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Morphologic Changes in the Dog's Adrenal Gland Following Anoxia.* (20038)

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Recently Selye(1) in his book on Stress summarized the literature on the adrenal gland. Apparently many observations have been made on the histochemical changes in the adrenal gland following periods of anoxia; however, few studies on morphology have been made. Rats exposed to chronic anoxia show hypertrophy of the cortex and a depletion of lipids in the zona fasciculata and zona reticularis(2-5). Since prolonged anoxia causes severe damage to the brain(6), it was of interest to ascertain whether the adrenal cortex also was damaged by anoxia. Because the adrenal gland has the greatest blood flow of any organ in the body, 5-8 ml/g/min.(7), it seemed likely that this reflected a high metabolism and that the gland therefore might be as susceptible as the cerebrum to anoxia. The present experiment gives the data obtained in this study, supplementing a previous preliminary report(8).

Experimental methods. The dogs were obtained from the pound and used soon thereafter. From their physical appearance their health was classified as excellent, good or poor. The method of producing anoxia and of resuscitating dogs has been described(9). Pure nitrogen was given unanesthetized animals to breathe by means of a special head mask. In this type of fulminating anoxia, the breathing failed within one to 2 minutes and the pulse pressure disappeared after 2 to 4 minutes. The dogs were resuscitated by two measures: periodic insufflation of the lungs with oxygen, using a special resuscitating valve for the head mask, and extrathoracic cardiac massage. This latter procedure was carried out with the dogs lying on their sides. The chest was compressed sharply between thumbs and fingers of both hands at a rate of approximately one hundred times per minute. This massage was stopped briefly every half-minute or so to ascertain whether the circulation had recovered. Soon after the blood pressure started to rise, respiration returned and quickly increased to normal pulmonary ventilation. During the first 24 hours following the induction of anoxia, episodes of decerebrate rigidity, running movements and ululation were common. The animals frequently flung themselves around the cage in apparent frenzy, with fits of "sham rage" often apparent. All grades of delirium and stupor occurred. The dogs then progressed into coma, and death occurred within two to three days. The few dogs that recovered did so slowly, showing ataxic attempts to stand and slow return of vision and hearing; after 4 or 5 days they appeared

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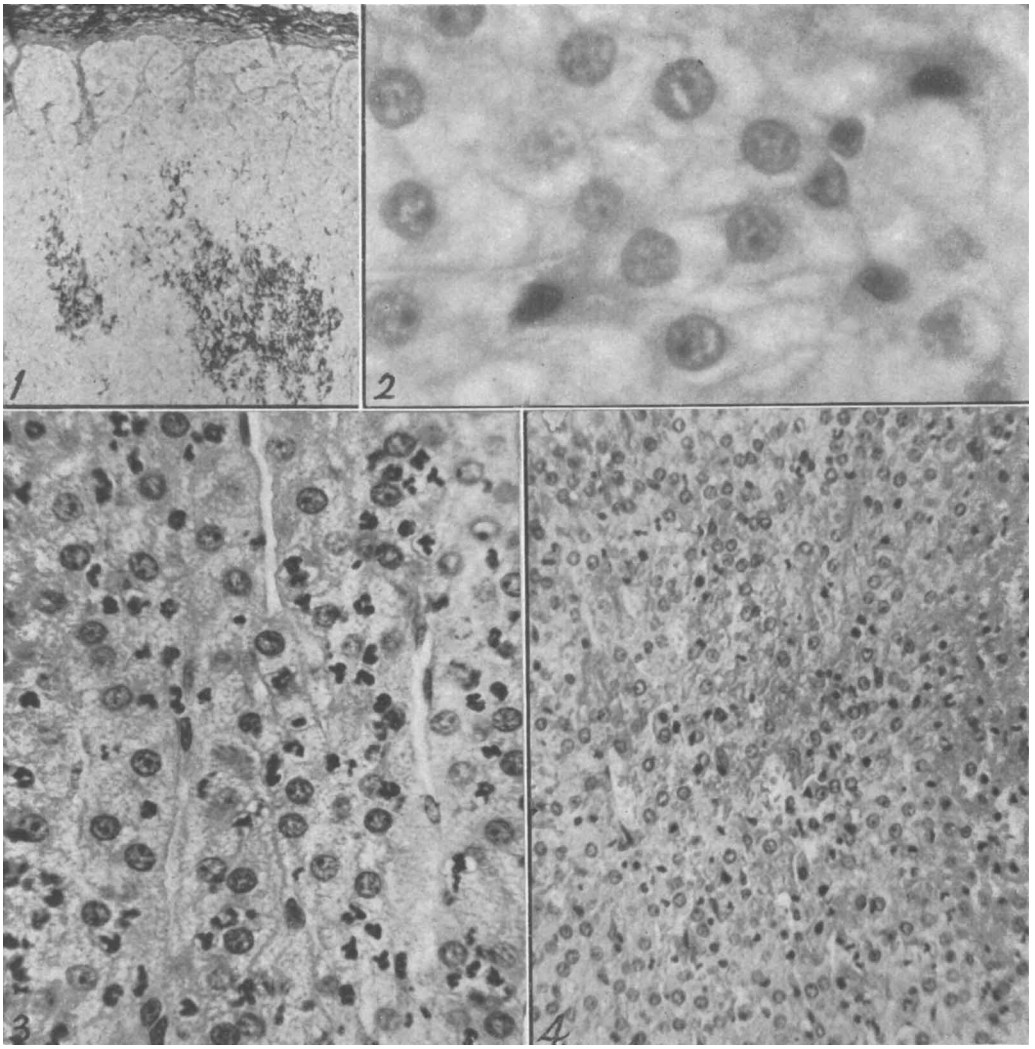

FIG. 1. Length of anoxia and interval before death for each dog is given in Table I. Dog 188, focal areas of hemorrhage in the cortex of the adrenal. Azo-Carmine stain × 75.

FIG. 2. Dog 196. Pyknotic nuclei and coagulated cytoplasm apparently are the minimal lesions occurring in the adrenal. H & P × 1026.

FIG. 3. Dog 172. Polymorphonuclear leucocytes frequently infiltrate the adrenal cortex of these anoxic dogs. H & P × 559.

FIG. 4. Dog 188. The pyknosis and coagulation necrosis occurring in the cortex may be diffuse. H & P × 440.

normal. All animals were given good nursing care; water and a starch solution were given by stomach tube. The ability to drink and eat spontaneously returned only after about 5 days. The method of classification of the clinical symptoms which we employed is slightly modified from that suggested by Delarue(10) from his studies on traumatic unconsciousness. These classifications are: 1) Conscious; 2) confusion; 3) delirium; 4) stupor; 5) semi-coma; 6)coma; and 7) death. Twenty-six dogs subjected to from 5½to 8½ minutes of fulminating anoxia and 4 controls were used. They were sacrificed either by electric shock or exsanguination and autopsied immediately. All the viscera were examined macroscopically; however, only specific tissues were kept for histological study, except

TABLE I. Effect of Anoxia on the Adrenal Gland.

Animal No.	Physical state	Length of anoxia, min.	Interval before sacrifice, hr	Clinical manifestation when sacrificed	Degree pathologic change
206	Excellent	6.5	6	Confusion	0
207	Poor	"	"	Delirium	+
209	Excellent	"	"	Semi-coma	+++
205	"	"	24	Coma	+
208	Good	"	"	Stupor	+
210	"	"	"	Coma	+
102	Excellent	8	"	"	+
30	"	8.5	"	"	+
173	Poor	6.5	"	Death*	++
82–2	Good	8	"	Coma	++
82–3	Excellent	"	"	"	++
192	"	6.5	"	Semi-coma	+++
188	"	"	"	"	++++
172	Good	"	"	Coma	++++
99	"	8	"	"	++++
219	Poor	6.5	48	Stupor	0
220	Excellent	"	"	Confusion	0
14	"	"	"	Stupor	+
15	Good	"	72	Conscious	0
16	"	"	"	"	+
180	Excellent	"	96	Delirium	0
214	"	"	"	Conscious	0
215	"	"	"	Coma	0
174	"	"	"	Stupor	+
184	"	5.5	"	Conscious	+
196	"	6.5	120	Delirium	+

* Autopsied immediately after death.

for the adrenals which were carefully examined in every animal. Tissues for microscopic study were fixed in a dilute solution of formaldehyde; paraffin sections were prepared and stained routinely with hematoxylin and phloxine. Some sections were stained by Mallory's aniline blue method and by the periodic acid technic.

Experimental. The period of anoxia and the interval before death for each dog is given in Table I. At autopsy no attempt was made to determine the relative size of the adrenal glands since the animals were mongrels and varied much in size, weight and state of nutrition.

The adrenal gland on each side was similar. Dilated blood vessels and focal areas of macroscopic hemorrhage were present in the adrenals of a few dogs (Fig. 1). The earliest microscopic change occurred in the nucleus and cytoplasm of the epithelial cells in the zona fasciculata and zona reticularis. The nucleus in the injured cells was pyknotic and the cytoplasm appeared to be coagulated, staining deeply with phloxine (Fig. 2). The number of such injured cells varied; however, the lesion was diffuse. This cytological change is recorded as one plus in Table I. Polymorphonuclear leucocytes infiltrated the zona fasciculata and zona reticularis but were not found in the zona glomerulosa (Fig. 3). The number of leucocytes varied as indicated by 2+, 3+ and 4+ in Table I. Only occasionally were they found in groups. Usually the leucocytes were restricted to the zona fasciculata and zona reticularis; however, in three dogs (99, 172 and 173) they were also present in the medulla of the adrenal. Extensive necrosis was observed in only one dog, No. 188. This occurred in the zona fasciculata and zona reticularis and was a diffuse lesion. This necrosis was characterized by a coagulation process not associated with an abnormal number of phagocytic cells (Fig. 4).

The data in Table I are arranged to show the increased length of time between the period of anoxia and the time of death. It may be seen in this table that the maximum injury to the adrenal gland occurs in dogs sacrificed between 6 and 24 hours. Each of 12 dogs sacrificed at 24 hours showed pathologic lesions; only 5 showed a 1+ and 7 a

2+ lesion or more. Of the 11 dogs sacrificed after 48 hours, 6 did not show any pathologic changes in the adrenal and 5 showed only a one plus lesion.

The correlation between the degree of pathologic change and the clinical symptoms at the time of death is not close. Many animals that were comatose had only minor degenerative changes in the adrenals. These observations would suggest that death in this group of anoxic dogs is not the direct result of adrenal gland injury. Dog 215 was comatose at the end of 96 hours, but no lesions were found in the adrenals. There is nothing in this experiment to indicate that dogs sacrificed after 48 hours had had a more severe pathologic change during the earlier part of the experiment and the lesions then subsided. The cerebral damage resulting from the anoxia, no doubt, is a significant factor in the mechanism of coma and death in these animals.

Summarizing the clinical changes in 36 dogs made anoxic, 10 died (they are not included in the pathologic study). Of 24 made anoxic for 6½ minutes, 4 recovered or would have recovered if not sacrificed; of 6 made anoxic for 8 minutes, 4 were comatose when sacrificed for histologic study and 2 died; of 5 made anoxic for 8½ minutes, 2 were comatose when sacrificed for autopsy and 3 died.

Many of the dogs had pulmonary edema, petechiae and bronchopneumonia. A few had small lacerations in the liver. Sometimes the brain was edematous grossly; microscopically, extensive neurological changes were present in a majority of the animals. The pituitary and the thyroid glands were studied in many of these anoxic dogs but no significant morphologic changes were found.

Discussion. The morphological studies on the adrenal gland of anoxic dogs show changes that may be irreversible. However, as the gland has considerable power of growth and regeneration, this is not thought likely. The infiltration of polymorphonuclear leucocytes through the cortex is indicative of severe injury. It is of interest to observe that this injury occurs in the zona fasciculata and zona reticularis. No morphological changes were noted in the zona glomerulosa. Polymorphonuclear leucocytes were observed in the medulla of only 3 dogs and these animals also showed very severe lesions in the cortex.

In considering the pathogenesis of these adrenal lesions, it was originally thought that the effect might be secondary to a change within the pituitary gland. No morphologic changes were observed, however, in the pituitary gland of any dog. The facts that the adrenal gland has such a great flow of blood through it, that it apparently secretes such large quantities of hormone(11), and that it has been shown here to be susceptible to anoxic damage—all these facts appear to be related to one characteristic: that is the tissue is metabolically very active. Haldane (12) originally pointed out that anoxia not only slows the bodily machine, it causes irreparable damage to living structure as well. One would expect, therefore, that a tissue with high metabolic activity would suffer irreparable damage sooner than another tissue with low metabolic activity. The adrenal cortex follows this rule: its susceptibility to anoxic damage is apparently exceeded by only one other organ in the body, *viz.*, the brain. The clinical manifestations of anoxia in these dogs appear to be related primarily to the cerebral anoxia rather than to adrenal insufficiency. In support of this hypothesis, the clinical symptoms in four anoxic dogs were not affected by large doses of cortisone† given intramuscularly following the period of anoxia.

The variation in the degree of pathologic changes in the adrenals of dogs made anoxic for some length of time would suggest chemical differences within the glands. If the lesions could be inhibited, the agent might be of therapeutic value in cases of anoxia in man. It would seem necessary, however, to use other animals than mongrel dogs, as we have done, for such a study. The occurrence of pathologic lesions in the adrenal of dogs as the result of anoxia is significant when one recalls that many cases have been studied where death occurred in both man and animal following anoxemia and such lesions as we have observed were not present. It is suggested that the conditions of this experiment

† Supplied by Merck & Co., Rahway, N. J.

are such that one would not likely encounter its counterpart in either human cases or other experiments. To produce it, the anoxia must be of long duration—so long, in fact, that the victim dies of circulatory failure and never lives to show morphologically the anoxic insult to the adrenal cortex. This may account for the absence of reported pathologic changes even in uncomplicated acute anoxic death, such as reported by Kritzler(13). Life must persist in the dog for at least 6 hours for these lesions to develop. Animals surviving longer than 48 hours after this period of anoxia are not likely to show any more than mild cytological changes in the epithelial cells of the adrenal cortex.

It was concluded in a previous report by one of us (H.G.S.) that an irreversible cerebral insult was produced in dogs by 6½ minutes of fulminating anoxia(9). The present study shows that this estimate is a little too short, for 4 out of 24 (17%) made apparently complete recovery from this length of exposure. At 8 minutes, however, all dogs either died or would have succumbed. These figures are very similar to those of Kabat, Dennis and Baker(14). Undoubtedly an irreversible cerebral insult takes place in both cats and dogs after approximately seven minutes of fulminating anoxia.

Summary. Dogs were given pure nitrogen to breathe and then resuscitated by lung insufflation and cardiac massage about 6½ minutes after the onset of anoxia. This was at a time when breathing and circulation had failed. These animals were sacrificed at various intervals thereafter. The earliest morphological changes that occur in the adrenal glands are pyknosis of the nucleus and coagulation of the cytoplasm of epithelial cells in the zona fasciculata and zona reticularis. Subsequently, polymorphonuclear leucocytes infiltrate these areas of the cortex. A few petechiae occur. Clinical manifestations in such anoxic dogs appear to be the result of cerebral injury and not due to adrenal insufficiency.

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Antagonism of Mercurial Poisoning in Growing Cells Investigated with Onion Root Tips. (20039)

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Growing root tips of the bulbs of onions (*Allium cepa L.*) are highly sensitive to the organic mercurials phenylmercuric hydroxide (PMOH) and basic phenylmercuric nitrate (PMN), and the gross reactions provide delicate indicators for a range of very low concentrations(7,8,10). Aqueous solutions of alcoholic extracts of beef spleen and of yeast protected roots during short exposure to lethal concentrations of PMN(9), but did not pre-