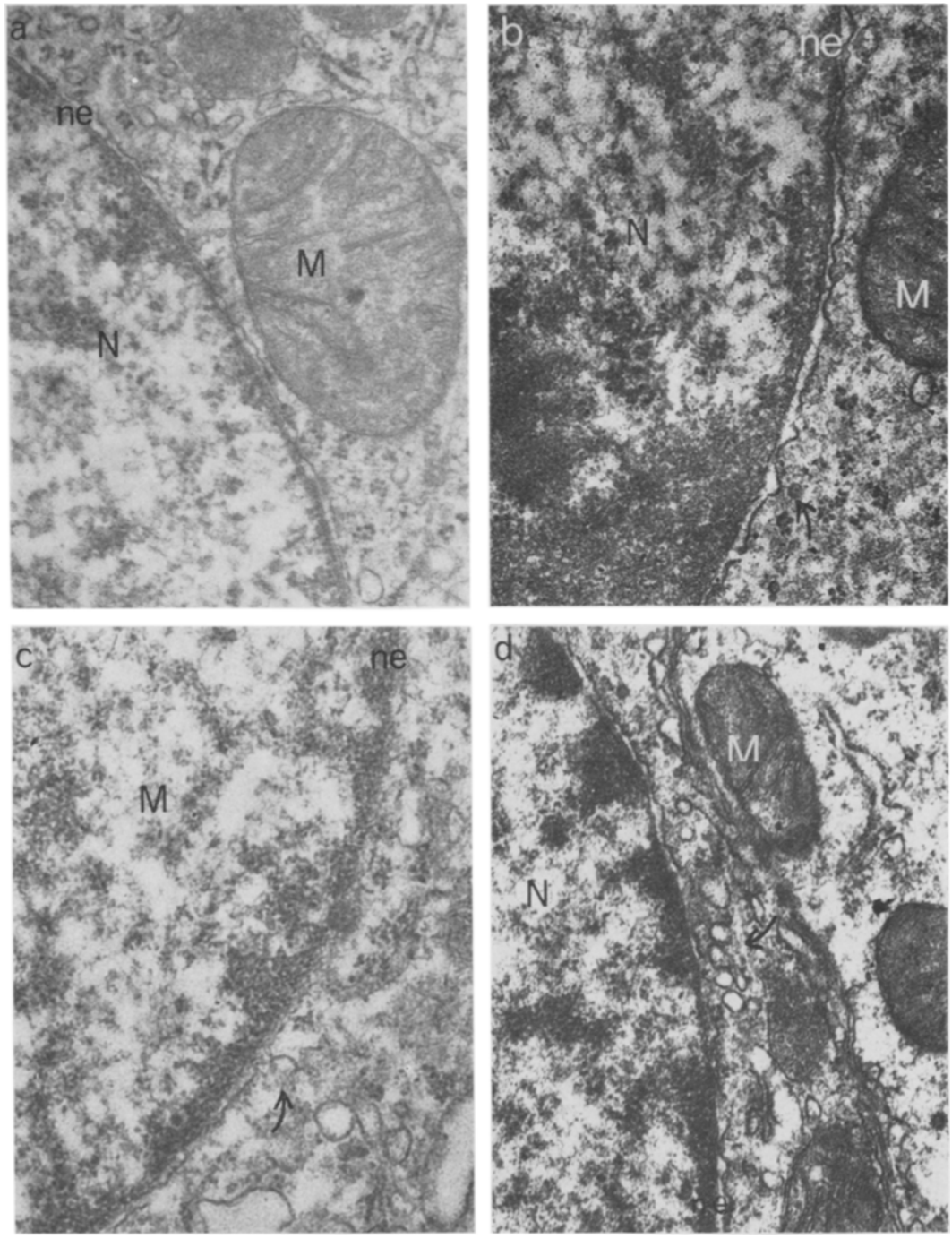


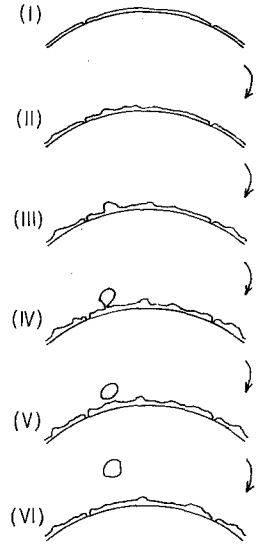
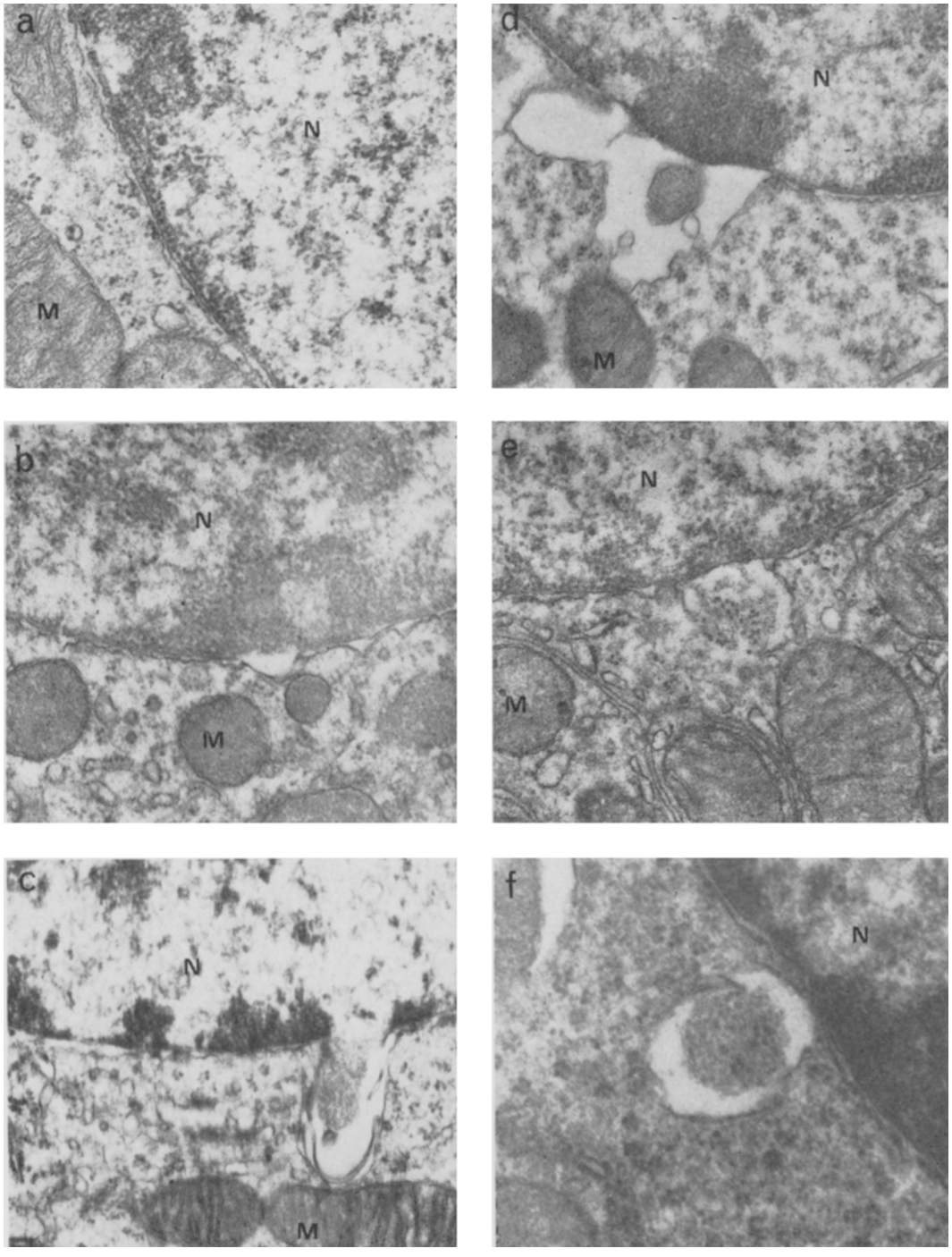




Fig. 1 and 2. Stages in the postulated development of a small vesicle from the outer membrane of the nuclear enveloppe.



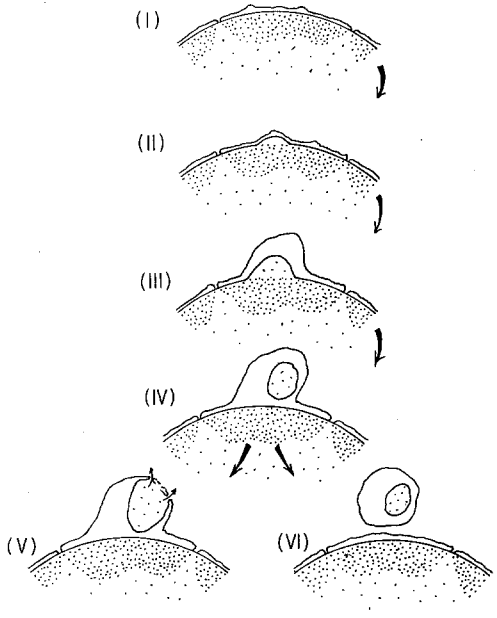




Fig. 3 and 4. The development of membrane protrusions from the nuclear envelope of kidney cells.

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.

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## 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

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