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STUDIES ON SOME FETAL RAT ORGANS FOLLOWING MATERNAL HYPERTHERMIA

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دراسات على بعض أعضاء أجنة الجرذ الأبيض نتيجة لارتفاع درجة حرارة الأمهات

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مما لا شك فيه أن تعرض السيدات الحوامل للارتفاع المفاجىء في درجة الحرارة نتيجة للاصابة بالانفلونزا أو للعدوى بالأمراض المختلفة أمر وارد خصوصاً في الأشهر الأولى من الحمل .

وفي هذا البحث تم دراسة تأثير ارتفاع درجة حرارة الجسم لامهات الجرذ الأبيض في اليوم السابع من الحمل عل المخ والكبد والكلية للأجنة .

وقد قسمت الأمهات الحوامل إلى مجموعتين أحدهما عرضت لدرجة حرارة ٤٥ْم لمدة ساعة (متوسط درجة حرارة الجسم ٤١ْم) أما المجموعة الأخرى فقد عرضت لدرجة حرارة ٥٠ْم لمدة ٢٥ دقيقة فقط (متوسط درجة حرارة الجسم ٤٢,٦ْم) .

وقد وجد أن الارتفاع الكبير في درجة حرارة الجسم لفترة قصيرة له تأثير واضح على الأجنة من الارتفاع الأقل في درجة الحرارة ولكن لفترة أطول . كما لوحظ أن الارتفاع في درجة حرارة الجسم للأمهات له تأثير على عدد ووزن الأجنة بالإضافة إلي ظهور بعض التشوهات في كثير من الأجنة .

وبالدراسات الهستولوجية وجد أن الارتفاع في درجة حرارة الجسم للأمهات أدى إلى صغر سمك القشرة المخية وتحطيم خلايا الطبقة المبطنة للأوعية الدموية المنتشرة بها مما أدى إلى ظهور نزيف دموي ، كما ظهر تكسير واضح في خلايا الكبد والخلايا المبطنة للأيبيبات البولية لهذه الأجنة نتيجة لإرتفاع درجة حرارة الأمهات .

Key Words: Rat, Fetuses, Histological changes, Temperature

ABSTRACT

The present investigation was carried out to determine the histological changes in brain, liver and kidneys of rat fetuses maternally heatstressed at early stage of pregnancy to either high "spiking" temperature of short duration or low temperature of long duration. The number of viable fetuses as well as the fetal weight of the heatstressed groups was significantly reduced compared with corresponding controls. Edema and microphthalmia are the only malformations detected among the viable 18 days fetuses of the two heatstressed groups. The thickness of cerebral cortex of maternally heatstressed fetuses was markedly reduced. Hyperthermia destroyed the endothelial cells of blood capillaries thus resulting a disruptive appearance of the neuroepithelial tissue. Hyperthermia exerted degeneration of hepatocytes, dilation of sinusoids and destruction of vascular endothelium of developing liver. Moreover, server changes were observed in the cortical region of the kidneys of maternally heatstressed fetuses. Some glomeruli were atrophied while the tubular system showed fatty degeneration, clowdy swelling and necrosis of tubular epithelium.

INTRODUCTION

Maternal hyperthermia has been shown to disrupt normal embryonic development in different species (Warkany, 1986). The central nervous system is particularly sensitive to elevated temperature. Studies dealing with the effect of hyperthermia are confined largely to the neural tube defects and/or embryo/fetal malfunctions in humans (Hakosalo and Saxen, 1971; Chance and Smith, 1978; Halperin and Wilroy, 1978; Miller *et al.*, 1978; Smith *et al.*, 1978; Fisher and Smith, 1981; Shiota, 1982; Spraggett and Fraser, 1982), rats (Skreb and Frank, 1963; Arora *et al.*, 1979; German *et al.*, 1985; Mirkes,

1985; Webster *et al.*, 1985; Walsh *et al.*, 1987; El-Shabaka and Emad, 1990, mice (Finnell *et al.*, 1983, Webster and Edwards, 1984; Chernoff and Golden, 1983), guinea pigs (Edwards, 1967, 1969 a and b), hamsters (Kilham and Ferm, 1976; Umpierre and Dukelow, 1977), chickens (Nilsen, 1984) and Bonnet monkeys (Hendrickx *et al.*, 1979).

The highest incidence of neural tube defects was reported in Egypt (Myrianthopoulos, 1979). However, the contributing factors involved in the neural tube defects remain poorly understood. Maternal hyperthermia, especially at early stage of pregnancy, due to viral infection, acute febrill illness, urinary tract infection, working in laundries, saunas or even the high temperature of summer season, may be one of the many factors responsible for normal tube defects.

Many parameters have been used by several investigators to determine teratogenicity of hyperthermia. These are temperature elevation, duration of elevation and the number of spacing of exposures. Among these parameters, the temperature elevation/duration combination is the most important (Germain *et al.*, 1985). Moreover, gastrulation is necessary not only for the formation of three-layered embryo but for induction of the central nervous system and related ectodermal placodes of the head which contribute to the sense organs and elements of the peripheral nervous system (Webster *et al.*, 1985). hence, the present investigation is carried out to determine the histological changes in some fetal organs following exposure of rats at an early critical stage of pregnancy (pregastrulation stage) to either high "spiking: temperature of short duration or low temperature of long duration.

MATERIAL AND METHODS

Virgin white rats of about 250 gm were used. Females were kept with males for 2 hours in the early morning. Vaginal smears were then examined and the presence of sperms indicated that mating had occurred and the date was considered as the 0 hour on day 0 of gestation.

According to the previous result (El-Shabaka and Emad, 1990), the 7th day of gestation is the critical stage to hyperthermia in rats. At the same stage, pregnant rats were divided into 2 groups 12-14 each. The first group (12 pregnant rats) was heatstressed for 60 minutes in an electric oven adjusted at 45°C (then mean poststressing rectal temperature was 41.3°C). The second group (14 pregnant rats) was heatstressed for only 25 minutes in the same electric oven set at 50°C (the mean poststressing temperature was 42.6° C). In addition, 20 control rats for groups I and II (10 pregnant rats each) are exposed to a normal temperature (38°C) in the oven for 60 and 25 minutes (the mean poststressing temperatures were 38.1°C and 38°C respectively). The rectal temperature for the two experimental groups as well as controls was measured immediately before and after each heating period.

On day 18 of gestation, the mothers were sacrificed by decapitation, dissected and the fetuses removed. The surviving and dead fetuses as well as resorptions were counted. Most survivors were weighed and examined for external abnormalities. Some living fetuses were dissected and brain, liver and kidneys are fixed in Bouin's fluid for 24 hours. Fixed tissues were dehydrated, cleared, embedded in paraffin, sectioned at 7 microns and stained with hematoxylin and eosin.

The significance of the differences between controls and heatstressed embryos was determined using student t-test.

RESULTS

Survival, resorption and fetal weight:

Table (1) summarizes the data obtained after 18 days of gestation in both control and maternally heatstressed fetuses of groups I and II. The number of viable fetuses obtained after 18 days of gestations from maternally heatstressed dams (groups I and II) was significantly reduced ($P<0.001$) as compared with that of corresponding control rats. Moreover, the resorption incidence was significantly higher ($P<0.001$) in heatstressed dams (groups I and II) as compared with that of corresponding controls. In addition, the mean fetal weight of groups I and II was significantly reduced ($P<0.001$) as compared with that of corresponding controls (Table 1).

Incidence of malformations:

In the two control groups, no malformation patterns were detected among 18 day fetuses. However, in maternally heatstressed fetuses (groups I and II) a significant number of malformations ($P<0.001$) were detected. Edema and microphthalmia are the only malformations detected among the viable 18 days fetuses of the two maternally heatstressed groups (Table 1).

Histology:

Cerebral cortex:

Transverse sections through the cerebral hemispheres of control and maternally heatstressed 18 days rat fetuses are shown in Fig. (1). The thickness of cerebral cortex of heatstressed fetuses (groups I and II) was markedly reduced as compared with that of corresponding controls. Hyperthermia destroyed virtually the endothelial cells of blood capillaries thus resulting in disruptive appearance of the neuroepithelial

Table 1

Effect of low temperature of long duration and high "spiking" temperature of short duration on developing rat embryos.

	No. of rats	Poststressing temp. (mean)	Dead rats	Resorptions (mean±SE)	No. of viable fetuses	Fetal weight (g)	No. of malformation incidence
Control	10	38.1	-	0±00	9.6±0.34	1.4±0.033	-
heatstressed	12	41.0	2	2.2±0.32 (p 0.001)	5.6±0.38 (p 0.001)	0.91±0.012 (p 0.001)	1.6±0.22 (p 0.001)
control	10	38	-	0±00	10±0.26	1.4±0.033	-
heatstressed	14	42.6	6	38±0.3 (p 0.001)	3.36±0.28 (p 0.001)	0.89±0.015 (p 0.001)	2.25±0.33 (p 0.001)

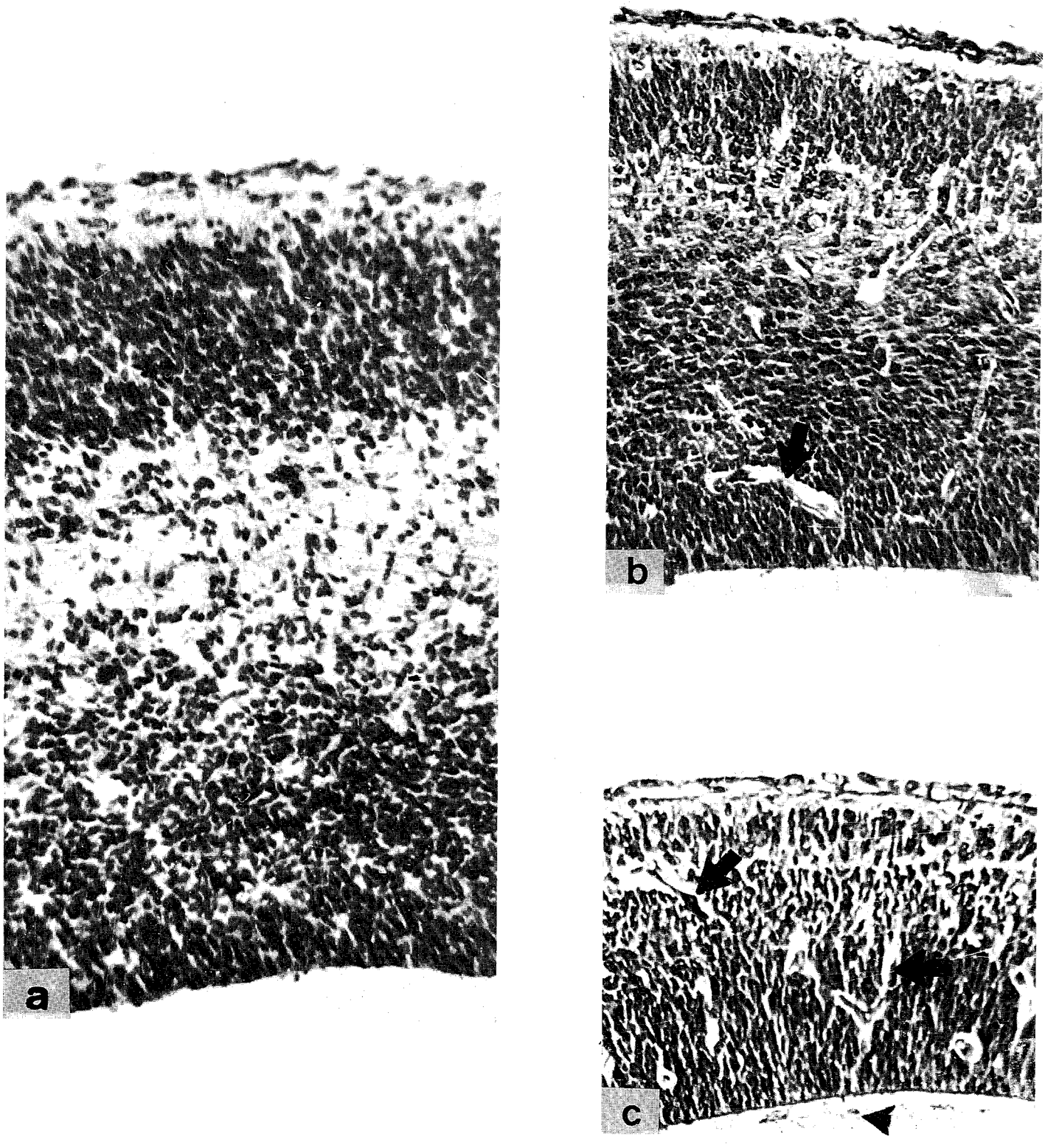

Fig. 1: T.S. through the cerebral cortex of 18 day old rat fetuses.
a. Control fetus.
b. Maternally heatstressed fetus (group I) showing destruction of neuroepithelial capillary (arrow).
c. Maternally heatstressed fetus (group II) showing destructed neuroepithelial capillaries (arrows) and evidence of hemorrhage (arrowhead).

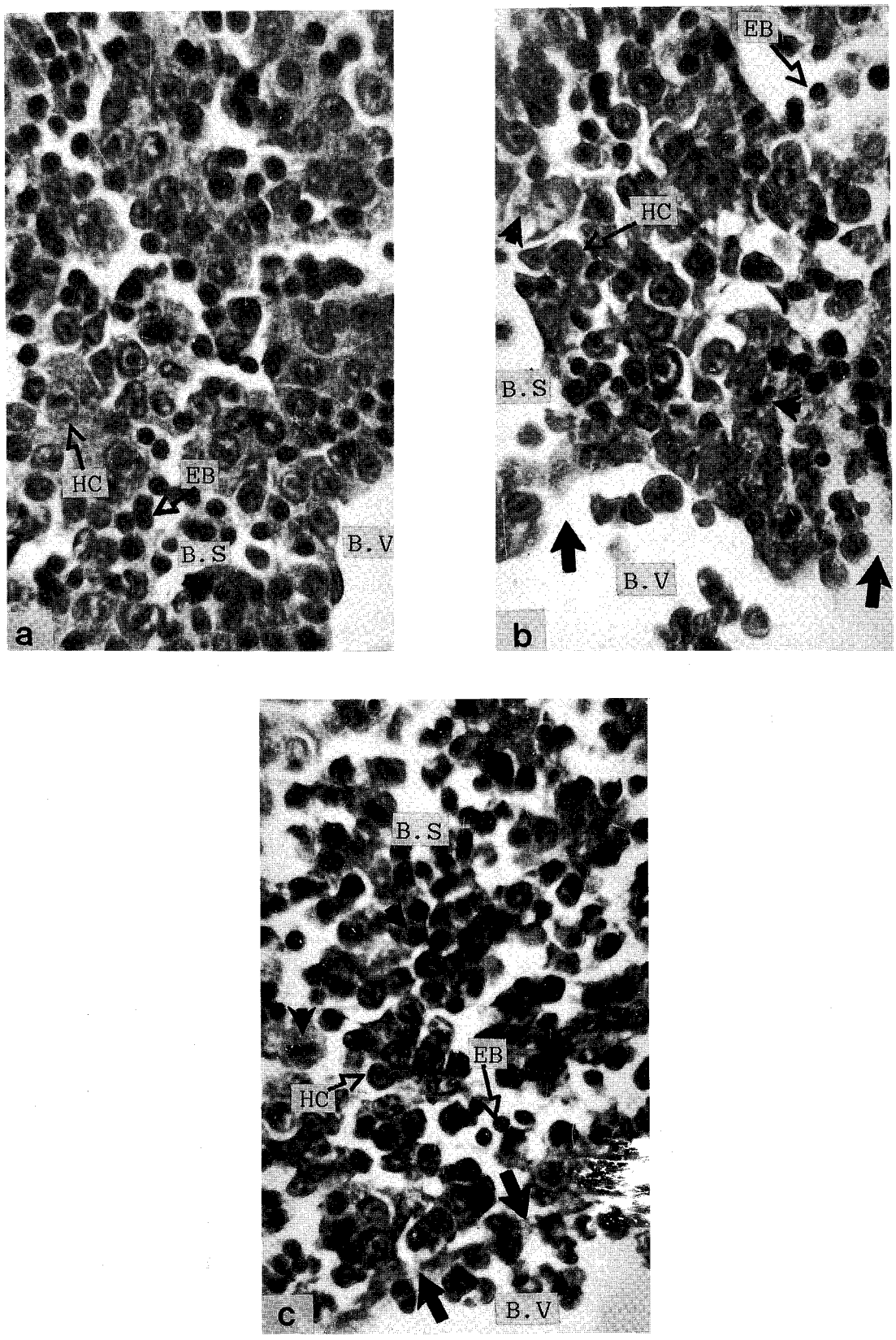

Fig. 2: S. through the liver of 18 day old rat fetuses showing blood sinusoids, B. S.; blood vessels, B. V.; erythroblasts, E. B. and hepatocytes, H.C.

a. Control fetus.
b. Maternally heatstressed fetus (group I) showing destruction of hepatocytes (arrowheads) and destructed vascular endothelium (thick arrows).
c. Maternally heatstressed fetus (group II) showing destruction of hepatocytes (arrowheads) and destructed vascular epithelium (thick arrows).

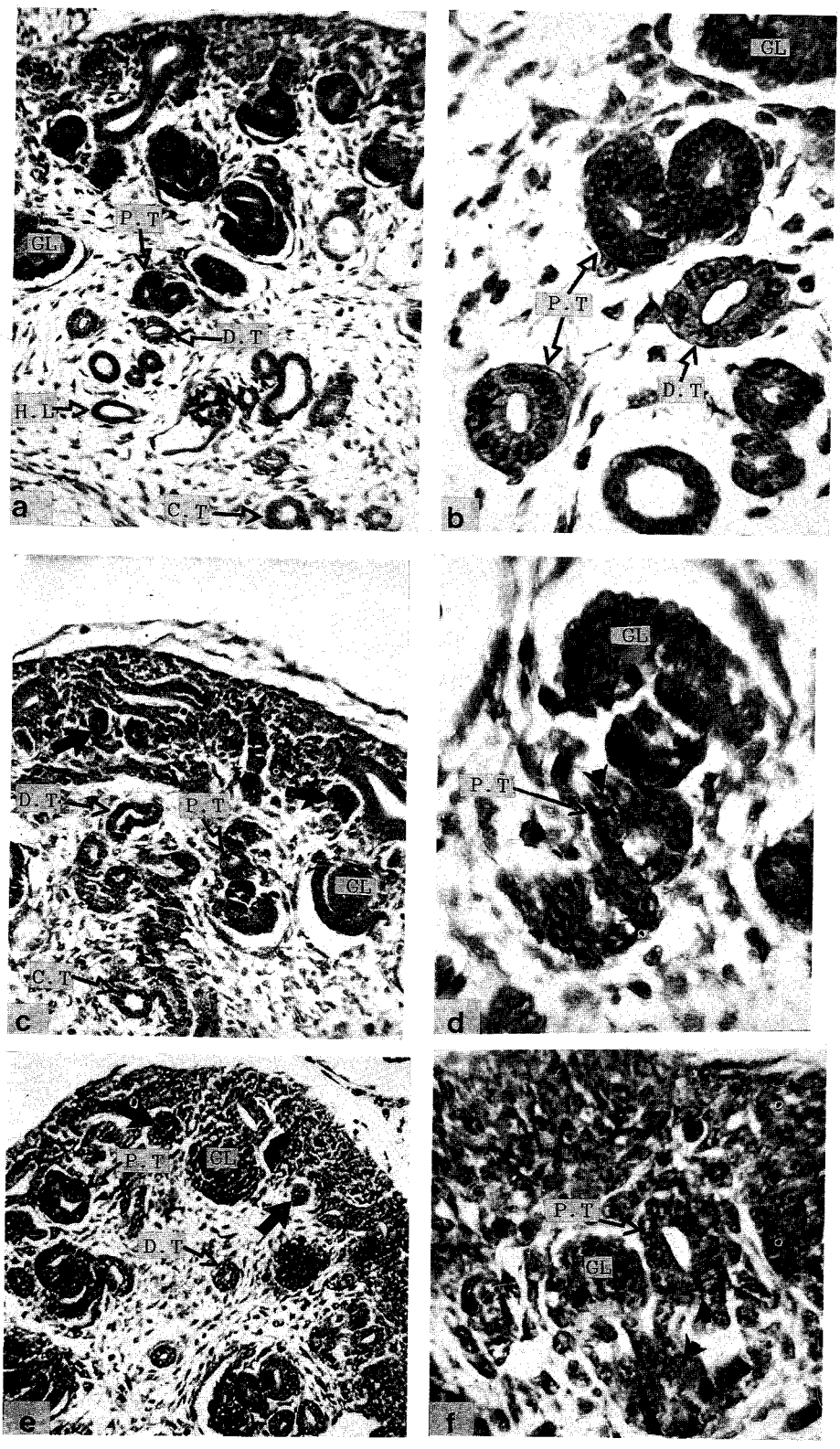

Fig. 3: T.S. through the kidneys of 18 day old rat fetuses showing collecting tubule, C. T.; distal tubule, D. T.; glomerulus, GL; Henle's loop, H. L. and proximal tubule, P. T.

a, c and e are transverse sections through the kidneys of control, maternally heatstressed fetuses of groups I and II respectively. Some glomeruli of heatstressed fetuses are atrophied or hypertrophied (thick arrows).

X 160

b, d and f represent the enlarged portions of the kidneys of control, maternally heatstressed fetuses of groups I and II respectively. The tubular system of heatstressed fetuses shows fatty degeneration, clowdy swelling and necrosis of tubular epithelium (arrowheads).

X 320

tissue (Fig. 1, b and c) in contrast to the normal state in the control fetuses (Fig. 1a). Destruction of neuroepithelial capillaries was more pronounced in fetuses of groups II than those of group I. Moreover, hyperthermia increased the permeability of some blood capillaries in the neuroepithelium and some evidence of hemorrhage, especially in group II, was noticed. In addition, few nerve cells of cerebral appeared swollen with pyknotic nuclei in heatstressed fetuses of the two groups.

Liver:

The liver of 18 days control fetuses is composed of hepatic cords of 1-3 hepatocytes thick (Fig. 2a). A number of mature erythroblasts, mainly orthochromatic erythroblasts, with darkly stained nuclei were observed in the blood sinusoids between hepatic cords. However, the majority of circulating erythroblasts had lost their nuclei.

Hyperthermia exerts degeneration of some hepatocytes, dilation of sinusoids and destruction of vascular endothelium (Fig. 2 b, c) through which erythroblasts escape producing hemorrhagic foci in the liver tissue. The hepatocytes of group I were somewhat similar to those of controls, though few cells exhibit vacuolated cytoplasm and either pyknotic or fragmented nuclei. In group II, most hepatocytes were shrunken and contain strikingly hyaline cytoplasm and collapsed, pyknotic or completely lysed nuclei. The majority of erythroid cells in sinusoids of heatstressed groups were polychromatic while a few orthochromatic erythroblasts were encountered (Fig. 2b, c).

Kidney:

The kidneys of maternally heatstressed 18 days fetuses were reduced in size and slightly congested compared with those of control.

Transverse sections through kidneys of control and maternally heatstressed 18 days fetuses are shown in Fig. (3). Severe changes were observed in the cortical region of the kidneys of maternally heatstressed fetuses of groups I and II (Fig. 3 c, e) compared with that of controls (Fig. 3a). In most heatstressed fetuses, some glomeruli were atrophied or hypertrophied structures containing narrow capsular spaces (Fig. 3, c & e). These glomeruli were gradually replaced by a small amount of dense connective tissue.

The tubular system of maternally heatstressed fetuses showed fatty degeneration, clowdy swelling and necrosis of tubular epithelium (Fig. 3d & f) compared with normal tubular structure of control fetuses (Fig. 3 b).

The interstitial tissue throughout cortex and medulla of maternally heatstressed fetuses of group II was slightly increased and congested and contain numerous foamy cells (Fig. 3 f).

DISCUSSION

Elevation of maternal body temperature of about 3.2°C for 60 minutes or about 4.4°C for 25 minutes on day 7 of gestation induced a high incidence of fetal resorption compared with the corresponding controls. However, a spiking temperature of short duration is more highly effective in producing a significant increase of resorption incidence than the low temperature of long duration. This was tentatively attributed to the fact that fetal temperature increase in parallel with that of the mother during exposure (Edwards, 1968, a). High resorption incidence was previously reported by Edwards, (1968), Edwards and Wanner (1977) and El-Shabaka and Emad (1990) following maternal hyperthermia.

The number of viable fetuses was significantly decreased in hyperthermic mothers, especially in groups II, compared to the corresponding controls. This may be due to the fact that hyperthermia in early gestation leads to early abortion. According to Devereux (1955), hyperthermia is still used by some African tribes as an abortifacient by applying heated water, ashes and stones to the belly.

The weight of viable fetuses of the two maternally heatstressed groups was significantly smaller than that of the corresponding controls. However, there was no marked difference in the mean fetal weight of the two heatstressed groups. Hyperthermia exerts dwarfing of lambs maternally heatstressed to 40.6°C for 8-9 hours/day during the last two thirds of pregnancy (Ryle and Morris, 1961) and growth retardation of rat embryos or fetuses maternally heatstressed in an incubator that raised deep body temperature to either 42.7° for 40-60 minutes (Edwards, 1968) or to 40°C for 20 minutes (El-Shabaka and Emad, 1990).

In the present study, edema and microphthalmia were the common malformations detected among the viable 18 day fetuses of maternally heatstressed groups. A spiking temperature of short duration produced an increase (but not significant) in the number of malformative fetuses compared with the low temperature of long duration. Previous studies reported that, hyperthermia produced a wide variety of abnormalities in 12 and 15 days rat fetuses maternally heatstressed on the 7th day of gestation (El-Shabaka and Emad, 1990), 18 days rat fetuses maternally heatstressed at 9 days, 12 hours of gestation (German *et al.*, 1985) and 20 days rat fetuses maternally heatstressed on day 4, 6, 8 or 10 of gestation (Arora *et. al.*, 1979). In addition, Germain *et al.*, (1985) reported that microphthalmia is the most common malformation at all effective temperatures and is frequently the only malformation seen at the shortest exposures for a particular temperature. I was unable to explain why other malformations were not obtained among viable 18 days fetuses heatstressed while passing through the sensitive stage of development (7th day of gestation). This may be attributed to the failure of severely affected fetuses to live, hence they die before they reach 18th day of gestation and/or due to the fact that the critical period for abnormality induction vary in duration and sensitivity to hyperthermia with the genotype of mother and embryo (Edwards and Webster, 1982; Chernoff and Golden, 1983; Webster and Edwards, 1984; Webster *et. al.*, 1985; Finnell *et. al.*, 1986). The mechanism by which hyperthermia induces anomalies has not yet been evaluated. However, Germain (1983) had hypothetized that hyperthermia may, through the induction of the heat shock response, alter the normal developmental program (transcription) thereby leading to a number of deleterious outcomes including cell death, abnormal cell-cell interaction and altered cell migrations all of which culminate in abnormal development.

According to Mirkes (1985), the mitotically active cells are particularly susceptible to hyperthermia-induced cell death. In part, this is confirmed by the present investigation since the neuroepithelium of the heatstressed rat fetuses was particularly thinner than that of the controls. The endothelial cells of the cerebral blood vessels of heatstressed fetuses appeared thinner and may easily rupture resulting in hemorrhage so common in these fetuses. The high mortality incidence in these fetuses may be attributed to the vascular hemorrhage in the neuroepithelium and / or degeneration of the neuroepithelial cells. In previous studies, the brain of developing fetuses was

shown to suffer a marked decrease in thickness of cerebral cortex (El-Shabaka and Emad, 1990). Cell death, pyknosis and clumping of nuclear chromatin following maternal hyperthermia have also been reported by Edwards *et. al.,* (1974) and El-Shabaka and Emad (1990). A number of investigators studied the incidence of central nervous system disorders in children born to mothers who had influenza symptoms during the first trimester. Some studied reported a positive correlation (Saxen *et. al.,* 1960; Coffey and Jessop, 1963; Hakosalo and Saxen, 1971) while others found no association (Campell. 1953; Wilson and Stein, 1969; Leck, 1971; Mackenzie and Houghton, 1974).

Hyperthermia causes degeneration as well as nuclear pyknosis of some hepatocytes. A high spiking temperature of short duration is highly effective in producing more degenerative action on hepatocytes. Consequently, degeneration of hepatocytes as a result of hyperthermia leads to a marked dilation of blood sinusoids and delayed maturation of erythroblasts. Moreover, hyperthermia also resulted in a marked destruction of vascular endothelium producing extensive disseminated hemorrhages in the liver.

Hyperthermia produced atrophied or hypertrophied glomeruli which may lead to albuminurea and be responsible for the fetal midget. The cells lining the proximal, distal and collecting tubules show the features of cloudy swelling and contain abundant vacuoles. These vacuoles may be attributed to deposits of fat in the form of granules or globules. The interstitial tissues throughout the cortex and medulla was slightly increased in kidneys of maternally heatstressed fetuses which may be a reaction of repairing the previously destructed cells.

It may be concluded that, a spiking temperature for a short duration is more effective in producing anomalies and some organ lesions than the low temperature for long duration. The present findings support the hypothesis that maternal fever (or some associated factors) in early pregnancy can increase the probability of developmental disorder.

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