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Effect of Cold Stress on Ultrastructure of Adrenaline - and Noradrenaline-Storing Cells in the Domestic Fowl[1/]

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ABSTRACT

Sixteen Brown-Leghorn cockerels were fed in temperature controllable chamber at 30°C for 10 days before being immediately subjected to low temperature at 3°C. The ultrastructure of adrenomedullary cells following the change of temperature at various time intervals (0, 1, 3, 6, 9, 12 hours and 1, 2, 3, 4, 7, 10, 14 days) were observed. The noradrenaline-storing (NA) cells were more intensely affected than adrenaline-storing (A) cells, resulting in decrease their secretory granules. It is suggested that the cold stress induces secretory granules of both A-and NA-storing cells degranulation via a release mechanism other than exocytosis in the common sense as previous findings. The domestic fowl could adapt to chronic cold environment.

INTRODUCTION

Many morphological studies have found a decrease in the number of chromaffin granules with an electron-dense core after stimulation through the sympathoadrenal system. Many Stimuli, such as drugs hormones and stress, have been used for exciting secretory activity of adrenomedullary cells (Hopwood, 1971). The stressful stimuli is one of the most using stimulation secretory activity of adrenomedullary cells. The depletion of adrenal catecholamines have been investigated in rats on exposure to excercise (Eränkö and Harkonen, 1961 ; Morisawa, 1968), immobilization (Corrdi et al., 1968 ; Kobayashi and Serizawa, 1979), crowding (Welch and Welch, 1968), heat (Corrodi et al., 1967), and cold stress (Young and Landsberg, 1981 ; in Syrian hamster, Al-lami and Farman, 1975)

In avian species, it has a little studies on the effect of temperature stress on adrenal catecholamines (Lin and Stukies, 1968; El-Halamani et. al., 1973). In addition, the releasing mechanism of catecholamines from adrenal medulla of the domestic fowl has also not clearly elucidated. Although the previous works (Chungsamarnyart et al., 1981 b, 1982) had found the existence of release mechanism of secretory granules other than exocytosis, but they were in the emergency stage of insulin-induced hypoglycemia and reserpine-induced stimulation.

Therefore, the present work is an attempt to confirm the previous works about the releasing mechanism of catecholamines from adrenomedullary cells in the domestic fowl, and to study the ultrastructural appearance of these cells on the cold stress-induced stage.

MATERIALS AND METHODS

Sixteen Brown Leghorn cockerels (about 1.0 kg in body weight) were fed with free access to food and water in temperature controllable chamber (Koitotron HN) at 30 C. for 10 days. One fowl was used as control which it was sacrificed just before subjected to low temperature. The other were immediately subjected to low temperature at 3 C. The temperature change took 15 min for the temperature becoming stabilization. At various time intervals (1, 3, 6, 9, 12 hr and 1, 2, 3, 4, 7, 10, 14 days) following subjected to low temperature, one or two fowls were anesthetized with sodium pentobarbiturate (Nembutal) and

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perfused with modified Karnovsky's fixative solution containing s-collidine (Chungsamarnyart et al, 1981 a), or the glands were quickly excised and immersed in the same fixative solution.

The adrenal glands of all the specimens were sliced in fixative solution into cubes about 1 mm thick and then left in fresh fixative solution for 2 h. After tissues were rinsed several times for 30 min with 0.14 M veronal acetate buffer (pH 7.4), they were postfixed in a solution of 1% osmium tetroxide in 0.14 M veronal-acetate buffer (pH 7.4) for 2 h. and then stained en bloc with 0.5% uranyl acetate in 0.14 M veronal-acetate buffer for 1 h. Then tissue blocks were dehydrated in series of ethanol and embedded in Epon 812. The thin sections (60-80 nm) were double stained with 2% uranyl acetate and modified lead staining solution (Sato, 1968). Thick sections (about 1 um) were stained with 1% toluidine blue in borax (pH 9.1).

For quantitative studies of A-and NA-storing-cell organelles, ten photographs of each cell which showed a nucleus and was clear of contamination, were randomly taken at a fixed magnification of 4,000, and then enlarged in print to x12,000. The 100 test-point frame was used for counting of some cellular organelles according to Weibel et al. (1966). Secretory granules, granule-sized vesicles, vacuoles, and mitochondria, were counted in two opposite cytoplasmic areas, avoiding the Golgi complex and any accumulation of rough endoplasmic reticulum except during the extensively degranulated stage when the Golgi complex and rough endoplasmic reticulum were dispersed over the cytoplasm. The mean values of the elements per unit volume for each group were plotted against the number of hours after cold exposure.

RESULTS

Light microscopic observation

In thick sections of control adrenal tissues stained with 1% toluidine blue, the NA-cells exhibited blue-green colored granularity in the cytoplasm, while the cytoplasm of the A-cells was metachromatically and homogeneously stained deep violet, as reported in the intact fowls in previous works (Chungsamarnyart et al., 1981 a). In the cold exposed fowls, the whole of each A-and NA-cells was not equally stained with toluidine blue, especially the NA-cells in the fowls following cold exposure at 1, 3, 6, 9 and 12 h.

Electron microscopic observation

The general ultrastructural appearance of A - and NA-cells in control fowl (Fig. 2 a) resembled that of the intact fowl (Unsicker, 1973: Chungsamarnyart et al., 1981 a). Therefore, a brief description is as follow; A-and NA-cell cytoplasm was filled with secretory granules of 225-550 nm and 200-600 nm in diameter, respectively. The secretory granules of A-cells were always spherical or elongated membrane-bound, filled with fine grains or reticular material of moderate electron density. The NA-cell secretory granules were spherical membrane-bound with an eccentrically located core of high electron density surrounded by granular subunits (15-37 nm in diameter). Small amount of tubular cristae type of mitochondria, rough ER, granule-sized vesicles, free ribosomes and polysomes were often dispersed uniformly throughout the cell. A small Golgi complex consisting of several flattened cisternae and vesicular components was observed in para-nuclear position. Dense bodies always bounded by single mambrane and a little larger in size than secretory granules often occurred near the Golgi area.

In cold exposed fowls, the NA-cells were more intensly affected than the A-cells with respect to decreasing of secretory granules. The quantitative studies (Fig. 1) showed that both A-and NA-cells were affected within the first hour after cold exposure. At this initial depletion stage, the number of A-and NA-granules in the cells of cold stress-induced fowl had decreased by approximately 30% and 26% respectively when compared with the control fowl. At the other times interval after cold exposure, the number of A-granules had still approximately decrease in the same level as in that first hour. The number of NA-granules continued more decreasing at 6 and 9h after cold subjected, and after that they gradually increased to the same level of the first hour until 10 days after cold

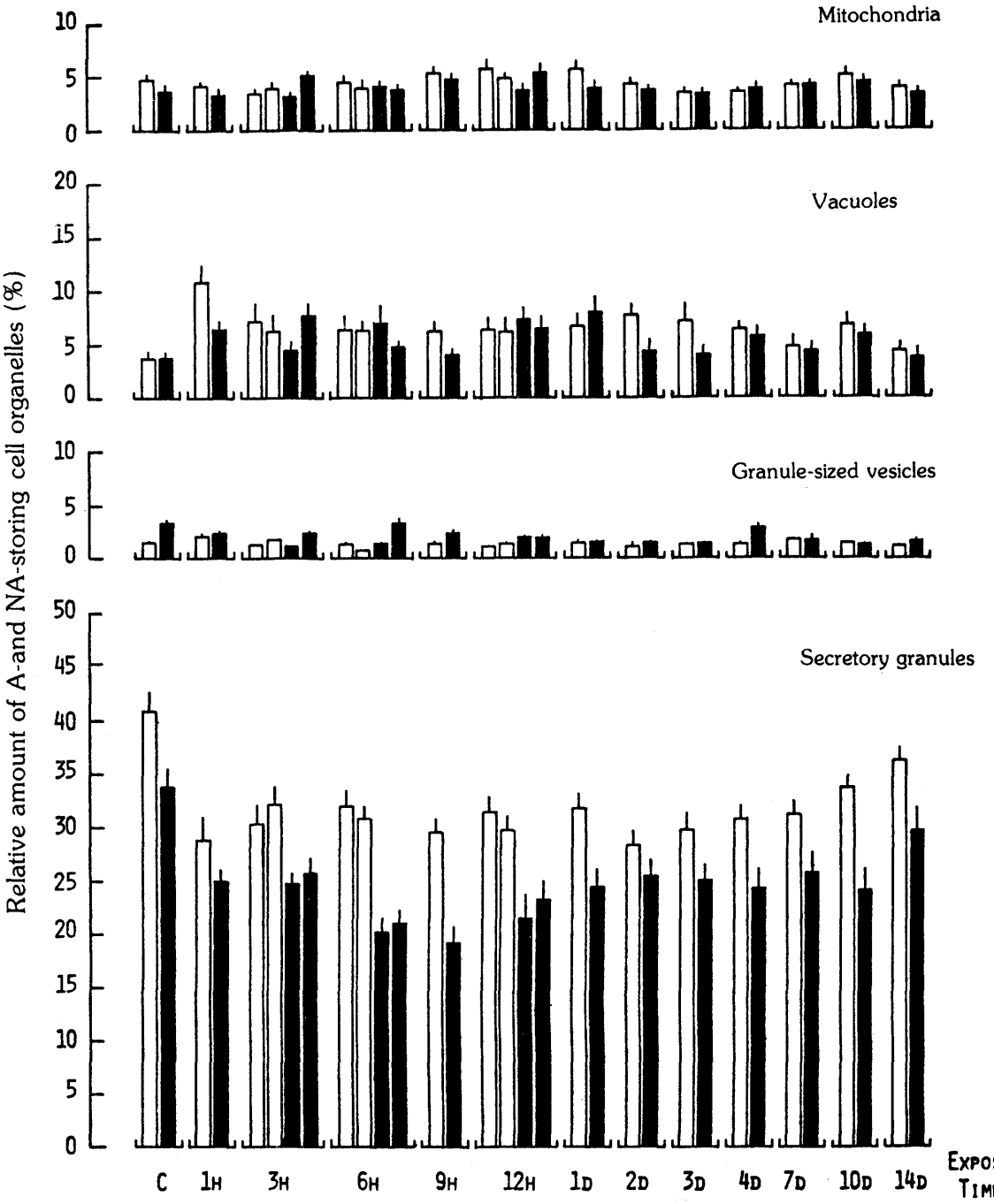

Fig. 1 Effect of cold stress on the amount of Adrenaline (A)-and Noradrenaline (NA)-storing cell organelles. The values (mean ± standard error of 20 determinations in 10 cells) were estimated by multipurpose 100 test-points (Weibel et al., 1966). C : Control fowl; □: A-storing cells ; ■: NA-storing cells.

exposure. At those most affected stage, the number of NA-granules had decreased by approximately 40% compared with the control fowl. The amount of both A-and NA-granules had restored at 14 days after cold exposure. During depletion stage, the number of granule-sized vesicles and mitochondria in both A-and NA-cells were not significantly changed, but the vacuoles were significantly increased in both sized and number. During cold stress, the secretory granules of A-and NA-cells decreased considerably in number (Fig. 1), but profiles indicative of exocytosis,which were rarely found in the control, did not seem to increase. The ultrastructural change of the secretory granules and other cell organelles was observed as follows:

a) In the affected cells of both types, some secretory granules were found to contain a small vesicle or an electron-lucent area. These granules will hereafter be referred to as "modified secretory granules". In addition to these modified granules, some secretory granules were enlarged. The enlarged secretory granules in A-cells contained flocculent, low electron-dense core (Figs. 3b, 3c, 5f, 6c). In NA-cells they contained loosely dispersed subunits or flocculent low electron-dense material with a small electron-dense core (Figs. 2b, 3a, 3d, 4a, 4c, 5a). These modified and enlarged secretory granules were also occasionally found in the control, but to a much lesser degree than in the cold subjected fowls. The opening of an unmodified secretory granule into an enlarged granule (Fig. 5c, d) or the joining of enlarged granules with each other was occasionally found in affected cells (Fig. 5a).

b) Modified, enlarged and unmodified secretory granules often appeared in contact with or open to the vacuoles, in both the perfusion and immersion specimens (Figs. 3a, 3c-e, 4a, c, d, 5b, c, e, 6a-e). Vacuoles of different sized were found to be much more numerous in the cold stress-induced adrenomedullary cells than in the control. They were often located at the periphery of the cell, particularly the larger ones and contained granule-like, electron-dense mass and flocculent material of low electron density (Figs. 2b, 3a, 4a, b, 5d, f, 6d, e). The coalescence of two or three small vacuoles into one was also observed (Fig. 6e). The vacuoles sometimes opened into the intercellular or extracellular spaces (Figs. 2b, 3a, 6d). These opening vacuoles often contained loosely flocculent low electron-dense material only and often observed the micropinocytotic pits on the membrane of vacuoles (Fig. 6d).

c) Both of affected A-and NA-cells especially NA-cells, had numerous rough endoplasmic reticulum in form of accumulation or dispersion, abundant free,ribosomes and polysomes, and a great enlargement of the Golgi complex (Fig. 7). The Golgi complex was rich in both cisternae and vesicular components. Some terminal saccules of Golgi cisternae contained electron-dense material. In the Golgi zone, spherical membrane-bounded granules were found in a much greater number than in the control fowl. They contained a small electron-dense core and homogeneous low electron-dense material or only an electron-dense core. They were probably newly-formed secretory granules, which appeared to detach from the terminal succule of the Golgi cisternae containing electron-dense material (Fig. 7).

d) The ultrastructural appearance of most A-and NA-cells at 14 days after cold exposure were similar appearance to that in the control fowl.

DISCUSSION

The quantitative and ultrastructural studies revealed that both A-and NA-cells were affected by cold exposed stress. This finding showed that the domestic fowl and mammals have some what different responsibility for cold-exposed stress, since in the rat and syrian hamster only noradrenaline was released after cold stress (Al-Lami and Farman, 1975; Storm et al., 1981). However, the amount of NA-granules were more slightly decreased than A-granules on 6-12 h after cold exposure (Fig. 1). This results agrees with previous work (El-Halawani et al., 1973) that in the 6 h cold (7°C) -expose turkeys, both adrenaline and noradrenaline increased depletion (64% and 73% respectively) after the turkeys was pretreated

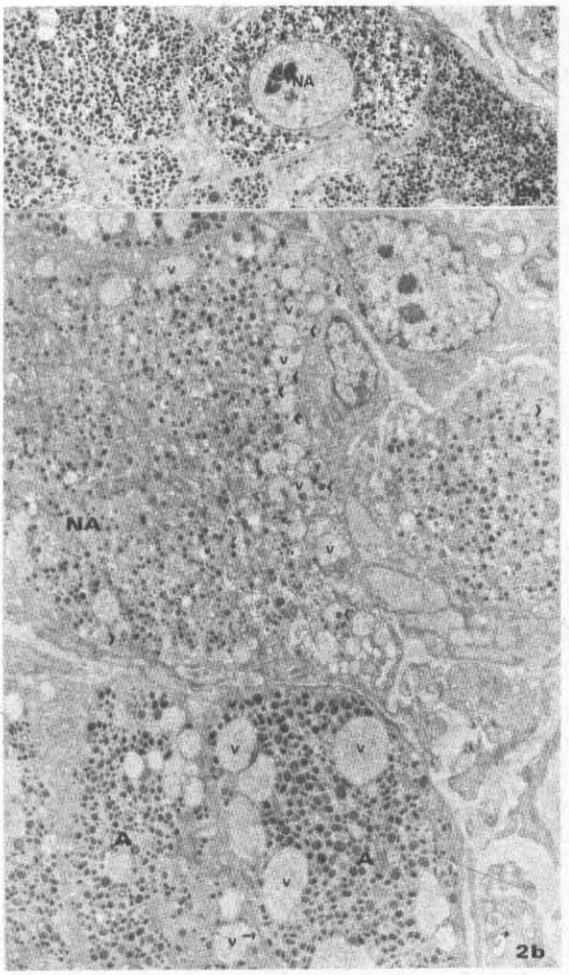

Figure 2 Adrenomedullary cells of the control and 3h cold exposed fowls. Figure 2a, Adrenaline and noradrenaline-storing cells (A-and NA-cells). X 3,000. Figure 2b, A-and NA-cells of 3h cold exposed fowl showing numerous enlarged granules (arrow heads) and vacuoles of various size (V) at periphery of the cells. The vacuoles contain moderate electron-dense mass and flocculent low electron-dense material. One vacuole opening into intercellular space (arrow). X 5,000

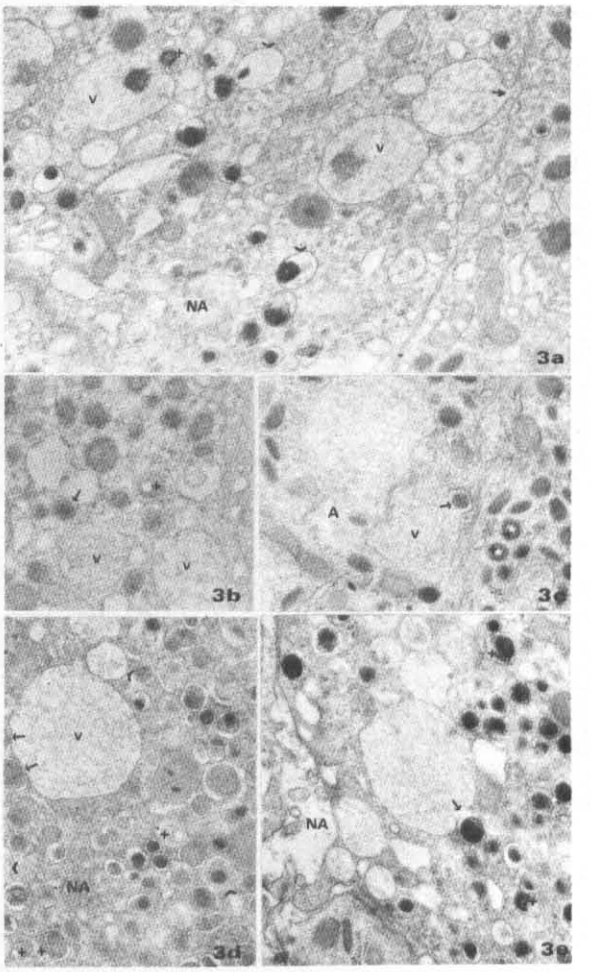

Figure 3 A-and NA-cells of the fowls after cold exposure 1h (Figs 3a, 3b), 3h (Figure 3d, 3e) and 6h (Figure 3c) showing modified (+) and enlarged secretory granules (arrow heads), large vacuoles (V) contain moderate electron-dense mass and flocculent low electron-dense material which probably represent the released content of secretory granules. One vacuole opening into intercellular space (arrow). Unmodified secretory granule contacts with the vacuole (Figure 3c; arrow) and one is opening into the large vacuole (Figure 3c and 3e; arrows) and small vacuole (Figure 3b; arrow). X 20,000.

with an inhibitor of tyrosine hydroxylase. It suggests that cold exposed stress increases the activity of sympathoadrenal system.

Based on the return of ultrastructural appearance of A-and NA-cells similar to the control fowl following prolonged cold exposure was observed at 14 days. It also agrees with El-Halawani et al. (1973) in the turkeys. Therefore, it may be suggested that the domestic fowl could adapt to chronic cold environment in 2 weeks.

The phenomena observed in the affected A-and NA-cells were similar to those observed in

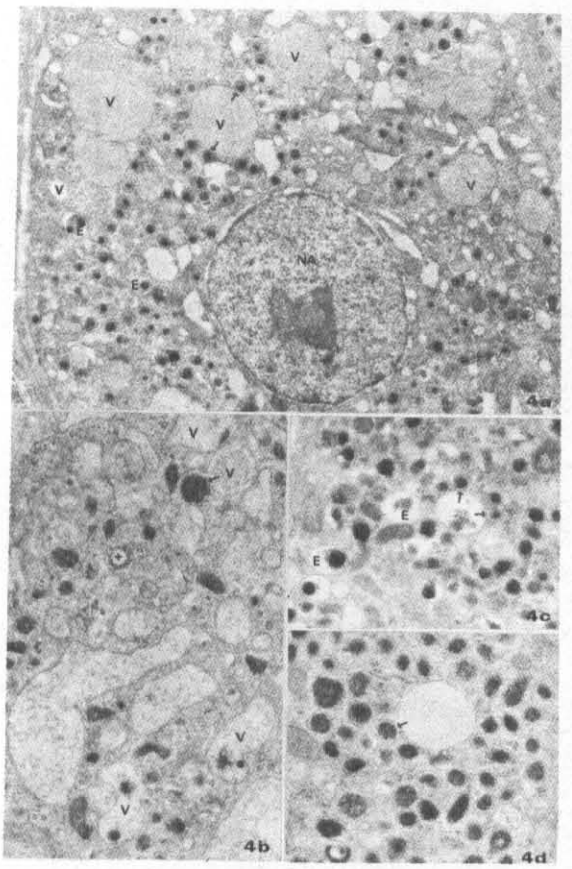

Figure 4 A-and NA-cells of the fowls after cold exposure 6h (Figure 4a; X10,000) and 9h (Figure 4b-d; X 20,000) showing numerous vacuoles of various size (V) with some vacuoles contain granule-like electron dense mass (Figure 4b), enlarged granules (E). Some unmodified granules contact and fuse with membrane of vacuole (Figure 4a, 4b and 4c; contact and fuse with membrane of vacuole (Figs. 4a, 4b and 4c; arrows) and one is opening into vacuole (Fig. 4c; arrow).

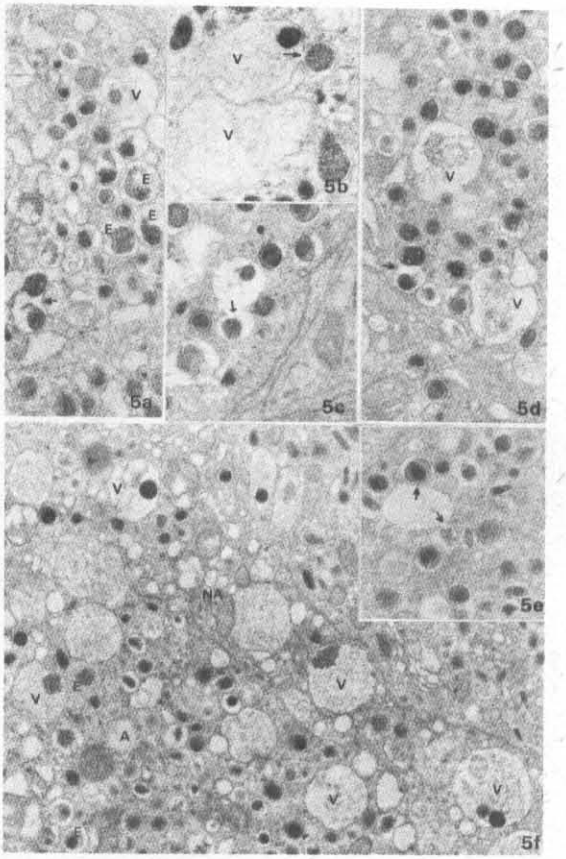

Figure 5 A-and NA-cells of the fowls after cold exposure 12h (Figure 5a, b) 24h (Figure 5d-f), and 10 days (Figure 5c). Figure 5a, 5c and 5d show the unmodified or enlarged secretory granules of NA-cells uniting to each other (arrows). X 20,000 Figure 5b shows one secretory granule opening into vacuole containing granule-like electron mass. X 20,000. Figure 5e, Unmodified secretory granule in contact with and one opening into small vacuole (arrows). X 20,000. Figure 5f shows the general ultrastructural change of affected A-and NA-cells having numerous enlarged secretory granule (E), and vacuoles of various size (V) which contain granule-like electron-dense mass. X 16,000.

the previous insulin- (Chungsamarnyart et al., 1981 b) and reserpine-induced degranulation (Chungsamarnyart et al., 1982). Thus the present observation suggests that cold-exposed stress produces a depletion phenomena of A-and NA-cells in the same way as in the case of insulin-induced hypoglycemia which induces reflex neurogenic stimulation of the secretory activity, and in the case of reserpine-induced degranulation. Therefore, only a brief description will be given here.

During cold exposed-stress induced degranulation of A-and NA-cells, A-and NA-granule contents altered to contain an electron-lucent area or a small vesicle, probably due to some initial change in the construction of storage complex of catecholamine, ATP and carrier protein (Figs. 3 a - e, 4b). These modified

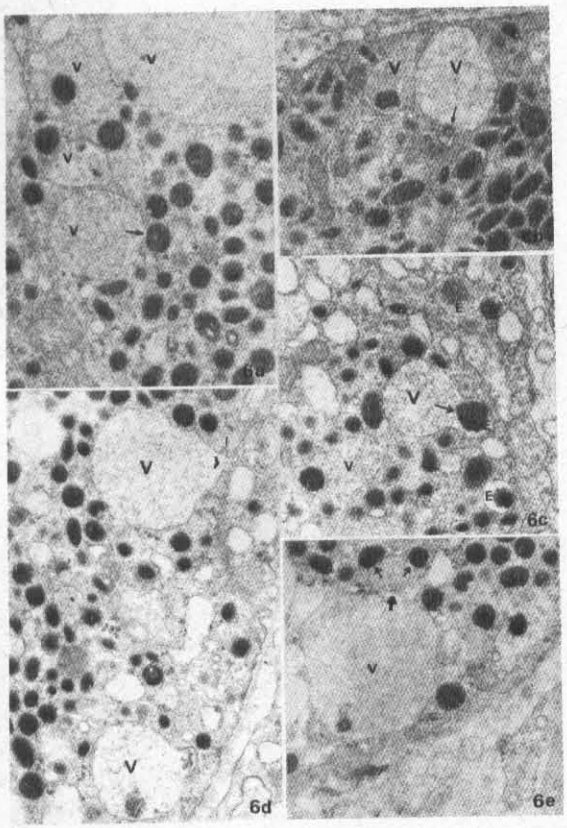

Figure 6 A-cells of the fowls after cold exposure 2 days (Figure 6a), 3 days (Figure 6b), 4 days (Figure 6c and 6d) and 7 days (Figure 6e) showing unmodified and enlarged secretory granules opening into the vacuole (arrows). One vacuole opening into intercellular space (arrow head) with having micropinocytotic pit (!). X 20,000.

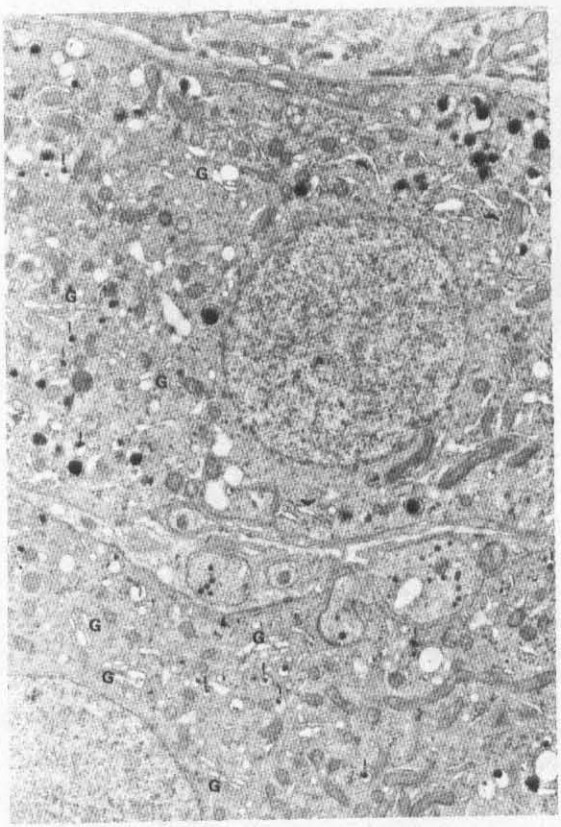

Figure 7 Affected NA-cells of the fowl after exposure 12h showing small amount of secretory granules, numerous free ribosomes and polysomes, discrete rough endoplasmic reticulum (arrow heads), enlarged Golgi complex (G) which consists of abundant Golgi cisternae and vesicular components, and numerous propably newly-formed secretory granules (!). X 10,000.

granules then become enlarged with a small electron-dense core and flocculent radiated materials or a loose arrangement of contents (Figs. 2b, 3a, 4a, 5a, 5f, 6c). These enlarged granules showed a tendency to unite together to form small vacuoles (Figs. 5a, 5c). Unmodified secretory granules, as well as enlarged granules, released their contents into there small vacuoles to form larger vacuoles (Figs. 3b, 4b, 4c, 6e). The granule-like, electron-dense mass in the vacuole probably represents the released contents of granules (Figs. 2b, 3a, 4b, 5b, d, f, 6a, b, d, e). Unmodified secretory granules also appeared to discharge their contents into the vacuole (Figs. 3c, d, e, 6e). Large vacuoles with flocculent low electron-dense materials and granule-like electron-dense mass come to occupy the periphery of the cell, contact and fuse with the plasma membrane and eventually open to discharge their contents into the intercellular or extracellular spaces. Since the phenomena of the opening these vacuoles was similar in those in insulin and reserpine treatments, the present studies was not observed these vacuoles on serial thin sections. The previous observation showed that the large vacuoles to be closed in round, oval or elongated shapes in the cytoplasm, and not, therefore, the indentation of plasmmalema, although some vacuoles were in the process of opening to

outside of the cell (Figs. 2b, 3a, 6d). In addition, the present studies also found that the vacuoles opening to the outside of the cell were not found to contain the granule-like electron-dense mass that vacuoles in the cytoplasm did. The vacuoles increased during the degranulation stage but not during the regranulation stage of the cells. The idea that the vacuole arose from the indentation of plasma membrane and subsequently became closed vacuoles in the cells is, therefore, unlikely suggestion.

According the present work was also found similar degranulation phenomena of A-and NA-cells as in the insulin and reserpine experiments (Changsamarnyart et al., 1981 b; Chungsamarnyart and Fujioka, 1982). It might be suggested that A-and NA-cells in adrenal domestic fowl release their secretory granules via this possible degranulation mechanism which it differed from the exocytosis in common sense. By this possible release mechanism, the entire contents of the secretory granules were also simultaneously discharged and left the secretory membrane in the form of opened vacuoles. Many micropinocytotic pits occurring on the opened vacuoles may be signs of a retrieval process of the secretory granule membrane.

In the affected cells that their secretory granules were much depleted, had numerous free ribosomes and large amounts of accumulated or discrete rough endoplasmic reticulum. It is possible that these numerous structures could participate in producing the precursors of the carrier-protein of secretory granules, which subsequently appeared within the Golgi complex (Coupland et al. 1976; Benchimol and Cantin 1978; Frydman and Geffen 1973). The occurrence of spherical granules containing a small electron-dense core and homogeneous low electron-dense material or only a small electron-dense core, were found much more than in the cells of the control fowls. These structures are regarded as the detached vesicles from terminals of Golgi cisternae, in agreement with the suggestion of Elvin et al. (1966), Benedeczky (1967) and Elfvin (1967).

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LITERATURE CITED

Al-lami, F. and N. Farman. 1975. Ultrastructural and histochemical study of the adrenal medulla in normal and cold-stressed syrian hamsters. Anat. Rec. 181: 113-130.

Benchimol, S. and M. Cantin. 1978. Ultrastructural radioautography of the incorporation of tritiated leucine by the rat adrenal medulla in vivo. Cell Tissue Res. 193: 179-199.

Benedeczky, I. 1967. Ultrastructural analysis of adrenaline synthesis following insulin treatment. Acta. Morphol. Acad. Sci. Hung 15: 23-37.

Chungsamarnyart, N., T. Fujioka and J. Kitoh. 1981 a. Correlative light, fluorescence and electron microscopic studies of catecholamine-storing cells in the adrenal medulla of the domestic fowl. Cell Tissue Res. 217: 471-477.

Chungsamarnyart, N., T. Watanabe and T. Fujioka. 1981 b. A possible mode of release of secretory granules from adrenomedullary cells in domestic fowl. Jpn. J. Vet. Sci. 43: 951-955.

Chungsamarnyart, N. and T. Fujioka. 1982. Ultrastructural studies on the Degranulation and Regranulation of adrenal catecholamine-storing cells in the domestic fowl after reserpine treatment. Jpn. J. Vet. Sci. 44: 439-457.

Corrodi, H., K. Fuxe and T. Hokfelt. 1967. A possible role played by central monoamine neurons in thermo-regulation. Acta. Physiol. Scand. 71: 224-232.

Corrodi, H., K. Fuxe and T. Hokfelt. 1968. The effect of immobilization stress on the activity of central monoamine neurons. Life Sci. 7: 107-112.

Coupland, R.E., S. Kobayashi and C. Kent. 1976. Observations on the localization of

recently synthesized catecholamines in chromaffin cells after the injection of L-(2, 5, 6, -3H) DOPA. J. Endocrinol. 69: 139-148.

El-Halawani, M.E., P.E. Waibel, J.R. Appel and A.L. Good. 1973. Effects of temperature stress on catecholamines and corticosterone of male turkeys. 224: 384-388.

Elfvin, L-G. 1967. The development of the secretory granules in the rat adrenal medulla. J. Ultrastruct. Res. 17: 45-62.

Elfvin, L-G., L.E. Appelgren and S.Ullberg. 1966. High-resolution autoradiography of the adrenal medulla after injection of tritiated dihydroxypheny-alanine (Dopa). J. Ultrastruct. Res. 14: 277-293.

Eränkö, O. and M. Härkönen. 1961. Distribution and concentration of adrenaline and noradrenaline in the adrenal medulla of the rat following depletion induced by muscular work. Acta. physiol. scand. 51: 247-253.

Frydman, R. and L.B. Geffin. 1973. Depletion and repletion of adrenal dopamine-β-hydoxylase after reserpine. Immunohistochemical and fine structural correlates. J. Histochem. Cytochem. 21: 166-174.

Hopwood, D. 1971. The histochemistry and electron histochemistry of chromaffin tissue. Progr. Histochem. Cytochem. 3: 1-66.

Kobayashi, S. and Y. Serizawa. 1979. Stress-induced degranulation accompanied by vesicle formation in the adrenal chromaffin cells of the mouse. Arch. histol. jap. 42: 375-388.

Lin, Yu-C. and P.D. Sturkie. 1968. Effect of environmental temperatures on the catecholamines of chicken. Am. J. Physiol. 214: 237-240.

Morisawa, T. 1968. On the noradrenaline reaction of the adrenal especially in spontaneously hypertensive rats III. Effects of various experimental conditions on the noradrenaline storing cell islets of the adrenal medulla in spontaneously hypertensive rats. Jap. circul. J. 32: 177-193.

Sato, T. 1968. A modified method for lead staining of thin sections. J. Electron Micros. 17: 158-159.

Storm, H., C. van Hardeveld and A.A.H. Kassenaar. 1981. Thyroid hormone-catecholamine interrelationships during exposure to cold. Acta Endocrinologica 97: 91-97.

Unsicker, K. 1973. Fine structure and innervation of the avian adrenal gland. I. Fine structure of adrenal chromaffin cells and ganglion cells. Z. Zellforsch. 145: 389-416.

Weibel, E.R., G.S. Kistler and W.F. Scherle. 1966. Practical stereological methods for morphometric cytology. J. Cell Biol. 30: 23-38.

Welch, B.L. and A.S. Welch. 1968. Greater lowering of brain and adrenal catecholamines in group-housed than in individually-housed mice administered DL-α-methyltyrosine. J. Pharm. Pharmacol. 20: 244-246.

Young J.B. and L. Landsberg. 1981. Effect of concomitant fasting and cold exposure on sympathoadrenal activity in rats. Am. J. Physiol. 314-319.