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CARDIOVASCULAR, HEPATIC AND RENAL LESIONS IN MICE RECEIVING CORTISONE, ESTRONE AND PROGESTERONE†

In mice receiving large amounts of cortisone for one week the total incidence of pericarditis, myocarditis, and of abscesses in kidneys, liver and myocardium was approximately 20 per cent. Simultaneous administration of cortisone and androgen increased the incidence of such lesions.[1] In these experiments myocardial necrosis occurred almost entirely in association with the inflammatory lesions mentioned above.[1]

This report considers effects of cortisone, estrone and progesterone, and of combinations of cortisone and these ovarian hormones. The cortisone plus estrone combination produced a high incidence of myocardial and arterial lesions which was not associated in a majority of the cases with concurrent pericarditis or abscesses within the heart or other organs.

MATERIALS AND METHODS

Animals: Young adult male and female (nulliparous) albino mice (Balb/C and Taconic Swiss) with initial body weights of 22-26 gm. were used. An adequate commercial diet and water were available at all times. Mice were weighed daily at the time of injections and immediately before being killed by cervical compression.

Injection of hormones: Daily subcutaneous injections of the hormones‡ (as aqueous suspensions) were made at 9 to 10 a.m. as follows:

(1). 50 mice: cortisone acetate, 2.5 mg. for 7 days.
(2). 49 mice: estrone, 1 mg., 9 days.
(3). 60 mice: progesterone, 1 mg., 9 days.
(4). 57 mice: estrone 9 days, plus cortisone during the last 7 days, both as above.
(5). 50 mice: progesterone 9 days, plus cortisone during the last 7 days, both as above.

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* National Institutes of Health, U.S. Public Health Service (2G-287) Predoctoral Trainee in the Anatomical Sciences. A portion of these data will be included in material to be submitted to the Faculty of the Graduate School of the University of Mississippi as a dissertation in candidacy for the degree of Doctor of Philosophy.

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† Supported by grant H-4052 (C2) from the National Institutes of Health, U.S. Public Health Service.

‡ Hormones were generously supplied by the Schering Corporation.

Received for publication 19 July 1962.

When two hormones were administered (as above) the mice were exposed to the actions of estrone or progesterone for 48 hours prior to receiving the initial injection of cortisone. Body weights (Table 1) are based upon changes during the entire 7 days of injection of cortisone (alone) and those during the last 7 days of injection(s) in the other four groups (groups 2-5, above).

Histological methods: Mice were killed 24 hours after the last injection and organs were fixed in Lavdowsky's solution or 10 per cent formaldehyde. The technics used were the same as those described previously[1] and followed procedures described by Lillie[2] and Gurr.[3]

TABLE 1. CHANGES IN BODY WEIGHTS OF MICE RECEIVING CORTISONE (C), PROGESTERONE (P), AND ESTRONE (E)

	Cortisone (C)	*Proges-terone (P)*	*Estrone (E)*	*C+P*	*C+E*
I. Total no. of mice	50	60	49	50	57
II. Without extensive inflammatory lesions	78%	97%	98%	84%	88%
A. Wt. change*	—10.8%	+5.8%	—5.2%	—5.4%	—14.4%
	±4.3	±4.4	±5.4	±4.1	±6.7
	0.76	0.58	0.81	0.62	0.94
(1) % gained wt.	0	79%	22%	10%	2%
(2) % lost wt.	100	7%	67%	80%	82%
III. With extensive inflammatory lesions	22%	3%	2%	16%	12%
A. Wt. change	—14.6%	+11.6%	—7.5%	—12.2%	—19.6%
	±4.8	**	**	±8.4	±7.35
	1.6			2.9	2.7
(1) % gained wt.	0	100	0	0	0
(2) % lost wt.	100	0	100%	100%	100%

* Standard errors are underlined.

** No statistical analyses of these small groups.

The study was based on specimens prepared as follows:

(1). Complete frontal sections of heart (with great vessels at base, and also containing thymus, fat, nerves, ganglia, lymph nodes, bronchi and esophagus): H. and E., PAS (with and without diastase-hydrolysis and oxidation with periodic acid), van Gieson (for fibrosis), Sudan black (for ceroid pigment), von Kossa or alizarin (for minerals), and thionin[3] (for metachromasia of acid mucopolysaccharides).

(2). Kidneys and lungs: PAS and H. and E.

(3). Liver: PAS, H. and E. toluidine blue (with and without ribonuclease-hydrolysis to show RNA), and formalin-fixed frozen sections stained with oil red O to show fat.

The PAS procedure was used extensively because of its capacity, when properly controlled and modified,[2,3,4] to show glycogen, fibrosis, hyalin and/or fibrinoid, precise renal glomerular structure, elastic membranes of vessels, and under certain circumstances, myocardial necrosis.[5,6,7] The Gram and Ziehl-Neelsen methods were used to show microorganisms.

OBSERVATIONS

Mice of the two stocks and of the age used here show no significant incidence of spontaneous disease.[1] Excluding reactions of reproductive systems there were no changes that seemed related to the sex, or to the stocks of mice used.

TABLE 2. INCIDENCE (%) OF INFLAMMATORY LESIONS OF HEARTS, LIVERS AND KIDNEYS, AND OF PNEUMONIA IN MICE RECEIVING CORTISONE (C), PROGESTERONE (P) AND ESTRONE (E)

Types of lesions	*C*	*P*	*E*	*C+P*	*C+E*
Pericarditis	4%	3%	2%	2%	1.8%*
Myocardial abscesses	2%	0	0	6%	7%
Hepatic abscesses	8%	0	0	0	1.8%
Renal abscesses	18%	1.7%	0	14%	3.5%
Hepatic necrosis	0	1.7%	4%	0	3.5%
Pneumonia:					
(a) Total incidence	60%	15%	18%	34%	7%
(b) As only inflammatory lesion	43%	15%	18%	22%	7%

* In one mouse receiving cortisone and estrone there was peritonitis; in another, abscesses within one adrenal cortex.

Changes in body weight

These data are presented in Table 1. Progesterone (alone) increased body weight, and reduced weight loss in mice receiving cortisone. Estrone (alone) produced weight loss, and accentuated that of mice also injected with cortisone. In general, weight loss was increased in mice with abscesses (Table 1).

Gross findings at autopsy

Vaginae and uteri were enlarged in mice receiving estrone and cortisone plus estrone. Livers and spleens were enlarged and distended with blood in mice receiving estrone alone, and also cortisone and estrone. In the cortisone-plus-estrogen group subcutaneous edema, ascites and hydrothorax were

also observed. The renal and hepatic abscesses were obvious. The same was frequently true for pericarditis. The gross appearance of lungs often suggested edema and vascular congestion, or pneumonia.

Pericarditis, and abscesses of heart, liver and kidneys

A significant incidence was limited to mice receiving cortisone alone (Table 2). Non-purulent pericarditis and well localized myocardial, renal (cortical and medullary) and hepatic abscesses accounted for all such lesions. Microorganisms, chiefly Gram-negative, were observed in abscesses. Excluding decreases in renal abscesses, neither estrone nor progesterone altered significantly the incidence of lesions of this type from that produced by cortisone alone (Tables 1 and 2).

OTHER CARDIAC LESIONS AND MURAL HYALINIZATION OF BLOOD VESSELS

Epicardium and endocardium: Except for the pericarditis described earlier these layers were normal.

Myocardium: Except as is stated later, lesions were limited to mice receiving cortisone plus estrone (Table 3). The designation of the lesions as necrosis is based on the observations presented below, including the intense positive staining with PAS (with and without prior diastase-hydrolysis) of myocardial fibers described as necrotic. Reports of several studies have concluded that this type of positive PAS reaction indicates (or demonstrates) early myocardial necrosis.[1,5-8] The myocardial muscle fibers considered to be necrotic were non-striated, afibrillar, often non-nucleated or had pyknotic nuclei, and were hyperacidophilic (eosin) and

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All sections (paraffin-embedded) were stained by PAS plus hematoxylin following diastase-hydrolysis. Specimens are myocardium of mice receiving cortisone and estrone.

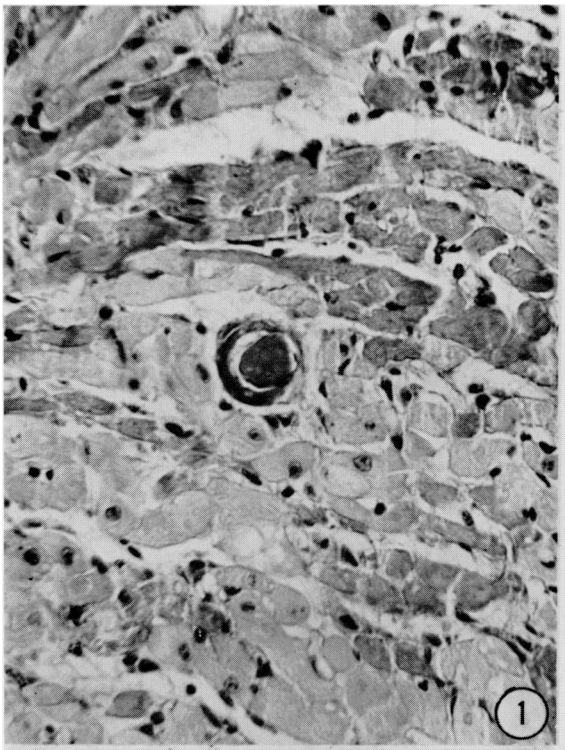
FIGS. 1 and 2. Similar areas from the same heart showing typical myocardial necrosis and mural hyalinization and thrombosis of small arteries. PAS-positive (darkly stained) myocardial muscle fibers are interpreted as necrotic. x400.

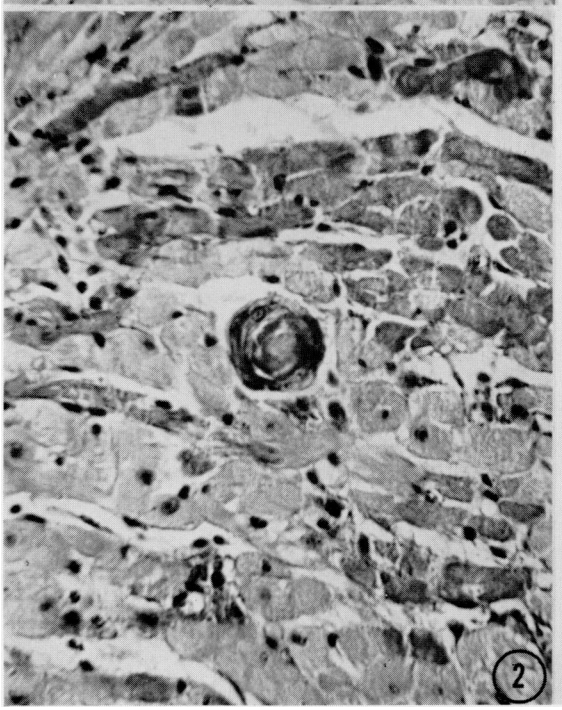
FIG. 3. Hyalinized artery. The adjacent necrotic myocardium was located to the left of this vessel. x685.

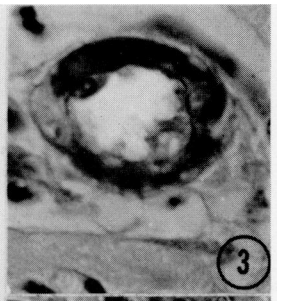
FIG. 4. Hyalinized, thrombosed and extremely dilated artery. In other sections the mural structure showed this vessel to be an artery and the thrombus to be more compact and homogeneous. No neutrophils or stainable microorganisms were present. This and other thrombi looked as if lipid might have been dissolved from them. Necrotic myocardium is not in the Figure, but was located above the vessel as shown here. x400.

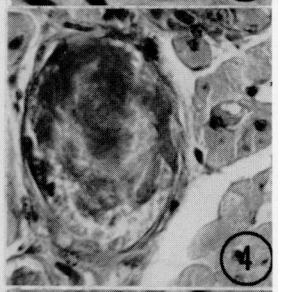
FIG. 5. Artery at upper right and the smaller arteries at lower left are hyalinized. Myocardial necrosis at left, but only a trace is shown. x500.

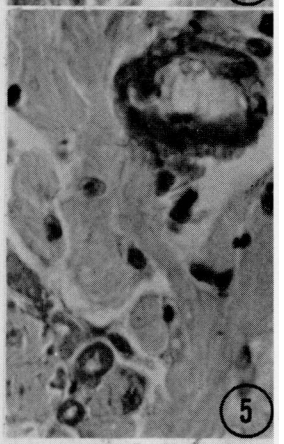
FIG. 6. Upper left artery is hyalinized. Myocardial necrosis (very PAS positive) is shown at lower right. x550.

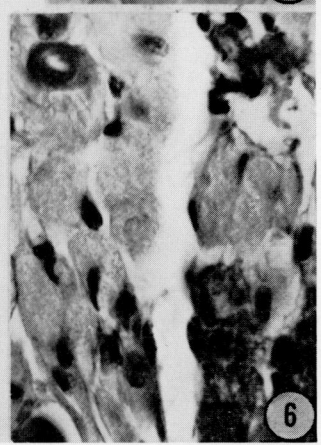












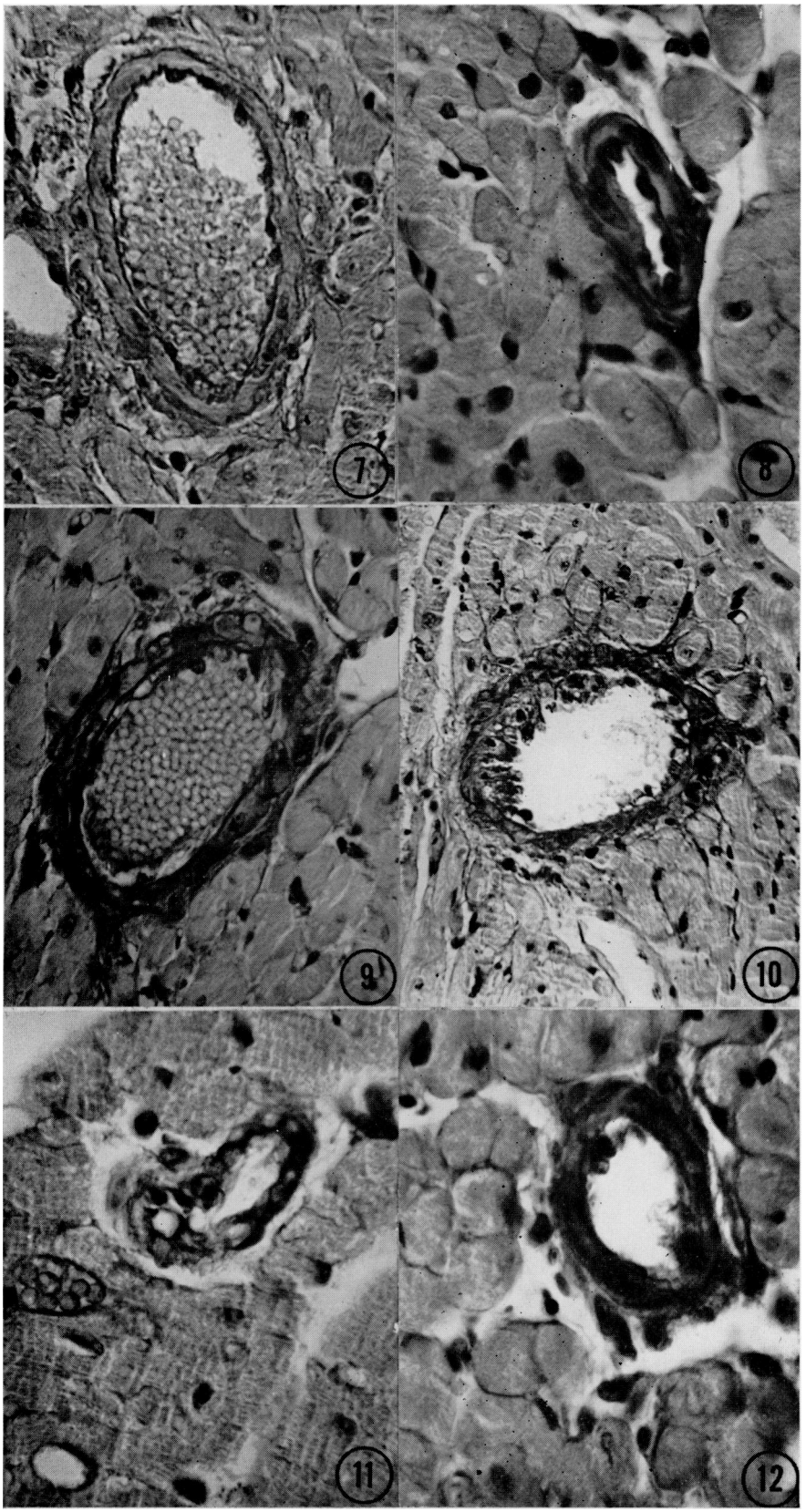

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TABLE 3. INCIDENCE (%) OF MYOCARDIAL NECROSIS IN RELATION TO HYALINIZATION OF CORONARY ARTERIES AND OTHER LESIONS IN MICE RECEIVING CORTISONE (C), PROGESTERONE (P) AND ESTRONE (E)

Types of lesions	*C*	*P*	*E*	*C+P*	*C+E*
Myocardial necrosis *without* arterial lesions	4%	0	0	4%	0
Myocardial necrosis *with* arterial lesions	0	0	0	0	55%
Incidence of inflammatory lesions of hearts, kidneys and livers (Table 2) in mice *with* myocardial necrosis	0	0	0	0	19%
Incidence of pneumonia as sole inflammatory lesion in mice *with* myocardial necrosis	50%	50%	0	100%	6%

strongly PAS positive (Figs. 1, 2, 6). These fibers were slightly hyperbasophilic when stained with thionin, but were not metachromatic. The PAS-positive material within sarcoplasm was granular (Fig. 6), the positive staining was dependent upon prior oxidation with periodic acid, and was not altered by diastase-hydrolysis. There was indistinct and limited vacuolation of sarcoplasm which may have been due to dissolved lipid, but methods to show fat were not applied to the hearts. In these areas of necrosis there was no hemorrhage. Also there were no neutrophils, lymphocytes, or cells that could be identified as macrophages. In about 10 per cent of the lesions an early fibrosis consisted of fibroblasts and of very small connective tissue fibers that were moderately PAS positive and were stained red by the van Gieson technic. The changes just described

All sections shown are specimens of myocardium from the same set of mice and with the same staining as those in Figs. 1-6.

FIG. 7. Normal artery. Note lack of PAS-positive stain except in elastic membranes. x300.

FIG. 8. Very heavily stained section. The artery shows only a trace of medial PAS-positive stain. No myocardial necrosis appeared adjacent to the vessels shown in Figs. 7 and 8. x550.

FIGS. 9 (x400), 10 (x200), 11 (x550) and 12 (x550) demonstrate a considerable range in mural hyalinization of arteries. In Fig. 9 the circulation within this vessel would seem normal. Endothelium seems swollen, foamy, and perhaps hyperplastic in Fig. 10. Fig. 11 shows mural vacuolation as well as hyalin. Hyalinization is advanced in Fig. 12. In Figs. 11 and 12 the perivascular space is no greater than frequently seen in normal myocardium of the mouse.

were limited to the three groups of mice given cortisone. The incidence of myocardial necrosis was 4 per cent or less except in mice receiving cortisone plus estrone where it was 55 per cent (Table 3). The predominant site of necrosis was ventricular. Atria were involved in only 15 per cent of the cases.

Myocardial vessels: Within all hearts of cortisone-plus-estrone mice showing myocardial necrosis (as described above) there was mural hyalinization of small arteries and of arterioles (Figs. 1-12). Non-granular, amorphous material that was eosinophilic and PAS positive was located in all layers of these vessels, but it was seen most obviously in media where it had filled, or replaced, or at least blotted out the smooth muscle fibers. There was a range from small deposits of hyalin to complete replacement of mural layers by this material (Figs. 7-12). When intima was extensively hyalinized the endothelium either could not be recognized or had pyknotic nuclei. When the hyalinization was chiefly medial, endothelium was present and in some instances appeared swollen and foamy (Fig. 10). The smooth muscle fibers of incompletely hyalinized media were swollen (Fig. 9). Adventitia was least involved by hyalinization. Thrombosis of these damaged vessels was common and is described later. Hyalinization had not involved lymphatics and was seen in only two instances within the extremely thin-walled veins typical of mouse myocardium. In three hearts major branches of the coronary artery contained very limited deposits of hyalin within media.

What is termed hyalin here could probably be designated as fibrinoid. Because of the relative nonspecificity and breadth of the latter term there seems no real objection to its use.[4, 9, 10] Here the staining of mural hyalin was identical in color to that of thyroid colloid. Its PAS-positive stain was red rather than the magenta color of glycogen and cartilage matrix, and was not granular as was that within the necrotic myocardial muscle fibers described earlier. When stained by the van Gieson technic the hyalin was the same color as muscle. The hyalin was neither basophilic nor metachromatic. The PAS-positive reaction of hyalin was dependent upon oxidation (periodic acid).

Other thoracic vessels: The sections of hearts included portions of aorta, and of pulmonary vein and artery. At the bases of 32 hearts with myocardial necrosis, all from cortisone-plus-estrone mice, extensive hyalinization was seen in intima and media of three aortas, one pulmonary artery, and one pulmonary vein. Unilaterally in ten longitudinal sections of aorta (Fig. 14) the entire wall consisted chiefly of hyalin (intima and much of

media) and a thick layer of dense fibrous connective tissue which had replaced the remainder of the media and the adventitia. The opposite wall of this aorta was normal in the same sections.

The intramural hyalin did not appear to be fibrin nor was there other evidence of clots forming within vessel walls. However, in two instances there was hemorrhage between elastic membranes of thoracic aortas, but no hyalin or fibrin.

Other vessels: Only a very limited portion of the vascular system was sampled. There was no material taken from intracranial vessels or the carotid-vertebral system. In addition to the lesions described above, mural hyalinization was seen in "chance" sections from mice receiving cortisone plus estrone as follows: abdominal aorta, 4 of 9 specimens; renal artery, 2 of 5 (Figs. 15, 16); artery in perirenal fat, 3 of 57. The only intravisceral site of such hyalinized vessels found was within myocardium. Vessels within kidneys, alimentary tract, liver, adrenals, reproductive tracts, lungs, bronchi, spleen, mammary glands and lymph nodes were normal. In thymus a few hyalinized vessels (apparently small arteries) were present, but these changes seemed related to the normal involution of this organ since they were observed in control mice and in all of the injection groups.

Except for thicker adventitias, the small arteries within skeletal muscle of mice are very similar to those of myocardium. Specimens of muscle (thigh, psoas or diaphragm) were available from one-half (16 of 32) of the cortisone-plus-estrone group showing lesions of myocardial vessels. All vessels within these muscles were normal.

Thrombosis: Thrombosis was common in the hyalinized myocardial arteries described earlier (Figs. 1, 2, 4). The thrombi showed the intense PAS-positive stain and other characteristics of the intramural hyalin. Some thrombi were vacuolated as if lipid had been dissolved (Fig. 4). Thrombosis was not observed in extramyocardial vessels. In three hearts with extensive ventricular myocardial necrosis (all from cortisone-plus-estrone mice) large thrombi occurred within atrial lumina; one in the right, two in the left. The abundant fibrin was weakly PAS positive. There was myocardial necrosis within these atria.

Periarteritis, arteritis, calcification, and ceroid pigment: No cells or other reactions indicating inflammation appeared within or adjacent to hyalinized and normal myocardial arteries. In one mouse there was adventitial arteritis in hyalinized and fibrotic areas of both the thoracic and abdominal aorta (Fig. 14). In another, a major proximal division of

the renal artery was similarly involved (Fig. 15). Calcification and ceroid pigment were not observed in the cardiovascular system or other sites.

CHANGES IN OTHER ORGANS

Liver: In mice receiving cortisone the parenchymal cells (cytoplasm) were frequently enlarged. Their cytoplasm contained fat, increased glycogen and decreased amounts of basophilic granulation. These are typical reactions to cortisone.[8] Estrone and progesterone did not alter these actions of cortisone on the liver. Coagulative necrosis of parenchyma was observed in a few livers (Table 2). This necrosis was not associated with hepatic abscesses. When only estrone had been injected the sinusoids were dilated and filled with erythrocytes, lymphocytes, and a few neutrophils. Concurrent injection of cortisone with estrone reduced, but did not eliminate, this congestion and leucocytic reaction (Fig. 17).

Kidneys: The abscesses were well localized and produced no apparent damage elsewhere in the kidneys. In all mice receiving cortisone, casts (eosinophilic and PAS positive) were fairly frequent in tubular lumina. Also there was a slight amount of amorphous PAS-positive material apparently within glomerular capillaries. With these exceptions, all of the vascular, tubular and stromal elements within kidneys were normal in all mice, including those with severe cardiovascular damage.

Lungs: In mice it is difficult to evaluate the relation of pneumonia to the injection of cortisone. The incidence here (Table 2) and in similar experiments was relatively high.[1] Pneumonia is also frequent in mice when the stress of the experiment does not include injection of cortisone or

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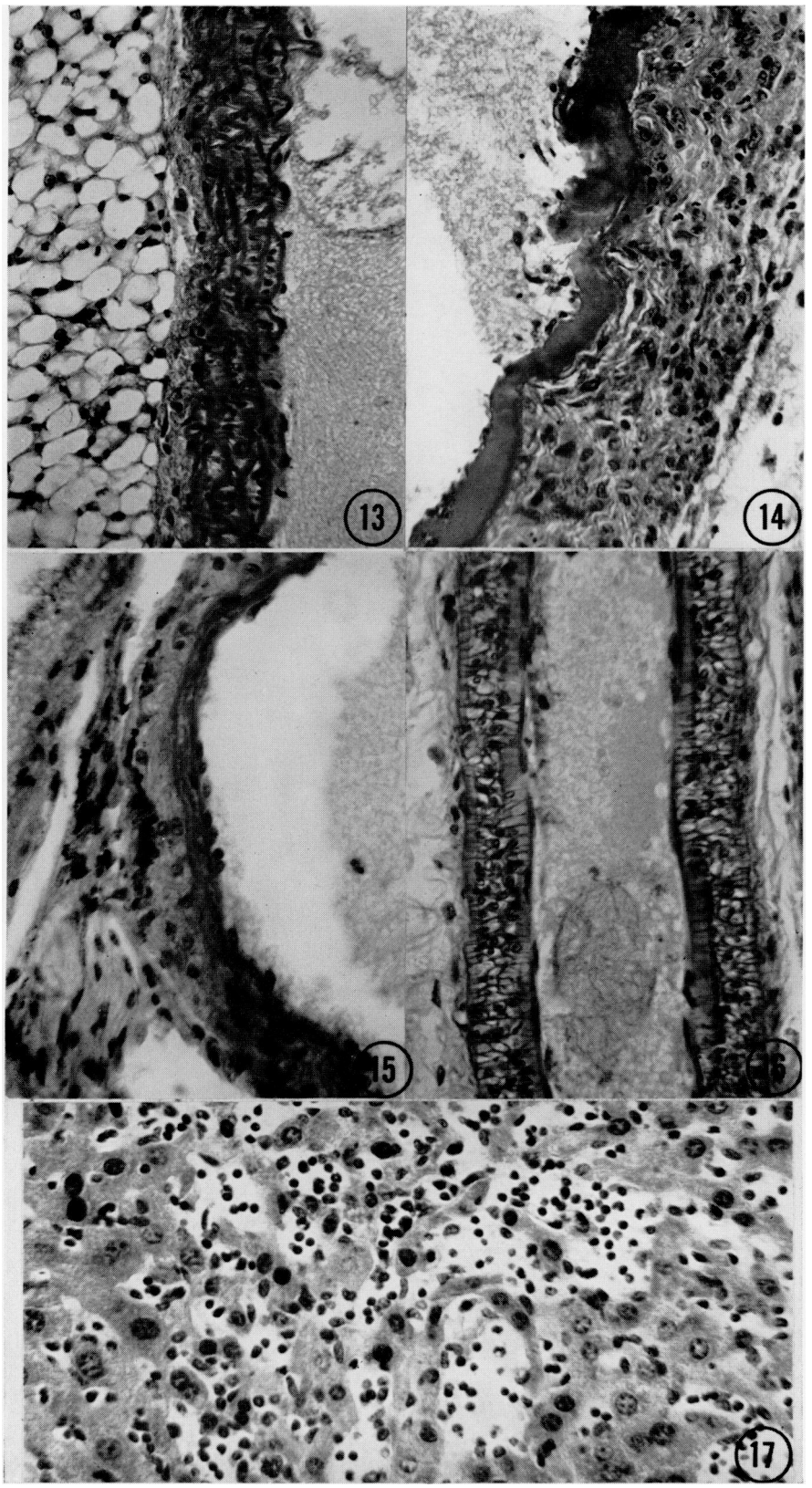
All specimens are from mice receiving cortisone and estrone. All except Fig. 17 (H. and E.) were stained by PAS as in Figs. 1-12.

FIGS. 13 and 14 (x200). Both show portions of the same section of a thoracic aorta. On the left (in the section) the wall was normal (Fig. 13). On the right (Fig. 14) there was intimal and medial hyalinization, and fibrosis of media and adventitia. The fibrosis extended into periadventitial fat. A few neutrophils in the vessel wall may indicate inflammation. Fibroblasts are abundant in the area shown in Fig. 14, where there is also unidentified pigment. The vessel was not thrombosed.

FIG. 15. Hyalinized and fibrosed portion of a primary superior (extrarenal) branch of the renal artery. A few neutrophils can be identified in the adventitia and perivascular area. The very dark staining material (adventitial and periadventitial) is unidentified pigment. The vessel was not thrombosed and the kidney was normal. x400.

FIG. 16. Apparently normal primary inferior branch of same renal artery as in Fig. 15. x400.

FIG. 17. Liver with dilated and congested sinusoids containing erythrocytes, lymphocytes and a few neutrophils. Some nuclei are enlarged, but the parenchymal cells in general are not as swollen, vacuolated or hypobasophilic as is usual in mice receiving cortisone. Some of the cells are enlarged and others are shrunken. x 400.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other hormones.[7, 11] When it was the only lesion, the mice appeared healthy and maintained body weight. It is possible that serial sections of both lungs would have revealed some areas of pneumonia in all of the animals receiving cortisone. There was no correlation between the frequency of pneumonia and that of cardiovascular or other lesions (Table 3).

In the mice receiving cortisone and estrone, especially those with myocardial necrosis, other changes were common in lungs. Large areas of edema and hemorrhage were frequent, and within them there were macrophages containing a bronze (H. and E. stain) pigment resembling hemosiderin. Aveolar capillaries (septal) were frequently distended with erythrocytes. The occurrence of hydrothorax has been mentioned earlier.

Reproductive system: Uteri, vaginae and mammary glands were hyperplastic in mice receiving estrone. Cortisone accentuated these changes, producing uterine edema (basal portion of lamina propria) and some mammary secretion. The ovaries and the male reproductive tract were normal.

Alimentary tract: Esophagus was seen in all animals and was normal. In mice receiving cortisone, ulceration of the gastric mucosa was seen only once, and the lesion was small. Other than at autopsy, the small intestine was studied in only 10 per cent of the mice, but appeared normal in these few sections. Sections of rectum were often included with female reproductive tract. These were normal.

Lymphoid organs: In cortisone-plus-estrone mice congestion of splenic sinuses with erythrocytes was frequent, and one thymus was severely depleted of lymphocytes. Otherwise the organs of this system, including lymph nodes, were normal.

Other organs and tissues: Skeletal muscle, peripheral ganglia and nerves, thyroid, and hyaline cartilage (of respiratory system) were normal. Fibrous connective tissue (except as described in blood vessels), areolar to dense, showed no changes. The central nervous system and bones were not studied. The abscesses within one adrenal cortex have been mentioned (Table 2). In 15 per cent of mice receiving progesterone, with or without cortisone, there was enlargement and increased cytoplasmic liposis of reticularis cells.

DISCUSSION

The limited effects of two ovarian hormones on body weights, when injected alone and also with cortisone, agree with studies showing disparity

in actions of progesterone and estrogens on body weights, growth and protein anabolism.[12-14]

In the mice receiving cortisone plus estrone or progesterone the incidence of pericarditis and of abscesses (hepatic, myocardial and renal) did not differ sufficiently from that found in mice given cortisone alone to warrant further discussion.[1]

Experimental methods for maximal production of arteriosclerosis do not commonly include injection of estrogens or glucocorticoids.[15-17] In rats, the injection of large amounts of ACTH produces arteriosclerosis, polyarteritis and arterial aneurysms.[18] In female rats receiving ACTH the severity of coronary arteriosclerosis was increased by a preceding and prolonged period of frequent breeding, and by unilateral nephrectomy.[19] In the same species cortisone reduced the amount of arterial necrosis resulting from renal injury.[20]

In mice the secretions of transplantable granulosa cell tumors seem to contribute to the production of a profound homeostatic derangement resulting in hypervolemia characterized by severe vascular congestion of many viscera. This hypervolemia is not produced solely by estrogenic and/or progestational substances.[21-23] In the present study significant cardiovascular damage occurred only in the mice given the estrone-plus-cortisone combination of hormones (Table 3).

The vascular lesions were characterized by mural hyalin (or fibrinoid) in arteries. A "hyalinosis syndrome" has been described in unilaterally nephrectomized rats receiving salt and mineralocorticoids.[24] Mural hyalin or fibrinoid has been observed in a wide range of blood vessels in association with several experimentally induced conditions including hypersensitivity and various types of stress.[4, 10, 25] Hyalinization (or fibrinoid necrosis) may contribute to mural changes resulting in arteriosclerosis in man.[26, 27]

In the mice receiving estrone plus cortisone the hyalin was frequent in media of small myocardial arteries, a layer normally composed of smooth muscle. Hyalin or fibrinoid may result from degeneration of smooth muscle.[28] In some of these mice, however, hyalin was also abundant in aortic media, consisting chiefly of elastic fibers. In two instances recent intermembranous (elastic) hemorrhage was apparent within aortic media, but no formation of fibrin or hyalin. The arterial mural lesions were similar to those in rats with experimentally induced hypertension and nephrosclerosis, but the restriction of intravisceral arterial injury to myocardial vessels was different from the widespread vascular damage in these hypertensive rats.[29]

SUMMARY

1. When injected in large amounts for 9 days, progesterone produced a slight increase in body weight, and estrone a slight decrease.

2. Administration of each of these ovarian hormones along with cortisone (for 7 days) did not alter significantly the total incidence of extensive inflammatory lesions (liver, kidneys and heart) from that produced by cortisone alone. Progesterone decreased and estrone increased loss in body weight in these mice.

3. In mice receiving cortisone, or estrone, or progesterone, or cortisone and progesterone the incidence of myocardial necrosis was 4 per cent or less. All blood vessels appeared normal.

4. In more than one-half of the mice receiving a combination of cortisone and estrone there was extensive mural hyalinization and thrombosis of the small arteries of the myocardium, and foci of myocardial necrosis. This cardiovascular damage in the cortisone-plus-estrone mice was associated with hydrothorax, ascites, and subcutaneous and pulmonary edema. There were also a few examples of hyalinization of aorta, major branches of coronary arteries, pulmonary artery and vein (extrapulmonary), and renal artery (extrarenal). Kidneys showed no evidence of vascular or other types of injury. Only one-fourth of the mice with cardiovascular damage showed inflammatory lesions of the heart or other organs.

5. In mice injected with estrone alone the sinusoidal systems of livers and spleens were congested with blood. These changes were less prominent in mice simultaneously injected with cortisone.

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