EFFECTS OF NICKEL SULPHATE ON RAT TESTES SEMINIFEROUS TUBULES: AN ULTRASTRUCTURAL INVESTIGATION

(Received 3 March, 1994)

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SUMMARY

This study investigated the ultrastructural effects of daily oral doses of nickel sulphate ($NiSO_4$) (12.5 mg/kg) on rat testis seminiferous tubules on days 15, 60 and 95 of the experiment. The latter constituted the recovery group. The seminiferous tubules were severely damaged on the 15th and 60th days. The major findings were the germ cell sloughing and decreased spermatogenesis. A disorder in germ cell alignment was evident. Sertoli cell's phagocytic activity was increased. Recovery which was aimed to be established on the 95th day following a 60 day-$NiSO_4$ administration could not be observed at the seminiferous tubules of that group.

Key Words: Testis, Nickel sulphate, Electronmicroscopy.

INTRODUCTION

Harmful effects of the nickel compounds were investigated in many studies (1-11). Nickel sulphate, nickel sulphide, nickel oxide, nickel chloride and nickel subsulphide were told as toxically effective agents. Those compounds administered intravenously, intramuscularly or by inhalation were investigated in many tissues such as kidney, lung and testes (1-4, 6-9,12). Nickel sulphate, one of the highly soluble nickel compounds, was told to cause severe tissue degeneration when exposed by inhalation especially on the respiratory system (2,7,8,11).

In our ultrastructural study, we wanted to investigate the effects of orally administered nickel sulphate on the rat testicular seminiferous tubules.

MATERIALS AND METHODS

Sixteen Wistar albino male rats of 200-220 gr. average body weight were used and accomodated in the some room, fed on a regular diet and water ad libitum. One control and three experimental groups were established. Control group rats (n=4) were administered orally 12.5 ml distilled water for 60 days. The rats in the experimental group were given orally $NiSO_4$ at a daily dose of 12.5 ml/kg dissolved in distilled water for 15 days (group I n=4) and 60 days (group II n=4). Group III rats constituted the recovery group and were allowed to survive for 35 days (fed on a regular diet and water ad libitum) following a 60 day- $NiSO_4$ administration (12.5 ml/kg). Group I, group II and group III rats were sacrificed on days 15, 30 and 95 of the experiment.

For transmission electronmicroscopical (TEM) investigation, testes materials from all groups were fixed in 2.5% phosphatebuffered glutaraldehyde and then postfixed in 1 % OsO_4 solution for one hour. The thin sections taken from Vestopal W blocks (400-600 A°) were contrasted with Reynold's uranyl acetate and lead citrate method and evaluated by JEOL 100 C electronmicroscope.

RESULTS

In the control group, ultrastuctural investigations revealed a normal appearance of seminiferous tubules with their germinal epithelium residing on the basal lamina. Spermatocytes, spermatids, spermatozoa and Sertoli cells were reflecting normal ultrastructure (Figs. 1,2).

In group I where the rats were given 12.5 ml/kg $NiSO_4$ for 15 days, a disorder in the germinal epithelial cell alignment and widened intercellular areas were noticed between Sertoli cells, spermatocytes and spermatids (Figs. 3,4,5). Sertoli cells' smooth endoplasmic reticulum membranes were widened (Fig. 3). Spermatozoa localization close to the basal part of the Sertoli cell's cytoplasm was an interesting finding (Fig. 3). Sloughing cells in the tubular lumen were among the interesting ultrastructural findings of that group (Fig. 5).

In group II where the rats were given 12.5 ml/kg $NiSO_4$ for 60 days, an increased cell sloughing was still observed. Desquamated spermatocytes and spermatozoa were observed in the lumen of seminiferous tubules (Figs 6, 7). Widened intercellular areas were observed between spermatogonia and spermatids (fig. 6) and a decrease in spermatocytes (Fig. 6) were interesting. Vacuolated Sertoli cells had widened smooth endoplasmic reticulum membranes (Fig.6).

In group III seminiferous tubules, spermatogonia, spermatocytes and spermatids did not reflect an obvious regeneration process. Widened intercellular areas were still present between spermatocytes, spermatogonia and the Sertoli cells (Fig. 8).

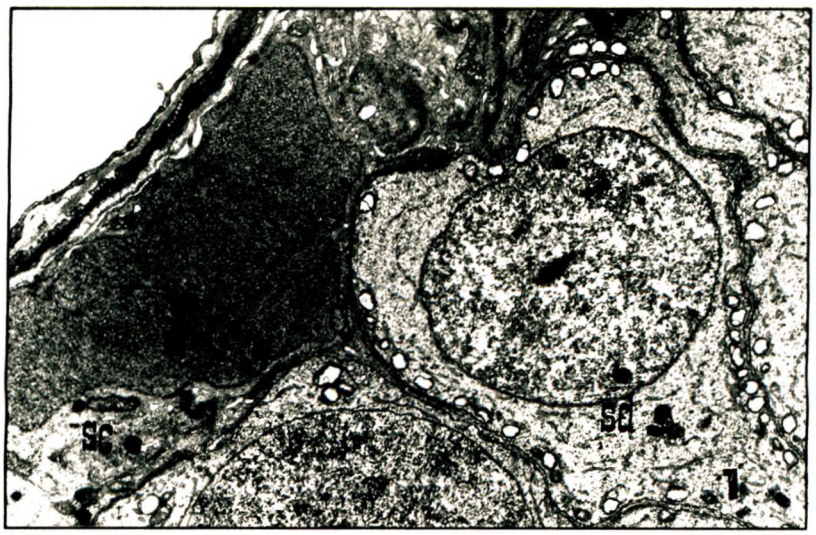

Fig 1. Control group electronmicrograph. sd: spermatid; sc: Sertoli cell. X 6600.

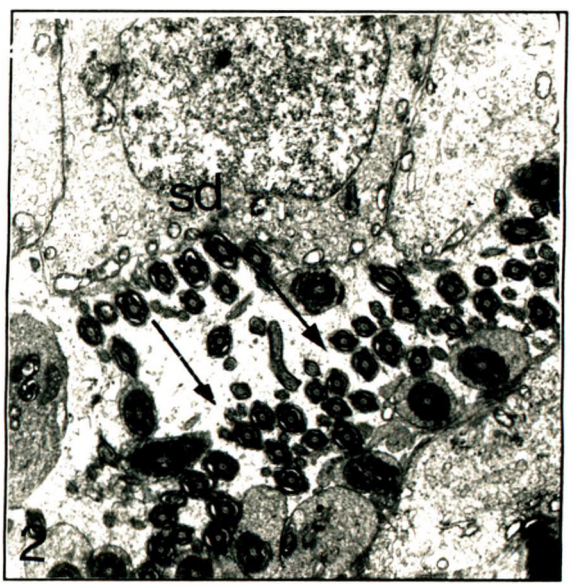

Fig 2. Control group electronmicrograph: sd: spermatid, arrow: longitudinal sections of the spermatozoon tails. X 6600

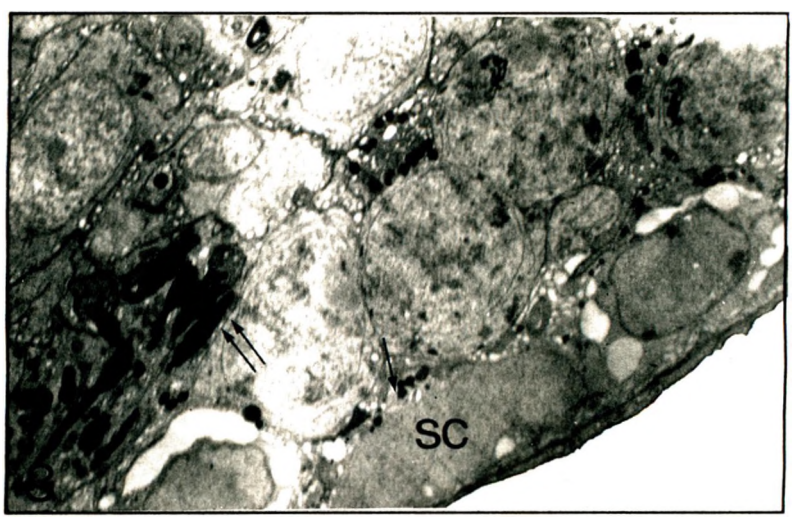

Fig 3. Group I seminiferous tubule ultrastructure: dilated smooth endoplasmic reticulum membranes (arrow) at Sertoli cell (sc). Double arrow: spermatozoon; X 3500.

Fig 4. Group I electronmicrograph: widened intercellular areas (arrow) and dilated smooth endoplasmic reticulum membranes (double arrow) at the Sertoli cell (sc). X 3500.

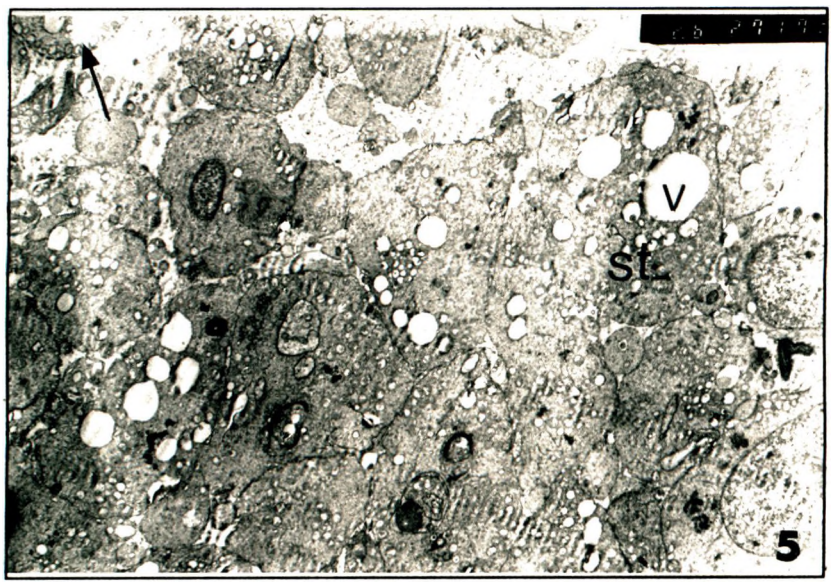

Fig 5. Vacuoles (V) at spermatocytes (st) and spermatids (arrow) on the seminiferous tubule wall of the group I. X 3500.

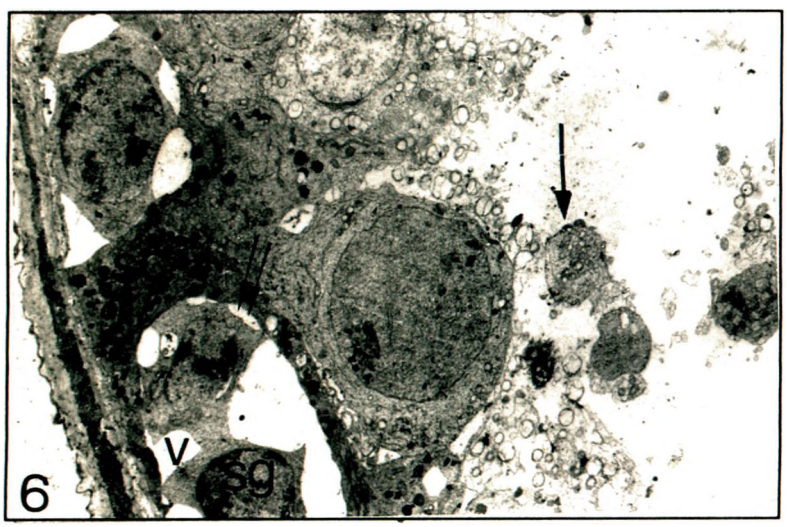

Fig 6. Seminiferous tubule fron the 60th day of the experiment: sloughed epithelial cells (arrow), widened intercellular areas (double arrow) between germinal epithelial cells and dilated smooth endoplasmic reticulum membranes at the Sertoli cell (sc). X 3500.

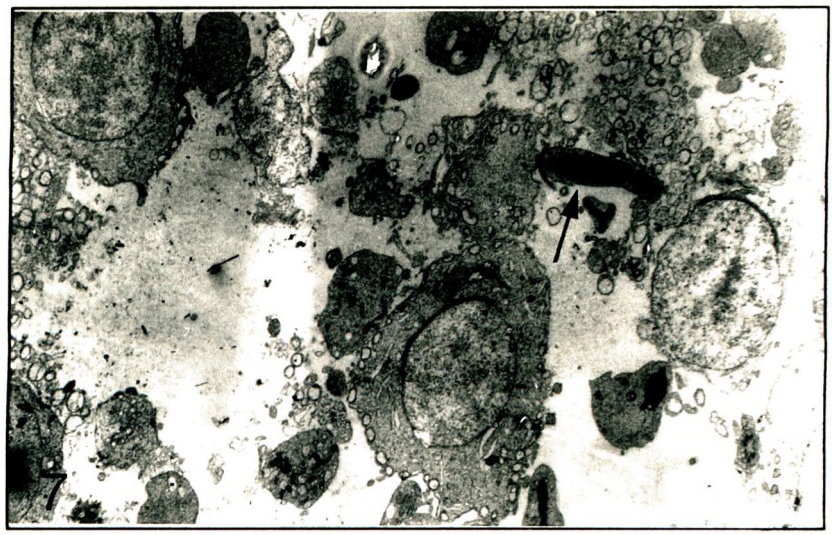

Fig 7. Seminiferous tubules of the IInd group: cellular sloughing and few spermatozoa (arrow) in the tubular lumen. X 3500.

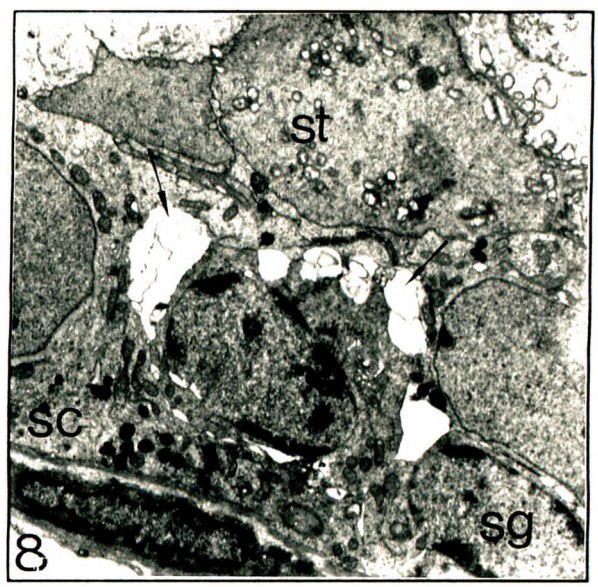

Fig 8. Recovery group electronmicrograph: Large intercellular areas (arrow) were noted. sc: Sertoli cell; sg: spermatogonium; st: spermatocyte. X 6600.

DISCUSSION

In the present study, ultrastructural changes at the rat testes by oral administration of $NiSO_4$ were investigated.

Nickel salts were told to cause a severe damage at the testicular seminiferous tubules together with an obvious delay in spermatogenesis (3, 13-16). Parallel to the mentioned studies, we observed disorganized germinal epithelia of the tubules. Widened intercellular areas between spermatogonia and spermatocytes and extensive cellular sloughing in the tubular lumen were among the observations at days 15th and 60th. Spermatocytes were not extremely affected at the end of 15th day. Decreased number of spermatocytes, as well as a germinal epithelium degeneration still persisted at the end of 60th day.

In literature, early spermatids were encountered in the seminiferous tubules of rats treated with toxic agents and it was regarded as a generalized response to the disorganized seminiferous epithelium (1). Parallel to those mentioned findings, we observed many sloughed cells in the tubular lumens.

Giant spermatids were observed in many studies (1,17-20) and were told as one of the common deformities caused by several types of treatments. They were expected as a result of spermatid degeneration and widening of the intercellular bridges. We could not observe any giant spermatids in our study.

Sertoli cells were usually observed damaged in many related studies (5, 21). We also observed vacuolated Sertoli cells. The vacuolization of the Sertoli cells was also mentioned in a study in which they were told to metabolize many toxic agents such as 1, 3 dinitrobenzene (1). Sertoli cell alterations were declared as a major cause in spermatogenetic delay (1, 21-23). Sertoli cell dysfunction was also monitored as a decline in serum androgen-binding protein (22). But in one study (21), the high phagocytic activity of the Sertoli cells was mentioned and their increased

number of lysosomes were correlated to the Sertoli cell's function in disposing of the altered germ cells.

It was reported that during the metabolism of toxic agents, a reactive intermediate was formed that moved into the germ cells and caused multiple types of degeneration (24). It was thought that those reactive intermediates might cause the formation of atrophic tubules.

In our study, we observed many degenerative changes both in the germ cells and Sertoli cells. Probably, nickel sulphate, as a toxic agent, caused all those ultrastructural changes. Intercellular widenings were among the interesting findings observed in our study. They were correlated with the studies (1, 25, 26) which mentioned that the toxic agents might damage normal cellular association possibly because the damaged Sertoli cells could not resynchronize germ cell development (1).

We could not observe regenerative structures at the end of a 35 day recovery period following the nickel sulphate administration for 60 days. Thus, we concluded that degenerative effects of $NiSO_4$ were irreversible at the end of 60th day where nearly all the stem cells were severely damaged.

As a result, both dose and time-related toxic effects of $NiSO_4$ on the rat testis seminiferous tubules were morphologically identified. Investigations on the recovery group did not reveal an obvious regeneration of the testicular seminiferous tubules.

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