Morphological studies of adrenal cortex and striated muscle in 9-fluoroprednisolone acetate treated golden hamsters (*Cricetus auratus*)

by

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INTRODUCTION

Porcine stress syndrome (PSS) is a condition in domestic pigs characterized by sudden deaths following certain stressful stimuli. Necropsy findings in such pigs are negligible. Certain muscles of such pigs often develop changes within a few hours after death which result in a condition described as "pale soft exudative" (PSE) muscle. These changes are accompanied by a rapid postmortem decline in skeletal muscle pH. Losses, both from the PSS, and from the adverse effects on pork quality in PSE muscle, result in economic losses for pork producers. Lack of criteria for identification of stress susceptible and stress resistant pigs and the relatively high cost of domestic pigs have retarded research in this area.

The studies described in this thesis were based on reports which associated PSS and PSE muscle with degenerative changes in the inner zones of the adrenal cortex. It was suggested that adrenal cortices of such animals might be functionally inadequate during episodes of stress, with this deficiency playing a role in the events leading to death. Exogenous glucocorticoids have been given to pigs to induce adrenocortical hypofunction, in attempts to induce PSS and PSE muscle experimentally.

Interest in the golden hamster as a possible model for study of experimental stress conditions and PSE muscle was

based on the fact that the hamster is unique among laboratory animals, having a lipid-poor adrenal cortex. In this respect, the hamster resembles the ungulates, including the pig.

Therefore, in these experiments 9-fluoroprednisolone acetate was administered via intramuscular injections to golden hamsters in order to induce adrenocortical hypofunction. Investigations were conducted on: 1) postmortem skeletal muscle pH; 2) histopathological features of skeletal and cardiac muscle; and 3) light and electron microscopic features of the adrenal cortex. If these studies had indicated similarities between the experimental condition and PSS and porcine PSE muscle, a more convenient and economical model for the study of PSS and PSE muscle would be attained.

LITERATURE REVIEW

The Porcine Stress Syndrome

The problem of porcine stress syndrome (PSS) has caused a serious economic loss in the pork industry[22, 79]. PSS is usually characterized by pale, soft, exudative (PSE) muscle seen on the carcass within 3-4 hr. after slaughter. This change is accompanied by a rapid muscle glycolysis and decline in muscle pH shortly after death, while the muscle has a temperature close to 37°C[9]. PSE muscle is found mainly in the loin and the ham muscles of short meaty carcasses[87]. PSS may result in dry dark muscle if the duration between stress stimulus and slaughter is relatively long[78]. In the field, PSS is seen as sudden death of mainly rapidly growing pigs, usually after a history of stress[79]. The clinical signs of these pigs may be acidosis, marked dypsnea, alternated blanched and reddened areas on the skin, muscle tremors (especially of the tail), stiffness, hyperthermia, cyanosis and ataxia just before death[79]. Stress stimuli may be transportation, overcrowding, fights, estrus, or extremes in environmental temperature or humidity, sudden severe exercise, or change in housing.

It has been noted that among rapidly growing pigs there are stress-susceptible and stress-resistant individuals. Formerly, it was acceptable to consider pigs which responded to mild stress by muscle tail tremors and overexcitement as

stress-susceptible pigs. Recently, the question of what a stress-susceptible pig is has been raised[78]. This question has not been fully answered yet. Christian concluded that "stress susceptibility is definitely under genetic control but the exact mode of inheritance is unclear"[22]. Some tests and observations have been suggested which might help the antemortem determination of stress susceptibility[22, 39]. It was stated that finding an economical test for stress susceptibility, especially in young pigs, is the present primary concern of the PSS problem[5].

Injection of aldosterone 30 minutes prior to slaughter produced PSE musculature, which was prevented by administration of aldosterone blocking agent (aldactazide)[57]. Further study is required in order to practically consider the implication of this report.

The adrenal cortex of pigs which developed PSE muscle contained a marked accumulation of large fatty masses, especially in the zona reticularis (ZR), which was interpreted as a degenerative process[16]. It was reported that the ZR of about 1/3 of the pigs which developed PSE muscle contained heavy fatty accumulation, while the remaining 2/3 contained only moderate amounts of stainable lipids, and only 2% contained small amounts of stainable lipids[6]. Furthermore, the ZR of about 10% of stress resistant pigs from a genetic background of stress susceptibility, contained marked lipid accumulation[6].

A correlation between high levels of lipid accumulation in the ZR and sharp decline in post slaughter muscle pH of stress-susceptible pigs was reported[16, 38]. Pigs which were reared in fluctuating temperatures or in low relative humidity had high accumulations of lipid droplets in the ZR[38].

Pigs which were injected with 100 mg. prednisolone daily for 10 days and exercised before slaughter had adrenocortical atrophy, increased susceptibility to exercise stress, high plasma lactate and low blood pH[52]. Environmental high humidity or temperature has been reported to cause increased plasma ACTH (adrenocorticotropic hormone) and decreased plasma corticoid levels in Poland China and Chester White pigs[50]. This increase in plasma ACTH levels was interpreted to be due to an increased use of plasma corticoids or to direct stress stimuli[50]. In the case of high humidity condition there was an indication of decreased response of the adrenal cortex to circulating ACTH[50]. It was suggested that plasma corticoids: plasma ACTH ratio should be measured for a more accurate picture of the pituitary-adrenocortex response to stress stimuli[49]. Furthermore, the value of this ratio was suggested as an index for stress susceptibility, where the stress susceptible pigs should have about 1/3-1/4 of the ratio of that of resistant pigs[51].

According to a recently forwarded theory, PSS involves repetitive episodes of stress stimuli which cause higher secretion of ACTH which in turn results in adrenocortical

hyperplasia. Ultimately, stress-susceptible pigs fail to respond or to adapt to the stress condition due to adrenocortical malfunction[6].

Postmortem lactate accumulation in normal porcine muscle was reported to be 0.3 μ moles per gm. per minute at 37°C, while in PSE muscle it was markedly increased with a maximum of 5.0 μ moles[68]. An important factor in this change was reported to be the levels of available adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), and probably AMP diaminase in muscle[68]. Fiber composition of the skeletal muscle was postulated to be an important cause of PSE muscle production in response to stress[23]. The muscle of stress susceptible pigs was reported to have more intermediate fibers and in turn less red fibers[23]. Certain intermediate fibers ("giant fibers") had more amylophosphorylase and ATPase activity[23]. Giant fibers were not found in all stress susceptible pigs, but were absent in stress resistant pigs[23]. Intermediate fibers are dependent on aerobic metabolism; their high ATP breakdown accelerates glycolysis rate, which in turn results in PSE muscle characteristics[23].

A discussion on the beneficial role of corticosteroids in stress conditions has been presented[78]. There, the role of adrenomedullary catecholamines in carbohydrate and fatty acid metabolism was also emphasized. The physiology of

the stress susceptible pig was summarized as follows:

> stress stimuli, especially exercise, results in acidosis; there are not enough corticosteroids to combat the acidosis; corticosteroid insufficiency may be due to adrenocortical insufficiency, but more likely due to higher corticosteroid turnover in stress susceptible pigs; the action of catecholamines is hindered in the low pH environment; the acidosis condition progresses and a state of shock develops.

Lipid and cholesterol accumulation as well as pigmentation in the ZR of the golden hamster was reported to occur in response to ageing[53]. This observation was cited in 2 reports which dealt with PSS[16, 38]. However no correlation between the ZR degenerative process of ageing and that related to stress susceptibility has as yet been suggested.

The Effect of Steroids on Striated Muscle

Two papers citing several references reported that either prolonged and/or high doses of adrenocorticoids produced skeletal myopathies and/or weakness in man and animals[3, 29].

Triamcinolone, as compared to other anti-inflammatory steroids, produced the greatest reduction in body weight and muscle fiber thickness in dogs[29]. In mice, as in dogs and humans, the most pronounced muscle change occurred in the posterior extremities; otherwise, no other histological myopathies were detected[29]. The fluorine radical on the steroid

was incriminated for the above changes in muscle and body weight[29]. Otherwise, the degree of change in mice[29] and in rats[89] depended on dosage, treatment duration, steroid of choice, and amount of exercise. Triamcinolone caused body weight loss in rats[58, 83]. This loss was more severe than in starvation or hydrocortisone treatment[83]. Corticosteroids produced body weight loss also in rabbits[3, 73].

In rats, skeletal muscle weakness was produced by 9 alpha-fluorocortisol and sodium phosphate[25]. The ultrastructural lesions were granular inclusions (calcium-like compounds) in the mitochondria, degeneration of the myofibrils and their replacement by structureless masses, and phagocytosis of the mitochondrial inclusions by macrophages[65]. Another study[89] reported that the ultrastructural lesions were slight or absent. Cortisol or stress conditions aggravated experimental disuse muscular atrophy in rats without producing necrotic lesions[4].

Hydrocortisone, triamcinolone or acute starvation produced in rats, muscle fiber atrophy accompanied by disruption of mitochondrial cristae, especially in the mitochondria-poor fibers (Type II)[83]. Triamcinolone acetonide produced in rat muscle, mitochondrial enlargement and ribosomal inability to incorporate amino acids <u>in vitro</u>[11]. Hydrocortisone affected muscle ribosomes similarly[10]. These 2 responses to the steroid were interpreted as being independent. Anabolic steroids, irrespective of their androgenic potency, counteracted the triam-

cinolone effect on ribosomes and to a lesser extent on mitochondria[10, 12]. Cortisone and denervation increased protein degradation and decreased protein synthesis in muscle of hypophysectomized rats[35]. Cortisone affected the breakdown of both myofibrillar and soluble proteins while denervation caused a catabolic effect in myofibrillar proteins only[35]. Denervation sensitized the soleus muscle to the catabolic effect of cortisone[35]. Apparently either corticoids, starvation or denervation could cause fiber atrophy, and could act synergistically when applied simultaneously.

The following produced glycogen accumulation in skeletal muscle: prednisolone in pigs[49], cortisone in granular and agranular fibers in diaphragms of rabbits[25], triamcinolone in other muscles of rabbits[73], denervation muscular atrophy in cats[40b].

The morphological absence of lysosomes in normal skeletal muscle has been generally known. Canonico and Bird cited several papers which reported on the presence of lysosomes in myopathies such as denervation muscle atrophy, trichinous myositis, vitamin E-deficiency and experimental myopathies[14]. A group of "lysosomes" thought to be of muscle fiber origin had the following characteristics[14]: rich in cathepsin D and acid phosphatases; poor in acid ribonuclease, β-glucuronidase and arylphosphatase acitivity; insensitive to corticosterone, Dextran 500 and Triton WR-1339. These authors supported the idea that normal skeletal muscle lysosomes are part of the

sarcotubular system, and secondary (morphologically identifiable) lysosomes are formed in pathological conditions. Isolated sarcotubular fragments (possibly lysosomes) and mitochondria from rats treated with triamcinolone had no biochemical alterations[58]. These investigators[58] suggested that the effect of the corticoid in the muscle should be sought somewhere else.

Triamcinolone and cortisone produced skeletal myopathies in rabbits[3, 25, 28, 73]. The lesions consisted of severe progressive atrophy, especially of the quadriceps femoris, coagulative necrosis, dystrophic calcification, phagocytosis, thickening of the sarcolemma and absence of an inflammatory response[4, 18]. Moreover, the muscle regenerated after drug withdrawal. The investigators concluded that steroids produce primary myopathy[4,18]. Potassium deficiency produced similar muscle lesions, as did cortisone[27, 28]. The chemical skeletal muscle lesions included accumulation of lipid, water and sodium accumulation and depletion of potassium[27], and these were absent in the myocardium. Adding potassium to the diet failed to reverse cortisone-induced skeletal myopathies[28]. Moreover, the cortisone effect on skeletal muscle was independent of potassium deficiency, vitamin E-deficiency or infection[28]. Cortisone increased the granular fiber diameter in rabbit diaphragms[25]; other lesions were characterized by marked accumulation of fat droplets, clumping of degenerated myofibrils

and appearance of pale staining sarcoplasmic components. Triamcinolone produced a patchy loss of phosphorylases and an increase in mitochondrial activity in the rabbit muscle white fibers[73]. Necrotic fibers of these rabbits contained acid phosphatases. Smith stated that myopathies were produced by the antiepinephrine effect of the glucocorticoid which blocked the activation of phosphorylases[73].

The variety of ways of skeletal muscle reaction to irritants is limited. Steroid-induced myopathies in rabbits arehistogically similar to that produced by vitamin E-deficiency, potassium deficiency and mice Coxsackie virus infection[28]. West and Mason pointed out the value of the golden hamster in studies of muscular dystrophy associated with vitamin E-deficiency[84]. This dystrophy was characterized by a noninflammatory muscular necrosis followed by muscular regeneration after removal of the deficiency[85].

Mouse cardiac muscle was more resistant to the effect of steroids than skeletal muscle[29]. In rats, however, ultrastructural myocardial lesions were described while those of skeletal muscle were negligible or absent[89]. These myocardial lesions were fatty accumulation, mitochondrial disruption and deformation of the myofibrils. Myocardial necrosis in rats which otherwise would be produced by corticoids, stress and excessive sodium, was prevented by amiloride which is a non-steroid potassium-sparing agent[70]. Potassium deficiency

produced myocardial necrosis in rabbits, while cortisone treatment failed to do so[28]. Myocardial lesions were absent in rabbits treated with cortisone or triamcinolone[73]. Adrenocorticoadenoma or carcinoma (and other spontaneous tumors) were accompanied by endocardial alterations, myocardial necrosis, ascites and hydrothorax in the golden hamster[31].

Cortisol increased ribonucleic acid (RNA) synthesis in cultured rat heart cells[71]. The reinitiation and protein synthesis effect of cortisol on cultured heart cells was suggested to be at a cytoplasmic site[71]. Low corticol doses prolonged and high doses decreased the survival of isolated mouse hearts in organ culture[86].

The Adrenal Cortex of the Golden Hamster

The literature (up to the mid 1960's) concerning the adrenal cortex of the Syrian golden hamster has been reviewed[60]. These authors state that although the adrenal cortex of the golden hamster is morphologically similar to that of other common mammalian species, histochemically it is lipid poor and stains negatively for cholesterol. The male adrenal gland weighs more than that of the female, and this is unique among laboratory animal species.

Although the reports concerning the major corticosteroid secreted were conflicting[60], there was most support for hydrocortisone[66]. Cortisol percentage increased after stress[66]. Apparently, blood flow rate altered the cortisol percentages[32].

ACTH increased blood flow through the adrenal gland and ovary in hamsters[80] but only through the adrenal gland in rats. Lowest adrenocortical secretion rate per unit body weight was recorded in the hamster, as compared to other common mammalian species[66]. Blood flow was similar in all species[66]. A lack of storage of precursors or preformed steroids in the adrenal gland was suspected[66]. Hamsters survived only about 2 weeks after bilateral adrenalectomy[75]. Survival of females was generally longer, and progesterone treatment prolonged the survival[75]. Corticoids were essential for survival while addition of 1% of sodium chloride did not maintain the adrenalectomized animals[76].

Histological features of young adult male and female golden hamsters was described[41, 53]. The zona glomerulosa (ZG) lies under a thin connective tissue capsule. The ZG is a thin layer which consists of cell nests, in which the individual cells are faintly basophilic, somewhat elongated and contain large spherical or elliptical nuclei[53]. The presence of fatty droplets in the ZG cells was reported[41]. A thin layer (sometimes inconspicuous) of flattened cells parallel to the capsule separates the ZG and the zona fasciculata (ZF). This layer was described as a transitional layer[41]. The ZF is a thick layer of cells with eosinophilic cytoplasm and deep basophilic spherical nuclei. These cells are spherical and arranged in parallel columns[53]. Few intra- and intercellular vacuoles were

present in the ZF[53], and Knigge[41] reported them absent. The ZR consists of polygonal cells arranged singly or in nests. These are similar to the ZF cells, but some contain shrunken nuclei and vacuoles[53]. Knigge[41] reported sexual dimorphism in the ZR. The female ZR contained smaller and more packed cells with more degenerative processes than the male ZR. Also in the female, a thin layer of connective tissue demarcated the corticomedullary junction. This tissue was absent in the male ZR[41]. The presence of phospholipids was reported in all cortical layers and of liposomes in the ZF and ZR only[55].

With ageing the following increased gradually[53]:

1. vacuolation, especially in the inner cortical area; this is more pronounced in the male.
2. pigmentation in the parenchymal and reticuloendothelial cells, especially in the ZR with radiation outwards but without reaching the ZG or the hyperplastic nodular cells.
3. lipid and cholesterol accumulation, starting in the ZR and radiating outwards.
4. nodular hyperplasia, especially near the capsular area, where the nodules contain either ZG cells only or both ZG and ZF cells.
5. invasion of the cortex by connective tissue elements from the capsule.

Degenerative processes such as the appearance of nuclear

vacuoles, foamy cytoplasm, necrosis, hyperemia, hemorrhages and leukocytosis characterized the most advanced stages of ageing[53]. X-irradiation produced similar adrenocortical changes and it was concluded that irradiation accelerated ageing[64]. The ZR and ZF of hamsters with leishmaniasis[42], spontaneous tumors[31], and implanted mouse sarcoma 180[1] were characterized by lipid and cholesterol accumulation and various other degenerative processes.

Hypophysectomy increased pigmentation and the number of multivacuolated cells (without cellular degeneration) in the inner cortical zones[41]. Also, hypophysectomy increased the number of liposomes and decreased that of mitochondria in the ZF without changing the content of cholesterol[41]. Decreased numbers of mitochondria after hypophysectomy were also seen by the electron microscope[44]. Hypophysectomy also resulted in atrophy of the ZF and ZR but not in the ZG[41]. An electron microscopic study reported that ACTH increased numbers of mitochondria in the ZF and ZR, but to a lesser extent in the ZG[44]. These studies indicated that in hamsters the functions of the ZF and ZR are dependent on the hypophysis while the function of the ZG is much less so. However, this border line is rather simplified.

The adrenal cortex of male cardiomyopathic hamsters (strain BIO 14.6) with mild congestive heart failure was characterized by increase in the thickness and accumulation of lipid in the ZG, accumulation of lipid in the inner cortical

layer but not in the outer ZF (OZF), and increased function of the ZG and decreased that of the ZF and ZR[15]. In severe congestive heart failure, degeneration of the inner cortical zones with pigmentation in the ZR occurred[15].

DOCA (desoxycorticosterone acetate) reduced the thickness of the ZG without producing other histological changes in the cortex[2]. DES (diethystilbesterol) caused increased pigmentation and cholesterol content in the male ZR[2]. Alpert interpreted this as a degenerative effect of DES[2]. ACTH increased the male adrenal weight[2]. Alpert interpreted the response of the adrenal cortex to ACTH in hamsters to be slower than in rats and guinea pigs[50].

The function of the adrenal cortex was not changed by either prepuberal ovariectomy or estradiol[33]. However, ovariectomy reduced adrenal weight, and either estradiol or testosterone reversed this change[33]. Prepuberal orchiectomy reduced adrenal weight as well as the *in vivo* and *in vitro* adrenal secretion[33]. Testosterone stimulated adrenocortical secretion and prevented or reversed the effect of orchiectomy[33]. Testosterone also stimulated *in vivo* and *in vitro* adrenocortical function of ovariectomized hamsters[33]. In contrast to rats, testosterone stimulated the adrenal cortex in hamsters while estradiol had no effect on the gland[33].

Some aspects concerning the ultrastructure of the golden hamster adrenal cortex have been reported[26, 43, 44]. The following will relate to these reports only. Several reports

concerning the ultrastructure of the adrenal cortex in other species have been published. These may have implications on the adrenal gland of the golden hamster and will be discussed in the discussion of experiment 2.

The space between the parenchymal and endothelial cells (subendothelial space) was said to be similar to the space of Disse[43]. Projections of parenchymal cells, and, to a lesser extent, those endothelial cells, were seen in this space[43]. Cells referred to as "light cells" were more common in the ZR[43, 44]. Light cells as compared to dark cells had more expanded cytoplasm[43, 44].

The mitochondria were irregular in shape throughout the cortex[26, 43, 44]. Cortical cells of all layers were rich in mitochondria and poor in liposomes[26]. While one report[26] claimed that there was always a distinct border between the mitochondria and liposomes, Lever[43,44] supported the idea that mitochondria may be a source of lipid droplets. Moreover, intermediate stages between mitochondria and lipid granules were reported and some mitochondria contained osmiophilic material[43, 44]. Normal mitochondria, especially those of the deep cortical zones were described to undergo changes such as flattening, multilamination, loss of normal tubular structure and formation of multichondrial chondriospheres[26]. The mitochondrial cristae of ZG cells were parallel, while those of the IZF and ZR were saccular[44]. The idea of a

possible continuity between the mitochondria and the cytoplasm was supported[44]. A possible interchange between mitochondrial and Golgi figures was also reported[44]. Golgi-like multilaminar mitochondria figures were seen more after ACTH stimulation. Lever suggested that Golgi figures curl and bud in as a possible process of mitochondrigenesis[44].

Other organelles in the cortical cells, such as dense bodies, endoplasmic reticulum and large vacuoles were reported[26]. De Robertis and Sabatini[26] suggested that the large vacuoles were communicating canaliculi to the surface membrane.

EXPERIMENT 1

Materials and Methods

Sixty-seven male Syrian golden hamsters (Cricetus auratus)*, 8 wk. old, weighing 80-100 gm. were used. They were divided randomly into 8 groups of 6-10 hamsters each. Each group was housed in a commercial cage, in which wood shavings were used as litter. Environmental temperature was held at approximately 20°C. The hamsters were fed fresh Wayne Lab-Blox** and fresh green lettuce ad libitum. Treatment groups were identified by ear notching. They were allowed 1 wk. of rest for adjustment to the new environment.

Nine-fluoroprednisolone acetate (Predef 2X)*** was obtained in the form of an aqueous suspension. The drug was further diluted in a sterile saline solution prior to administration.

Administration of the drug was via intramuscular injection. Injections were alternated among pelvic and thoracic limbs in order to minimize trauma.

The hamsters were divided into many experimental groups of 1-2 hamsters per group. For simplicity, groups which reacted similarly to treatment were considered as one group.

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* ENG:ALA (Syr.) strain, Engle's Laboratory Animals, Farmersburg, Indiana.

** Allied Mills, Inc., Chicago, Illinois.

*** Upjohn Company, Kalamazoo, Michigan.

Group I (low dosage group)

Sixteen hamsters (9 wk. old) were injected twice a week with 0.5 ml. of solution, which contained 15 µg. or 30 µg. of Predef 2X for 1.5, 2.5 and 3.5 wk. The hamsters were killed 3, 4, and 7 days after drug withdrawal. These hamsters and controls corresponding to treatment were not weighed. Treatments were given in April, 1971.

Group II (high dosage group)

Twenty-one hamsters (13 wk. old) were injected daily with 0.5 ml. solution which contained 0.4 mg. or 0.8 mg. of Predef 2X for 6, 7 or 10 days, and were killed from 1-4 days after drug withdrawal. These hamsters and controls corresponding to treatment were weighed daily. Treatments were given in late April to early May, 1971.

Group III (high dosage - older group)

Six hamsters (19 wk. old) were injected daily with 0.5 ml. of solution which contained 0.2 mg. of Predef 2X for 10 days. The hamsters were killed after drug withdrawal as follows: 1 at 6 hr., 2 at 1 day, 2 at 4 days, and 1 at 7 days after the last injection. These hamsters and controls corresponding to treatment were weighed daily. Treatments were given during June, 1971.

Group IV (controls)

Twenty-four hamsters (9-19 wk. old) were injected with 0.5 ml. of sterile saline. Age, injection intervals, and the

killing time after cessation of saline injections corresponded to those of the hamsters in Groups I, II, and III.

Tissue preparation and study

Each hamster was euthanized with ether in a closed chamber. Abdominal skin was incised immediately and the left adrenal gland was placed into cold glutaraldehyde (in Millonig's buffer[40a]), in which it was minced immediately. Tissue samples from the left ventricle and from the left longissimus dorsi muscle were treated similarly. The following tissues were fixed in 10% neutral formalin: right adrenal, heart, liver, spleen, and samples from the right longissimus dorsi, left long digital extensor and left masseter muscles. Animals found dead were subjected to the usual necropsy procedures, except that electron microscopic studies were omitted.

The muscle, liver, and tissue representing approximately 1/2 of the length of the spleen, and 1/2 of the right adrenal gland were embedded in paraffin. Histological sections 6 μ thick were prepared and stained with hematoxylin and eosin (H&E). A section representing half the length of the spleen was mounted on a glass slide. The other half of the right adrenal gland was sectioned in a cryostat and stained with oil red O (ORO) in propylene glycol[47].

Tissues from control animals were studied first. Subsequent evaluations were made from slides identified only

by numbers, so that the viewer did not know the treatment used. Lipid accumulation in the adrenal cortex stained with ORO was graded as follows for each layer of the cortex: 0 = none or negligible (or lipid poor), 1 = slight, 2 = moderate, 3 = marked. The length of spleen represented in each section was estimated and graded as follows: 1 < 0.5 cm., 2 = 0.5-1.0 cm., 3 > 1.0 cm. The number of lymphatic follicles in each section of spleen was termed low if it was 8 or less, and high if it was 9 or more.

After fixation in glutaraldehyde for 1 hr., tissues destined for examination by electron microscopy were washed in cold Millonig's phosphate buffer for at least 1/2 hr.[40a]. They were then fixed and stained in cold 1% osmium tetroxide[40a] for 1 hr. before being dehydrated through graded alcohols and propylene oxide and embedded in Epon[40a].

Adrenal cortices of 6 hamsters (2 controls, 4 treated - Group II) were studied. The status of the 4 treated animals was as follows:

1 received 10 daily injections of 0.4 mg. of Predef 2X and was killed 1 day after withdrawal of the drug;

1 received 6 daily injections of 0.4 mg. of Predef 2X and was killed 2 days after withdrawal;

1 received 7 daily injections of 0.8 mg. of Predef 2X and was killed 4 days after withdrawal;

1 received 10 daily injections of 0.4 mg. of Predef 2X and was killed 4 days after withdrawal.

A thick section of tissue from the block was cut with a razor blade and mounted on glass. This was examined under the light microscope for orientation. Tissue was termed ZG and OZF if the capsule was seen, ZR and IZF if the medulla was seen, and ZF if neither the capsule nor the medulla were seen. The block was trimmed and faced. An LKB ultramicrotome and glass knives were used to obtain ultrathin sections (gray-silver). These were mounted on 100 x 400 mesh copper grids and double stained with 8% uranyl acetate in methanol and Reynold's lead citrate[40a]. Sections were examined using an HS-6 or HU-11A electron microscope.

Results

General observations

A few hamsters were depressed upon arrival but recovered by the next day.

Control hamsters remained healthy throughout the experiment. No noticeable changes were seen throughout the experiment in animals of Group I (given 15-30 µg. Predef 2X). Hamsters in Group II (given 0.4-0.8 mg. Predef 2X) and in Group III (given 0.8 mg. Predef 2X) were depressed and weak by the 4th day following daily injections. Other clinical signs were: rough hair coat, photophobia, conjunctivitis, anorexia, and in severe cases ataxia and death. Seven of the 23 hamsters in Group II (given 0.4-0.8 mg. Predef 2X) were found dead. No animal died, however, in Group III

(given 0.2 mg. Predef 2X). The hamster killed at 6 hr. after withdrawal was moribund.

Signs of recovery were not apparent by day 4 after drug withdrawal in Group II (given 0.4-0.8 mg. Predef 2X). However, recovery signs were apparent in Group III (given 0.2 mg. Predef 2X) on day 4 after withdrawal and thereafter, as evidenced by improvement in hair and general appearance.

Fatty changes in the liver with or without multifocal abscesses were a common finding in animals of Groups II and III. A narrow, pale streak of myocardial necrosis was seen in hamsters of Groups II and III. Hydrothorax and/or ascites were less consistent. Gastroenteritis was rather common in dead animals of Group II.

Body weight (Data summarized in Tables 1 and 2 on pages 34 and 35.)

In controls, only those hamsters corresponding to Groups II and III were weighed and these gained weight (Tables 1 and 2). Body weight data were not obtained in Group I or on controls corresponding to Group I. Visual assessment indicated no marked body weight difference between the treated hamsters (Group I) and controls. Hamsters in Group II (given 0.4-0.8 mg. Predef 2X) and in Group III (given 0.2 mg. Predef 2X) lost about 20% of their body weight (Tables 1 and 2). Moreover, the difference between treated and control hamsters was obvious.

Histopathological features of skeletal muscle

No change was noticed in Group I (given 15-30 µg. Predef 2X) as compared to controls. In Group II, mild loss of striation and pale cytoplasm were occasionally seen.

In most animals of Group III various stages of muscle necrosis were seen, varying from loss of striation to mineralization. This was most marked and consistent in the *longissimus dorsi* muscle.

Histopathological features of cardiac muscle

No changes were seen in Group I as compared to control hamsters. Multifocal necrosis with infiltration of polymorphonuclear (PMN) white blood cells was seen in Group III. Cytoplasm of remnant muscle cells was often foamy. These pathological changes were more marked or consistent in animals which received higher doses or extended periods of treatment than in low dose, or briefly treated hamsters.

The adrenal cortex

H&E stain The histological features of all control hamsters were similar to those described for corresponding ages by Meyers and Charipper[53]. Some clear vacuoles were seen in the ZG of a few adrenal cortices, and these were interpreted as possible lipid vacuoles. There was no major change in Group I.

Degenerative processes were seen in cortical layers of Group II and Group III. These were mainly clear vacuoles,

most of which were in the ZG, IZF, and ZR. These were interpreted as possible lipid droplets. Nuclear hyperchromasia, cytoplasmic hypereosinophilia, and some necrotic cells were seen. Autolysis was the change most often seen in adrenal cortices from hamsters which were found dead.

ORO stain Cortical layers of control hamsters were generally lipid-poor. Very few droplets were seen in the ZG, and fewer still in the other cortical layers. Those present tended to be concentrated in a few focal sites. No major change was seen in Group I as compared to the controls. Moderate multifocal lipid accumulation was seen, especially in adrenal cortices of animals given high doses or longer periods of treatment.

Moderate to marked diffuse lipid accumulation throughout the ZG, IZF, and ZR was seen in Group II. In the OZF, lipid accumulation was less marked. Lipid accumulation in cortices of animals found dead was generally more marked than in killed animals. The least marked accumulation in this group was seen in adrenals of animals on shorter treatment schedules.

Marked lipid accumulation was seen in the OZF as well as in the IZF of Group III. Slight to moderate accumulation was seen in the ZG and was variable in the ZR of this group.

Ultrastructure of control hamsters Generally, all cortical layers of controls contained lipid-poor, mitochondria-rich cells. Some ZG cells contained a few vacuoles containing

osmiophilic material. They were interpreted as lipid droplets. Such droplets were rarely seen in the ZR and were almost completely absent in the ZF.

Profiles of mitochondria in the ZG were generally spheroidal, but some were slightly elongated. All contained sparse, parallel, leaf-like cristae. The ER was usually smooth, and saccular rather than tubular. However, this shape might be a result of improper fixation. Membrane bound dense bodies were seen occasionally. Golgi figures were usually juxtanuclear.

The ZF mitochondria were spheroidal, elongated or very irregular in shape. Both lamellar and tubular cristae were seen, sometimes in parallel array, more often arranged randomly. Several mitochondria contained amorphous, electron dense material. The amount of this material in the mitochondrial matrix varied. Sometimes a mitochondrion surrounded cytoplasmic structures such as ER saccules, ER tubules or vacuolated areas. Several mitochondria were surrounded by smooth multilaminar membranous figures.

ER in the ZF was usually smooth, and more saccular than tubular. However, this might be caused by improper fixation. ER cisternae varied in electron density. Some were completely transparent and resembled shallow vacuoles. The juxtanuclear ER was more saccular and its cisternae were wider than those of the rest of the cell. Golgi figures were occasionally

seen and were usually juxtanuclear. Ribosomes were dispersed abundantly in the cytoplasm.

Cellular content of the cells of the ZR was similar to that of cells in the ZF.

Ultrastructure of treated hamsters Within 24 to 48 hr. after drug withdrawal, heavy lipid accumulation was seen, characterized by several intracellular lipid droplets per cell in the cortical layers. Electron transparency of lipid droplets varied. Some contained remnants of membranous figures. Some droplets impinged on mitochondria and on other organelles.

Several cells contained electron transparent vacuoles (cavities) which were usually lined with few to several parallel and closely aligned membranes. Several vacuoles were divided by membranous structures which occasionally appeared to be continued with lining membranes. Cavities impinged on adjacent mitochondria.

No major changes were seen in mitochondria. However, mitochondrial figures presumably in different stages of dissolution were more common in treated ZF cells than those of controls.

Whorled membranes were seen more frequently in ZF and ZR cells than in controls. Electron dense structures resembling myelin figures were occasionally seen.

Cellular destruction, characterized by loss of cytoplasmic organization, was occasionally seen. About 1/4 of

the cells examined throughout the cortical layers were unaffected by Predef 2X treatment.

On day 4 after drug withdrawal, signs indicative of cellular recovery became apparent. Prominant among such signs was a decrease in number of lipid droplets. These droplets were usually smaller and more electron dense than those seen on day 1 and 2. Increase in mitochondrial numbers was marked. Cellular cavities were still common. Generally, parenchymal cells similar to those seen in controls were more common, and degenerate cells were fewer, than 1 and 2 days after drug withdrawal.

Histopathological features in the spleen

Splenic length of controls was usually graded as 3 (more than 2 cm. *in vivo*). Sections usually contained 9-12 lymphatic follicles. In Group I (given 15-30 μg. Predef 2X), length of 6 spleens was graded 2 (1-2 cm. *in vivo*) and 1 spleen was graded 1 (less than 1 cm. *in vivo*). The decrease in the number of lymphatic follicles per section was very slight in Group I. Follicular diameter was visually assessed and compared to controls. A slight decrease in diameter was observed.

Of 22 spleens examined in Group II (given 0.4-0.8 mg. Predef 2X), 7 were graded 2 and 7 were graded 1. The decrease in numbers of lymphatic follicles and their diameter was marked in this group.

Number and diameter of lymphatic follicles of all 6 spleens were decreased in Group III (given 0.2 mg. Predef 2X). However, only 2 spleens were graded 2 and none graded 1.

Histopathological features of the liver

Histologically, the liver of control hamsters was divided into hexagonal lobules with a central venule. Sometimes up to 3/4 of the periphery of the lobule contained cells with cytoplasm which had a rarified appearance and clear vacuoles. These vacuoles may be fat droplets. No change was seen in Group I.

Multifocal necrosis was the most prominent hepatic change in Group II. Necrotic areas contained neutrophils, macrophages, and cellular debris. Sometimes, interlobular connective tissue was increased. However, the above changes were absent in about 1/3 of livers examined. In Group III multifocal necrosis was more marked and consistent than in Group II.

Discussion

Results of this pilot experiment provided preliminary information for this thesis. High doses of 9-fluoroprednisolone (0.2-0.8 mg. daily for 6-10 days) were toxic while low doses (15-30 μg. every 3-4 days for 2 wk.) were not. Toxic doses caused death in 7 out of 29 hamsters treated. Other signs of toxicity were weight loss, rough hair coat, photophobia, depression, multifocal liver necrosis, atrophy

of the spleen or its lymphatic follicles, myocardial necrosis and accumulation of lipid in the adrenal cortex. Apparently, clinical recovery from toxicity starts about 4 days after drug withdrawal. It was decided that a dose of 0.4 mg. of Predef 2X daily for a period between 6 and 10 days would be used in experiment 2. It was also learned that weight and clinical signs of the hamster should be closely followed during treatment.

In this experiment, a highly potent glucocorticoid (9-fluoroprednisolone acetate) was chosen for treatment. Data of a study not presented in this thesis indicated that postmortem muscle color, pH, and expressible moisture in Group I were not significantly different from those in control hamsters corresponding to treatment. In porcine PSE muscle there is a decrease in postmortem muscle pH, an increase in expressible moisture, and paleness. Results of these parameters in hamsters were discouraging as far as the future use of the young-adult male golden hamster as a laboratory model for PSS and porcine PSE muscle. However, studies were continued and refined in experiment 2, in order to allow a more definite conclusion.

Toxic doses induced myocardial necrosis (Groups II and III) while low doses (Group I) did not. In this respect, the hamster is apparently similar to the rat, but not to rabbit and mouse.

The effect of Predef 2X on skeletal muscle was variable. No major change occurred in Group II, while in Group III necrosis was observed. In Group III, hamsters were older than those in Group II (19 vs. 13 wk.). Also, animals in Group III lacked exercise and appeared obese. Average body weight was 146 ± 10 gm. vs. 104 ± 24 gm. for the same age group reported by Meyers and Charipper[53]. It is possible that older age, lack of exercise, obesity or some combination of these factors aggravated the steroid effect in Group III. Necrosis was found most often in the longissimus dorsi muscle. Muscle lesions induced by this treatment were similar to those induced in rabbits by steroids[3, 25, 28, 73].

The longissimus dorsi muscle was examined in experiment 2, in order to varify the above observations. If skeletal muscle of the hamster is also susceptible to steroid-induced myopathy, as cardiac muscle is, then the hamster would be valuable for comparative studies of steroid-induced myopathies of striated muscle. No reports of cases where steroids induced muscular necrosis in both heart and skeletal muscle in the same laboratory animal species could be found.

The most pronounced adrenocortical change in Groups II and III was lipid accumulation. In Group II there was a difference between OZF and IZF, while in Group III there was not. The ultrastructural study was far from conclusive, due to the limited number of animals per group and tissue samples

per animal. Also, accuracy of differentiation between the OZF and IZF was poor. In experiment 2, more animals per group and more tissue samples per animal were studied with the electron microscope. Also, a technique was developed by which one could differentiate OZF from IZF. This helped to establish whether there is a difference between the OZF and IZF.

It is difficult to determine how many animals per group, blocks per specific tissue, grids per block, and electron-micrographs per grid should be studied ultrastructurally, in order to obtain adequate representation of a given tissue. This sampling problem is particularly difficult when dealing with a heterogeneous tissue, and even more so in pathologically altered tissue. This sampling problem has not been solved by statistical methods (Professor D. F. Cox, Department of Statistics, I. S. U., Personal Communication). Where possible, 3 animals per group, 3 blocks per adrenocortical layer, and 3 grids per block were studied as the minimum.

Table I. The effect of 9-fluoroprednisolone acetate on the body weight of hamsters (in gm.) Experiment 1, Group II and Corresponding Controls

Date	Saline	0.4 mg. Predef 2X/injection	0.8 mg. Predef 2X/injection
4/22	116.5 (11)[a]	116.5 (12)	114.0 (11)
4/27[b]	120.5 (11)	120.5 (12)	117.0 (11)
4/28	122.5 (11)	116.0 (12)	113.5 (11)
4/29	120.0 (11)	113.5 (12)	111.5 (11)
4/30	123.0 (11)	114.5 (12)	108.0 (11)
5/1	120.5 (11)	110.5 (12)	108.0 (11)
5/2	122.5 (11)	105.5 (12)	102.0 (11)
5/3	124.0 (11)	102.5 (12)	98.0 (11)
5/4	126.0 (11)	102.5 (11)	99.0 (10)
5/5	126.0 (9)	101.0 (9)	97.0 (7)
5/6	123.0 (9)	99.5 (8)	98.5 (6)
5/7	123.5 (9)	98.0 (8)	96.5 (3)
5/8	129.5 (4)	103.2 (5)	108.5 (3)

[a] Number in parentheses = number of hamsters/mean value.

[b] 4/27 = injections started and continued daily 6-10 days.

Table II. The effect of 9-fluoroprednisolone acetate on the body weight of hamsters (in gm.) Experiment 1, Group III and Corresponding Controls

Date	Saline	0.2 mg. Predef 2X/injection
5/16	137.0 (9)[a]	137.0 (9)
6/10[b]	142.5 (6)	154.0 (3)
6/11	138.5 (6)	154.0 (3)
6/12	136.0 (6)	151.0 (3)
6/13	135.5 (6)	151.5 (3)
6/14	130.0 (6)	151.5 (3)
6/15	130.0 (6)	152.5 (3)
6/16	129.5 (6)	156.5 (3)
6/17	130.0 (6)	153.0 (3)
6/18	127.5 (6)	152.5 (3)
6/19[c]	110.5 (6)	153.5 (3)
6/20	110.0 (5)	154.5 (3)
6/21	112.5 (3)	154.5 (3)
6/22	112.5 (3)	154.0 (2)
6/23	113.5 (3)	153.5 (2)
6/24	113.0 (1)	158.0 (2)
6/25	115.0 (1)	160.0 (1)
6/26	111.0 (1)	158.0 (1)

[a] Number in parentheses = number of hamsters/mean value.

[b] 6/10 = injections started and continued daily.

[c] 6/19 = last injection was given.

EXPERIMENT 2

Materials and Methods

Forty male, Syrian golden hamsters (Cricetus auratus)*, 12 wk. old were divided randomly into 6 groups (6-7 hamsters/group). The hamsters were identified individually by ear notching. Each group was housed in a commercial cage in which wood shavings were used as litter. Room temperature was influenced by fluctuating outdoor temperature (Ames, Iowa, August, 1972). Hamsters were fed fresh Wayne Lab-Blox** and green lettuce ad libitum. They were kept in their surroundings for 1 wk. before experiments were started, in order to allow them to adjust to their environment.

During the week preceding treatment, hamsters were weighed every other day. Average body weight one day before treatment started was 102.0 gm. ± 7.5 gm. (T_{95} distribution[74], with a median = 101.6 gm.). Body weight was recorded daily during treatment. Treatment and weighing were done at about 4:00 P.M. each day.

Thirty-one hamsters were treated and 9 served as controls. Treatment included 8 daily intramuscular injections of 0.5 ml. of 0.4 mg. 9-fluoroprednisolone acetate (Predef 2X)***.

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* ENG:ALA (Syr.) strain, Engle's Laboratory Animals, Farmersburg, Indiana.

** Allied Mills, Inc., Chicago, Illinois.

*** Upjohn Company, Kalamazoo, Michigan. The drug was obtained in aqueous suspension and was further diluted in a sterile saline solution prior to administration.

Injections were alternated among thoracic and pelvic limbs in order to minimize trauma. Control hamsters received 8 injections of 0.5 ml. of sterile saline solution similar to the regime used for experimental hamsters.

Twelve hamsters died during treatment. Nineteen were killed after drug withdrawal in groups of 2-3 hamsters per group, as follows: 3 in 2 hr., 3 in 8 hr., 3 on day 1 (24 hr.), 3 on day 2, 2 on day 3, 2 on day 4, and 3 on day 7. Control hamsters were killed after cessation of saline injections as follows: 3 on day 1, 3 on day 4, and 3 on day 7.

Tissue preparation and study

Each hamster was killed with ether in a closed chamber. Abdominal skin was incised quickly, and the left adrenal gland was placed into cold, buffered glutaraldehyde[40a] and immediately incised into halves. A special effort was made to perform the last 2 stages as quickly as possible, in order to minimize adrenal capillary collapse. Tissue samples from the left ventricle and left *longissimus dorsi* muscle were placed into cold gluteraldehyde, where they were minced immediately. Skeletal muscle was minced, as nearly as possible, along lines parallel to the long axis of muscle fibers, so that small rectangular pieces of tissue resulted. The right adrenal, heart, and a sample from right *longissimus dorsi* muscle were then fixed in 10% neutral formalin. Animals found

dead were subjected to the usual necropsy procedures except that electron microscopic studies were omitted.

Carcasses of killed animals were refrigerated at 4°C for 18-24 hr. Carcasses of animals which were found dead were refrigerated at 4°C for 18-24 hr. minus the roughly estimated time between death and refrigeration.

The right adrenal was divided into halves. One half of this adrenal gland, selected skeletal muscle and heart were embedded in paraffin blocks. Histological sections of 6 μ thickness were prepared and stained with routine hematoxylin and eosin (H&E). The other half of the adrenal was sectioned in a cryostat, where 12 μ thick sections were obtained. These were stained with oil red O (ORO) in propylene glycol[47].

Light microscopy evaluations were made from slides identified only with numbers, so that the viewer did not know the treatment. Grading was used for statistical analysis*. For skeletal muscle, 0 = normal, 1 = slight, occasional eosinophilia and loss of striations, 2 = moderate necrosis, and 3 = severe focal or multifocal necrosis with or without mineralization. For severity of skeletal muscle edema, 0 = none or normal, 1 = slight, 2 = moderate, and 3 = marked edema. For cardiac muscle, 0 = normal, 1 =

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* The aid of Dr. David F. Cox, Professor of Statistics, Iowa State University, Ames, Iowa, is acknowledged.

irregular striations, 2 = slight to moderate necrosis, and 3 = severe focal or multifocal necrosis with or without hemorrhage. For adrenal cortices, in each of the 4 layers (ZG, OZF, IZF, and ZR) several characteristics were graded as 0 = none or negligible, 1 = slight, 2 = moderate, and 3 = marked; these included: number of cavitated cells (with H&E stain), number of dark cells (cells with hyper-eosinophilic cytoplasm and hyperchromic nuclei, with H&E stain), and amount of lipid accumulation (with ORO stain). Adrenocortical hyperemia was graded 1 if present and 0 if absent. For part of the statistical study all animals were divided into 3 groups: 1) controls, 2) treated and killed, and 3) treated and found dead. Using these groups, analysis of variance was applied to body weight and to post-mortem skeletal muscle pH. Chi-square analysis was applied to the lesion grades.

Means of values obtained on the various treatments, on animals found dead on a certain day, and on control animals killed on a certain day, were subjected to graphical analysis. In this way, body weight, postmortem muscle pH, severity of skeletal and cardiac lesions, number of cavitated cells and amount of lipid accumulation in the various layers of the adrenal cortex were plotted against days of the experiment (figures 1, 2, 3, 12, and 13). Body weights of individual hamsters were adjusted to their mean group for simplified graphical analysis.

For ultrastructural study, adrenal gland halves and minced cardiac and skeletal muscles were fixed in glutaraldehyde for 1 hr., and washed in Millonig's phosphate buffer for at least 1/2 hr.[40a].

While immersed in cold buffer, adrenal halves were handled under the dissecting microscope as follows: a 1 mm. thick slide from each half was cut by a sharp razor blade; each slice was divided into 6-10 triangular pieces in which the adrenal capsule was at the curved (wide) end and medullary tissue was at the pointed end of each piece; similar triangular pieces were obtained from remaining adrenal tissue.

All tissues were post-fixed in 1% osmium tetroxide for 1 hr. before dehydration in graded alcohols and propylene oxide[40a].

Heart tissue was embedded in Epon[40a] blocks. Adrenal cortices and skeletal muscle tissues were embedded in Epon[40a] plates so that adrenal orientation and orientation of muscle fibers were visible.

Ultrastructure of 8 adrenal cortices were studied: 3 from control hamsters (1 killed on day 1, 1 on day 4, and 1 on day 7 after saline withdrawal) and 5 from treated hamsters (3 killed on day 1, and 2 on day 4 after drug withdrawal). Pieces of Epon plate which included adrenocortical tissue were glued onto dummy blocks. Thick sections (0.5-1.0 μ) were cut by an LKB ultramicrotome and examined under a light microscope until the whole cortex, from the capsule

to the medulla was visible. Then, the block was trimmed and 1 μ sections were collected for toludine blue (TB) staining. These sections were studied under the light microscope for quality of fixation. The parameters of satisfactory fixation were well-opened capillaries, integrity of capillary linings and adequate staining of cellular components. Only blocks with proper fixation were considered for further study. Exact topographical orientation, lipid accumulation, cellular cavitation and presence of other components such as phagocytes, white blood cells (WBC), necrotic cells, and other factors were noted. Two to 3 blocks from each control hamster and 3 blocks from each treated hamster were examined.

Ultrathin sections (gray-silver) were cut by the LKB ultramicrotome using glass knives. These were mounted on 200 mesh, parallel lines, copper Pelco grids* and double stained with 8% uranyl acetate in methanol and Reynold's lead citrate[40a]. Two to 4 grids from each block were prepared and examined under an HU-11A electron microscope. By keeping in mind the appearance of the tissue studied from TB stained thick sections, it was possible to have a fairly accurate topographic orientation under low magnification (about 3000X).

Postmortem skeletal muscle pH

Five gm. of epaxial and hind quarters muscles were removed from the foregoing carcasses which had been cooled.

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* Ted Pella Company, Tustin, California.

Then muscles were suspended in 50.0 ml. deionized H_2O. The mixture was galvanized for 20 sec., and the pH of galvanized suspension was measured.

Results

Treated hamsters became depressed and weak after 4 days of daily treatment. Other clinical signs were: rough hair coat, photophobia, conjunctivitis, anorexia, and in severe cases ataxia and death.

Twelve of 31 treated hamsters died: 1 after 6 days and 1 after 7 days of treatment, and 3 on the 1st day, 5 on the 2nd day, and 2 on the 3rd day after last treatment.

Recovery from treatment was seen in 2 of 5 hamsters on day 4 and in all hamsters by day 7 after drug withdrawal. Recovery signs were improvement in appearance of hair coat, increased alertness, and cessation of the daily weight loss caused by treatment.

At necropsy, fatty changes in the liver and whitish streaks within the myocardium were found in most treated animals. Gastroenteritis and ascites and hydrothorax were seen in most hamsters found dead.

Body weight (Data summarized and presented graphically in figure 1, page 72)

Control hamsters gained normally during the experiment. The first injection of Predef 2X resulted in a weight loss of 3-4 gm. per animal, as measured on the following day; the 2nd and 3rd injections did not affect weight of the hamsters.

The remaining 5 injections were followed by losses of 3-7 gm. per hamster each day in hamsters which subsequently died, and by losses of 1.5-3 gm. per hamster each day (until 3 days after drug withdrawal) in hamsters which were killed. Three days after drug withdrawal, treated hamsters either maintained body weight or gained about 1 gm./day/individual.

Postmortem skeletal muscle pH (Data summarized and presented graphically in figure 2, page 74)

Mean skeletal muscle pH 18-24 hr. postmortem was 6.34 in 9 control hamsters, 6.78 in 19 treated and killed hamsters, and 7.15 in 12 treated hamsters that were found dead. The mean of 3 treated hamsters killed on day 7 after drug withdrawal was 6.36, which indicated recovery.

Histopathologic features in skeletal muscle (Data summarized and presented graphically in figure 3, page 76)

Lesions were seen in treated hamsters in the form of necrosis, loss of striations, increased cytoplasmic eosinophilia in some fibers and pale cytoplasm in others, increased intercellular space, rowing of nuclei, infiltration of macrophages, and mineralization (figures 4-8). No lesions were seen in controls.

No edema was found in skeletal muscle of 9 control hamsters. Three of 19 treated and killed hamsters had slight edema while 4 of 12 treated hamsters found dead had slight edema, 3 moderate, and 1 severe. Since histological examination is a relatively poor method for detecting all but

pronounced edema, these results gave no more than a rough indication of severity of these findings.

Histopathologic features in the cardiac muscle (Data summarized and presented graphically in figure 3, page 76)

Predef 2X treatment caused focal and multifocal areas of myocardial necrosis which were characterized by fiber death, infiltration with polymorphonuclear leukocytes (PMN), phagocytes, and accumulation of cellular debris (figures 9-11). Sometimes, hemorrhages were seen in necrotic areas (figure 10). Papillary muscle was most affected. Ventricular wall and interventricular septum also contained lesions. Atrial walls contained pathologic alterations of a lesser extent. Severity of lesions was more marked in hearts of treated hamsters which died during the investigation than in those which survived until they were killed (figure 3). No lesions were seen in controls.

The adrenal cortex

Light microscopic features of control and treated hamsters (Data summarized and presented graphically in figures 12 and 13 on pages 86 and 87.) The zona glomerulosa (ZG) was covered by a thin, fibrous connective tissue capsule. Usually this zone was 4-5 cells thick (figures 14 and 22). ZG cells were usually elongated and arranged in nests (figure 21), separated by fine trabeculae of fine connective tissue. Wide capillaries were seen in or adjacent to trabeculae (figure 22), and within groups of parenchymal cells.

The cytoplasm of ZG cells was faintly basophilic (figure 14). Nuclei were usually elliptical, but sometimes spherical. With TB the cytoplasm of ZG cells stained faintly blue and the nuclei dark blue (figures 20-23). Several small, light-blue vesicles were scattered throughout the cytoplasm (figure 22). These were interpreted as mitochondria. In some ZG cells, 1-3 large yellow-green droplets were seen in the cytoplasm (figure 22). These were interpreted as lipid droplets, as they were easily distinguishable by oil red O (ORO), contrasting with the generally lipid-poor character of the ZG (figure 16).

A transitional layer was not detected by either H&E stain or ORO stain. With TB, a transitional layer 1-3 cells thick could be observed (figure 22). These cells were immediately under the ZG and parallel to the capsule. Transitional cells stained darker blue than the ZG cells but lighter than cells of the outer zona fasciculata (OZF) which were immediately under the transitional cells. Mitochondria of cells in the transitional layer were more obvious than those in the ZG cells, but less than those in OZF cells (figure 22). Lipid droplets were not seen in the transitional layer.

Predef 2X treatment caused a marked accumulation of lipid in the ZG (figures 17-19). It was more consistent in treated hamsters that were found dead than in treated and killed animals (figure 12). Marked accumulation of lipid droplets

was seen on TB stain sections. Lipid droplets were usually relatively large (up to 5-6 μ in diameter). Clear intracellular vacuoles were seen with the H&E stain, which were suspected as lipid droplets. Lipid accumulation decreased after the 4th day following drug withdrawal (figure 12). Dark cells (cells with hypereosinophilic cytoplasm and hyperchromic nuclei) and vacuolated cells were not seen in this layer (figure 13).

In control hamsters, the outer zona fasciculata (OZF) was not distinguishable from the inner zona fasciculata (IZF) by H&E or ORO stains. However, in TB stained 0.5-1.0 μ thick sections, the OZF was distinguishable topographically as a separate layer (figures 20 and 21). It was about 10-15 cells thick and occupied the outer 1/3 of what is usually considered the zona fasciculata (ZF) layer (figure 20). Instead of radiating cords, the cells of this layer were adjacent without a specific pattern (figures 20 and 21). Cells were spherical to polygonal in shape. Their cytoplasm stained intensely with TB. Mitochondria filled most of the cytoplasm (figure 22). Cavitated cells were rare and intracellular lipid droplets were absent in this layer. Examination of the outer 1/3 of the ZF layer by H&E revealed spheroidal parenchymal cells with eosinophilic cytoplasm, spherical basophilic nuclei and no cell cavitations (figure 14). The outer 1/3 of the ZF was lipid-poor, as seen by ORO stain (figure 16).

In treated and killed hamsters, Predef 2X treatment caused moderate accumulation of lipid in the OZF. Lipid accumulation reached its peak 8 hr. after drug withdrawal (figure 12), was slight 2 days and almost negligible 7 days after drug withdrawal. Lipid accumulation in the OZF was less marked than in other adrenocortical layers of treated hamsters (figure 12). The OZF recovered more rapidly than other adrenocortical layers from Predef 2X treatments (figure 12). Lipid accumulation was detected on TB stained sections as well as after ORO. Lipid droplets were smaller (1-2 μ diameter) than those seen in the ZG (5-6 μ diameter) (figures 18, 19 and 26).

In both killed hamsters and those found dead, cellular cavitation resulting from Predef 2X was slight, as seen by H&E (figure 13). No effect on number of dark cells in the OZF could be detected.

On TB stained sections the IZF (15-25 cells deep) occupied about 2/3 of the total ZF (figures 20 and 21). Cords of this layer were arranged in columns 1-2 cells in width. Cells were spheroidal or polygonal and their nuclei were spherical. There were several cavitated cells in this layer (figure 23). Mitochondria were abundant; they appeared as small, light-blue staining vesicles (figure 23). Lipid droplets were small (about 2 μ in diameter) and rare. In H&E stained sections, cells of the inner 2/3 of the ZF were usually similar to cells of the outer 1/3 of the layer;

however, more cavitated cells were seen (figure 14). With ORO, the inner 2/3 of the ZF was seen to be lipid-poor (figure 16).

In the IZF, Predef 2X treatment caused slight to moderate accumulation of lipid, which had reached its peak by 8 hr. after drug withdrawal (figure 12). Throughout the withdrawal period, this layer contained more stainable lipid than the OZF. Moreover, recovery was not noted 7 days after drug withdrawal, as it was in the OZF (figure 12).

Cellular degeneration, infiltration of PMNs and active phagocytosis could be detected with H&E and TB stains. An increase in cavitated cells was seen 3 days after withdrawal (figure 13). Evidence of recovery was seen 4 and 7 days following withdrawal in the form of a decrease in cavitated cells (figure 13).

The border between IZF and zona reticularis (ZR) was considered to be the site at which the cords of the IZF started to bifurcate to form a reticular network ("nests") (figures 14, 20, 21, and 24). Thickness of the ZR was usually 5-10 cells. Sometimes 2 concentric sets of reticular networks were present. ZR cells were sometimes found among adrenomedullary cells.

The ZR was lipid-poor in control hamsters (ORO stain, figure 16). With H&E, some ZR cells appeared similar to IZF cells but slightly more elongated. Elongated ZR cells

were obvious on TB stained sections (figure 25). Cell cavitations and membranes which sometimes divided them were also obvious (figures 24 and 25) using TB stains. Mitochondria were prominent in TB stained preparations. Infrequent lipid droplets were seen. These were about 2 μ in diameter.

Predef 2X caused moderate to marked accumulation of lipid (figure 12) which had reached its peak by 24 hr. after withdrawal. This feature was still present 7 days after withdrawal in treated and killed hamsters. Treated hamsters which died, usually had more stainable lipid in the ZR than treated and killed animals (figure 12). Increased cellular cavitation was seen in this layer and had reached a peak by day 3, then decreased at day 4 post-withdrawal and thereafter (figure 13). Cellular destruction, infiltration of neutrophils, and phagocytic activity were noted in the first 2 days after drug withdrawal (H&E and TB stains). Recovery 4 days after withdrawal was indicated by relative absence of these features.

Ultrastructural features of control hamsters

The ZG layer was 4-5 cells deep. Intercellular spaces were usually narrow, and cell surfaces were smooth. Where the intercellular space was wide, a few cellular microvilli projected into the space. The most characteristic ultrastructural feature of ZG cells was the mitochondrial morphology; profiles of these organelles were spherical or slightly elliptical (figures 29 and 30). These mitochondria contained

3-10 leaflet-like, parallel cristae. Mitochondria were relatively numerous (figures 29 and 30). Several mitochondria appeared in which cristae lay in different planes (figures 29 and 30).

Endoplasmic reticulum (ER) of the ZG cells was usually smooth (SER). In some cells, it occurred in the form of saccules (figure 29); however, this might have resulted from inadequate fixation. In other cells, the SER was tubular (figure 30). Juxtanuclear profiles of granular endoplasmic reticulum (GER) were occasionally seen (figure 30). One to 2 large lipid droplets were occasionally seen in a cell (figure 29). Free ribosomes were relatively numerous in the cytoplasm. Membrane bound dense bodies (lysosome-like bodies) were seen. Nuclei were usually elliptical (figure 29).

Ultrastructural features of the ZG cells changed gradually going from the capsule inwards. At about the 4th-7th cells from the capsule, transitional cells were seen (figure 30). These cells contained both features described earlier for ZG cells and some features of OZF cells. Only some mitochondria were spherical or elliptical; others were irregular in shape. Cristae were not always in leaflet-like parallel pattern, but a random pattern of tubular cristae was also obvious. Increased numbers of membranous figures surrounding mitochondria were seen (figure 30).

Intercellular space in the OZF contained microvilli (figure 35). Microvilli in subendothelial spaces were

contributed by both the parenchymal cells and the endothelial cells (figure 35).

Mitochondria were more frequent in OZF cells than in ZG or transitional cells (figures 35 and 36). Mitochondria were usually spheroidal to irregular in shape in the OZF (figures 35 and 36). Cristae were in the form of randomly arranged tubules. Several mitochondria had membranous layers parallel and adjacent to the outer and inner mitochondrial limiting membranes. Two mitochondria might be in close juxtaposition, or lie with one enveloping the other (figure 33). Content of the mitochondrial matrix varied from crowded cristae to an amorphous material with few cristae (figures 33 and 34). Small, empty appearing cavities were seen (figure 33). Rarely, lipid droplets were seen in OZF cells.

The Golgi body was usually juxtanuclear (figure 33). ER was predominantly smooth, and free ribosomes were numerous (figure 34). GER was rarely seen. Membrane-bound dense bodies were fairly common.

Cells of the IZF contained more mitochondria than those of the OZF, and mitochondrial morphology was much more irregular (figure 39). Arrangement of mitochondrial cristae varied from parallel to random (figure 39). Cristae were usually tubulovesicular. The mitochondrial matrix was relatively electron dense. In this layer mitochondria frequently enveloped other mitochondria or other cytoplasmic

parts (figure 39). Elaborate relationships of mitochondria enveloping one another were seen. Membrane-bound dense bodies were also seen. Electron transparent, large vacuoles (cavities) were seen in many cells. Some cells had a large cavity, around which cellular organelles were found in degenerative states. Electron density of the membrane-bound dense bodies was unusually great in such cells. It was possible to find membranous figures in the cavities.

The ultrastructure of ZR cells and inner IZF cells was similar (figure 42). Of the various adrenocortical layers, the ZR cells contained the most mitochondria (figure 42).

The ultrastructural features of treated hamsters

The effect of Predef 2X treatment on ZG cells was characterized by the presence of lipid droplets in the cytoplasm by 24 hr. after drug withdrawal. Endothelial cells remained intact at this stage. On day 4 after drug withdrawal, mitochondria were mostly irregular in shape (figures 31 and 32), with parallel cristae. The electron density of some of the membrane-bound dense bodies was greater than of those in ZG cells of control animals (figure 31).

Evidence of cellular destruction in the OZF was seen 24 hr. after drug withdrawal. PMNs were seen in the process of phagocytizing cellular debris. Parenchymal cells contained large cavities which were lined with several parallel smooth membranes (figure 35). These cavities contained lipid droplets, mitochondrial remnants and debris. Lipid droplets

were seen in parenchymal cells. By 4 days after drug withdrawal, evidence of cellular degeneration was rare. At this stage, parenchymal cells usually contained lipid droplets (figure 36). More parallel cristae were crowded within mitochondria than in cells examined 24 hr. after drug withdrawal or in controls (figure 36). Membrane-bound dense bodies were common and profiles of ribosomes associated with SER were seen occasionally (figure 38).

In some cells, mitochondria were sparse 4 days after drug withdrawal. The hyaloplasm was granular and the ER was smooth and sparse. Some mitochondria appeared elongated.

Twenty-four hr. after drug withdrawal, infiltration of PMNs and phagocytes was seen in the IZF. PMNs in process of active phagocytosis were observed. The phagocytized "debris" was interpreted as being related to lipofuscin bodies. Lipid droplets and dark membrane-bound dense bodies were seen in cortical cells.

By day 4 after drug withdrawal, lipid droplets were seldom found in the IZF. The number of degenerate cells was decreased. A notable decrease in phagocytes and phagocytized material was observed as compared to 24 hr. after drug withdrawal. Mitochondria in some cortical cells appeared to be in condensed states. Other mitochondria were elongated. Elaborate relationships between juxtaposed mitochondria were common (figure 41). Many free ribosomes were present (figure 40).

Twenty-four hr. after withdrawal, cellular cavities and lipid droplets were seen in ZR cells. Mitochondria enveloping other mitochondria were commonly seen.

By 4 days after drug withdrawal, some cortical cells contained lipid droplets. Phagocytes, adjacent to cortical cells, contained vacuoles, several GER figures, membrane-bound dense bodies and debris of varying electron density. The electron dense debris were believed to represent early lipofucsin granules (figure 44). Most cells in this layer contained cavities and mitochondria in elaborate juxtaposition one to another (figure 45).

Discussion

General observations

Body weight loss induced by corticoids has been reported in dogs and mice[29], in rats[59, 83, 89], and in rabbits[3, 73]. The first injection of Predef 2X caused a loss of about 4 gm. per hamster. This was explained as the initial catabolic effect of a very potent glucocorticoid. An adjustment period of 2 to 3 days followed, during which the effect of daily injection of Predef 2X on weight was negligible. Subsequent daily treatments caused a loss of 3-7 gm./hamster/day in those animals which subsequently died. Evidently, these hamsters were unable to survive the catabolic effect of Predef 2X. During the postadjustment periods, hamsters which subsequently were killed, lost 1.5-3.0 gm./hamster/day until 3 days after withdrawal. These hamsters were evidently more resistant

than hamsters found dead to Predef 2X effect. After 3 days of the withdrawal period, a recovery phase followed, in which treated hamsters maintained their weight or gained about 1 gm./day/animal.

The idea that hamsters which subsequently died were less resistant to catabolic effect of Predef 2X was supported by effect of the drug on postmortem muscle pH, skeletal and cardiac lesions, adrenocortical changes, clinical signs, and gross lesions. These changes were more pronounced in hamsters found dead than in hamsters subsequently killed. The impression of recovery from the catabolic effect of Predef 2X starting 3-4 days after drug withdrawal was supported by data on postmortem muscle pH, clinical observations, accumulation of adrenocortical lipid in OZF and cellular cavitation in the IZF and ZR. Five of 12 hamsters were found dead on day 2 and none on day 4 or thereafter after drug withdrawal. Apparently, days 2-3 post-withdrawal were crucial in the recovery process. On day 3, cavitations in the IZF and ZR reached a maximum. It is possible that the hypothalamus-anterior pituitary-adrenal cortex axis started to recover from glucocorticoid suppression on these days.

Skeletal and cardiac muscle recovery, if they occur, take place at a period later than 7 days after withdrawal. It is not known whether treated hamsters would have regained control weight levels, or whether the adrenal cortex and muscles would have completely recovered. It would be inter-

esting to study the course of weight gain and cellular adrenocortical and muscular events 7 days after withdrawal.

The adrenal cortex

Five different layers were detectable by light microscopy using toluidine blue (TB)-stained, 0.5-1.0 μ thick, Epon sections and correlating the findings with those seen in adjacent sections with electron microscope. These were zona glomerulosa (ZG), transitional layer, outer zona fasciculata (OZF), inner zona fasciculata (IZF), and zona reticularis (ZR). Only 3 layers were detectable in control adrenal cortices stained with H&E and ORO. These were ZG, zona fasciculata (ZF) and ZR. For statistical purposes, the outer 1/3 of the ZF was considered OZF and the inner 2/3 as IZF.

Sabatini and De Robertis[63] suggested a zonal theory for rat adrenal cortex based on subcellular morphology. Ultrastructural results in this study agreed with this theory. In control golden hamsters there was a gradual change in subcellular components from the ZG to the ZR characterized by an increase in number, size, irregularity in shape and electron density of the mitochondria; a decrease in mitochondrial matrix; an increase in tubulovesicularity and random arrangement of cristae; an increase in elaborate relationships of juxtaposed mitochondria; a decrease in GER; and an increase in cellular vacuoles. Size of lipid droplets decreased abruptly from diameters of about 5-6 μ in the ZG to 1-2 μ

in the OZF, IZF, and ZR. These relative sizes were observed in the few lipid droplets seen in control adrenal cortices as well as in droplets which accumulated in response to Predef 2X treatment. This zonal subcellular description, was correlated with the topographical findings in the adrenal cortex of control and treated hamsters seen in sections stained with TB. Support for the concept of zonal differences was also found in the different reactions of the various layers to Predef 2X treatment.

Morphological zonal theory and reactions of different layers to glucocorticoid treatment support the concept that enzymic zonal differences exist in the adrenal cortex of the golden hamster.

The adrenal cortex of young adult golden hamsters, has been reported to be lipid-poor and negative to cholesterol stains[2, 41, 53]. Results of this study using ORO, TB and ultrastructural observations agree with these observations. Presence of cholesterol crystals associated with lysosome-like bodies has been reported in the adrenal cortex of rats[61]. These increased under treatment conditions which suppress steroidogenesis, such as dexamethasone[61] or aniline[24] injection. Those workers concluded that cholesterol is not one of the direct precursors of corticoids in the adrenal cortex of hamsters. The same conclusion was reached by Reiter and Hoffman in their review on the biosynthesis of adrenocortical hormones in golden hamsters[60].

Spheroidal and irregularly shaped mitochondria have been described in ZG of rats[61, 63, 88] and man[48], and rod forms in opposum[46] and Rhesus monkeys[7]. In this study, ZG mitochondria were spheroidal or slightly elongated. In all foregoing species, mitochondrial cristae of ZG cells were tubular or shelf-like in shape.

The problems of zonal specificity in secretion of hormones with glucocorticoid or mineralocorticoid activity, and zonal specifity in response to ACTH stimulation have not been settled. Chester Jones and coworkers reported that ZG of mice and rats may be stimulated by ACTH, and could be a source of ZF and ZR under such stimulation[18, 19, 20, 21]. Moreover, these workers indicated that presence of the ZF is required for normal body control of salt electrolytes. In CBA strain of mice the ZG was reported to be reduced considerably after treatment with synthetic ACTH[72]. Sodium deficient diet caused the appearance of concentric membranous lamellae in the ZG cells[34]. ACTH was reported to play a role in controlling desoxycorticosterone secretion[77]. The ZG of hamsters responded to mineralocorticoid treatment more markedly than did the remainder of the adrenal cortex[2]. It is possible that zonal specifity is less obvious in the golden hamster, since several aspects of adrenocortical physiology of this species are unique.

Accumulation of lipid in the ZG cells following Predef 2X treatment in this experiment was believed to be due to

cardiac myopathy induced by the drug. This myopathy caused a condition of cardiac failure which probably resulted in Na^+ retention, which in turn caused inhibition of mineralocorticoid secretion. Lipid accumulation in ZG cells may have been influenced by degenerative processes in the remainder of the cortex. Lipid accumulation in cells of the ZG and IZF was similar to that described in hamsters with genetic cardiomyopathy[15].

Presence of a transitional layer has been reported in the adrenal cortex of hamsters[41]. This layer could be distinguished by light microscopy with iron hematoxylin staining[41]. In this study it was possible to detect this layer in Epon sections stained with TB and electron microscopic methods. The term "transitional" was suggested because cellular components of this layer contained characteristics which blended with those of neighboring ZG and ZF[41]. In this study, ultrastructural features of the transitional layer agreed with Knigge's description[41]. These cells were usually seen about 4 to 7 cells in from the capsule. A clarification of the role of this layer may shed light on the cell migration theory which has been suggested for the adrenal cortex of the rat and mouse[17, 21].

The terms outer zona fasciculata and inner zona fasciculata are not uncommonly encountered in the literature. In the rat, testosterone induced adrenocortical atrophy and decreased secretory activity, especially in the IZF and ZR[54].

Moreover, secretory activity of the OZF increased; this was explained as a compensating action of this layer, stimulated by IZF and ZR atrophy[54]. ACTH stimulated mitochondria in the OZF in the rat[55]. The pig has an OZF which responds differently than its IZF to prednisolone, Predef 2X, ACTH and possibly to stress (Dr. R. A. Ball, Associate Professor, Veterinary Medical Research Institute, Ames, Iowa, Personal Communication). In control hamsters, these layers could not be differentiated by H&E or ORO stain. The different reaction of the layers to Predef 2X in Experiment 1 suggested a functional difference between the layers. In Experiment 2, accumulation of lipids in experimental animals, architectural features seen in 1 μ sections, and ultrastructural features supported the concept that 2 layers were functionally distinct in the ZF. The OZF is apparently more responsive to ACTH stimulation and more resistant to suppression of the adrenal cortex. The IZF is more similar in this respect to the ZR.

In hamsters, factors which suppress the adrenal cortex caused any or a combination of the following changes in the ZR and the IZF: accumulation of lipids, increase in cellular cavitation and an accumulation of brown pigment. Severe suppression caused necrosis, increased phagocytosis, leukocytosis, hyperemia, and hemorrhages. Signs of adrenocortical suppression, similar to those produced in this experiment by Predef 2X, have been reported in hamsters in connection with

ageing[53], injection of estrogens[2], hypophysectomy[41], X-irradiation[64], leishmaniasis[42], spontaneous tumors[31], and implanted tumors[1]. Even though conditions which suppress the adrenal cortex affect the ZR and IZF more than the OZF, this ultrastructural study of ZR and IZF cells indicated that these cells appear active (possibly hyperactive) and not in a degenerated state. Similar findings were noted in porcine ZR (Dr. R. A. Ball, Personal Communication).

An increase in lipids has been associated with adrenocortical necrosis in mice[65]. An abnormal increase in lipids in adrenocortical cells may be associated with necrosis in Predef 2X-treated animals. These processes were possibly enhanced by the low levels of circulating ACTH, due to inhibitory feedback mechanisms acting upon the anterior pituitary gland. Aniline induced adrenocortical hyperplasia accompanied by accumulation of lipid and cholesterol in the rat[23]. Although aniline and Predef 2X both inhibit adrenocortical steroidogenesis, aniline does not act via the adrenocortical-hypothalamus-pituitary pathway. As a result the low level of circulating corticoids after aniline treatment, resulted in excessive ACTH activity, which in turn resulted in adrenocortical hyperplasia.

Negative feedback control mechanisms at the adrenocortical level in the rat were reported to be mediated by corticosterone-induced inhibition of both nuclear and mitochondrial RNA synthesis in ZF cells[56]. <u>In vitro</u> studies suggested that

in the rat, adrenal steroids inhibited adrenal protein synthesis and blocked ACTH-induced stimulation of corticosterone production, primarily through effects on respiratory and hydroxylation pathways in adrenocortical mitochondria[13]. The mechanism of the inhibitory effect of Predef 2X on adrenocortical cells of the golden hamster is not known.

Agranular cytoplasmic tubules (possibly SER) were reported to be in direct continuation with external mitochondrial membranes in adrenal cortices of control and experimental rats[81]. These interorganellar connections were interpreted as a possible means for facilitation of transport during corticosteroidogenesis. Mitochondrial matrix was seen in direct connection with the cytoplasmic matrix in the adrenal cortex of rats[59] and man[48].

Hypophysectomy induced mitochondrial gigantism in the rat, which was interpreted as a response to increased demands on the mitochondria[82]. Hypophysectomy in the rat induced disappearance of the ground substance and the membranous structures of the mitochondria, decrease in numbers of ribosomes and mitochondria and increased number of lipid vacuoles[8]. A decrease in ribosomes and ER and an increase in lipid have been reported following hypophysectomy in the rat[67]. These effects were reversed by ACTH administration. Aminoglutethimide and methylandrostenediol (inhibitors of adrenocortical activity) induced mitochondrial degeneration and cavitation[45, 59].

In this study, Predef 2X induced cellular destruction. In cells which survived, there were lipid droplets and degenerated mitochondria. It was difficult to determine whether changes occurred in dense bodies, ribosomes, and ER either 24 hr. or 4 days after withdrawal. Condensed mitochondria were seen 4 days after withdrawal. Mechanochemical changes, evidenced by morphological transformation and related to metabolic activity, have been reported in liver mitochondria[37]. It is possible that these metabolic changes were induced by ACTH stimulation. Degenerated mitochondria were seen surrounding other mitochondria. Whorled membranes resembling myelin formation have been reported during brown pigment accumulation related to adrenocortical ageing in mice[65]. It is possible that cells attempt to isolate or store their degenerated segments in whorled membranes, or this may be involved in an excretory process by which the cells eliminate debris.

Cellular vacuoles (cavities), especially those lined with several membranous structures, were interpreted as being associated with excessive abnormal lipid in adrenocortical cells. These reached their peak on day 3 after drug withdrawal, at which time presumably the adrenocortical hypothalamus-pituitary feedback mechanism recovered from the suppressing effect. Cavities were most commonly found in the IZF and the ZR where lipid droplets were found in larger amounts than in the OZF. These observations suggest that a

process, similar to that observed in rats[71], took place in experimental OZF, IZF and ZR. It is possible that in control adrenal cortex of hamsters such a process takes place but it is much less pronounced. A full understanding of the steroid secretion process in ZG cells of hamsters is lacking.

There were ultrastructural similarities between cellular debris in the parenchymal cortical cells and some phagocytic cells of the treated hamsters and early lipofuscin[30, 65]. These structures were also possibly related to excessive lipids in cells. Lipofuscin in adrenal cortex of mice was reported to be age-associated and to have a lipoidal source[65].

X-irradiation was reported to enhance age-associated adrenocortical changes[64]. Results of this study suggested that high doses of glucocorticoids cause similar changes. Aged rats were reported to have a substantial decrease in sensitivity of adrenal cortical control mechanism to feedback inhibition, which may play a substantial role in homeostatic alterations recognized to occur with increasing age[62]. It is possible that there is a relationship between accelerated ageing and the frequency of cycles of adrenocortical stimulation and suppression which the animal encounters during its life span. This concept might be useful in explaining adrenocortical ageing processes as related to stress susceptibility and resistance to disease. The golden hamster has been of value for adrenocortical ageing studies[53]. It might

also be useful for studies related to stress susceptibility and resistance to disease.

The skeletal and cardiac muscle

Induction of skeletal muscle necrosis by Predef 2X treatment was more consistent in Experiment 2 than in Experiment 1. Hamsters in Experiment 1 of same age group (12-13 wk. old) which received doses similar to those used in Experiment 2 (0.4 or 0.8 mg. daily) developed only very slight lesions. Reasons for this difference are not definitely known. In Experiment 2 the ambient temperature was much higher than in Experiment 1 (August vs. June) which may have aggravated the effects of steroids on skeletal muscle. It was concluded that skeletal myopathy characterized by necrosis could be induced in the hamster by Predef 2X and that additional stressors, such as high temperature, lack of exercise, obesity and older age may accentuate the corticoid effect.

Similar skeletal muscle necrosis was induced in rabbits by cortisone or triamcinolone[3, 25, 27, 28]. Potassium deficiency induced similar lesions in the rabbit[27, 28]. Ellis[28] and Awad et al.[3] concluded that steroids induce primary skeletal myopathy in the rabbit. Similar lesions were induced in hamsters by vitamin E-deficiency[85]. Hamsters used in this experiment may have had potassium deficiency (discussed later). It seems reasonable to assume that the hamster resembles the rabbit and differs from the rat, mouse, dog, pig, and

man. In the latter species, steroids do not induce skeletal muscle necrosis.

Cardiac and skeletal muscle necrosis was more severe in hamsters found dead than those killed. Cardiac lesions as compared to skeletal muscle lesions were greater in both treatment groups. Cardiac muscle necrosis was more severe and consistent than skeletal muscle necrosis in Experiment 1. It was concluded that cardiac muscle of the hamster is more sensitive than the skeletal muscle to steroid-induced myopathy. Support for such a conclusion was found in the studies of Fortner who reported myocardial necrosis, ascites and hydrothorax in golden hamsters with adrenocorticoadenoma and carcinoma[31].

Cardiac necrosis was induced in the rat by corticoid treatments[69, 70] and was prevented by amiloride, a nonsteroid potassium-sparing agent[69]. Cardiac necrosis was induced in the rabbit by potassium deficiency but was not induced by cortisone[28] or by triancinolone treatments[73]. The effect of corticoids on cardiac muscle of the hamster is similar to that in the rat and possibly in man, but differs from the effect in the rabbit and mouse.

Cardiac lesions in the hamsters may have been due to potassium deficiency. Hydrothorax and/or ascites were more common in the treated hamsters found dead than in those treated and killed. Hydrothorax and ascites accompany heart failure conditions. It is known that cardiac failure may be accom-

panied by Na^+ retention and K^+ depletion. Marked lipid accumulation in zona glomerulosa (ZG) cells of treated hamsters was found in these experiments. Similar findings were reported in a strain of hamsters with genetic cardiomyopathy[15]. This marked lipid accumulation could be associated with Na^+ retention (resulting from heart failure) and a consequent inhibition of secretion of mineralocorticoids from ZG cells. In Predef 2X treated animals a pathologic cycle may have developed in which the corticoid caused K^+ depletion and cardiac necrosis, resulting in heart failure condition, in turn causing Na^+ retention, further K^+ depletion, and further cardiac necrosis. One should also consider that the cardiac muscles which perform greater work were affected more by the treatment (papillary most, then ventricular, and atrial muscles least). Muscles under greater work loads may be more sensitive to corticoids and to K^+ deficiency.

Ultrastructural features of steroid-induced cardiac myopathy in the rat has been described as lipid accumulation, mitochondrial disruption and myofibrillar deformation[89]. Cardiac tissues were prepared in this experiment for ultrastructural investigation but time did not permit their study. Their examination would be fruitful.

After an extensive review of the literature, it appears that the golden hamster is the only laboratory animal species in which steroids induce muscular necrosis in both cardiac and skeletal muscles. Thus, the golden hamster is valuable

in the comparative study of steroid-induced myopathy of striated muscles, particularly so, because of not only widespread therapeutic use of these compounds in human medicine, but their increased use to treat valuable domestic animals.

Postmortem skeletal muscle pH as related to PSS

Postmortem muscle color, pH, and expressible moisture were found not to be changed in hamsters treated with low doses of Predef 2X (Experiment 1). High doses of Predef 2X elevated postmortem muscle pH. In stress-susceptible pigs, there is usually a marked decline in postmortem muscle pH[9].

The elevation in muscle pH of the treated hamsters may have been a reflection of plasma fluid (pH 7.3) which entered intercellular spaces or presence of basic residues from the breakdown of muscle proteins. pH elevation was greater in treated hamsters found dead than in killed hamsters. Although quantitative evaluation of edema by histological methods is impossible, one could say that edema was more severe in hamsters found dead than in those killed. Endothelial lesions were not detected in the muscle by light microscopy, but might be present at ultrastructural levels.

Goll et al. stated that pH of pig muscle at death is near 7.0 and of postmortem muscle is below 6.0[36]. Skeletal muscle of golden hamsters at death was not measured. The mean pH of postmortem skeletal muscle of control hamsters was 6.34. This difference between pig and hamster constitutes a limitation in the use of the golden hamster as a

laboratory model to induce PSE muscle.

The present trend is to regard PSE muscle as a unique reaction of stress susceptible pigs to stress stimuli. No other animal species is known to react to stress stimuli by PSE muscle. At the time this thesis was designed (1969-1970), it was believed that injection of potent glucocorticoids might afford an experimental method for induction of stress susceptibility in pigs. Presently, this belief is less popular among investigators.

The major factors thought to play a role in PSS and PSE muscle production were discussed in the literature review. Among these, muscle factors were prominent. High levels of available adenosine phosphates[68] and a relatively high ATPase activity caused by presence of intermediate fibers and lack of red fibers[23] are thought to be involved. The role of muscle glycogen breakdown is also important[78]. Short intervals between stress and slaughter produce PSE muscle in stress-susceptible pigs while dark dry muscle results, if the interval is relatively long[78].

Acidosis is a major factor in PSS. Production of large amounts of lactic acid from muscle glycogen is probably chiefly responsible. Adrenocortical insufficiency in stress susceptible pigs (which enhances the acidotic condition) is more likely caused by higher glucocorticoids turnover in stress susceptible pigs than by adrenocortical hyposecretion[78]. A correlation between porcine PSE muscle and fatty changes in

the ZR has been reported[16]. Ball et al.[6] found the same trend but the correlation was considerably less than that reported by Cassens et al.[16]. The difficulty partly lies in lack of precise differentiation in all cases between the stress susceptible and the stress resistant individual.

The golden hamster evidently has limited value as a model for studies of PSE muscle. However, similarities between the adrenal cortex of pigs and hamsters may warrant further exploration. The adrenal cortex of both species is relatively lipid-poor. The outer zona fasciculata (OZF) of the golden hamster is more responsive than the IZF to ACTH stimulation. Ultrastructural features of ZR cells indicated that these cells are active. Similar findings concerning the OZF and ZR have been noted in the pig (Dr. R. A. Ball, Personal Communication).

Figure 1. Effect of 9-fluoroprednisolone acetate on body weight of hamsters.

EFFECT OF 9-FLUOROPREDNISOLONE ACETATE ON BODY WEIGHT OF HAMSTERS

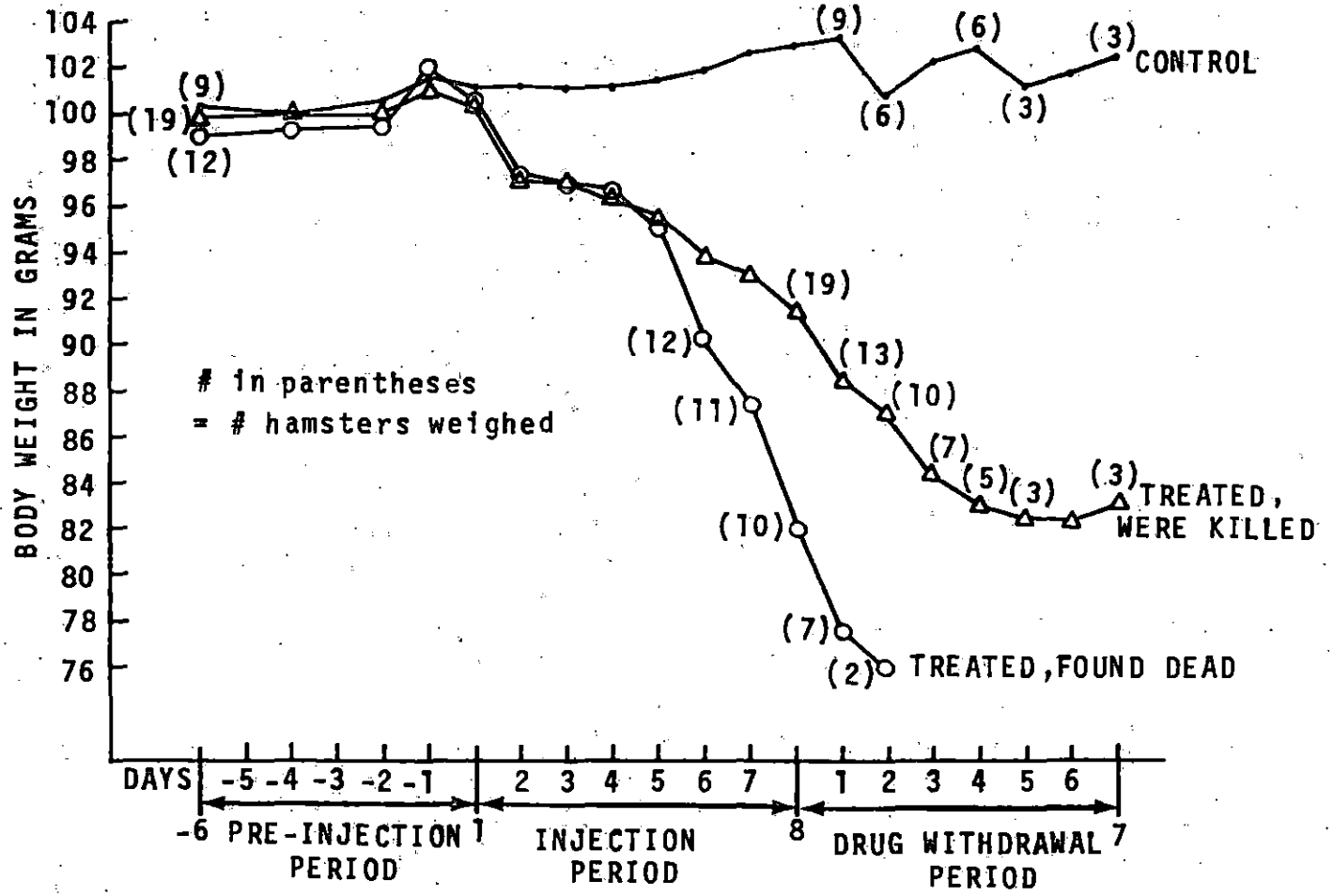


Figure 2. Effect of 9-fluoroprednisolone acetate on postmortem skeletal muscle pH of hamsters.

EFFECT OF 9-FLUOROPREDNISOLONE ACETATE ON POSTMORTEM SKELETAL MUSCLE pH OF HAMSTERS

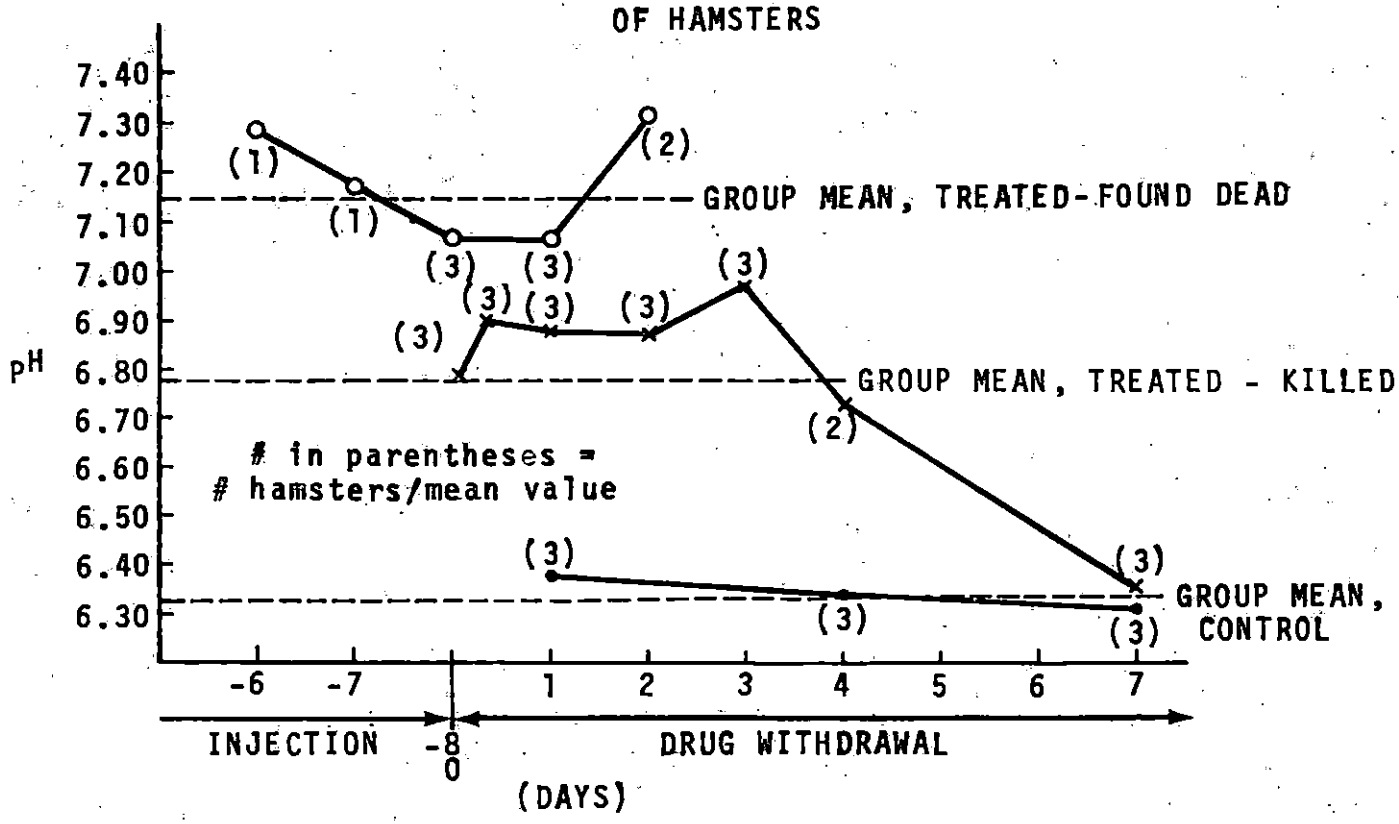


Figure 3. Skeletal and cardiac histomyopathies induced by 9-fluoroprednisolone acetate in hamsters (H&E stain).

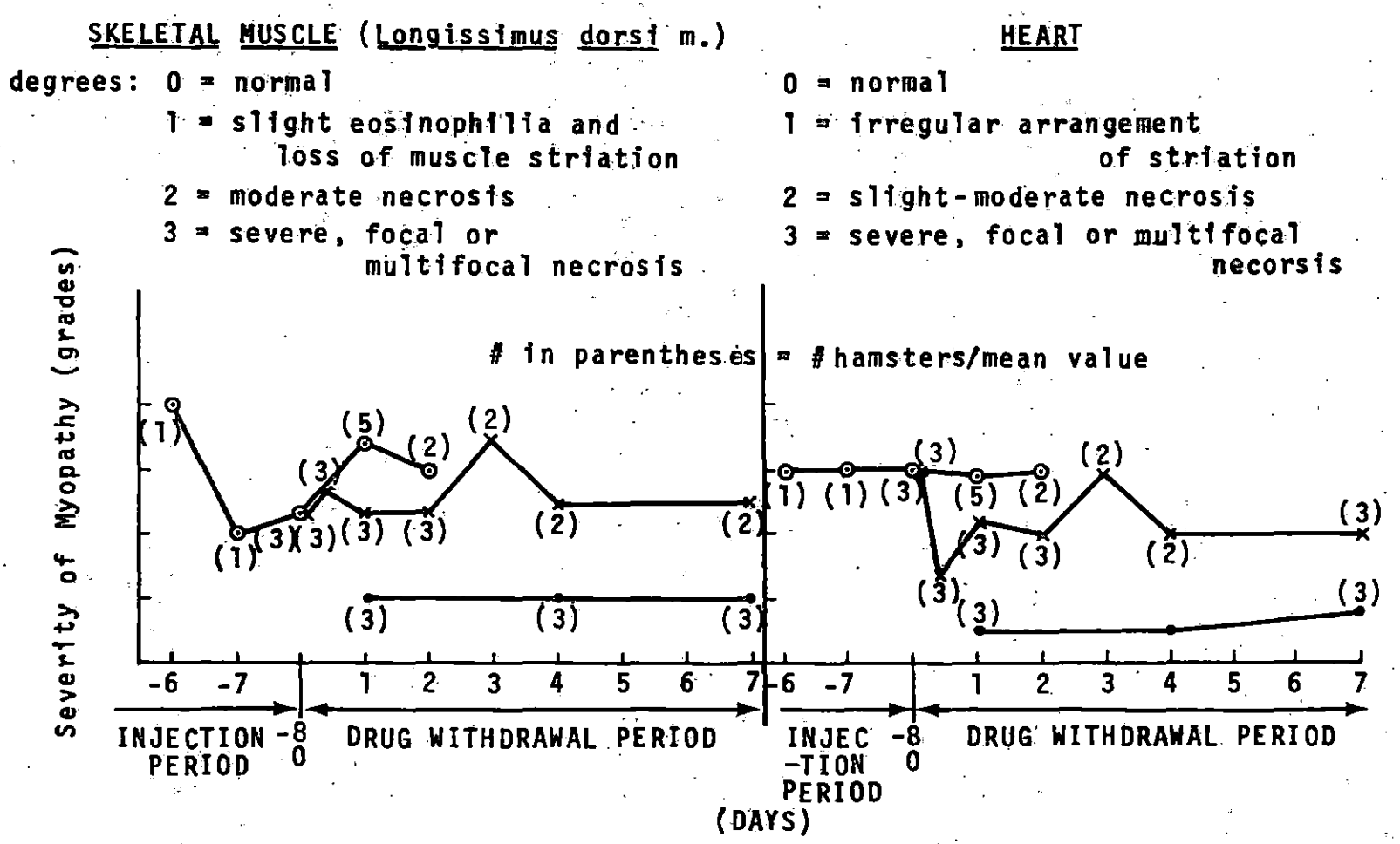
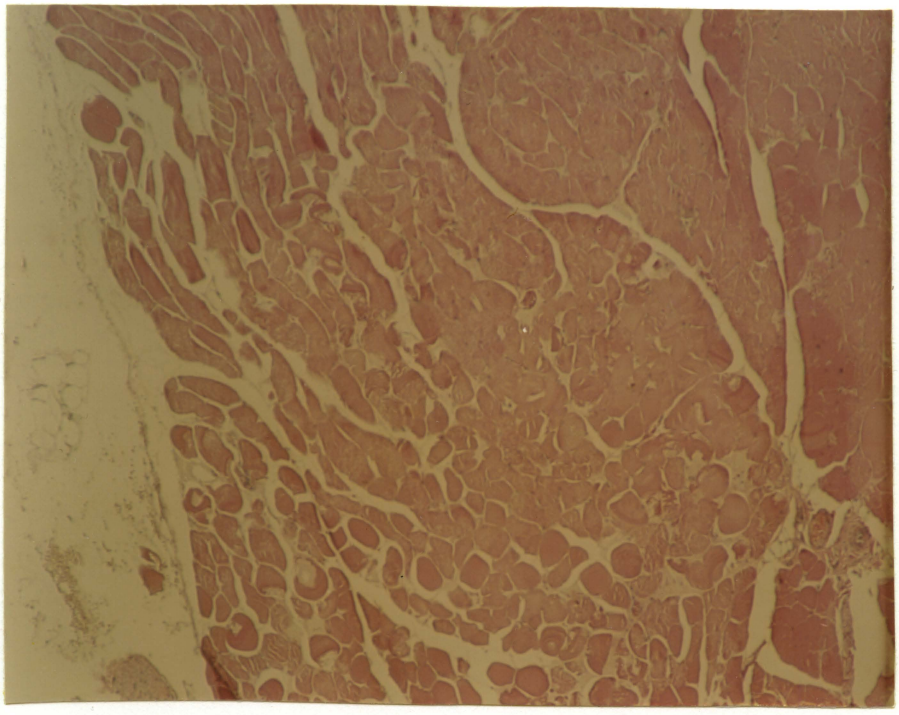

SKELETAL MUSCLE (Longissimus dorsi m.)
degrees: 0 = normal
1 = slight eosinophilia and loss of muscle striation
2 = moderate necrosis
3 = severe, focal or multifocal necrosis
HEART
0 = normal
1 = irregular arrangement of striation
2 = slight-moderate necrosis
3 = severe, focal or multifocal necorsis
# in parentheses = # hamsters/mean value
Severity of Myopathy (grades)
-6 -7 1 2 3 4 5 6 7
INJECTION PERIOD
-8 0
DRUG WITHDRAWAL PERIOD
(DAYS)
● Control
× Treated and killed
⊙ Treated and found dead

Figure 4. Longissimus dorsi muscle, fiber degeneration characterized by increased eosinophilia or pale cytoplasm in the different fibers. Hamster was found dead on day 2 after Predef 2X withdrawal, (H&E) 55X.

Figure 5. Longissimus dorsi muscle, 2 focal areas of mineralization (arrows) and loss of striation accompanied by nuclear degeneration (right and lower area in the field). Hamster was found dead 2 days after Predef 2X withdrawal, (H&E) 140X.



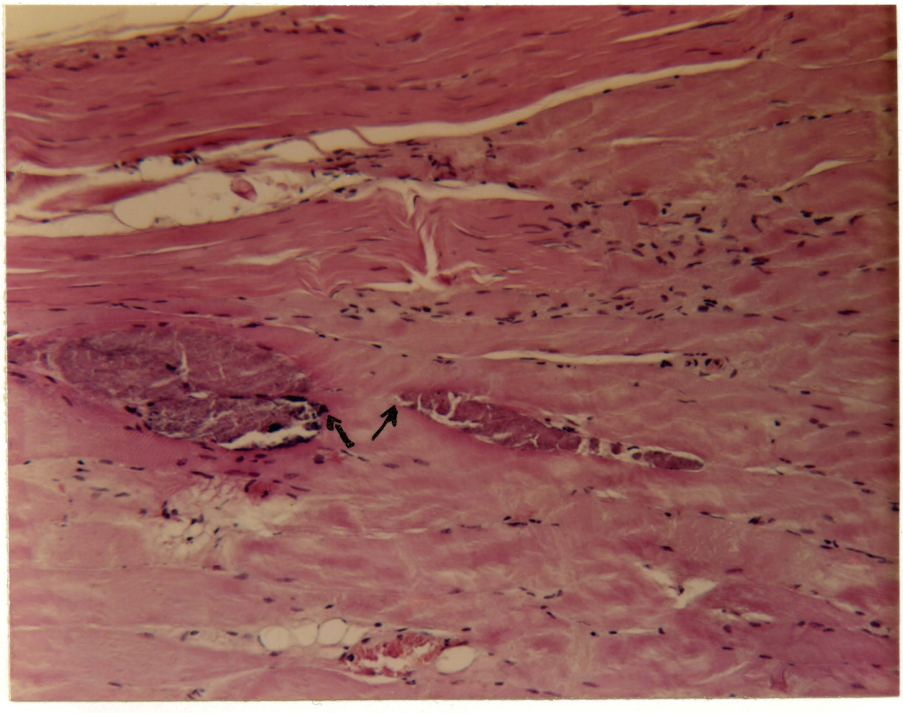
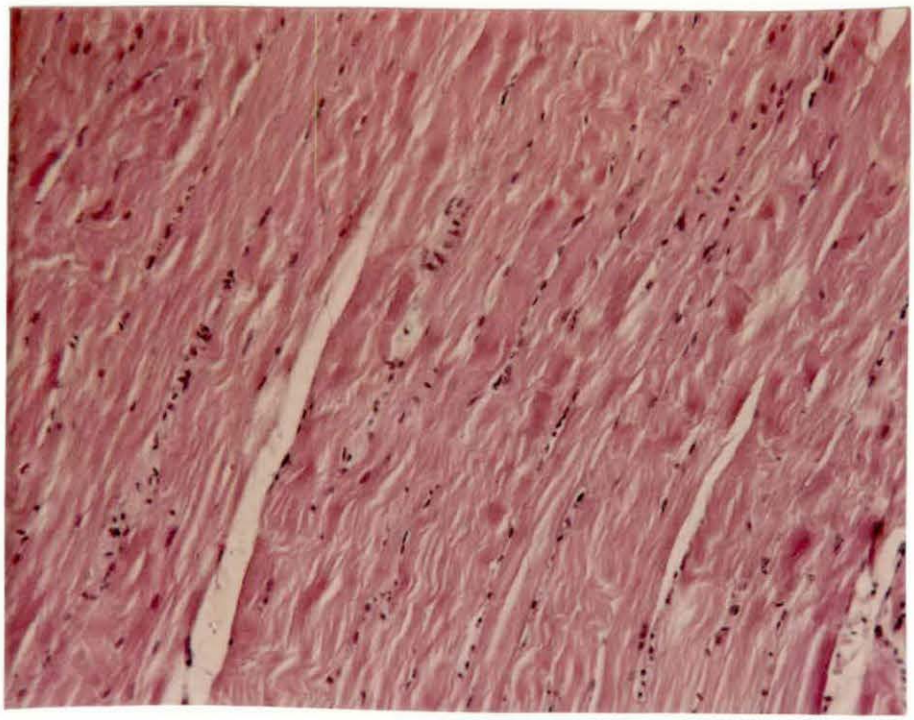


Figure 6. Longissimus dorsi muscle, rowing of nuclei. Hamster was killed 4 days post Predef 2X withdrawal, (H&E) 140X.

Figure 7. Longissimus dorsi muscle, infiltration by macrophages (M). Pale cytoplasm is seen in the left half and increased intercellular space in the right half of the field, (H&E) 55X.



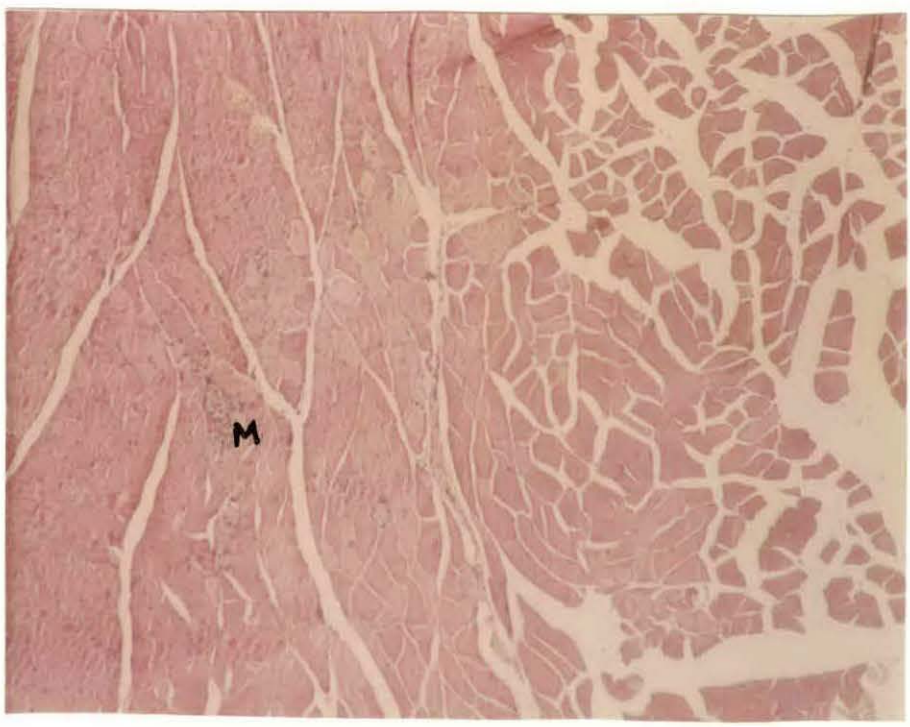
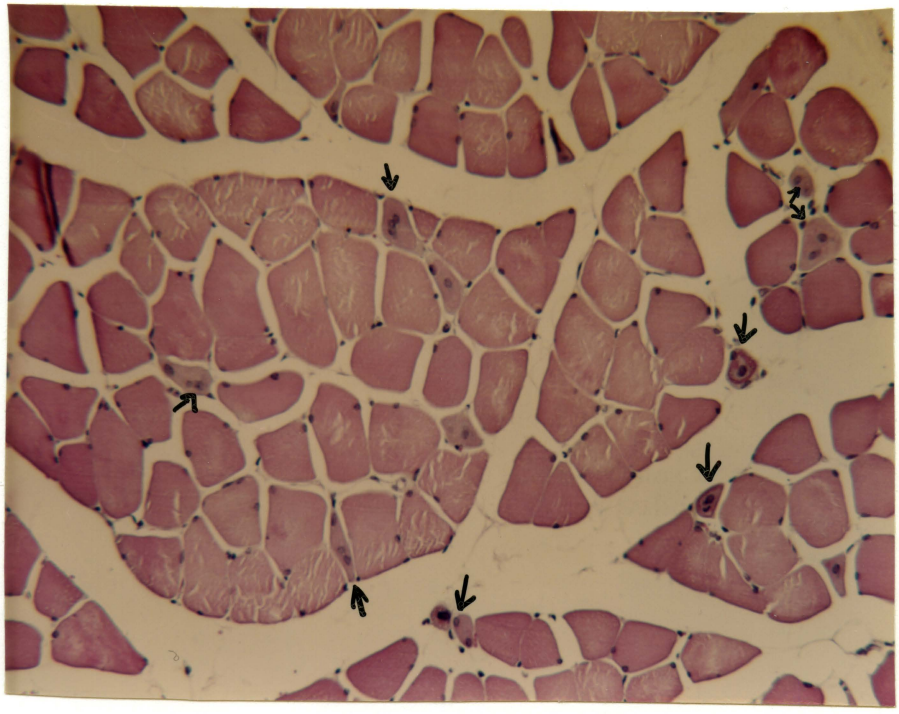

M

Figure 8. Longissumus dorsi muscle showing centralization nuclei in degenerated fibers (arrows) and increased intercellular space. Some fibers contain pale cytoplasm while others contain hypereosinophilic cytoplasm. Hamster was killed 7 days after Predef 2X withdrawal, (H&E) 140X.

Figure 9. Papillary muscle necrosis accompanied by infiltration by PMNs and macrophages. Hamster was killed 4 days after Predef 2X withdrawal, (H&E) 140X.



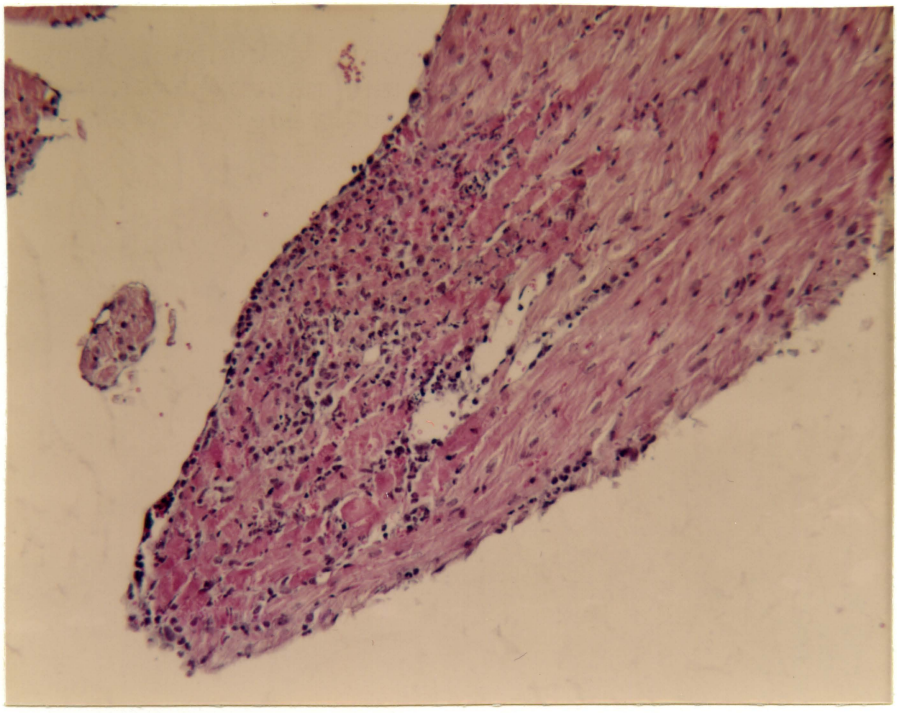
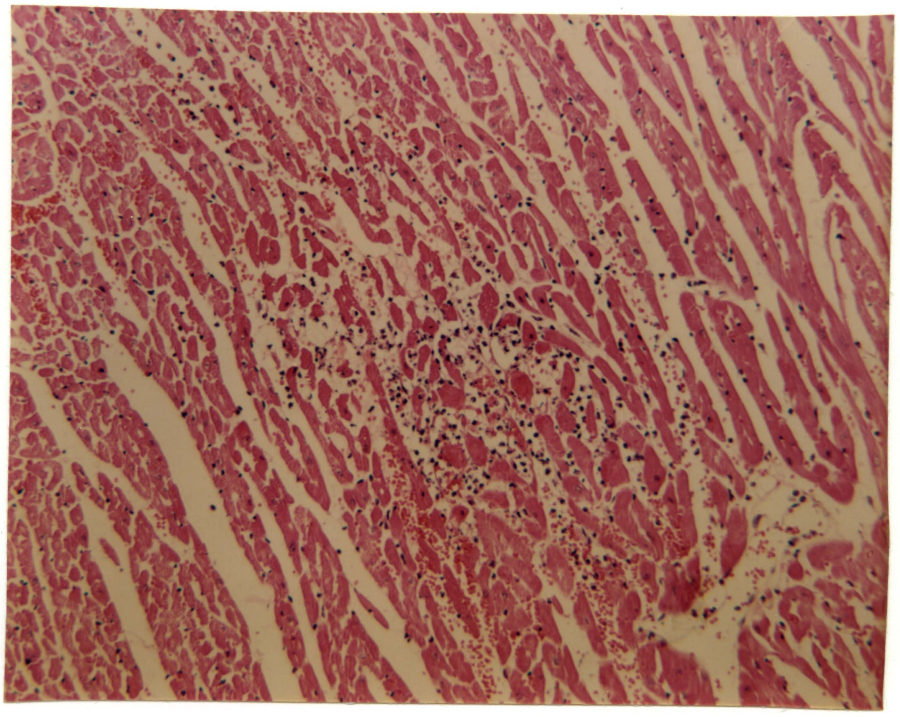
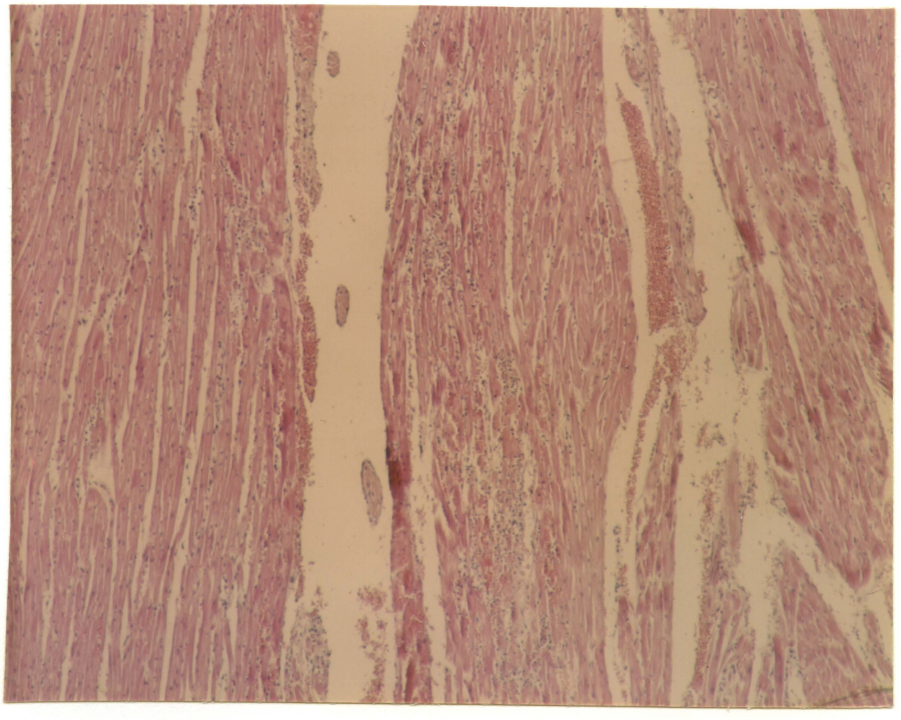


Figure 10. Ventricular necrosis accompanied by hemorrhage and infiltration by PMNs. Hamster was found dead on day 3 after Predef 2X withdrawal, (H&E) 140X.

Figure 11. Interventricular necrosis accompanied by hemorrhage and infiltration by PMNs. Hamster was found dead 2 days after Predef 2X withdrawal, (H&E) 55X.





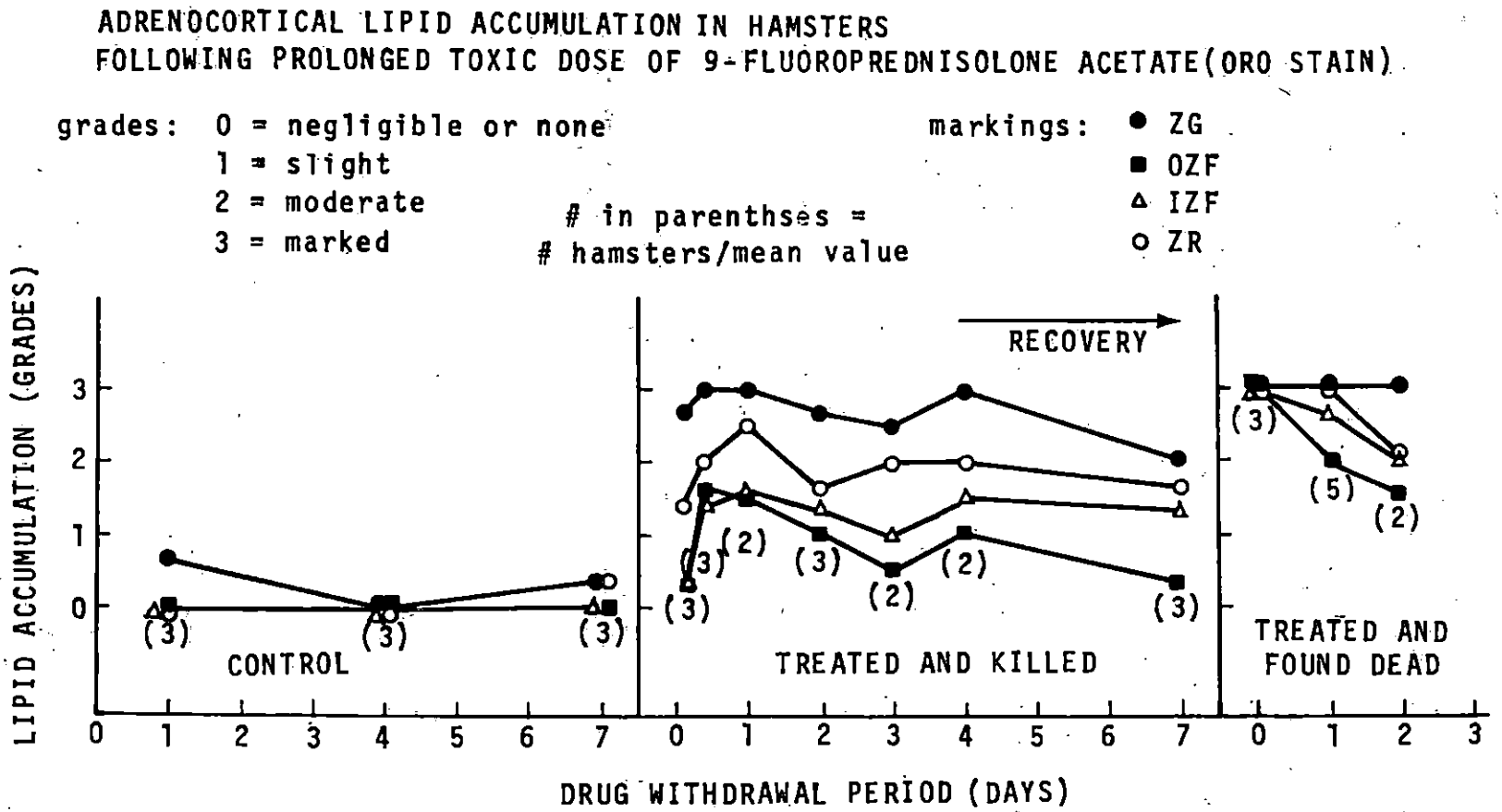


Figure 12. Adrenocortical lipid accumulation in hamsters following prolonged toxic dose of 9-fluoroprednisolone acetate (ORO stain)

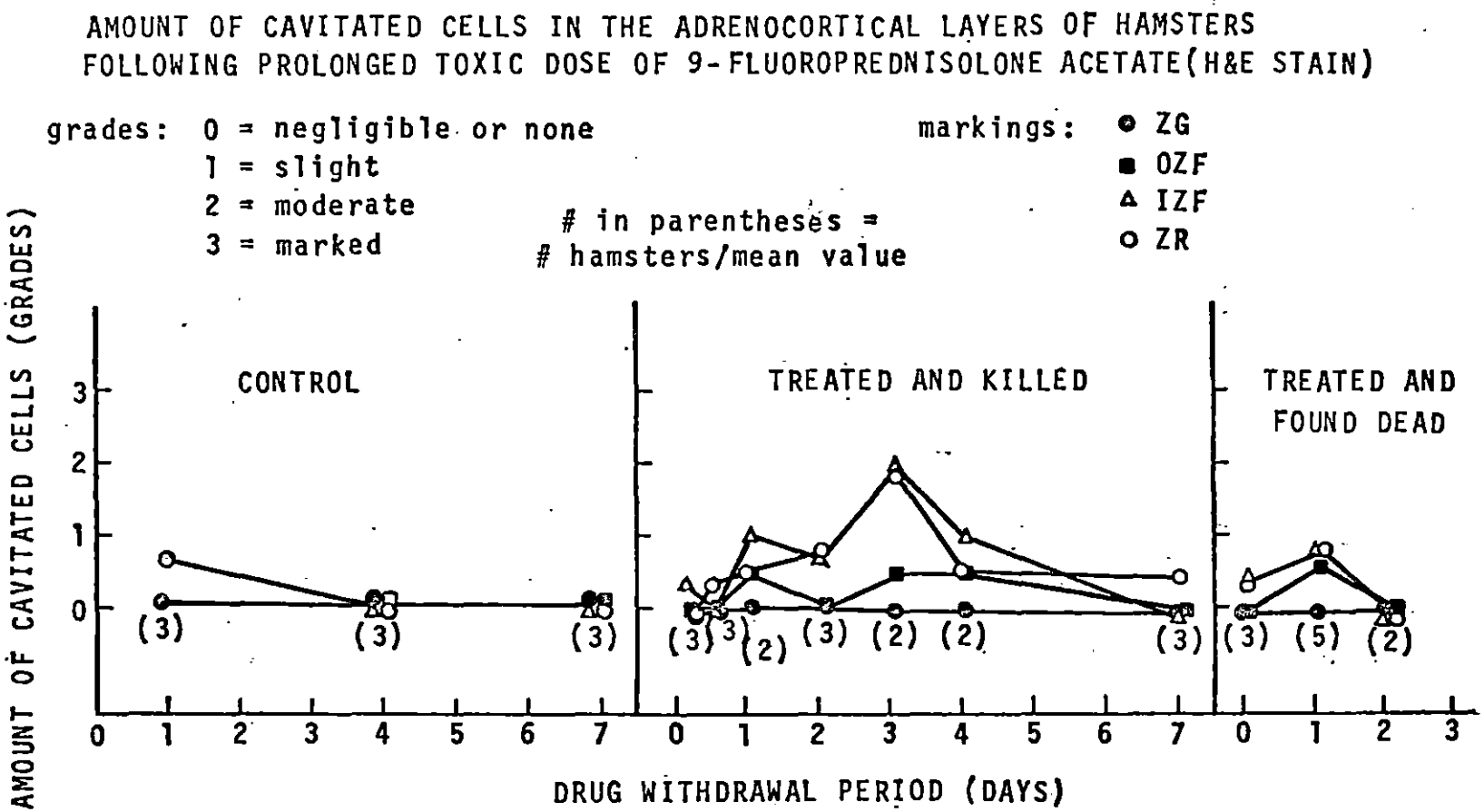
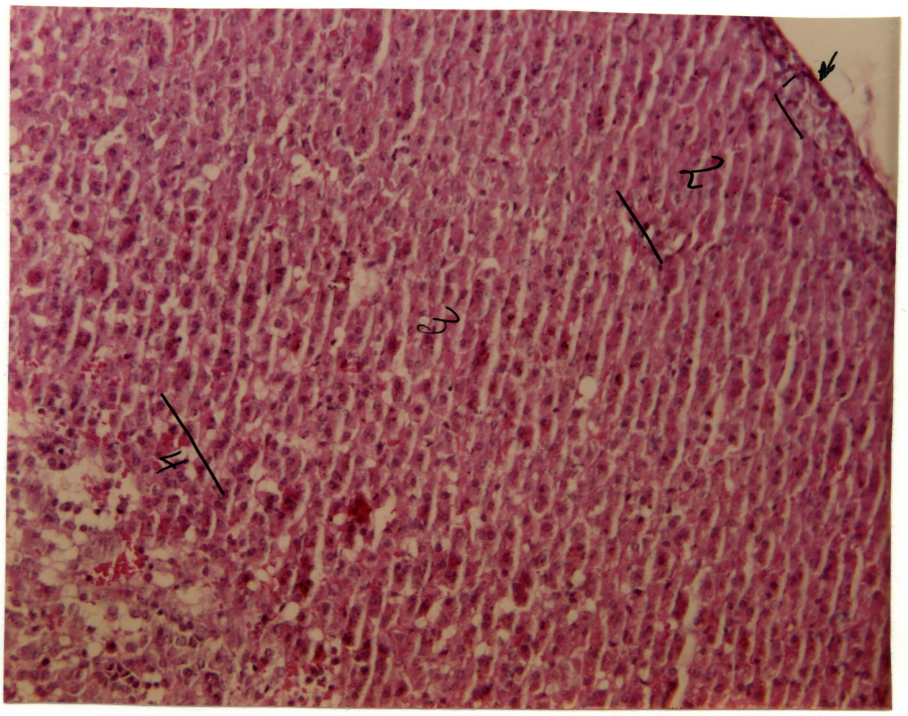


Figure 13. Amount of cavitated cells in the adrenocortical layers of hamsters following prolonged toxic dose of 9-fluoroprednisolone acetate (H&E stain)

Figure 14. Adrenal cortex of control hamster. Capsule (arrow), ZG (1), OZF or outer 1/3 of the ZF (2), IZF or inner 2/3 of the ZF (3), ZR (4), medulla (in the lower left corner) is somewhat basophilic. Cellular cavitations are seen in the IZF and ZR, (H&E) 140X.

Figure 15. Adrenal cortex. Hyperemia in the ZR and cellular degeneration is seen in all layers. Capsule is marked by arrow. Hamster was found dead on day 3 after Predef 2X withdrawal, (H&E) 140X.


1
2
3
4

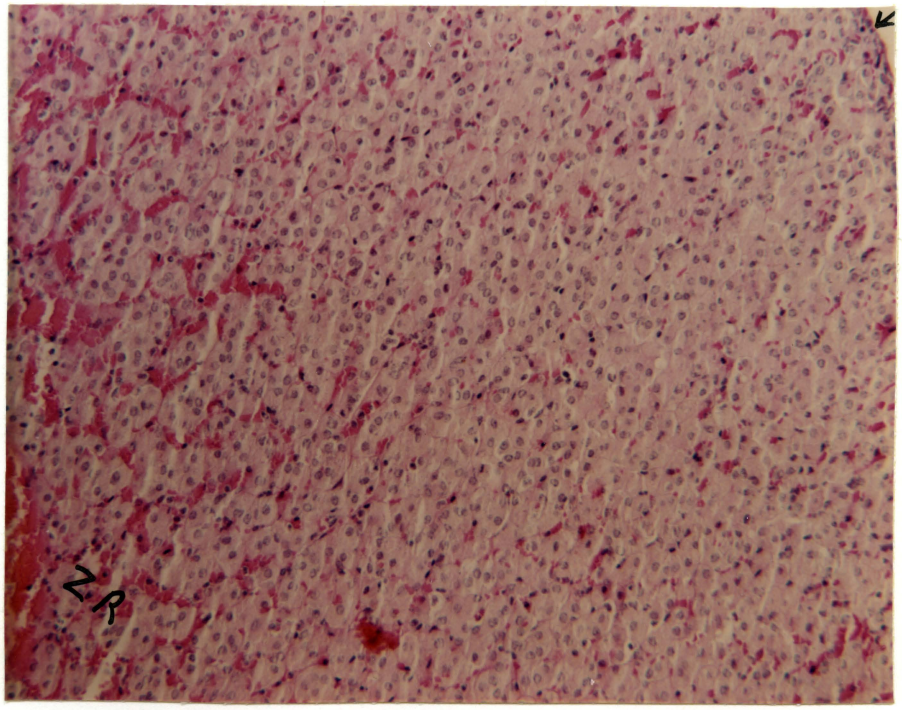
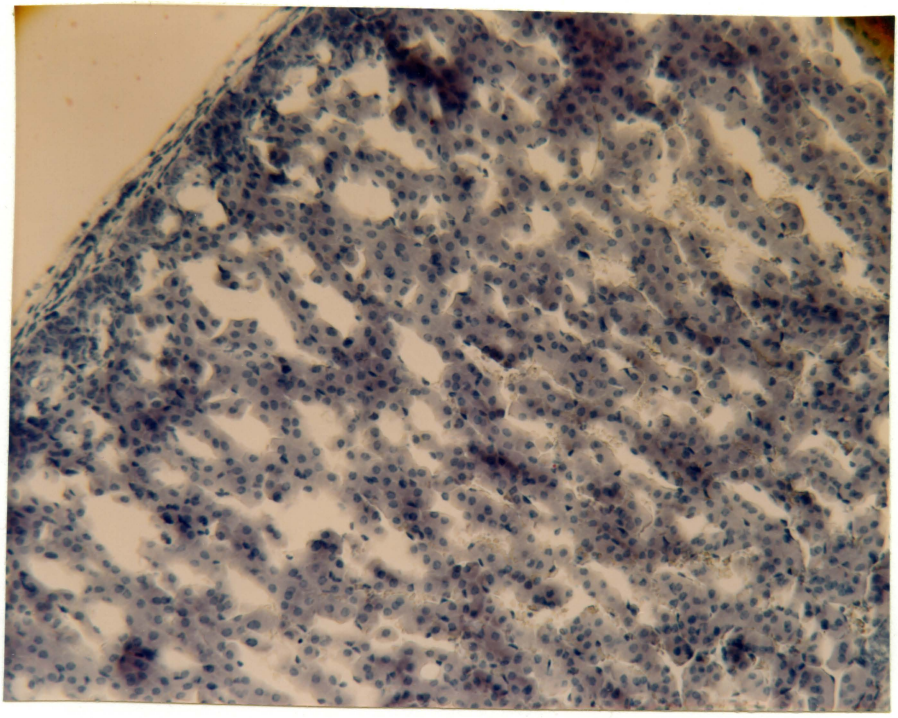

ZR

Figure 16. Lipid-poor adrenal cortex of control hamster. (ORO) 140X.

Figure 17. Lipid accumulation in the adrenal cortex. Marked in the ZG (1), negligible in the OZF (2), medium in the IZF (3) and slight in the ZR (4). Note larger lipid droplets (red) in the ZG than in the inner cortical layer. Hamster was killed 7 days after Predef 2X withdrawal, (ORO) 140X.



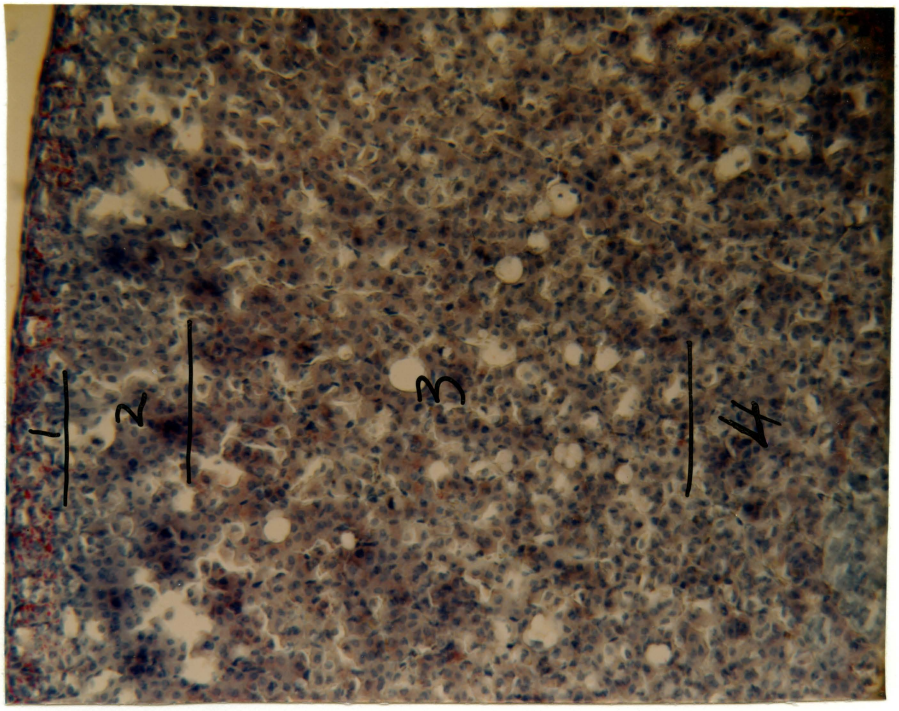
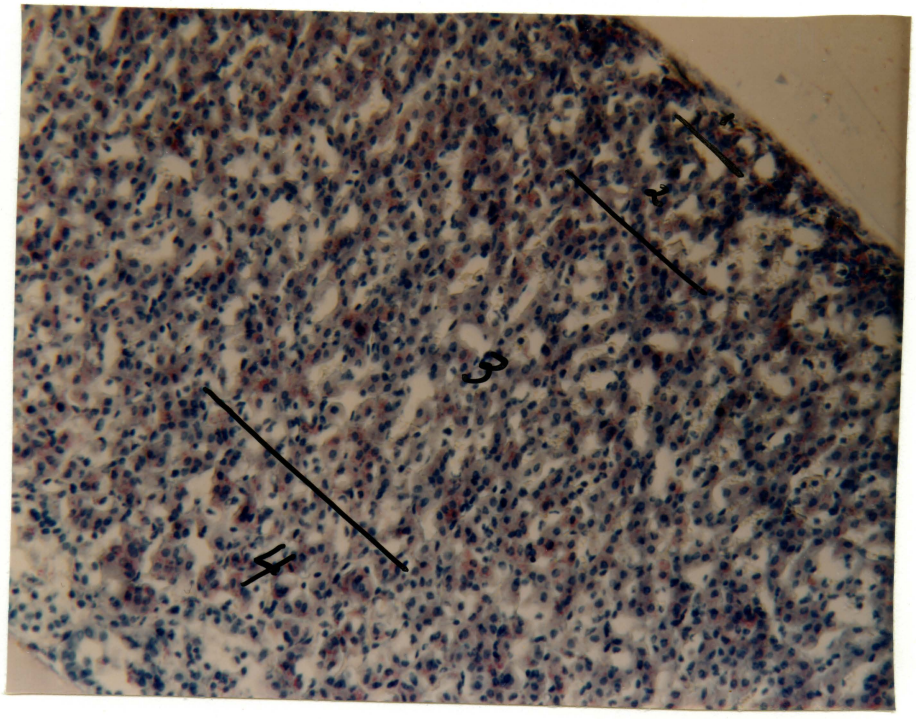

1
2
3
4

Figure 18. Lipid accumulation in the adrenal cortex. Medium in the ZG (1), slight in OZF (2), marked in the IZF (3) and the ZR (4). Hamster was found dead on day 1 after Predef 2X withdrawal, (ORO) 140X.

Figure 19. Lipid accumulation in the adrenal cortex. Marked in the ZG (1), medium in the OZF (2) and the IZF (3) and slight in the ZR (4). Hamster was killed on day 4 post Predef 2X withdrawal, (ORO) 365X.


1
2
3
4

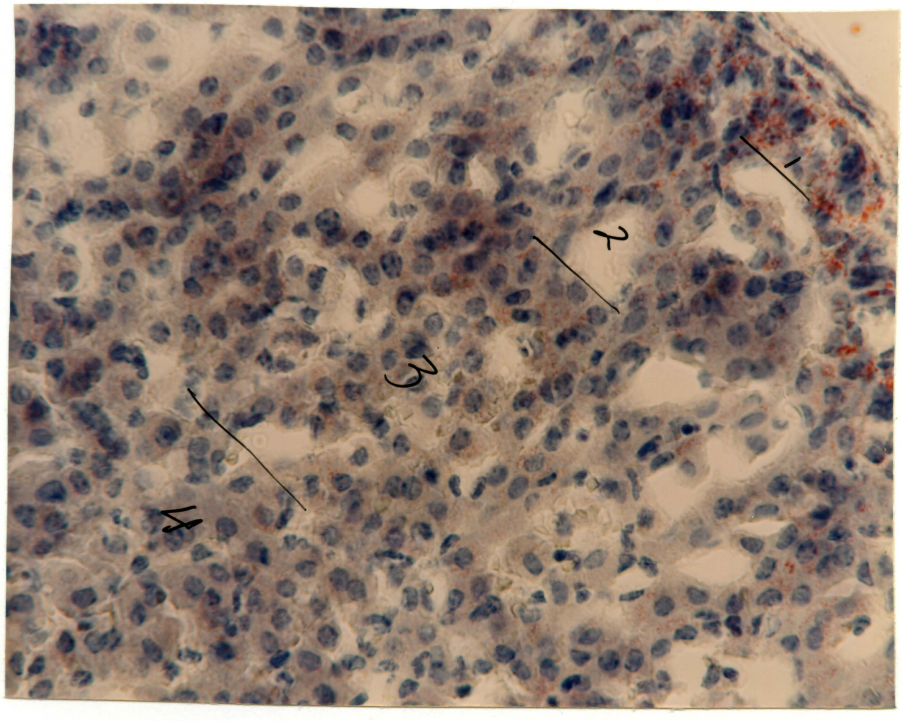
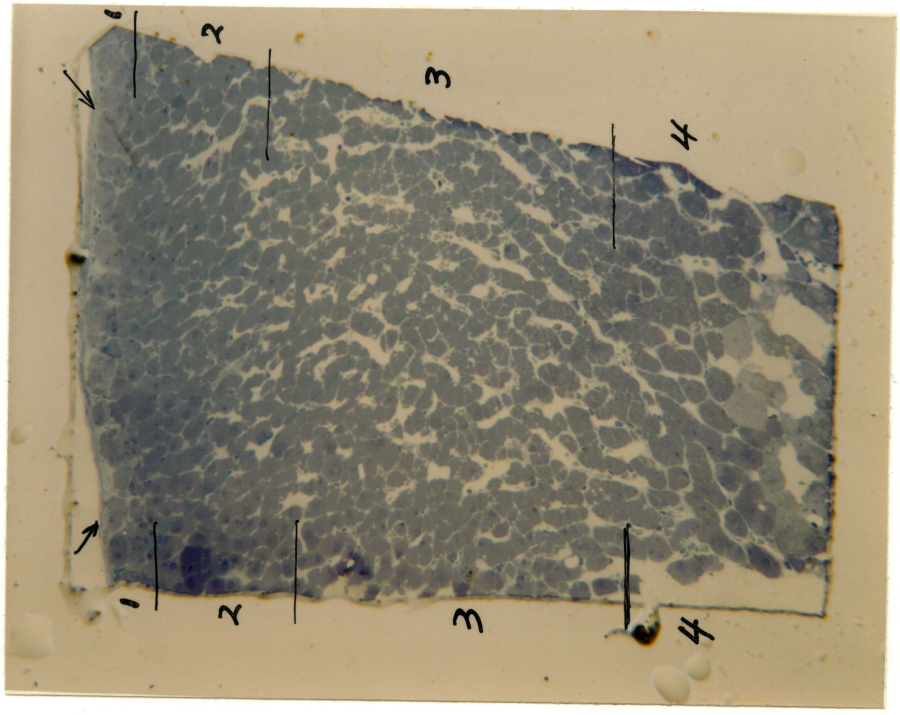

1
2
3
4

Figure 20. A topographic view of adrenal cortex as seen with TB stain 0.5-1.0 μ thick sections. Capsule (arrow), ZG (1), OZF (2) is relatively not corded, IZF is in cords and contains cavitated cells (3), ZR (4) is adjacent to the medulla (M), Epon embedded, 140X.

Figure 21. Higher magnification of Figure 20. Capsule (arrow), ZG (1), OZF (2), IZF (3), area of bifurcated cords in the IZF (4), 365X.


1
2
3
4
1
2
3
4

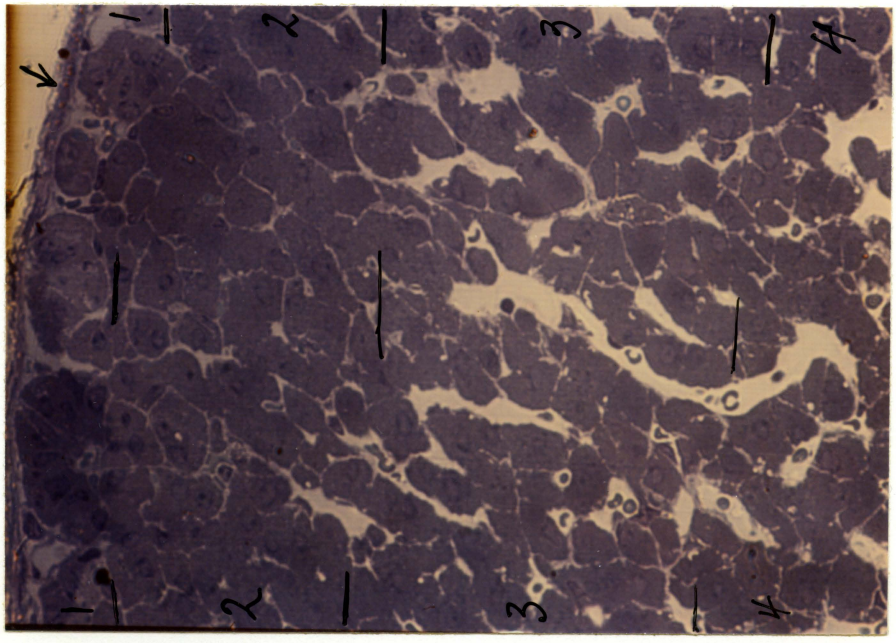
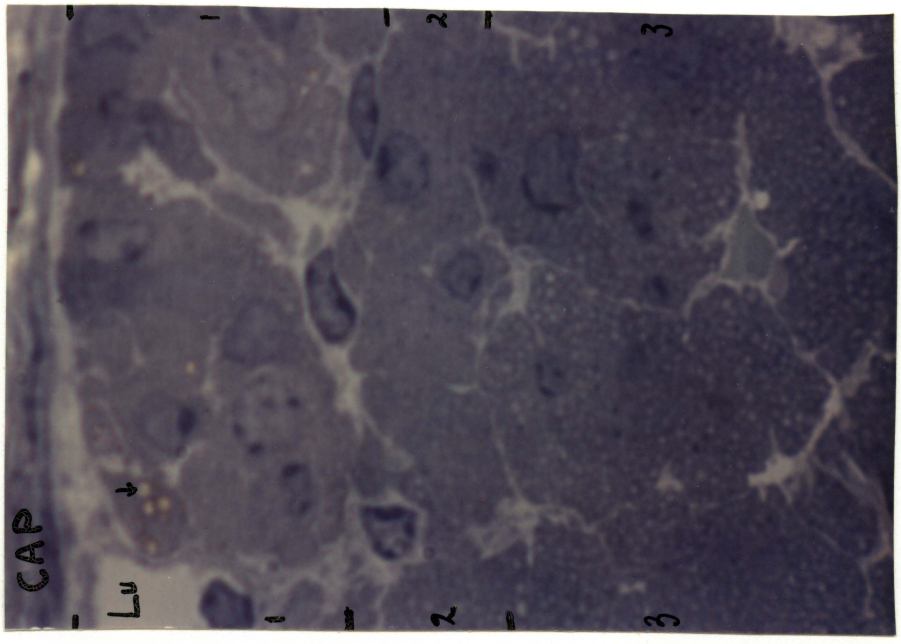

1
2
3
4
1
2
3
4

Figure 22. Higher magnification of Figure 21. Capsule (Cap), capillary lumen (Lu), ZG (1), transitional layer (2), OZF cells rich with mitochondria (2). Few lipid droplets are seen in the ZG (arrow), 960X.

Figure 23. Higher magnification of Figure 21. Cords of the IZF among which capillaries are wide open (Lu). Cavitation with inner membranous in a cortical cell is seen (arrow), 960X.


CAP
Lu

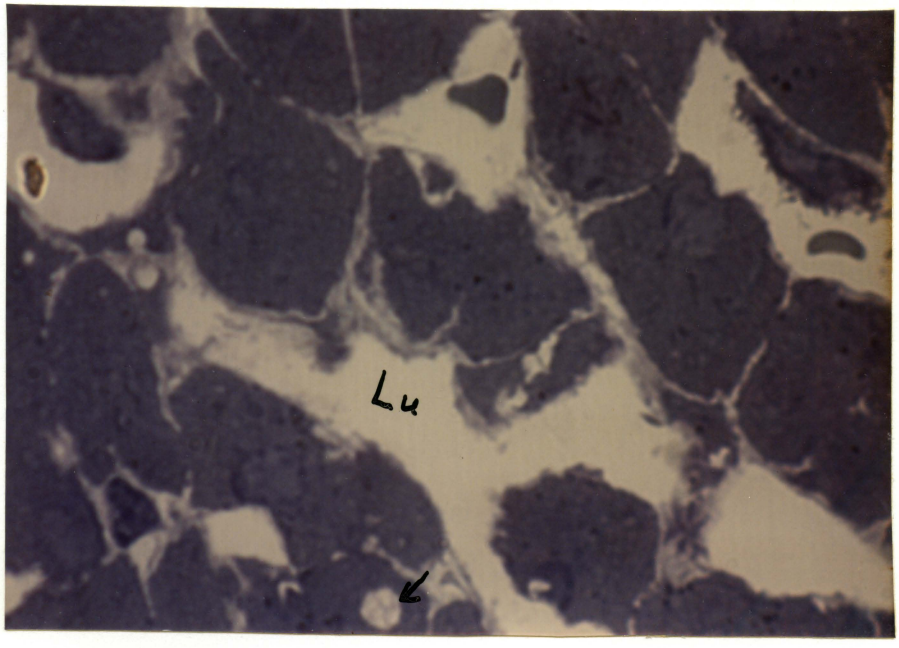
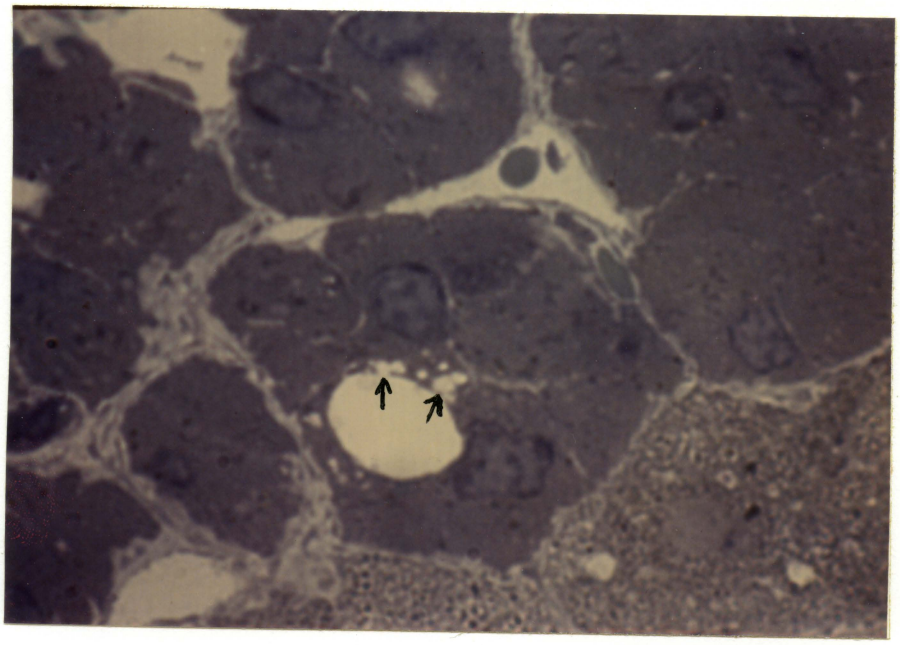

Lu

Figure 24. Higher magnification of Figure 21, ZR cells arranged in "nest" adjacent to the dark staining granules containing medullary cells. A cellular cavity is divided by membranous structures (arrows), 960X.

Figure 25. Higher magnification of Figure 21. Some of the ZR cells are long and narrow (arrows). A part of the medulla is seen in the right lower corner of the field, 960X.



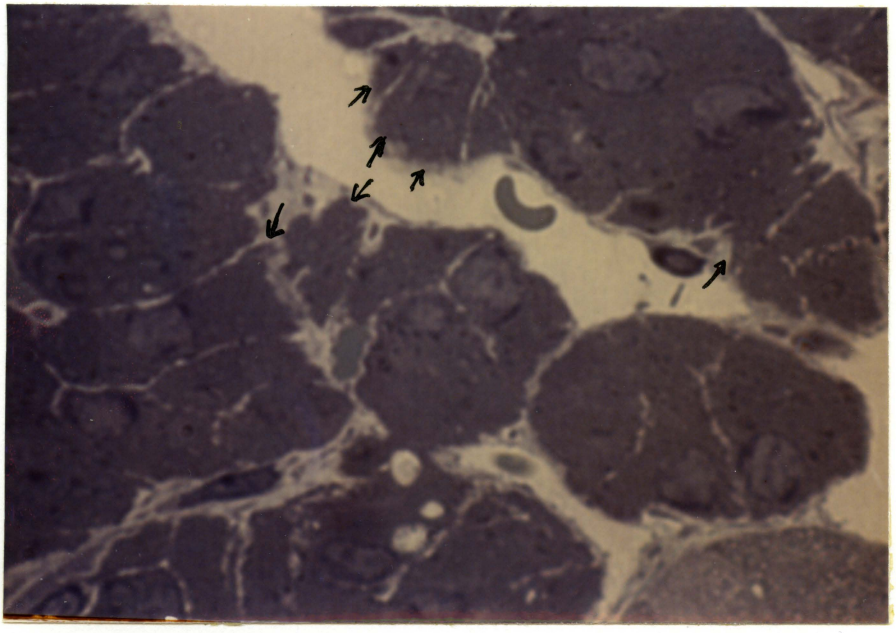


Figure 26. Epon embedded 0.5-1.0 μ section stained with TB of adrenal cortex from hamster killed 4 days after Predef 2X withdrawal. Capsule (1), ZG (2), transitional layer (3) and OZF (4). Lipid droplets are large in the ZG cells and almost absent in the OZF, 560X.

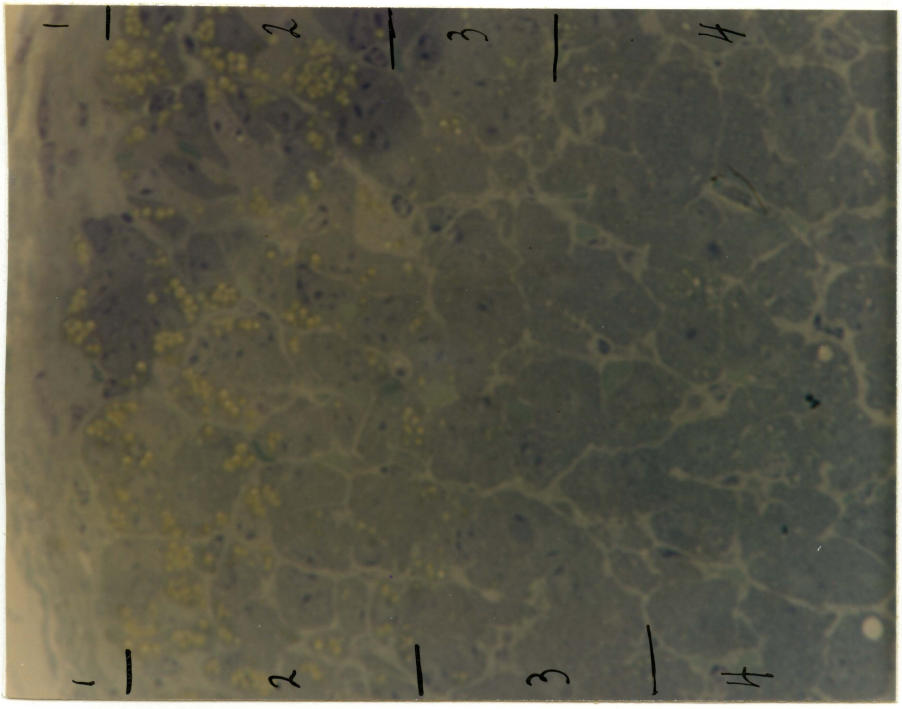
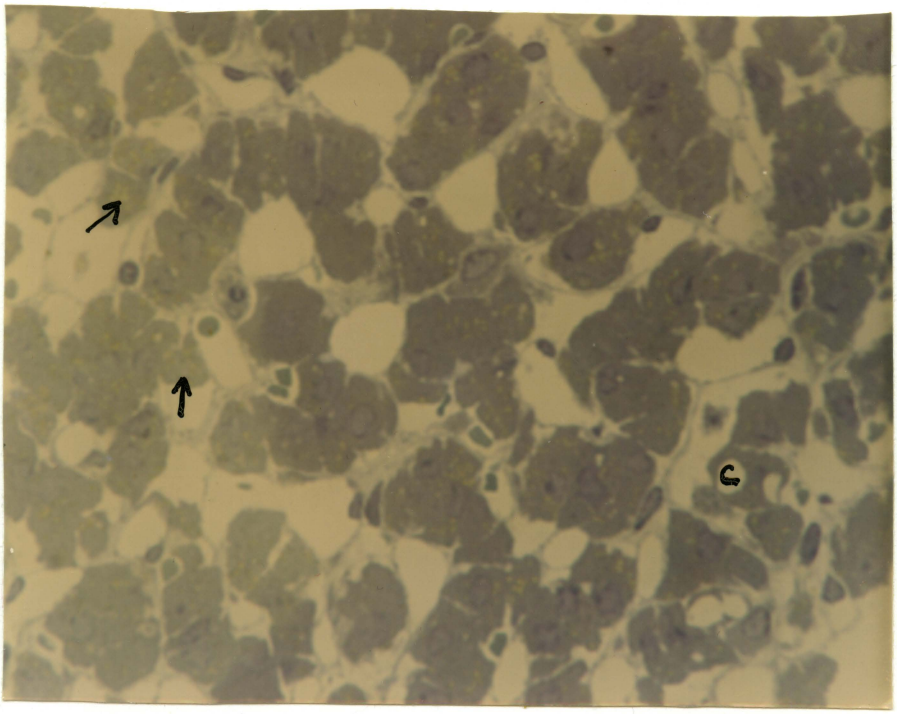
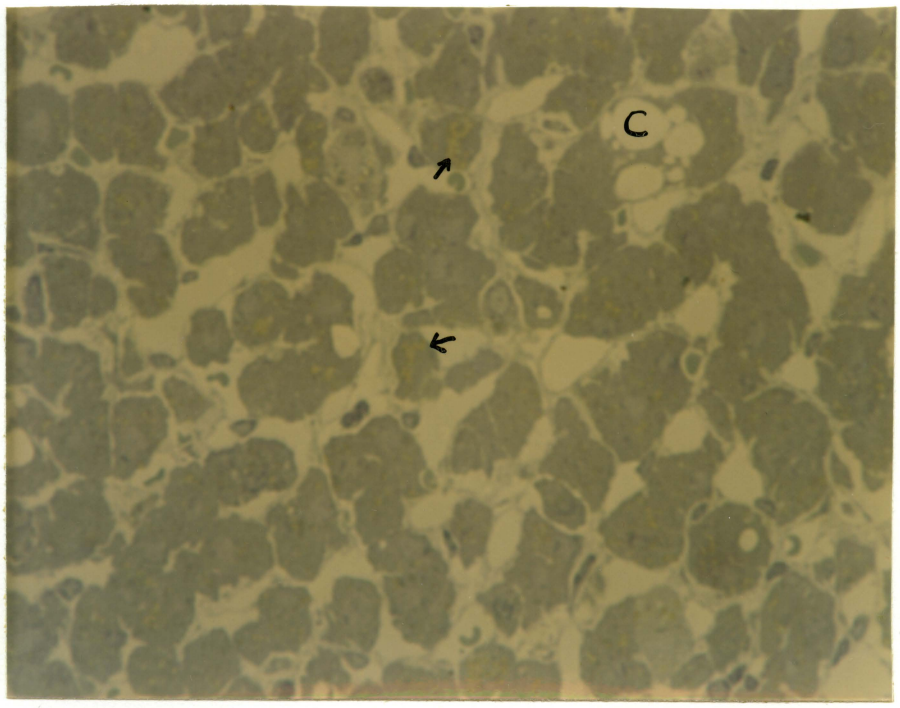

1
2
3
4
1
2
3
4

Figure 27. IZF cells from same section as in Figure 26. Cells contain cavities (C) and lipid droplets (arrows), 560X.

Figure 28. ZR cells from same section as in Figure 26. Cells contain cavities (C) and lipid droplets (arrows), 560X.


C


C

Index of Abbreviations Used in Electronmicrographs

D	Membrane bound dense body
En	Endothelial cell
G	Golgi body
GER	Granular endoplasmic reticulum
L	Lipid droplet
Lu	Capillary lumen
M	Mitochondrion
Mv	Microvilli (cellular projections)
N	Nucleus
R	Ribosomes
RBC	Red blood corpuscle
SER	Smooth endoplasmic reticulum
SES	Subendothelial space
V	Vacuole
W	Whorled membranous structure

Figure 29. An electronmicrograph of a zona glomerulosa cell from a control hamster. Mitochondria are abundant and seen in various planes of section (1, 2, 3, 4, 5). Mitochondria in juxtaposition with SER are indicated (small arrow heads). Fine basement membrane is seen (large arrow head), 13,000X.

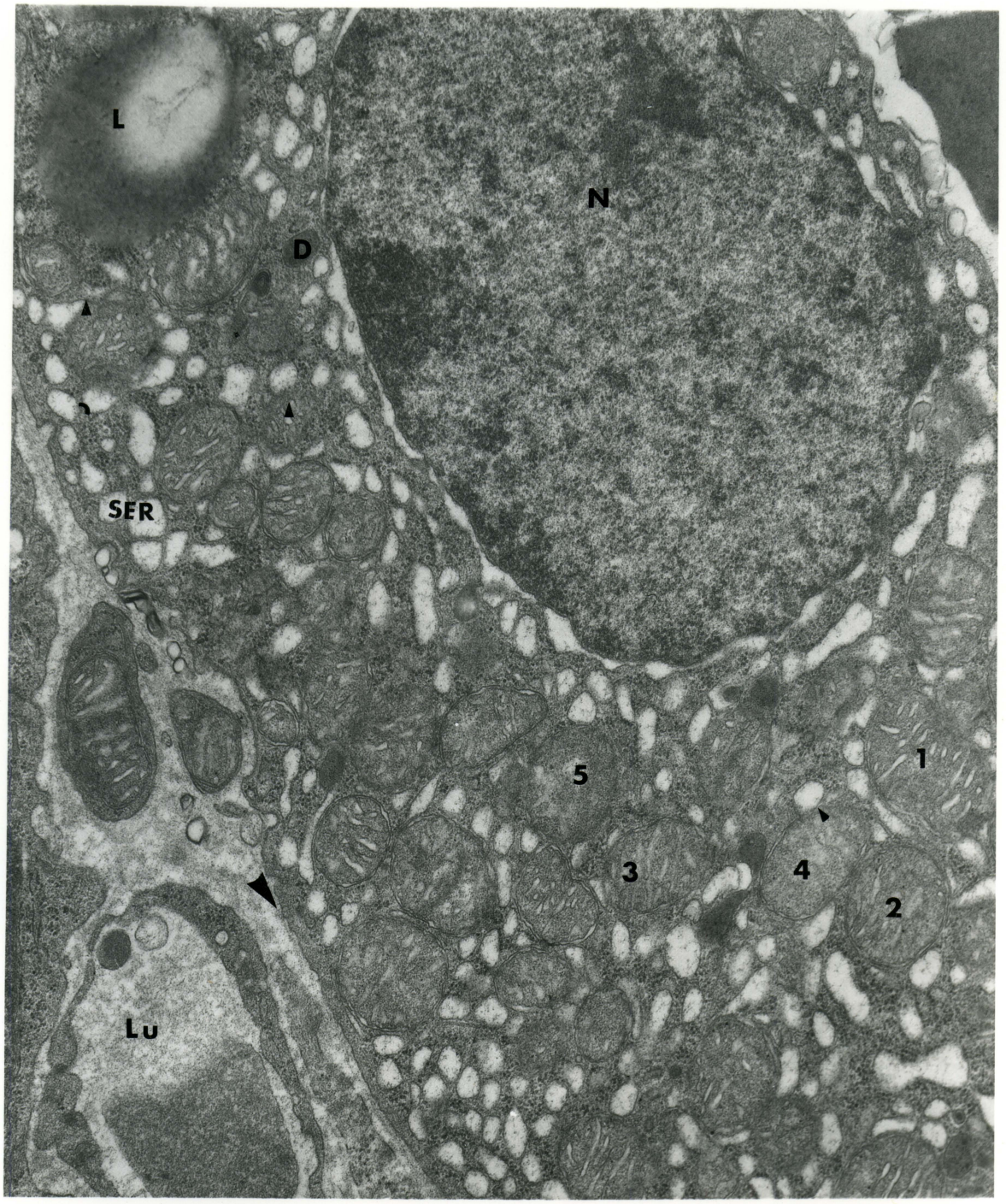

L
N
D
SER
5
1
3
4
2
Lu

Figure 30. An electronmicrograph of the transitional layer from a control hamster. Large arrows point away from the capsule. A gradual change in mitochondrial structure is shown. Mitochondrial shape is less regular, and mitochondrial cristae become more random in arrangement, with increasing numbers appearing tubulo-vesicular in form progressing from 1 through 5. Multilamination of membranous structures surrounding the mitochondria is seen (large arrow head). Relatively smooth and narrow intercellular space is seen between adjacent parenchymal cells (small arrow heads), 13,000X.

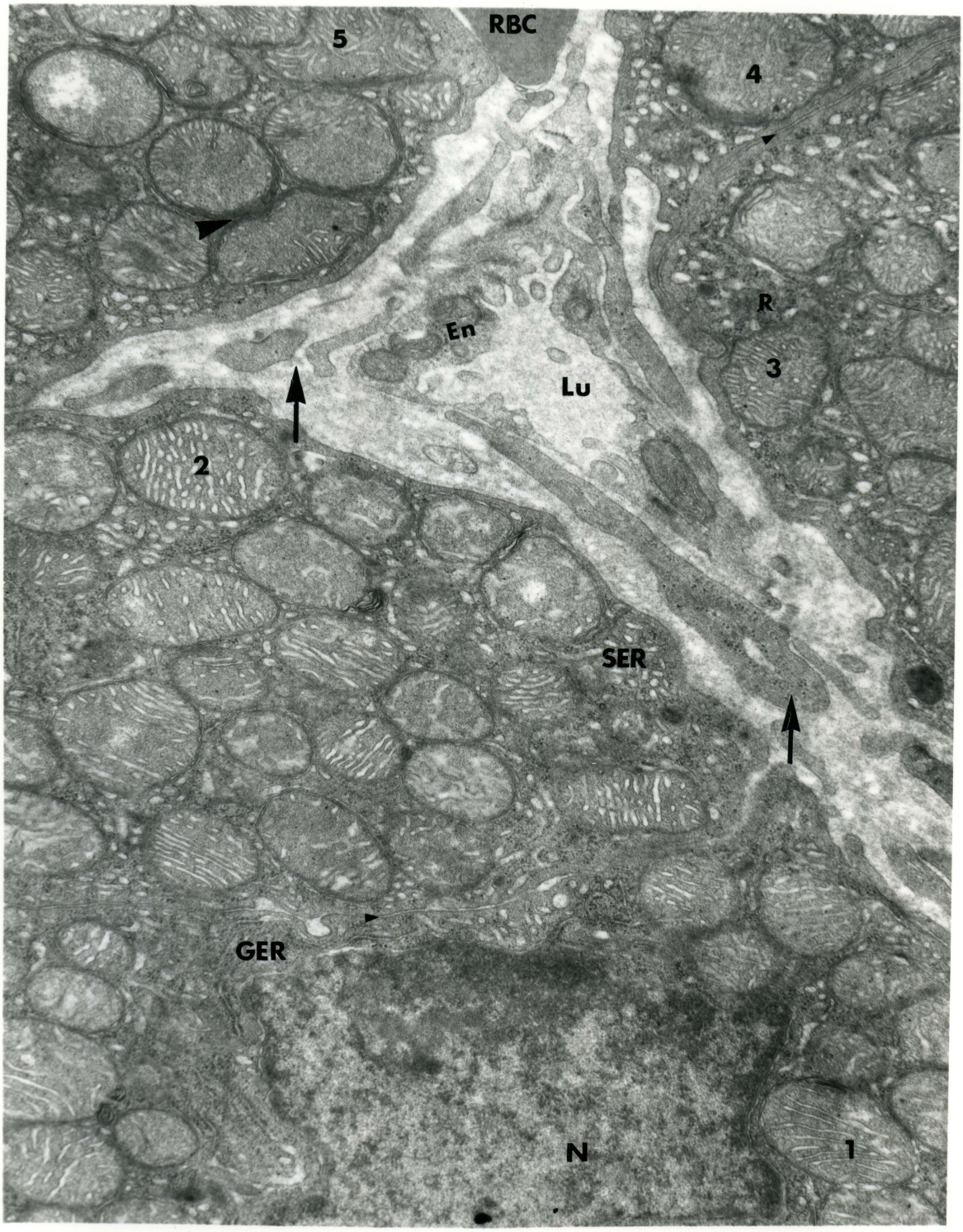

RBC
5
4
R
3
En
Lu
2
SER
GER
N
1

Figure 31. An electronmicrograph of ZG cell 4 days after Predef 2X withdrawal, showing great diversity in mitochondrial structure, 11,500X.

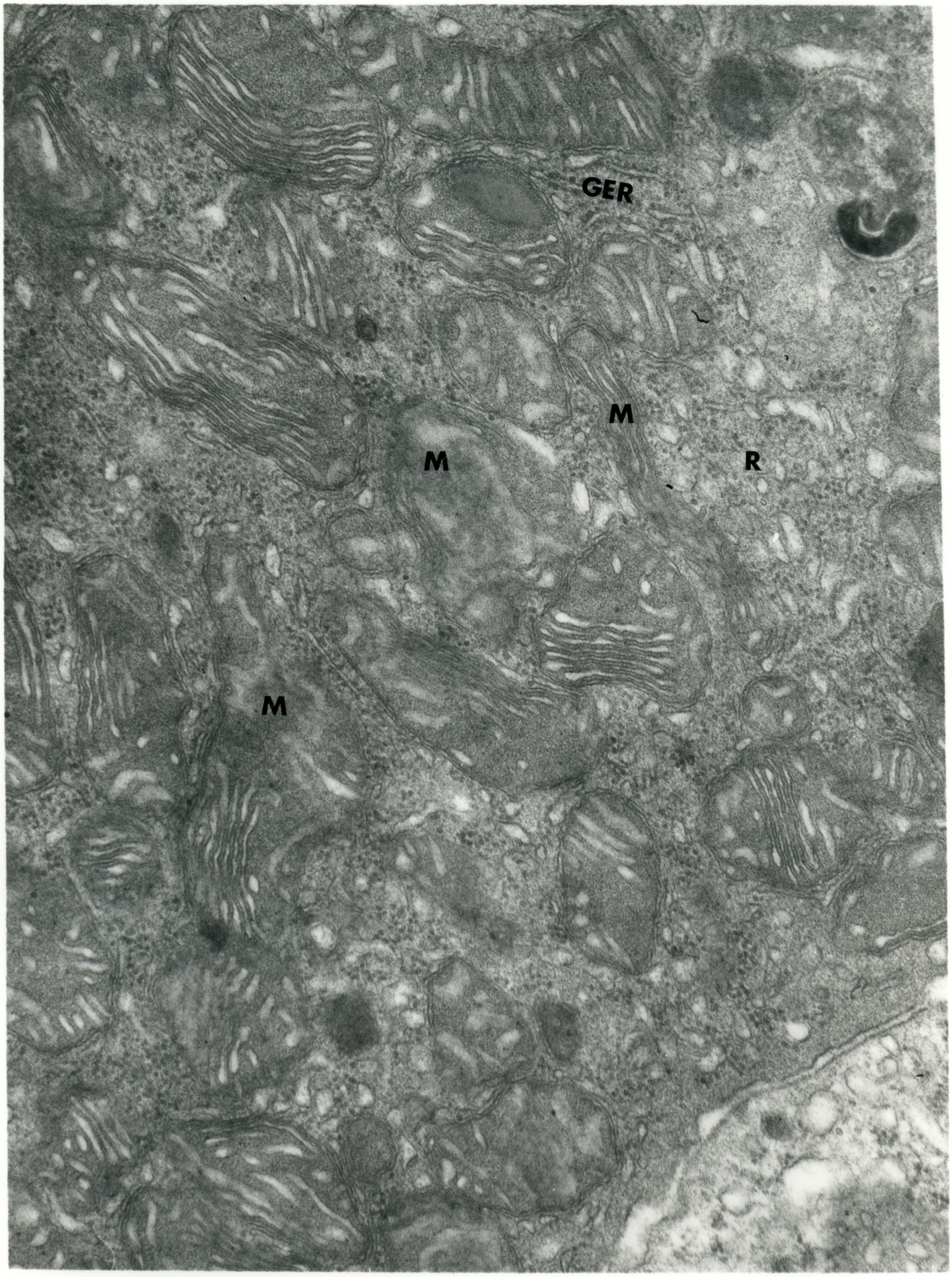

GER
M
M
R
M

Figure 32. An electronmicrograph of a ZG cell 4 days after Predef 2X withdrawal. A mitochondrion with cristae at right angles is seen in the central lower part of the field. Microtubules are indicated by small arrow heads, 23,900X.

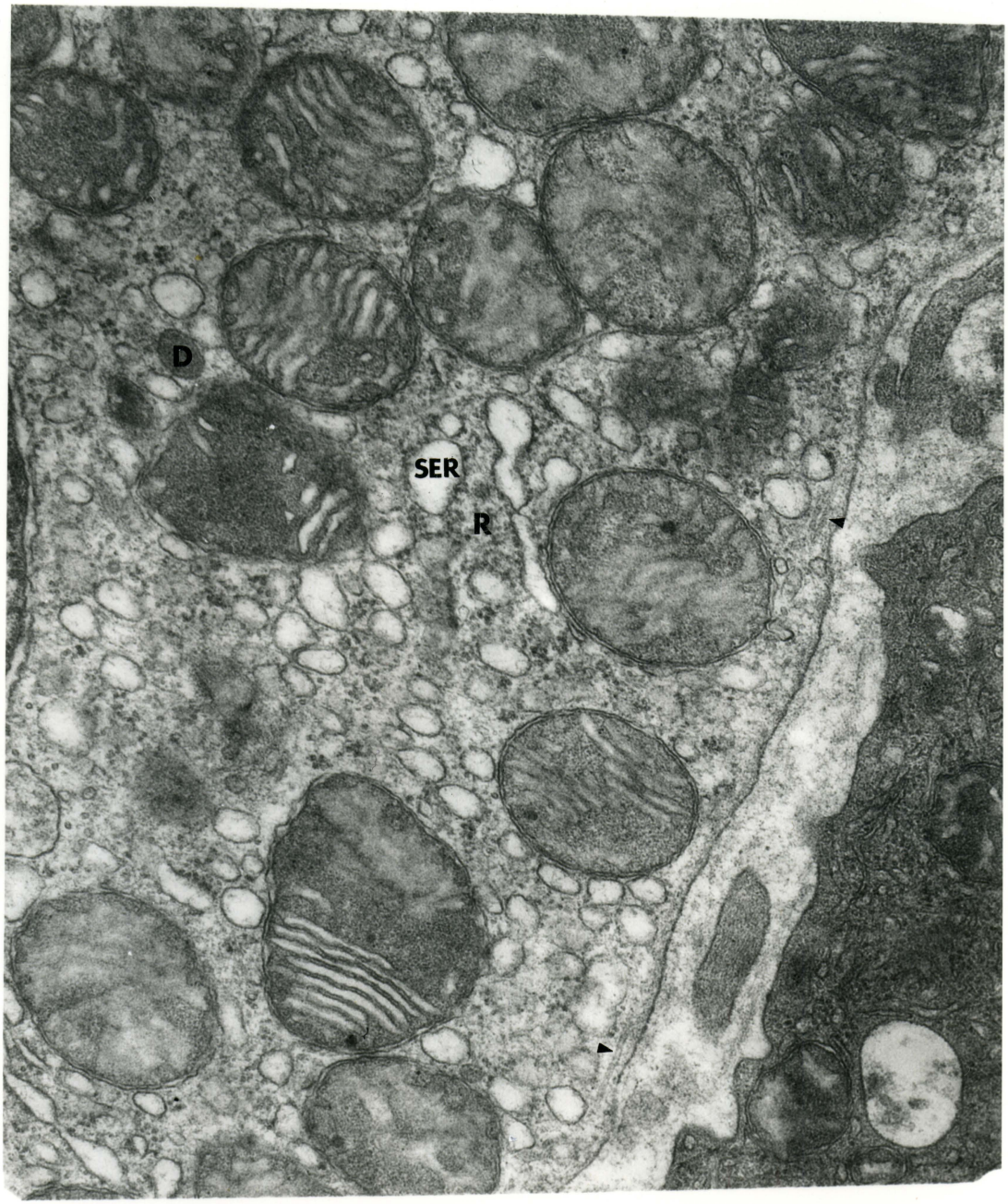

D
SER
R

Figure 33. An electronmicrograph of OZF cells from a control hamster. A corner of a cell which appears to be degenerating (DC) is seen. Most mitochondria are spheroidal but in many areas they are so crowded that their shape is distorted. In some cases one may be deeply indented by an adjacent mitochondrion (1, 2, 3, 4, 5). In some areas the intercellular space is dilated and contains microvilli (cellular projections), 4,980X.

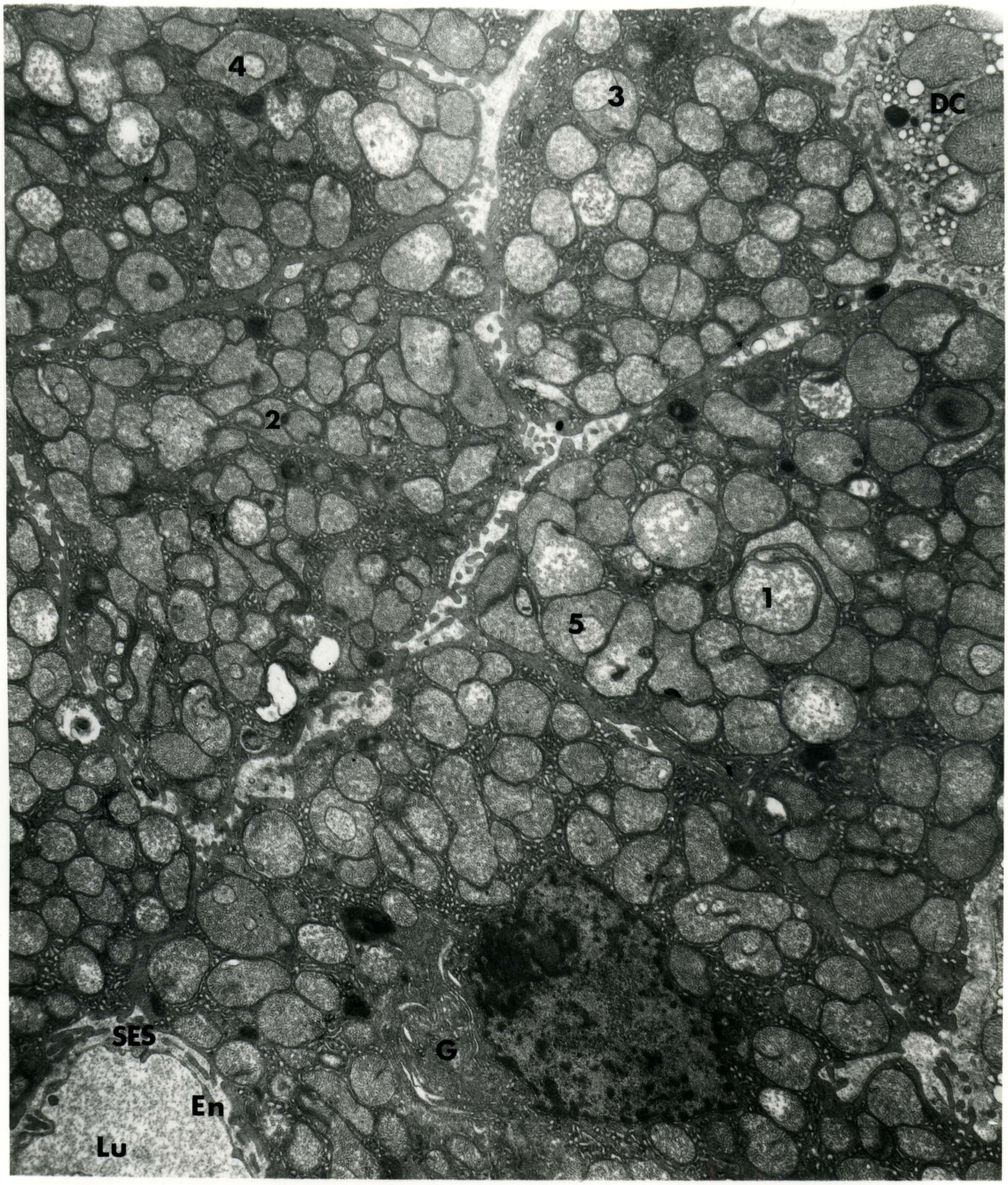

4
3
DC
2
1
5
SES
G
En
Lu

Figure 34. An electronmicrograph of an OZF cell from a control hamster. Profiles of membranes, presumably cristae, are aligned parallel to the outer and inner mitochondrial membranes (arrows). SER and free ribosomes are abundant, 23,400X.

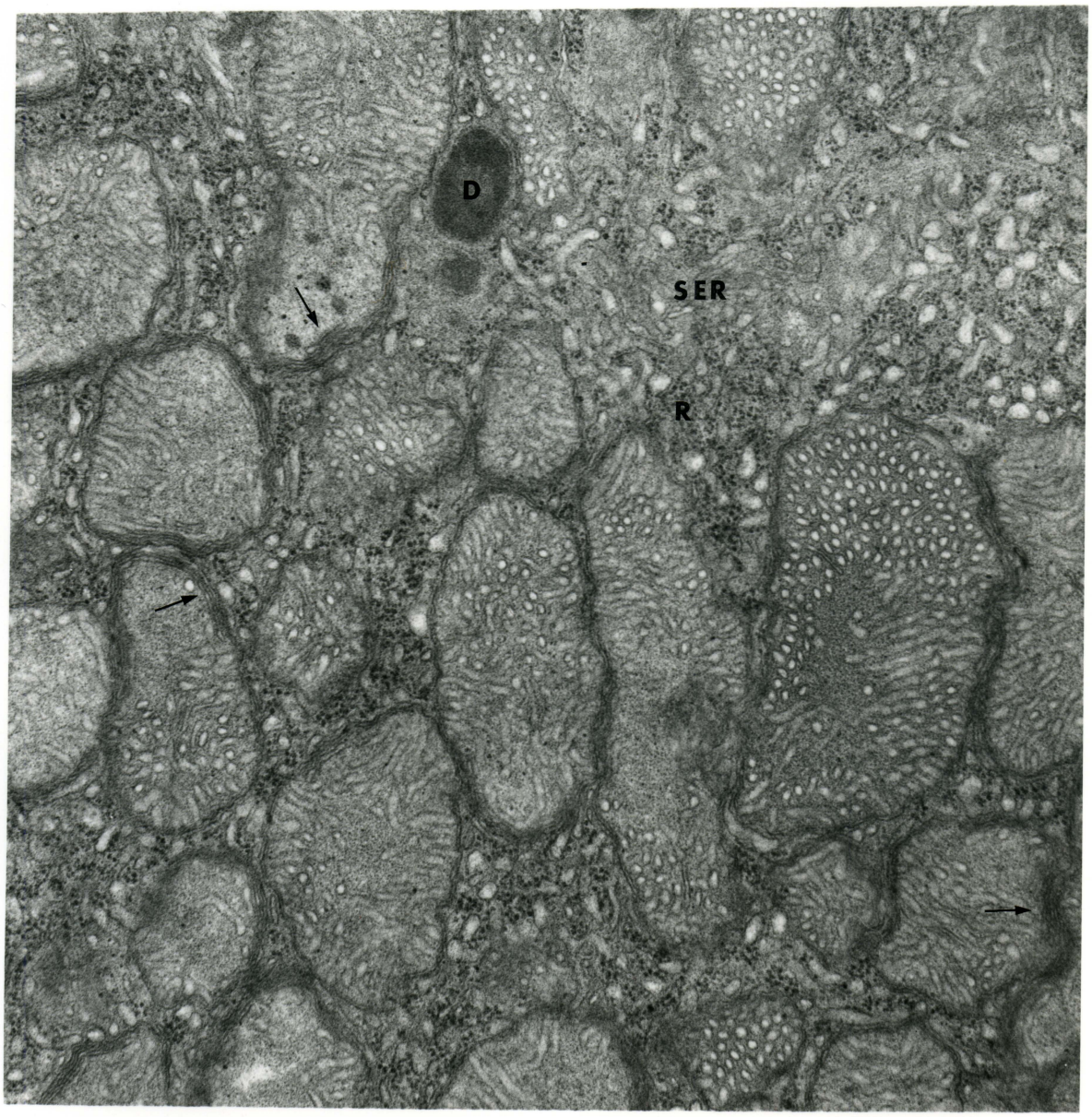

D
SER
R

Figure 35. An electronmicrograph of a vacuole characteristic of OZF cells 24 hr. after Predef 2X withdrawal. Whorled membranes line the structure, which contains a lipid droplet, a mitochondrion and debris, 36,000X.

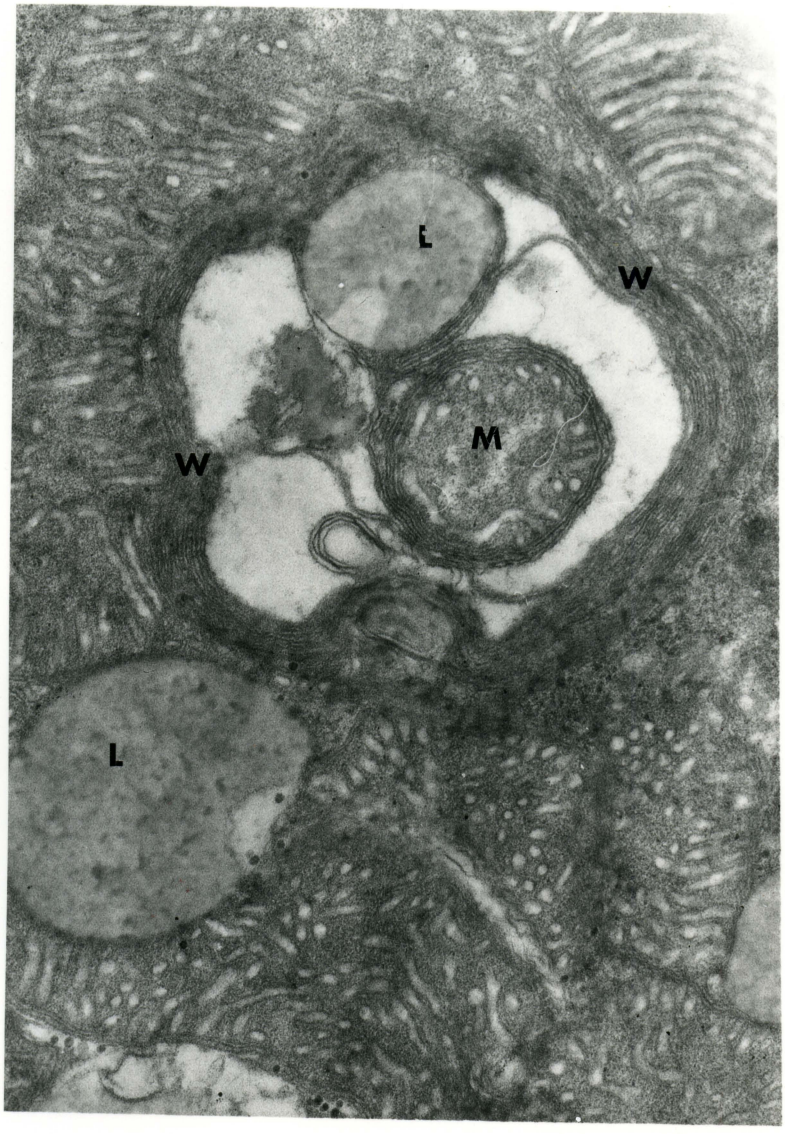

L
W
M
W
L

Figure 36. An electronmicrograph of cells in the OZF, 4 days after Predef 2X withdrawal. Few lipid droplets are seen. The intercellular space at lower right is slightly dilated. Stacks of cristae in parallel alignment are seen in many mitochondria, 10,600X.

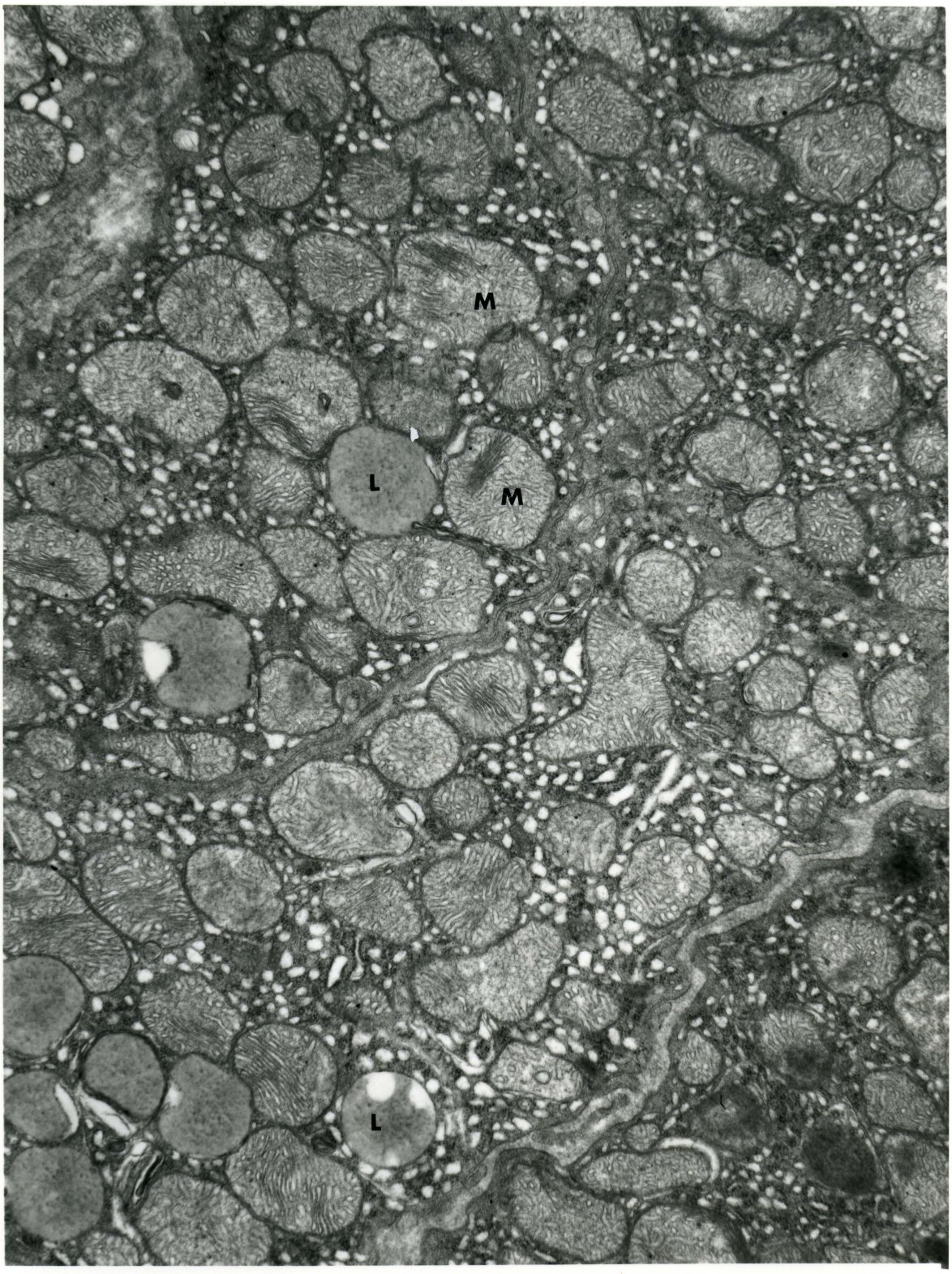

M
L
M
L

Figure 37. An electronmicrograph of an OZF cell, 4 days after Predef 2X withdrawal. The cytoplasm is rich with smooth endoplasmic reticulum and free ribosomes. Microvilli are seen in the intercellular space, 16,800X.

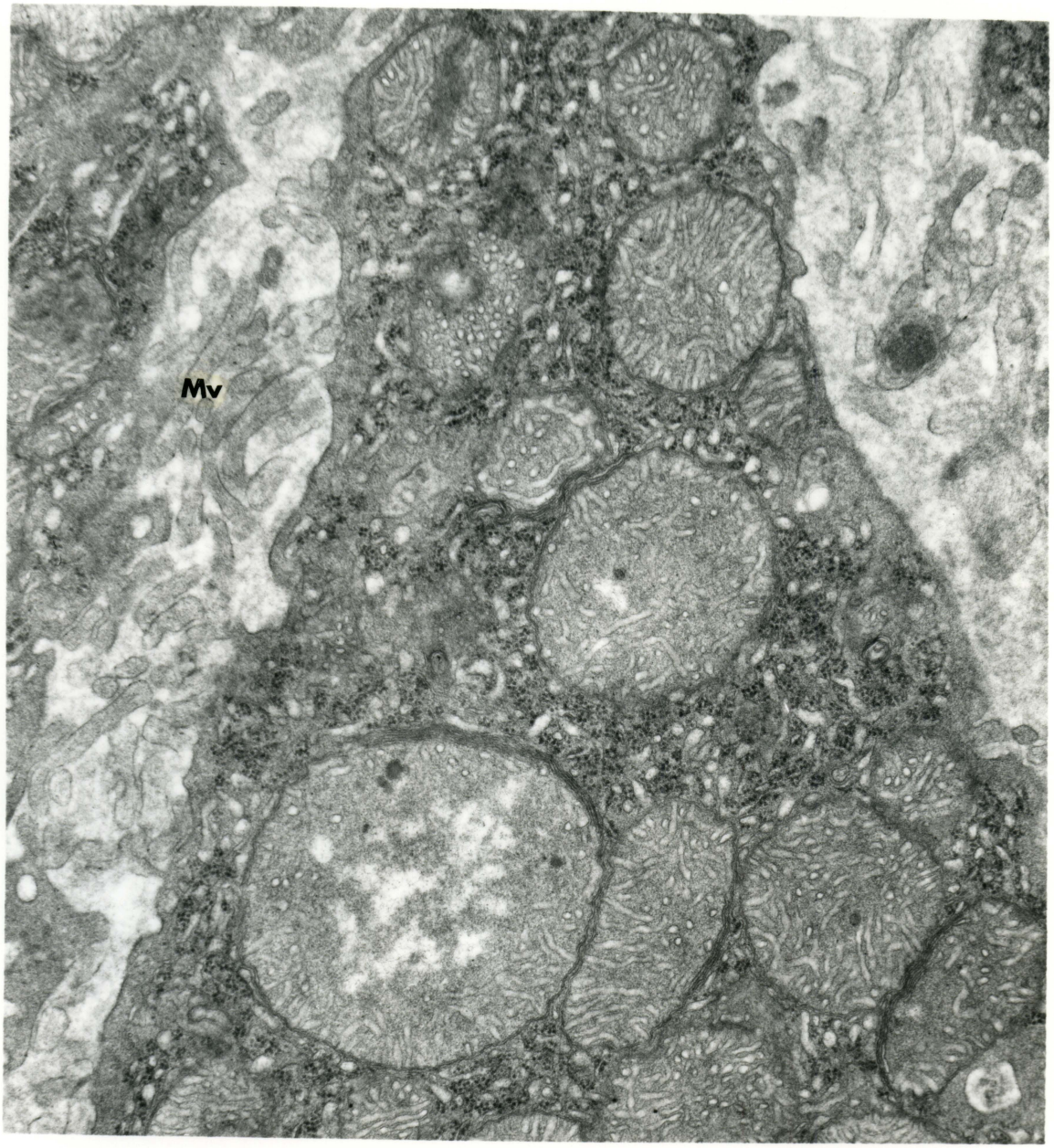

Mv

Figure 38. An electronmicrograph of an OZF cell on day 4 after Predef 2X withdrawal. Ribosomes associated with membranes of endoplasmic reticulum are indicated by an arrow. Mitochondria are crowded. Smooth endoplasmic reticulum, free ribosomes, lipid droplets and membrane bound dense bodies are seen, 23,100X.

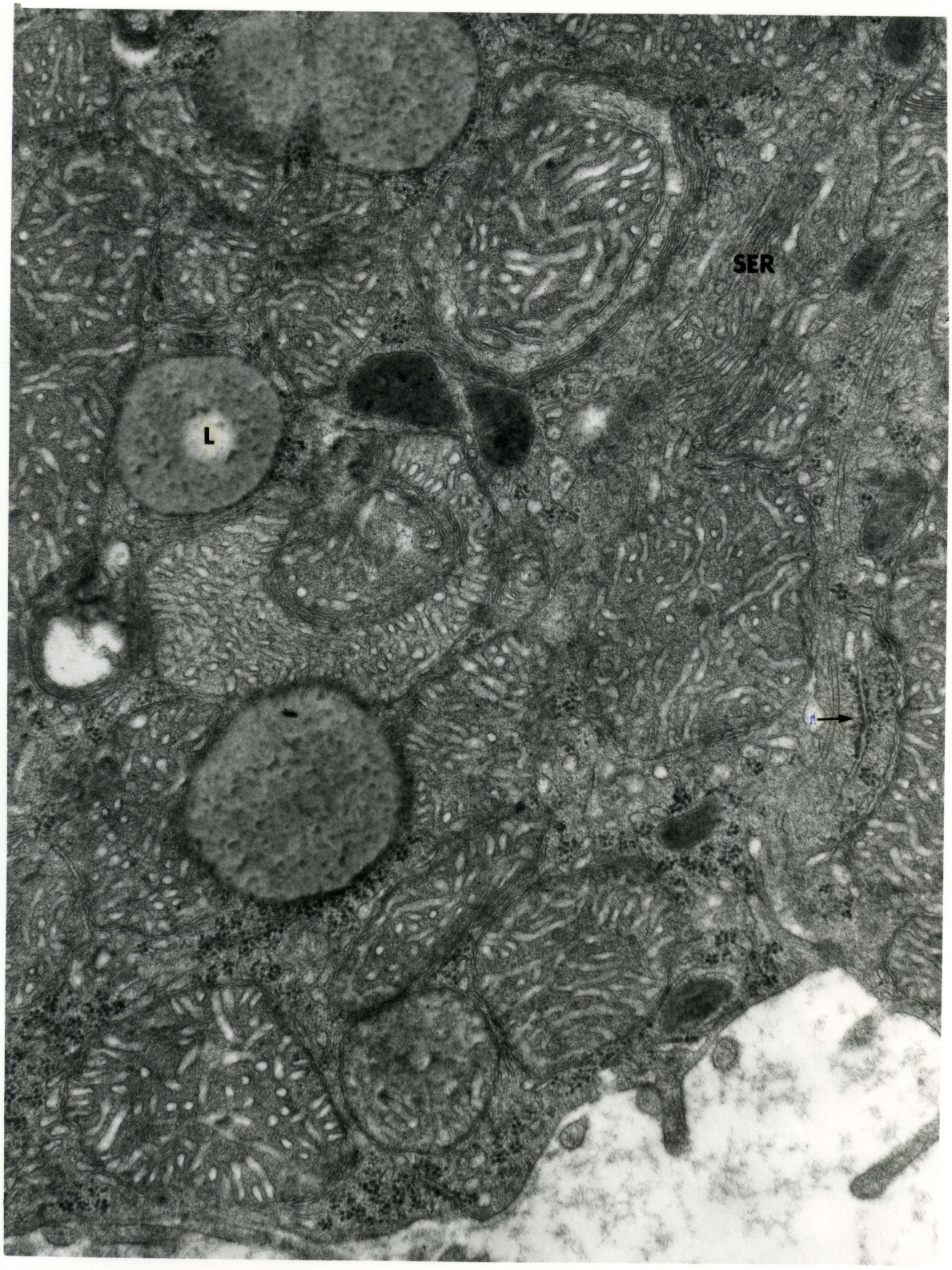

SER
L

Figure 39. An electronmicrograph of an IZF cell from a control hamster. Elaborate systems of parallel membranes of 2 or more adjacent mitochondria are peculiar to inner zones of the cortex. Such a relationship is seen just below the center of this field. The electron density of the mitochondrial matrix is greater than that in the OZF cells, 13,000X.

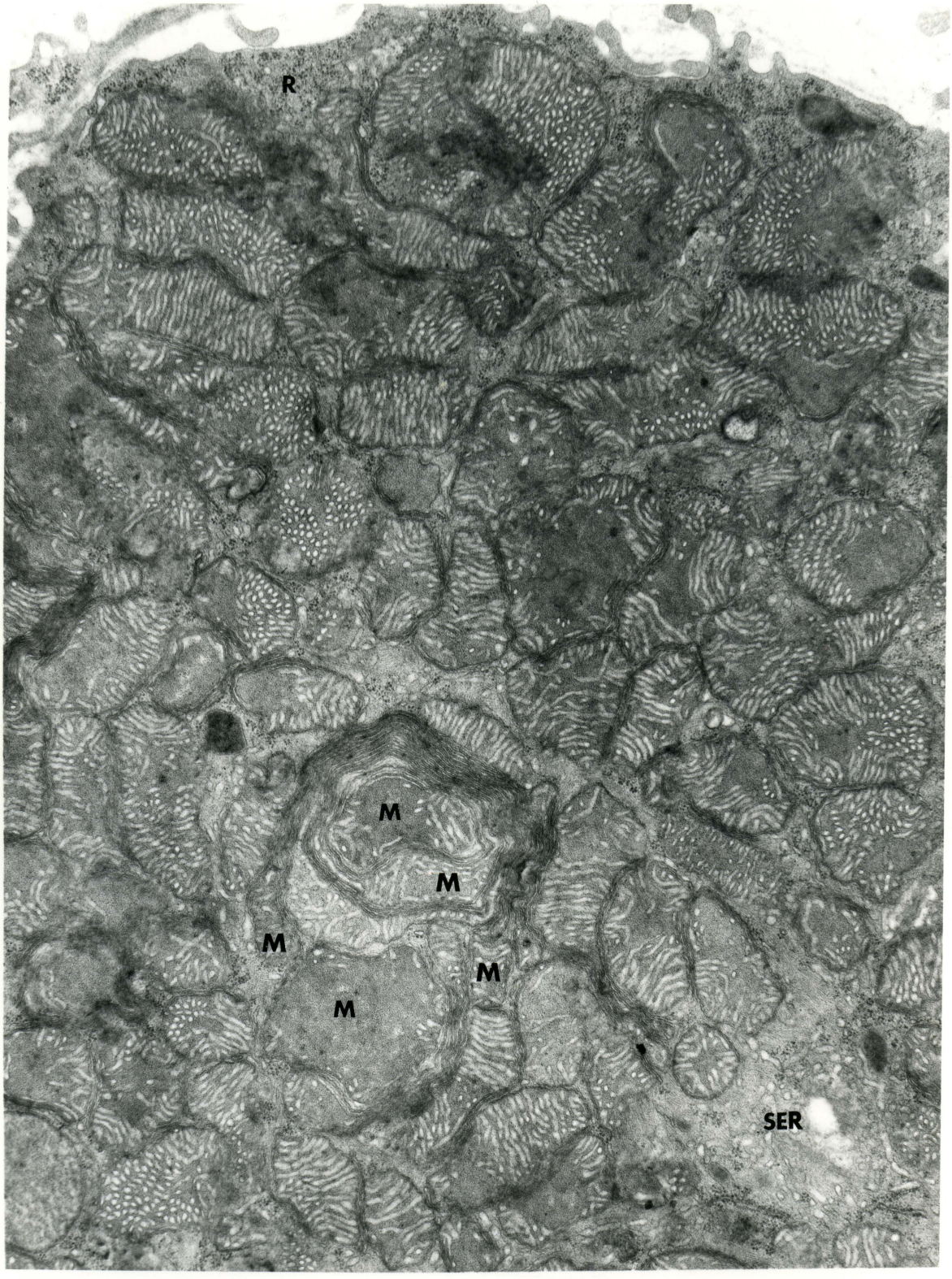

R
M
M
M
M
M
SER

Figure 40. An electronmicrograph of an IZF cell 4 days after Predef 2X withdrawal. Parallel membranes involving mitochondria encircle what is believed to be a mitochondrion cut tangentially through one of its cristae. The cytoplasm contains smooth endoplasmic reticulum and many free ribosomes, 20,000X.

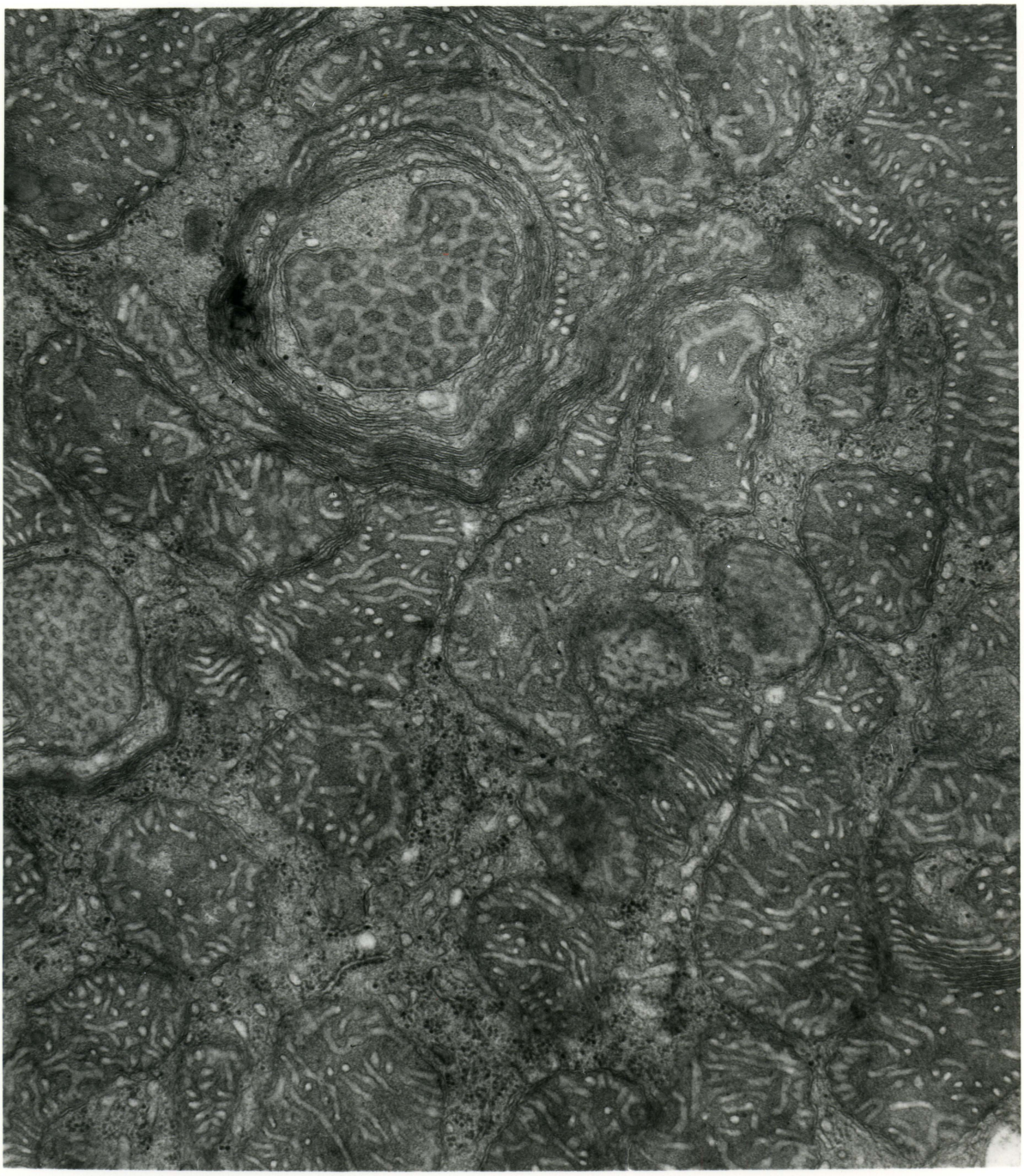


Figure 41. A high magnification of a portion of an IZF cell collected 4 days after Predef 2X withdrawal. The highly ordered relationships found between certain mitochondria in the inner zones of the cortex is shown, 38,600X.

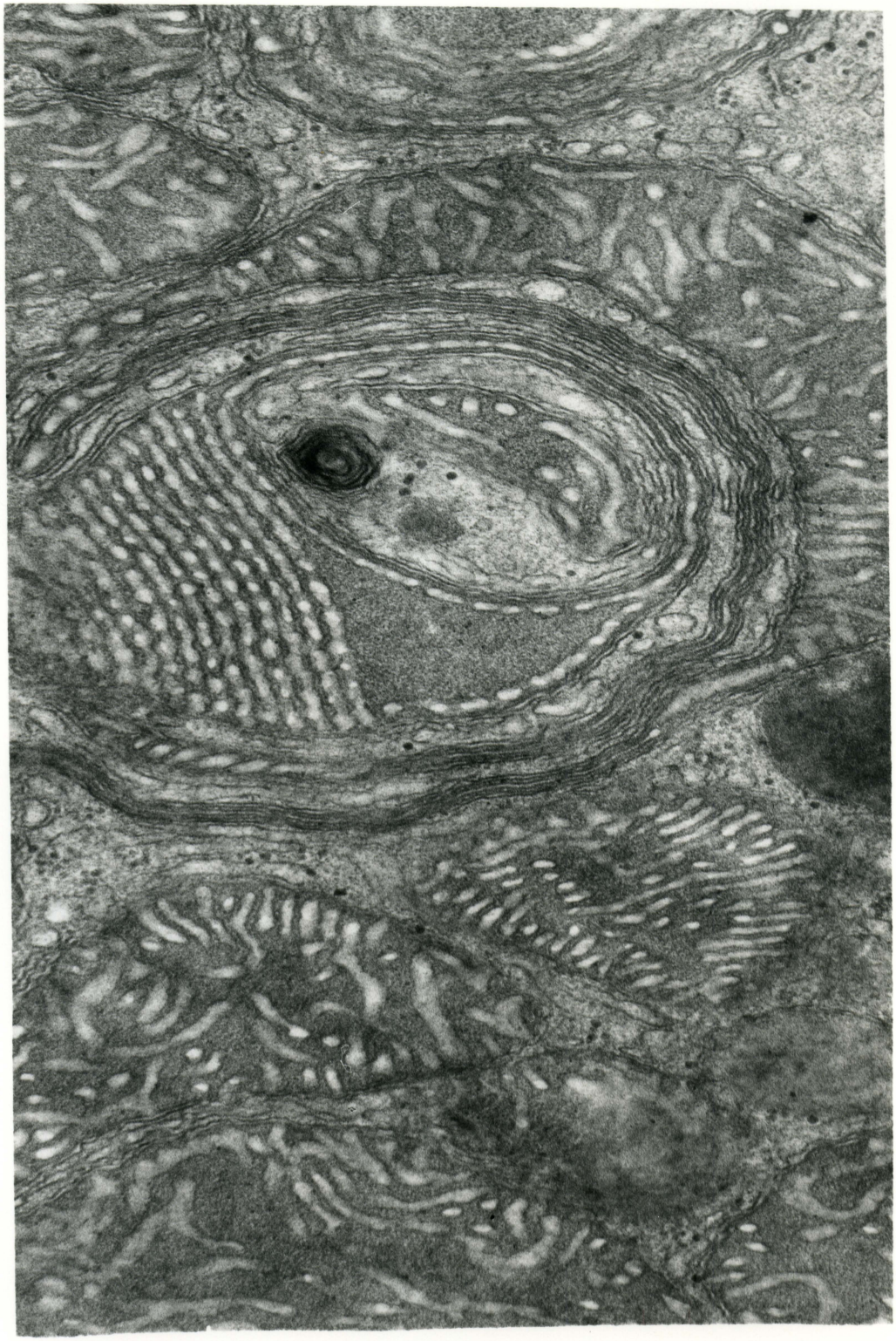


Figure 42. An electronmicrograph of a ZR cell from a control hamster. The mitochondria occupy a major portion of the cytoplasm, 12,750X.

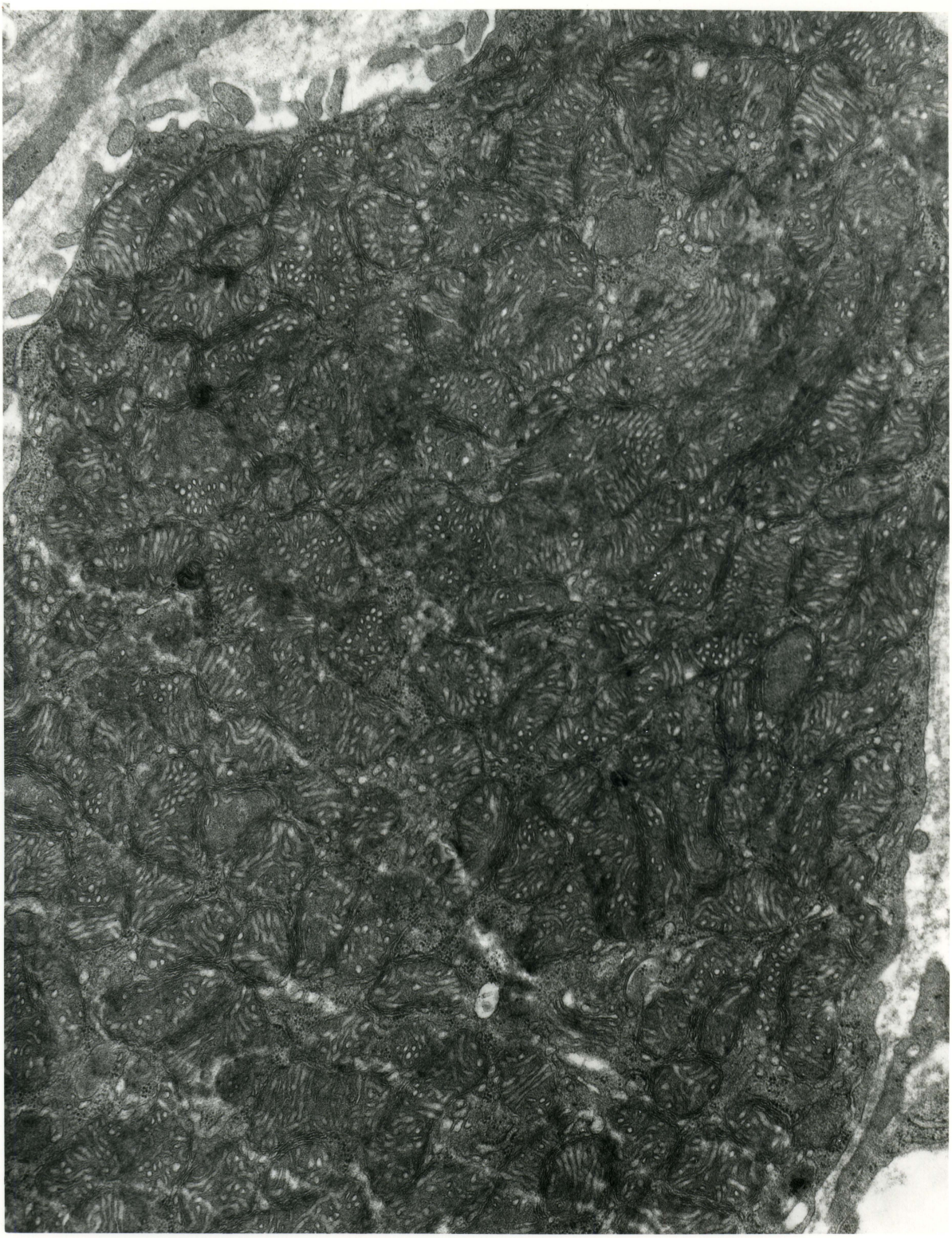


Figure 43. An electronmicrograph of a ZR cell 24 hr. after Predef 2X withdrawal. Lipid droplets are common. Areas resembling sites of focal degeneration are present in the right side of this field, 13,600X.

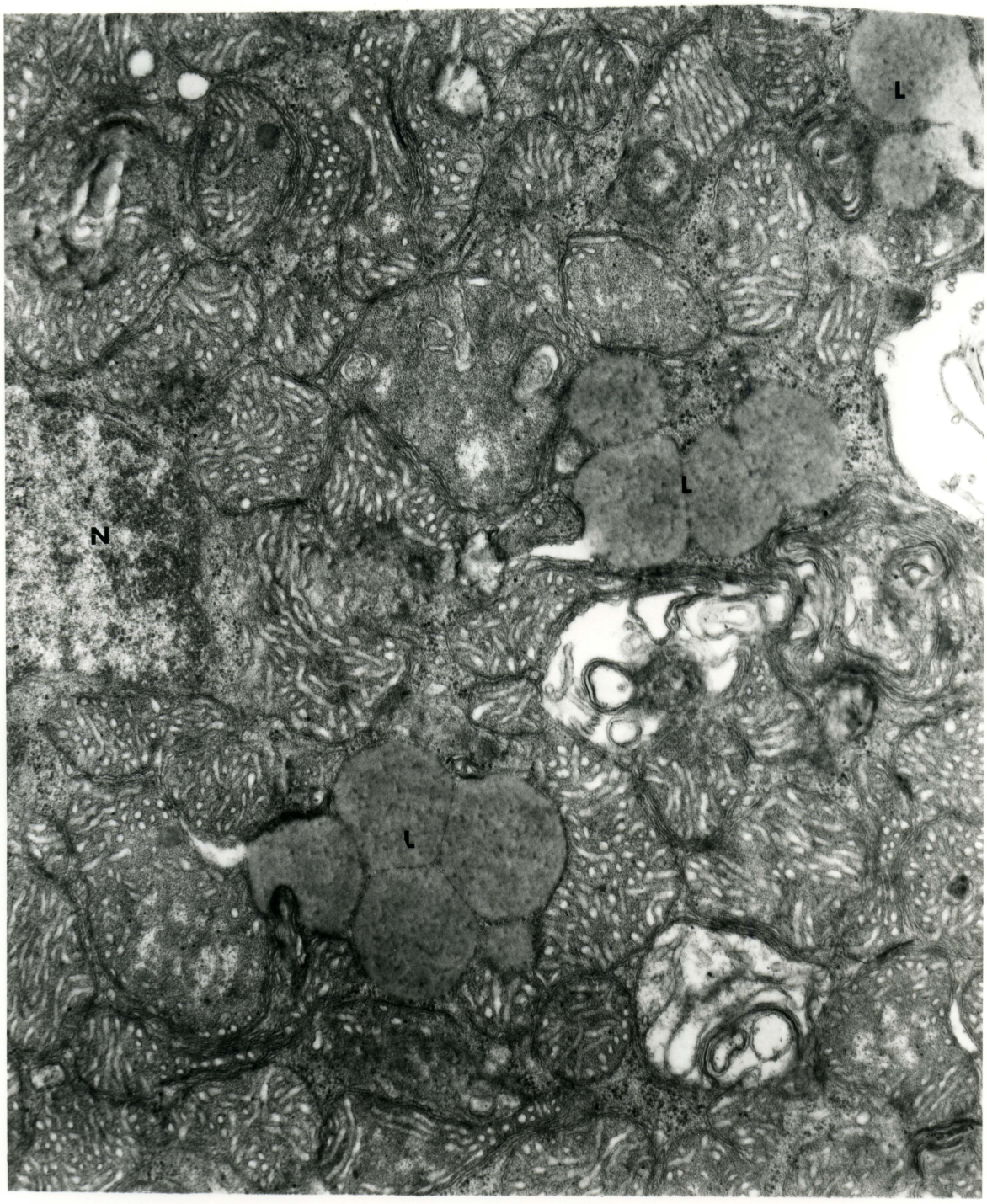
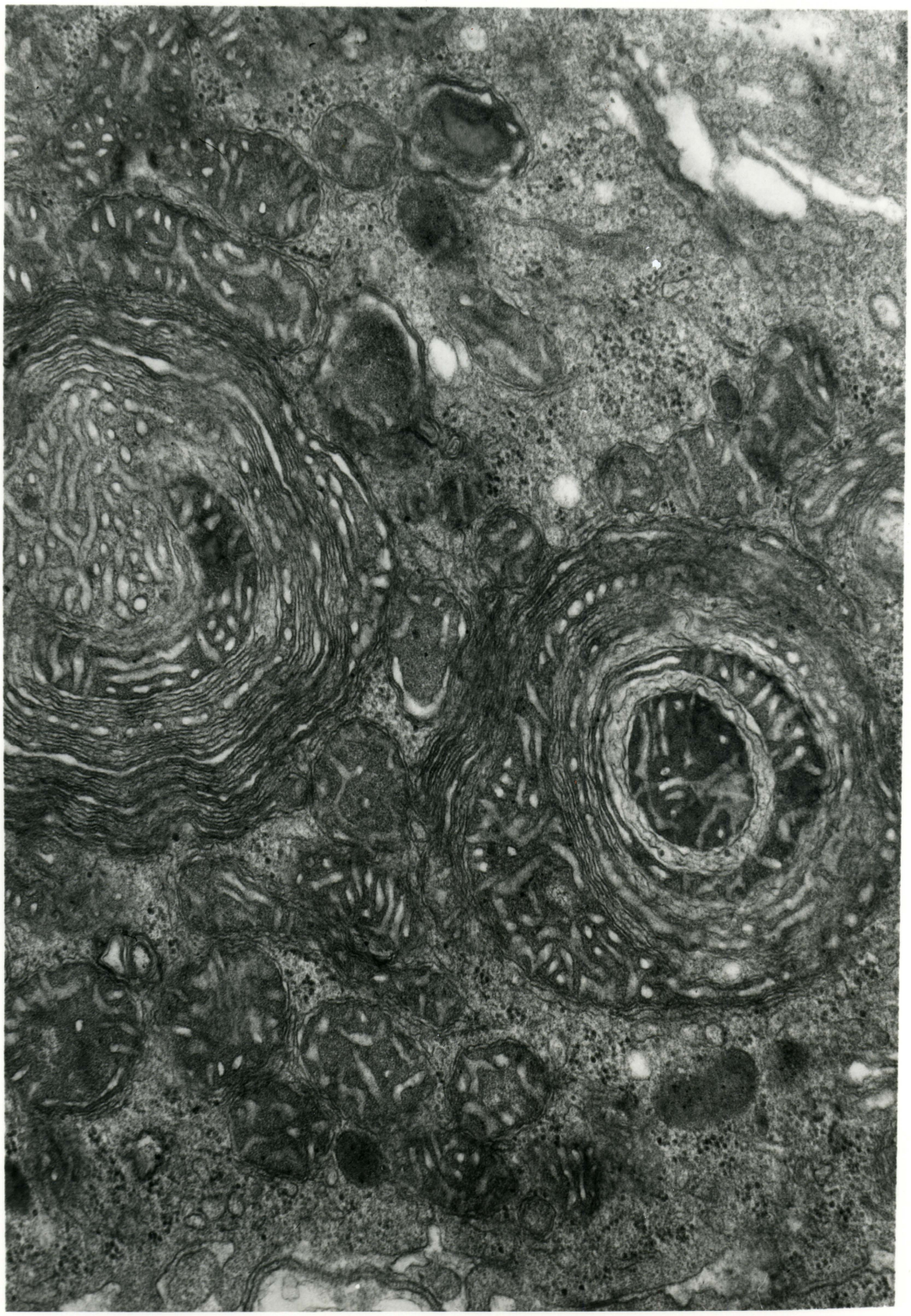

L
L
N
L

Figure 44. An electronmicrograph of the ZR on day 4 after Predef 2X withdrawal. A phagocytic cell (bottom 1/3) adjacent to a ZR parenchymal cell (upper 2/3) is shown. The phagocyte contains several vacuoles. Some are large and contain membranous structures. Material which may be early stages of lipofuscin (LF) is seen. The phagocyte has thin elongated projections (arrow heads) which are interpreted to be involved in phagocytosis, 16,240X.


G
V
LF
V
GER

Figure 45. An electronmicrograph of elaborate relationships between mitochondria in a ZR cell on day 4 after Predef 2X withdrawal is shown, 24,500X.



SUMMARY

Young-adult male Syrian golden hamsters (Cricetus auratus) received toxic treatments of 9-fluoroprednisolone acetate (Predef 2X) and the recovery period until day 7 post-withdrawal was studied. Steroid-induced changes in body weight, postmortem muscle pH, light microscopic features of the adrenal cortex, skeletal and cardiac muscle, and the ultrastructural features of the adrenal cortex were compared with those of control animals.

In both experimental and control hamsters five adrenocortical zones were distinguished in 0.5-1.0 μ thick Epon sections stained with toluidine blue and in adjacent ultrathin sections examined under the electron microscope. These were zona glomerulosa (ZG), transitional zone, outer zona fasciculata (OZF), inner zona fasciculata (IZF) and zona reticularis (ZR).

The treatment by the glucocorticoid was followed by an accumulation of lipid in the adrenal cortex. Accumulation was least in the OZF. Only this layer recovered from effect of the steroid. It was concluded that the OZF and the IZF are functionally distinct. The OZF was interpreted as being more susceptible to ACTH stimulation and more resistant to glucocorticoid suppression than the IZF.

Treatments were followed by adrenocortical necrosis, infiltration by polymorphonuclear cells, early deposition of a

substance believed to be lipofuscin and an increase in phagocytic activity and cellular cavities. These changes were more marked in the IZF and the ZR. The relative absence of these changes by day 4 post-withdrawal was interpreted as evidence of recovery. The possibility that high doses of glucocorticoids could accentuate adrenocortical changes related to ageing was suggested. The golden hamster may serve as an adequate laboratory model for stress susceptibility and resistance to disease as related to adrenocortical ageing change.

Cellular cavities were most evident on day 3 post-withdrawal. The presence of these cavities would seem to support the concept of endoplasmocrine process of steroid secretion, as put forth by Rhodin. The possibility that a much less vigorous endoplasmocrine secretory process exists in the OZF, IZF and ZR of control hamsters was suggested.

By correlating clinical signs and the observations on the adrenal cortex, it was concluded that days 2-3 post-withdrawal were crucial in the recovery from effect of the steroid. It was suggested that during these days the hypothalamus-anterior pituitary-adrenocortical axis started to recover from the glucocorticoid suppression.

The golden hamster is unique among laboratory animal species in that glucocorticoid treatment induced necrosis in both skeletal and cardiac muscles. The cardiac muscle was more sensitive to the drug.

The treatment induced elevation in postmortem muscle pH. These results were discouraging as far as further use of the golden hamster as a laboratory model for porcine stress syndrome studies and porcine pale soft exudative muscle is concerned.

Body weight loss was more marked in treated hamsters which subsequently died than in the killed ones. Other changes were also more marked in the animals which later died. It is believed that these changes and the fatal outcome are a reflection of the variations in resistance to the glucocorticoid drug.

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