rated on a 15-point multi-item scale derived from that of BUNNEY and HAMBURG[5]. The degree of depression ranged from moderately severe to severe with patients exhibiting symptoms of marked psychomotor retardation, sleep and appetite disturbance, depressive delusions, and suicidal ideation. Five of the depressed patients were bipolar, i.e., had histories of manic episodes. The 6 manic patients exhibited flight of ideas, pressure of speech, euphoric or angry mood and intrusive, demanding behavior. Four of the 6 manic patients required seclusion. A cross-over design was employed where rats receiving one type of serum were given a different serum during the trials on the next day.

Results. There were no significant differences in the maze behavior of rats following injection of serum from depressed and manic patients or normal controls (Figure 2).

In 6 trials the animals appeared acutely ill after injection – similar to the dogs injected with depressed serum described by POLIAKOVA. However, this syndrome occurred equally among groups receiving manic, depressed and control serum (2 in each). The rats became prostrate and tremulous with labored respiration, yet attempted to drag themselves into the correct alley. This syndrome lasted 5–10 min. After recovery all but 1 of the animals were able successfully to complete the maze, often running a successive trial in less than 10 sec. The exclusion of animals with this effect did not alter the results as summarized in Figure 2.

There was no difference in the number of maze errors among groups of rats receiving manic-depressive or control sera. In the cross-over design where the same animal received injections of many different sera, no individual rat showed a clear pattern of faster or slower runs with manic or depressed serum.

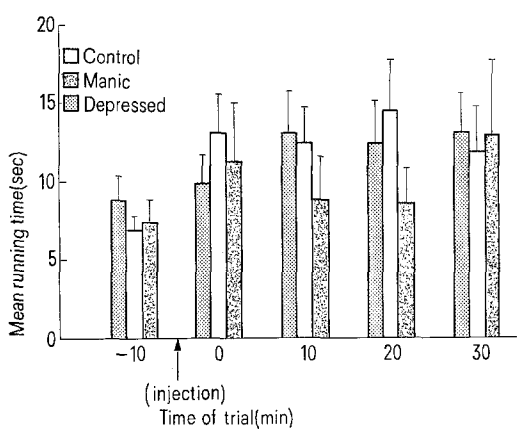

Fig. 2. Effects of manic and depressed serum on maze running time.

Discussion. Our failure to find any differences in the behavior of rats injected intravenously with serum from manic-depressive patients and controls raises questions about POLIAKOVA's findings, but does not rule out the possibility of a species specific effect in dogs.

As is evident in Fig. 2 all groups of animals showed an increase in maze running time following injection of serum. This may have been due to nonspecific serum effect or the stress of the injection procedure. Rats were immobilized in a restraining device before tail-vein injection. This procedure, equally stressful for experimental and control injections, could also have increased excitatory levels to the point where small differences in behavior mediated by a serum factor would not be evident.

No mention of medications is made in POLIAKOVA's study, while all of our patients were drug-free at least 1 week prior to sampling of serum. It is possible that psychotropic drug effects could explain POLIAKOVA's findings.

A number of animals, equally distributed between patient and control groups, appeared to have a toxic reaction to the injection of serum. This effect appears to be non-specific and was probably not related either to acute volume overload or anaphylaxis, as animals that had repeated injections did not have an increased likelihood of adverse reaction. It may be that this non-specific toxic reaction to injection of serum fortuitously accounted for the differences between depressed and control groups in POLIAKOVA's study.

Zusammenfassung. Serum von depressiven und manischen Patienten und von normalen Kontrollen wurde in die Venen von Ratten injiziert und deren Labyrinthlaufen beobachtet. Bedeutende Unterschiede bei Laufzeit und Fehlern wurden nicht festgestellt.

R. M. POST, J. KOTIN, M. MATZEN, C. C. JONES and F. K. GOODWIN

Section on Psychiatry, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda (Maryland, 20014, USA), 3 May 1972.

[5] W. E. BUNNEY JR. and D. A. HAMBURG, Arch. gen. Psychiat. *9*, 280 (1963).

Ring Shaped Nucleoli in the Primary Spermatocytes of Rats and Mice

Ring shaped nucleoli have been described in normal plasmocytes[1], oocytes[2–4], lymphocytes[5], smooth muscle and endothelial cells[1] as well as in hepatocytes during vital hepatitis[6], leukemic lymphoblasts[7] and Ehrlich ascitic cells[1].

In experimental studies ring nucleoli were obtained in a variety of cells under the influence of actinomycin D in vitro and in vivo[8–10] and chromomycine A_3 in vitro[1]. Starvation in some insects could also induce the appearance of ring nucleoli[11].

In the course of cytological study of spermatogenesis, using techniques described in previous papers[12–14], we commonly observed ring shaped nucleoli in primary spermatocytes of normal rat and mouse. It seemed therefore of interest to ascertain, whether the ring shaped nucleoli were a characteristic feature of these cells.

Material and method. Observations were made on testicles of 40 animals: 20 Wistar rats (Carworth Farm, N.Y.) weighing 120–240 g, and 20 Swiss mice CF-1 (Carworth Farm, N.Y.) weighing 20–40 g. All the animals were fed with standard Purina Chow (Ralson Purina Comp., St. Louis, Missouri) and water ad libitum.

Under general ether anesthesia the animals were unilateraly (right side) castrated. The testes were cut in halves with a razor blade and smears and imprints were prepared from the surface section.

After drying, the preparations were stained with the Giemsa-Ionescu solution[15], composed of: May-Grunwald

(dry powder), Giemsa (dry powder), Thionin (dry powder), 0.2 g each and Methanol 100 ml. The solution is ready for use after 5 days.

Slides were completely covered with the stain for 3 min, then an equal quantity of distilled water was added. The slides were kept under this diluted solution for 15–20 min, washed in tap water and dryed.

Results. The characteristic cell types observed in smears and imprints of testis have been previously described [12–14].

Examination by high magnification of the nucleoli of the primary spermatocytes revealed the presence of one, or less frequently, two nucleoli in about 50% of these cells. Some 30% of these nucleoli have an homogenous appearance. The remaining 70% showed a vacuolar, ring shaped form. The vacuole, 1–3 μm in diameter, was central or eccentric, and surrounded by more or less basophilic nucleolar substance.

The location of ring shaped nucleoli in relation to the sex vesicles was either a) in close proximity, b) at opposite sites, or c) in intermediate position.

In mice the most frequently observed aspect was the a) location, while in rats the b) and c) positions were seen often (Figures 1–3).

Discussion. Vacuolar nucleoli, often present in neoplastic cells, have been explained by 'metabolic anarchy' and hypertrophy of the nucleoli in these cells [1]. This interpretation, however, did not clarify the presence of this nucleolar feature in normal cells.

It can be assumed that RNA synthesis has stopped in ring shaped nucleoli which occur following administration of actynomycin D or other RNA inhibitors, as well as after starvation; thus the vaculoar features may reflect an inability of the nucleoli to rebuild or to maintain their homogenous compact structure.

In the seminiferous epithelium, the rapidly multiplying cells could also be unable to form regular nucleoli. Presumably, RNA production either cannot be completed during the continuous nuclear divisions [13, 14] or the available primary material is insufficient for RNA synthesis adequate to the fast cell reproduction. Accordingly, in spermatogenesis, nucleoli may appear as ring-shaped, vacuolar structures. This would be in line with the presence of nucleolar rings in cells with a high rate of multiplication such as tumor cells, lymphoblasts, regenerating cells [1, 5, 6] and correspondingly also in spermatocytes.

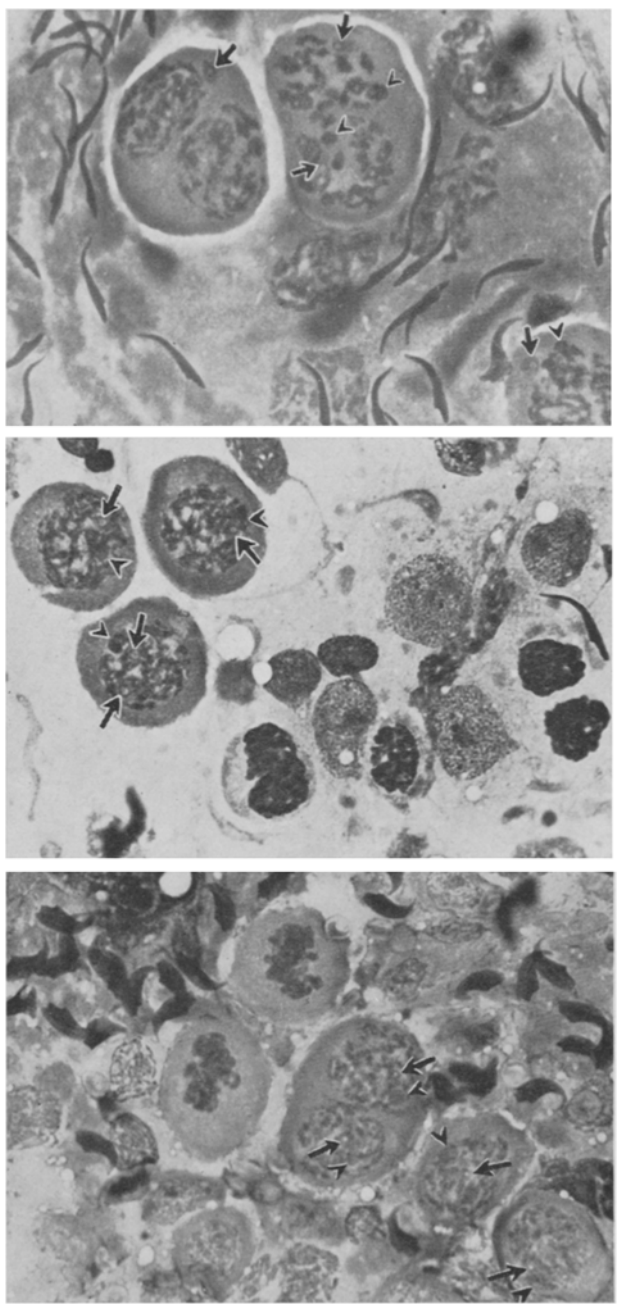

Fig. 3. Imprint from a mouse testis.

On all photographs the arrows indicate the ring-shaped nucleoli and the arrowheads indicate the sex vesicles. Fig. 1 and 2. Imprint from a rat testis.

Résumé. Des empreintes et frottis de testicule de rat et de souris ont été préparés et colorés par la solution de Giemsa-Ionescu. Des nucléoles annulaires ont été observés dans les spermatocytes primaires. Une corrélation possible est envisagée entre ces nucléoles annulaires et la multiplication rapide des cellules seminifères.

A. Rivenzon and L. Nebel

Department of Anatomy, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx (N. Y. 10461, USA); and Department of Embryology, Tel Aviv School of Medicine, Tel Aviv (Israel), 5 June 1972.

[1] H. Busch and K. Smetana, *The Nucleolus* (Academic Press, New York 1970), p. 61.
[2] R. R. Cowden, Z. Zellforsch. mikrosk. Anat. *60*, 399 (1963).
[3] H. Esper, Expl. Cell Res. *38*, 85 (1965).
[4] B. Scharrer and S. Wurzelman, Z. Zellforsch. mikrosk. Anat. *96*, 325 (1969).
[5] M. Potmesil and K. Smetana, Folia biol. Praha *15*, 300 (1969).
[6] L. Wasserman and S. Dolinescu, Stud. Invest. Inframicrobiol. *6*, 689 (1962).
[7] K. Smetana, M. Lane and H. Bush, Expl. Med. Path. *5*, 236 (1966).
[8] E. J. Journey and M. N. Goldstein, Cancer Res. *21*, 929 (1961).
[9] P. N. Panteleakis and G. M. Mateyko, J. Cell Biol. *31*, 154A (1966).
[10] K. Smetana and M. Potmesil, Z. Zellforsch. mikrosk. Anat. *92*, 62 (1968).
[11] A. Wessing, Z. Zellforsch. mikrosk. Anat. *65*, 445 (1965).
[12] A. Rivenzon, Med. Sect. Roumanian Acad. Scient. *6*, 1043 (1954).
[13] A. Rivenzon and M. Condoiu, Acta morph. hung. *9*, 1 (1959).
[14] A. Rivenzon and V. Comisel, Arch. microsc. Morph. exp. *55*, 55 (1966).
[15] V. Ionescu, *Tumor Cytology* (Medical Publishing House, Bucharest 1968).