STUDIES OF TOLERANCE OF SEVERAL KINCS OF ANIMALS TO HEAT, ELECTROSHOCK, HYDROGEN AND HYDROXYL IONS.

II. PATHOLOGICAL STUDY ON THE MICE EXPOSED TO HEAT.

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INTRODUCTION

Numerous mechanisms of heat death are suggested and no one mechanism operates for all animals. In higher animals, the heated tissues may liberate toxins which cause damage at a distance. It is clear that the high temperature lethal for an animals depends on duration of exposure to the elevated temperatures.

In the previous author's report of "Studies of Tolerance of Mice to Heat Stressor", 595 mice were subjected to dry hot environment of various air temperature of from 35 to 200°C, for periods of 6 seconds to 12 hours and data on the mortality and survival time were presented. It is suggested that morphological changes of organs of animal died from heat exposure may be different depend on the elevated temperature and duration of exposure. However, there has little information of those presented up to date.

The purpose of this study is a pathological observation upon the organs of mice died from exposure to heat considering the relation with the degree of temperature and duration of exposure.

MATERIAL AND METHOD

Healthy and non-pregnant mice weighing approximately 25 gms have been used and all mice were raised under observation for two weeks prior to the work.

Twenty mice were divided into 4 groups with 5 mice in each.

Group I: Five mice exposed in 40°C.
Group II: Five mice exposed in 60°C.
Group III: Five mice exposed in 80°C.
Group IV: Five mice exposed in 200°C.

Dry oven and incubator were used for heat chambers. Incubator was for 40°C group and dry oven for all other groups. They were controlled to keep a constant degree of heat.

When the temperature of the heat chamber became suitable, the mice were put into the chamber and the door was quickly and tightly closed.

When the visible respiration of the animal ceased in the heat chamber, it was considered to be the time of death. After the death, they were immediately taken out and autopsies were done.

Five normal and untreated mice were kept for control and they were sacrifieced by cutting the neck off with scissors and autopsy was made.

The tissues from skin, trachea, lungs, heart, gastrointestinal tract, liver, pancreas, spleen, adrenals, kidneys and brain were obtained and they were fixed in solution of 10% formalin, Orth's fluid and Bouin's fluid. Stains applied were hematoxylin-eosin, P. A. S., silver impragnation method of Masson, Bauer-Schiff, sudan III alcohol, toluidin blue and Spiel Mayer's. (4).

RESULT

The lengths of time from the beginning of exposure to heat till the moment of their deaths were varied according to the degree of heat as shown in the following table.

Group	Temperature	Lengths of time to death
I	40°C	2 ½–6 hrs
II	60°C	17–19 ½ minutes
III	80°C	8–8 ½ minutes
IV	200°C	70–95 seconds

A: Gross pathological findings.

Main gross findings observed in the mice exposed to heat were vascular changes of all organs without other remarkable findings.

Skin: In the auricular skin, the most severe change was seen in the group of 80°C. There were marked hyperemia and petechiae all over the auricular skin. The group of 200°C showed no appreciable hyperemia or petechia. In the group of 60°C, there were moderate hyperemia and scattered petechiae. The group of 40°C showed only mild hyperemia without petechia.

The abdominal skin revealed less degrees of changes than those of the auricle, and hyperemia and scattered petechiae in the group of 60°C., whereas the groups of 200°C. and 40°C. showed no gross changes. Only slight hyperemia without petechia was seen in the group of 80°C.

Lungs: The lung was one of the organs showing most severe vascular changes. The lungs of the 200°C. was most markedly involved and revealed severe gener-

alized congestion without hemorrhagic point. The minimal change was observed in the group of 60°C. showing slight congestion in focal areas. The groups of 80°C. and 40°C. showed moderate changes which were scattered in the former and diffuse congestion in the latter groups.

Gastro-intestional tract: No remarkable change was seen and even congestion was not appreciated.

Liver: Mild to moderate degree of diffuse congestion were present in the group of 80°C.. Both groups of 40°C. and 60°C. showed less and the group of 200°C. showed least changes which was localized and mild congestion at the peripheral portions of the livers.

Heart, Spleen and Kidneys: The spleen showed no remarkable changes in all groups, and the heart and kindeys were too small to make out any gross changes.

Cranial cap and Brain: The subscalpular areolar tissue was examined and no remarkable change was seen. Outer and cut surfaces of the brains were grossly negative.

B: Microscopical findings.

Skin: Auricular skin of all cases showed dilatation of capillaries with congestion. Particularly, in a case of the group of 80°C., died in 8 min. and 10 secds. There were seen extensive congestion and various sized hemorrhagic points. In the group of 200°C, coagulation of dermal collagen fibers was apparent.

Abdominal skin showed almost similar vascular changes to those of auricular skin, but it was generally less in degree and no evidences of collagen degeneration were noted.

In both auricular and abdominal skins, no remarkable changes of epidermis, hair follicles, sebaceous and sweat glands were seen.

Trachea: Marked capillary congestion in the submucosa was seen in all cases, and the cases died in high temperature in short duration showed minor hemorrhages. No appreciable changes were observed in the mucosal epithelium and mucus glands in all cases.

Lungs: The prominent changes were vascular reactions which were severe congestion and different degrees of intra-alveolar hemorrhages with mild edema.

Mice of both groups of 200°C. died in short duration and 40°C. in long duration as long as four to six hours showed the most severe congestions and hemorrhages.

Particularly, the cases of the group of 200°C. died in around $1\frac{1}{2}$ minutes revealed the bronchi and bronchioles to be constricted and the mucous secretions covering the epithelial surfaces were apparently dried up. Bronchial mucosal epithelium had no remarkable changes.

Localized pulmonary emphysema and atelectases were frequently observed in many cases without any relation with the degrees of heat and the lengths of time of exposures.

Heart: In all cases, there were no remarkable changes except for dilatation and congestion of capillaries and venules. There were no evidences of edema, hemorrhage or changes in the cardiac muscle per se.

Livre: There were marked dilatation and hyperemia of central veins and sinusoids of central zones. Endothelial cells (Kupffer cell) were swollen. The hepatic cord cells in the central zones were keeping their arrangement normally, but the cells themselves showed distinct cloudy degeneration of cytoplasm. In the peripheral zones, the cells showed irregular granular cytoplasm with coarse vacuolization.

In fat stain with sudan III alcohol solution, coarse fatty granules were observed to be increased in peripheral zone of the lobules in contrast to a small or no visible amount of fatty substance in the central zones.

In Bauer-Schiff stain there was completely disappearence of Schiff reagent positive material considered to be glycogen which was seen in a considerable amount in the liver of the control group.

The most severe changes were seen in the cases of the groups of 60°C. and 80°C. died in between 17 min. and 8 min.

In the groups of 40°C., died in longer duration, the changes were generally alike while less in degree.

In the group of 200°C. died in around $1\frac{1}{2}$ min., the changes were least, while still a minimal degree of fatty change.

Pancreas: Only mild to moderate degree of capillary and venous dilatations with congestions were present throughout the cases. There was no appreciable changes in the parenchymal cells.

Spleen and gastro-intestinal tract: No remarkable changes were observed in both organs of experimental groups.

Adrenals: In all cases, vascular changes were again prominent findings, and the scattered cases showed the walls of sinusoids of the medulla to be focally ruptured and few small points of bleedings were built up in the areas.

The cortex showed severe congestion of the sinusoids in all layers and it was most marked in the zona reticularis.

In the control group, the medulla showed two types of cells (5) in proportion of approximately same number and they were so called yellow cells and the polyhedral cells having lightly eosinophilic cytoplasmic granules. The yellow cells contained a large number of coarse chromaffin granules, in cytoplasm while the latter cells were smaller in size than that of yellow cells and contained more fine lightly eosinophilic granules. Except for only one mouse, all cases exposed to heat showed the yellow cells to be markedly increased in number whereas the latter cells were decreased.

Almost half of the cases in the proups of 60°C. and 80°C., there were seen few foci of cellular degeneration in zona reticularis, and each of foci were composed of a gronp of cells having rather clear and vacuolized cytoplasm. Numbers of the cells in each group were ranged from 4 to 5 and their nuclei were moderately pyknotic

and placed at the center of each cell.

In the groups of 60°C. and 80°C., the cells of zona reticularis and of lower fasciculata revealed singular or a few round vacuoles in the cytoplasm.

All of above changes were minimal in the group of 40°C. and were not present in rhe group of 200°C.

Appreciable change of the cells in zona fasciculata was observed mainly in the group of 40°C. and they showed fine vacuolization of cytoplasm. In the groups of 60°C. and 80°C., the cortical cells of zona fasciculata revealed slightly coarser eosinophilic cytoplasmic granules which were present in finer form in the control group.

Zona glomerulosa showed no remarkable changes in the cortical cells in all groups.

Staining with sudan III alcohol solution, the control group showed fine sudanophilic granules which were mainly seen in zona fasciculata.

These findings were coincided with those of cytoplasmic vacuoles of the cells seen in the hematoxylin-eosin stain in this zone.

Zona reticularis, in which very litile or none detectable amount of sudanophilic material present in the control group, contained almost same amount of coarse and massive sudanophilic granules as in zona fasciculata in the three groups. In other hand, the group of 40°C., died in between $2\frac{1}{2}$ hours, and 6 hours, showed marked diminish in amount of sudanophilic granules in zona fasciculata and only a small amount of sudanophilic granules in zona fasciculata and only a small amount was remained in the upper and mid fasciculata. Longer duration of exposure to heat resulted in more subsidence of the granules in the 40°C group.

Zona glomerulosa contained only a little amount of sudanophilic material in both experimental and control groups and no appreciable changes were observed after the heat exposure.

In silver impregnation method with Masson on the slides, the control group showed adrenal medulla to be composed of mainly black brown colored cells. The cortex showed the cells of zona fasciculata to contain brown granules, and the cells of zona reticularis as well as of zona glomerulosa to be light yellow in color. In the cells of light yellow color, the granularity of cytoplasm was not clear while in brown or black brown colored cells of zona fasciculata and medulla, the granularity was definitely made out.

In the experimental groups of 60°C., 80°C., and 200°C., there was a definite decrease in amount of the brown granules in the cells of zona fasciculata, while the degree of it was not constant in every cases.

The black brown granules in medullary cells were markedly decreased in large areas without any constant relation with the degree of heat and the duration of exposure.

In the group of 40°C with long exposure, the fine deeply brown colored granules in the cells of zona fasciculata became much coarser and occasionally formed small numbers of larger granules.

Kidneys: In all cases, dilatation and congestion of capillaries including glomerular capillaries were marked.

In the group of 40°C. with longer exposure, there was cloudy swelling of epithelial cells of proximal convoluted tubules with shrinkage and detachment of epithelial cells from basement membrane in collecting tubles.

These changes were not observed in the other groups.

P. A. S. and sudan III stains were made and no remarkable changes were obtained.

Brain: The most prominent change in the brain was also vascular reaction which was more marked in the group exposed to lower temperature for longer duration than that of higher temperature for shorter duration.

The vascular changes were shown by variable degree of congestion in meningeal and intra-cerebral capillaries, and dilatation of perivascular space (Virchow-Robin space) indicating brain edema. These congestion and edema could be seen in entire brain, though more marked in the hind brain, nuclei around the third ventricles and meninges.

Ganglion cells revealed very minimal changes, and only slight chromatolysis was observed in the group of 40°C.

No remarkable changes were demonstrate in glial cells and in axon fibers by hematoxylin-eosin and myelin stains.

SUMMARY

Twenty mice were divided into four groups with five mice in each. Mice of each group were placed in heat chambers under different temperatures of 40°C., 60°C., 80°C and 200°C. respectively. They died in the heat chambers in certain limitted time depend on the degree of temperatures. When a mouse expired, it was taken out and autopsy was performed immediately.

Pathological examinations upon the different organs of each mouse were made considering the relation with the temperature and duration of exposure.

The most prominent change was vascular reaction to the vital organs, and the reaction was manifested by dilatation and congestions of blood vessels, especially capillaries and veines, and it was learned that the degrees of the changes was much different depend on the organ even in a mouse.

The findings observed in the patients died from extensive burn recorded in the literatures (6) (7) (8) agreed with our findings in respect of vascular reaction.

Besides the vascular change, the appreciable changes were observed histopathologically in the adrenals and livers.

After the exposures to heat, the adrenal cortex showed the change in distribution of sudanophilic material which was considered to be mainly cholesterol. In the groups of 60°C., and 80°C., and 200°C., the zona fasciculata and zona reticularis showed sudanophilic granules to become much coarser. In the group of 40°C. in

which the animals died in between $2\frac{1}{2}$ and 6 hours, the granules were markedly decreased in amount, while of which the cases of longer duration of heat exposure showed more marked diminish in sudanophilic material.

Focal areas of cellular degeneration were seen in zona reticularis of almost half of the cases in the groups of 60°C. and 80°C.

All experimental groups revealed definite increase in numbers of yellow cells in the adrenal medulla. The fact of increase in number of these cells containing argentaffine granules is difficult to interprete. Whether or not these cells represent the acting forms of medullary cells in contrast to the other cells which do not possess the granules are the matter of debate. (5)

In the cortical cells, the silver stained granules which considered to be ascorbic acid (9) showed decrease in amount on exposure to heat, and it appeared to be fairly well correlated in parallel with the decrease in amount of sudanophilic material in the tissue. The facts suggest that the mice had been in condition of stress due to heat and the findings agreed with those described in other authers (9) (11) studying adrenal changes in stress.

The foci of cellular degeneration in the zona reticularis were not constant in all cases, while there were too many cases showing the lesions to neglect, though such changes were not described in the literatures searched by the author, and difficult to evaluate.

The liver showed cloudy degeneration of the hepatic cord cells in the central zones, and increase in number of coarse fatty granules in the cytoplasm of the cells at peripheral zones of the lobules. There was completed and diffuse disappearence of Schiff reagent positive material which was supposed to be glycogen from the liver cells.

These findings in the liver were most marked in the groups of 60°C. and 80°C. and the minimum in the group of 200°C. with the shortest duration of the exposure.

Except for glycogen, cellular changes in the liver were fairly well agreed with those described by Gillman and Gillman (6) in human cases of fatal burns. They described however, that the appearence of fat granules in the peripheral zones of lobules to be in between 36 and 72 hours after the accidents, and in our cases it was much shorter as to be $1\frac{1}{2}$ min. They observed atrophy of liver cells around the central veins in a liver 36 hours after burns, while in all our cases showed cloudy degeneration in the central zones which might go into atrophy later on.

Depletion or disappearence of glycogen was dramatic and this fact agrees again with that the increase in amount of blood sugar in stress as Hans Selye (11) and others (9) (12) described. From this finding it has to be considered that glycogen in the liver could be mobilized to blood stream within a short time, few minutes, by the stress of such as heat.

The lesions in the brain were mainly congestion and edema, though few cases of the group 40°C. showed ganglion cells having chromatolysis. It is well known in

human cases that the chromatolysis is one of the common findings of the brains in the patients died from extensive burns. (7) (8)

CONCLUSION

In this experimental study, mice were exposed to different degrees of heat till their deaths in the heat chambers and they were autopsied in order to study the morphological changes of various organs of them. Besides of main findings of vascular congestion and dilatation of all organs, there were well shown few findings which explain that the animals have been in stress condition before death.

It is clear that the definite mechanism of death involved in the mice exposed to heat is almost impossible to establish from the point of views of morphological pathology and it is rather appeared to be biochemical or simple functional character.

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Illustrations

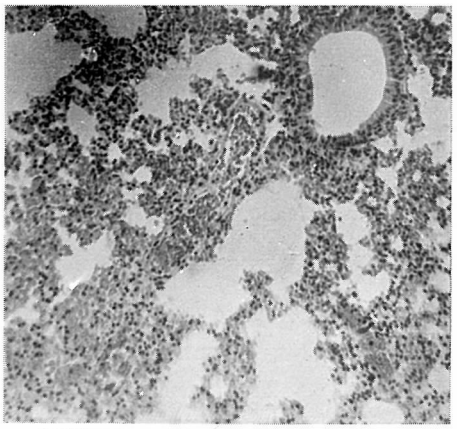

Fig. 1. Lung of mouse (200°C–80 sec.) marked congestion with irregular atelectasis and focal emphysema. H-E stain × 150

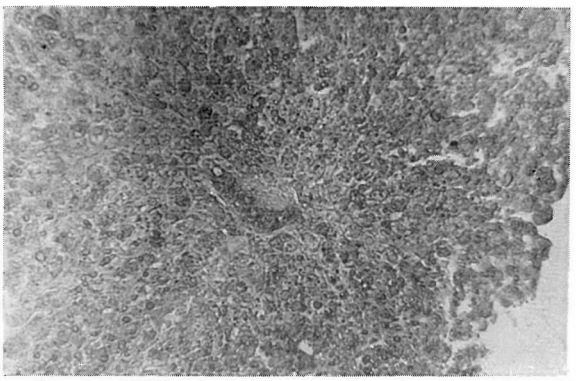

Fig. 2. Liver of mouse (60°C–18 min.) Appearence of coarse sudanophilic granules. Sudan III stain × 150

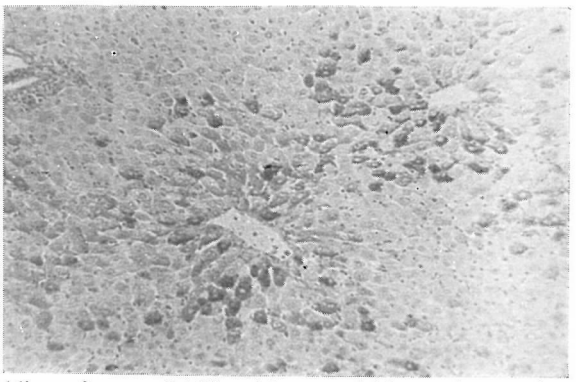

Fig. 3. Normal liver of mouse Schiff-positive material is seen in central zones of lobules. Bauer-Schiff stain × 150

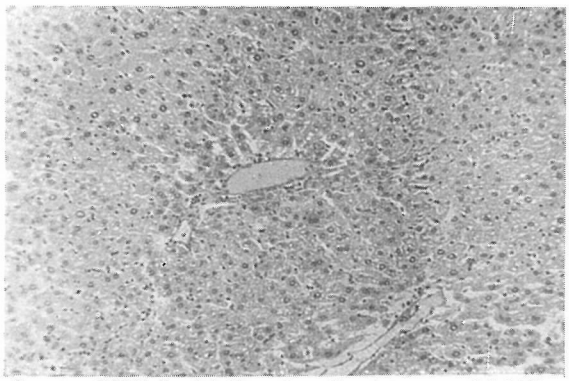

Fig. 4. Liver of mouse (80°C–8 min) Complete disappearence of Schiff-positive material. Bauer-Schiff stain × 150

Fig. 5. Normal adrenal of mouse. A large amount of silver-positive materials in cortex and medulla. Masson's silver method × 150

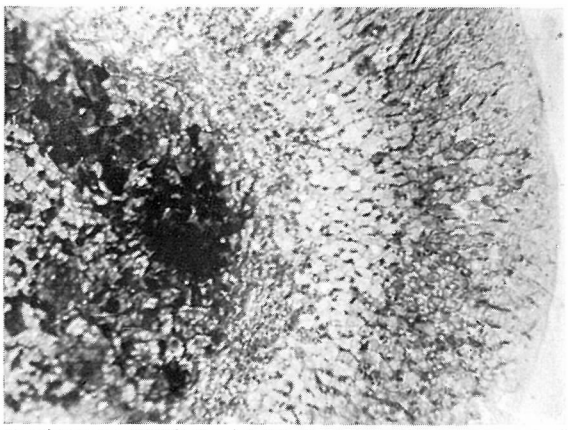

Fig. 6. Adrenal of mouse (80°C–8 min) Marked diminish in amount of silver-positive materiale from cortex and madulla. Masson's silver method × 150

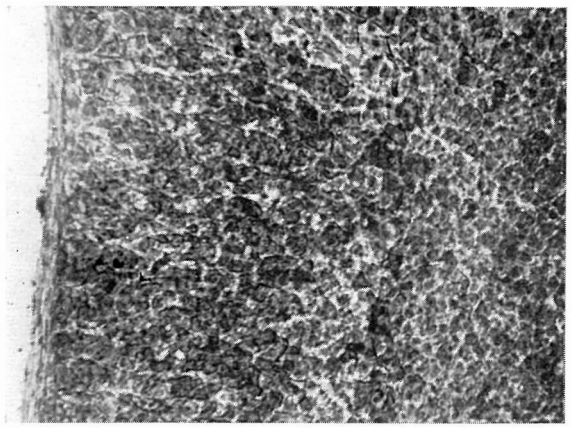

Fig. 7. Normal adrenal cortex of mouse. Fine granular sudanophilic materials in all zones of the cortex. Sudan III stain × 150

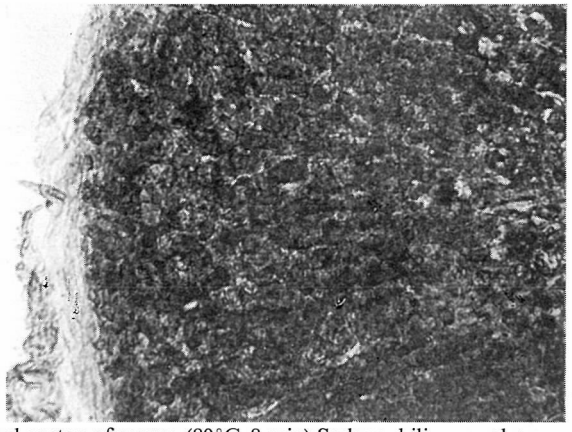

Fig. 8. Adrenal cortex of mouse (80°C–8 min) Sudanophilic granules are much corser in Zona fasciculata and reticularis. Sudan III stain × 150

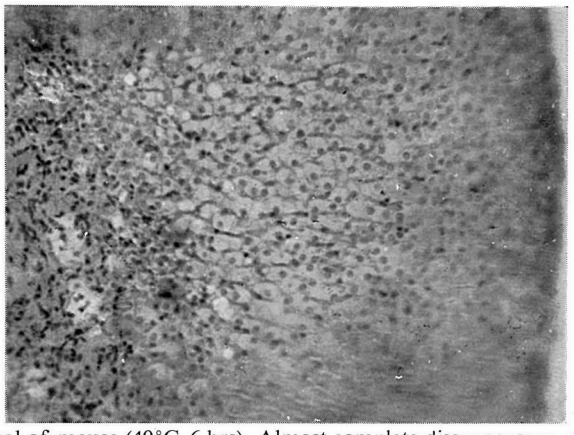

Fig. 9. Adrenal of mouse (40°C–6 hrs) Almost complete disappearonce of sudanophilic granules in the adrenal cortex. Sudan III stain × 150

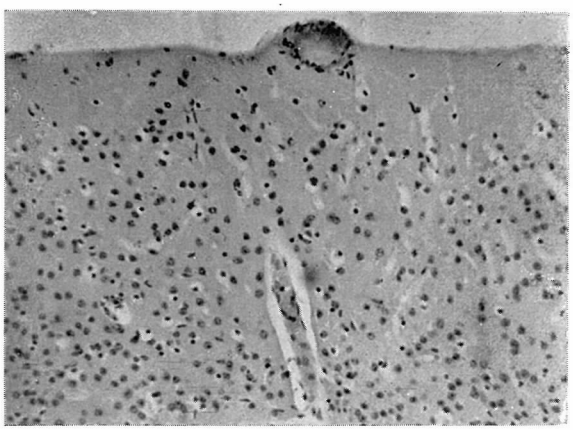

Fig. 10. Brain of mouse (40°C–4 hrs) Marked congestion and edema. H-E stain × 150