THE PATHOLOGY OF GOSSYPOL POISONING*

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The toxic effects of prolonged ingestion of cottonseed meal were recognized in animals as early as 1911.[1] Since this substance is produced in plentiful quantities as a by-product of the preparation of cottonseed oil, and since it is in other respects a valuable and concentrated source of protein for the feeding of livestock, its use for that purpose has continued and the limits of safety in the rations of domestic animals have received extensive study. Gossypol was reported to be the toxic component of cottonseed in 1916,[2] but for many years this was not universally accepted. One cause of confusion was the frequent concomitance of vitamin A deficiency in the diets which were used both commercially and experimentally, so that the claim persisted in some quarters that cottonseed poisoning was equivalent to avitaminosis A. It can now be accepted that gossypol is toxic to an important degree, especially in swine, which are more susceptible to it than other species.

The extraction of the oil from cottonseed is accomplished by several different processes. The gossypol remains in the meal and the processor endeavors to keep it at a level approximating 0.02 to 0.04 per cent but, owing to variations in temperature when processed by the hydraulic-press and to undesirable solvents in other processes, this amount may be greatly exceeded.

Our diagnostic routine afforded the opportunity to perform necropsies upon, and to examine the tissues of, pigs that died in the course of an experiment on the feeding of cottonseed meal which was conducted under the direction of Professors Fred Hale and Carl M. Lyman of the Animal Husbandry and Biochemistry Departments, respectively, of the Agricultural and Mechanical College of Texas. The details of this and other experiments in the feeding of cottonseed meal will be reported by Professors Hale and Lyman elsewhere. Since opportunities to study the effects of gossypol under accurately known and precisely controlled conditions are not numerous, and since pathologic criteria for the diagnosis of fatal poisoning have been questioned, it is desired to describe in this paper the pathologic changes encountered in 18 pigs which died in the course of the experiment.

* Received for publication, May 21, 1956.

Methods

The pigs were fed a diet provided with adequate amounts of all known essential nutrients, including vitamin A. Cottonseed meal of previously determined gossypol content was added so that different lots of pigs received diets which contained free gossypol in amounts ranging from zero to 0.03 per cent of the total ration. The animals that died (the subjects of this report) came from lots receiving from 0.02 to 0.03 per cent. In accord with the usual experience of the above-named investigators, amounts of free gossypol smaller than 0.01 per cent were invariably harmless; amounts between that figure and 0.02 per cent seldom produced specific symptoms but did lead to unsatisfactory growth.[8]

Two of the pigs on 0.01 per cent of free gossypol were studied in detail as controls for comparison with the 18 which died. After 70 days on this level of gossypol, during which they consumed respectively 22 and 24 gm. of gossypol and made excellent growth, these pigs were slaughtered for food, all organs being normal grossly and microscopically.

Results

As shown in Table I, the animals that died had been on the gossypol ration from 38 to 79 days, with the exception of 2 which survived until the 93rd day. It should be understood that even the highest level of gossypol used was not necessarily lethal, considerable numbers of pigs showing only retarded growth when marketed at the usual age of 6 to 8 months.

In the seriously poisoned animals signs of illness were typically apparent for 2 to 6 days, or, exceptionally, as long as 1 month. The outstanding symptom was always dyspnea, with violently labored respirations which stockmen

Table I

Animals Dying During the Experiment

Animal number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number of days on gossypol	38	43	46	50	57	60	62	74	77	93	93	45	79	45	48	63	76	77
Gossypol in ration, per cent	0.03	0.03	0.027	0.03	0.02	0.0225	0.0225	0.027	0.027	0.027	0.0225	0.0225	0.0225	0.0225	0.028	0.0225	0.0225	0.028
Number of days sick	3	12	5	10	7	8	?	?	?	?	?	18	3	4	14	18	27	29

? = Information not available.

call thumping. Progressive weakness and emaciation were accompanied by a good appetite almost until death.

The gross and microscopic lesions in the 18 pigs are believed to be characteristic of cumulative poisoning by gossypol. As can be seen from Tables II and III, widespread congestion and edema were salient

TABLE II
Gross Pathologic Lesions

Animal number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Subcutaneous edema	+				+		+			+			+				+	+
Hydrothorax	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion and edema of lungs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydropericardium	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dilatation of heart	+	+	+	+	+	+	+	+	+	+		+	+		+			+
Hypertrophy of heart					+	+			+			+	+					
Hydroperitoneum	+			+	+	+	+	+	+	+		+				+	+	+
Edema of gallbladder		+	+		+	+	+	+	+				+					
Edema of lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+
Congestion (and necrosis?) of liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congestion of kidney	+		+	+		+	+		+	+	+	+	+		+			+
"White muscles"	+	+			+	+	+	+	+	+	+							+
Icterus														+			+	+

features. The lungs and liver were markedly congested in all cases; the kidneys, spleen, and lymph nodes, in 75 per cent; and various other sites not included in the tables, such as the ventral belly wall, the adrenal and thyroid glands, frequently shared in the excess of venous blood.

Large amounts of fluid often were encountered in the pleural, pericardial, and peritoneal cavities. Edema of the lungs, present in all cases, was commonly so extensive that a frothy fluid was visible in the trachea. While the alveoli contained their share of fluid, microscopically much of the edema in the lungs was found in the interstitial tissues, the interlobular septa and subpleural layers being greatly distended by accumulated fluid. Edematous lymph nodes could usually be found in the body cavities, as well as in the cervical region and elsewhere. Subcutaneous edema listed in Table II involved the ventral abdominal wall or the distal parts of the limbs, or both, in certain animals. In addition to the sites included in Table II, divers other organs, such as the urinary bladder and thyroid gland, were frequently edematous. In the liver the separation of reticulum from hepatic cords which is usually interpreted as edema was observed frequently in

areas where it was not obscured by the more destructive changes to be described.

In accordance with accepted principles, the edema was considered to have been dependent on passive congestion and venous stasis. The latter conditions were attributed to a progressively failing heart on the basis of both symptoms and lesions. In all but 4 of the animals (77 per cent) the heart was conspicuously flabby and dilated. Degenerative changes consisting of partial dissolution (cytoplasmolysis)

TABLE III
Histopathologic Lesions

Animal number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Lung, congestion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Edema	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thickening of alveolar walls											+					+	+	
"Epithelioid" phagocytes		+		+	+	+				+		1		+			+	+
Alveolar hemorrhage																+	+	
Pneumonia of limited extent			+		+										+	2	+	+
Heart, degeneration	+	+	+			+	+	+	+	+	+		+			?	+	3
Hypertrophy	+	+	+			+	+	+	+	+	m	+	+	m	+	?	m	+
Intestine, enteritis				+			+							+	+			
Liver, severe congestion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loss of parenchyma	+	+	½	+	+	½	+	+	+	+	+	½	+	½	+	½	+	+
Spleen, congestion	?	+	+	+	+					+	+	+	+	+	+	?	+	+
Increased reticulo-endothelium	?		+		+	+								+		?		+
Atrophy	?		+		+	+	+		+	+		+	+		+	?	+	
Lymph node, edema	+	+	+	+	+	+	+	+	+	?	+	?	+	4	+	+	+	+
Congestion			+	+	+		+			?	½	?	+	4	+	+	+	+
Hemorrhage				+	+		+			?		?	+		+		+	+
Lymphoid atrophy			+				+	+		?	+	?					+	+
Kidney, congestion	?		+	+	+	+		+	+	+	+	+	+	+		+	+	+
Cloudy swelling (necrosis)	?		+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Lipidosis	?				+			+	+			+						
Edema	?										+							

+ = Present and distinct.
½ = Present, but less marked than usual.
m = Minimal or doubtful.
? = No data available.

1 = Phagocytic reticulo-endothelial cells, not epithelioid in appearance.
2 = Chronic pneumonitis.
3 = Degeneration of myocardium in region of an inflammatory endocardial lesion only.
4 = Lymphoid hyperplasia and exhaustion, increase of reticulo-endothelial cells.

of some muscle fibers, extreme atrophy of others, and rarely hyalinization gave microscopic support to the gross findings in most cases (Fig. 1). Five of the hearts were hypertrophied also, as judged at necropsy. Microscopic changes interpreted as compensatory hypertrophy were found to some degree in practically all of the hearts. These changes consisted of increased size or number of muscle nuclei, or both, sometimes accompanied by the presence of muscle fibers of unusually large diameter (Figs. 2 and 3).

In order to evaluate the accuracy of our judgment of the microscopic picture in these cases, both the size and the number of the nuclei in several hearts were determined by means of a micrometer eyepiece, and compared with hearts from the control pigs. The dimensions of the nuclei were measured directly, using the largest nuclei encountered in the myocardium of the animal in question and making no allowance for shrinkage which doubtless occurred in the preparation of the microscopic section. In determining the number of nuclei, all nuclei, whether in muscle fibers or in the endomysial connective tissue, were counted in an area which, for convenience, was 67 by 225 μ or, practically speaking, 15,000 sq. μ. An effort was made to use sections equal in thickness in all cases, as nearly as possible. No attempt was made to deduce means or averages lest a personal factor intervene through an unconscious selection of the fields to be counted.

TABLE IV
Analysis of Myocardial Hypertrophy

Animal number	5	7	10	Control	Control
Largest nuclei, μ					
Usual size	8 x 15	6 x 15	5 x 13	6 x 10	4 x 12
Exceptional size		10 x 25	6 x 18	7 x 11.5	
Nuclei, per 15,000 sq. μ					
Longitudinal section:					
Highest count	92	109	107	87	72
Lowest count	69	100	95	67	42
Cross section:					
Highest count	114	125	93	84	61
Lowest count	78	83	86	58	48

The results are given in Table IV and amply support the original microscopic impressions.

All of the livers were congested grossly, with at least a suspicion of intralobular necrosis or other degenerative changes. Generally speaking, the livers were reddish with the lobular architecture possibly

more prominent than normal. (In the pig this is normally conspicuous because of the distinct interlobular septa.) Microscopic examination revealed a startling change in all of the livers. While in 5 pigs the destruction was only partial, the remaining 13 had almost no viable parenchymal cells. In these only a narrow rim of hepatic cells remained at the extreme periphery of each lobule (Fig. 5). The remainder of the lobular space was filled with blood (Fig. 5), although a scattered reticulum of Kupffer cells appeared to be intact. While many poisons cause central or more extensive necrosis of the liver, usually with some degree of cloudy swelling or fatty change, I am not acquainted with any other intoxication which presents this extreme destruction.

In addition to the congestion and edema to which allusion has already been made, the lungs frequently displayed a very noticeable infiltration of the alveoli with macrophages of the well known "epithelioid" appearance. These, however, did not contain hemosiderin as might have been expected from the congested state of the lungs. Instead, their presence close to the alveolar walls, their greater frequency in peribronchial regions, and the co-existence of a slight thickening of the alveolar walls suggested that they were probably due to terminal infection. There were areas of genuine red hepatization in 5 animals, but these were never extensive and can be attributed to infection of previously devitalized pulmonary tissue. The pulmonary hemorrhages recorded in Table III were multiple in the 2 pigs affected but each involved less than 2 cc. of tissue.

In addition to congestion, the red pulp of the spleens of 10 animals was notably atrophic and deficient in lymphoid cells, possibly because of congestive anoxia. In several, however, the reticulo-endothelial and fibrous tissue was increased. The splenic nodules (malpighian corpuscles) suffered much less. Two spleens were considered edematous and in 2 the walls of arterioles appeared thickened. Atrophy of the lymphoid tissue in the congested and edematous lymph nodes was observed in 6 animals. Contrary to what was seen in the spleen, the follicles of the lymph nodes were not spared.

The renal lipidosis indicated in Table III was either in the proximal convoluted tubules or in the ascending loops of Henle, varying in different cases.

While extensive areas of the skeletal muscles frequently were pale or almost white, it was difficult to demonstrate microscopically any change beyond an abnormal variation in the size of certain fibers, some being atrophied, others hypertrophied (Fig. 4). Reproductive

and endocrine organs and the central nervous system revealed nothing of importance although they shared to some extent in the generalized congestion and edema.

Discussion

The lesions portrayed above are nearly identical to the lesions described by West[4] in dogs fed poisonous amounts of cottonseed meal except that gastro-enteritis was more prominent in the dogs and petechial and ecchymotic hemorrhages were numerous. The centrilobular destruction of liver cells was less pronounced in the dogs.

In contemplating the almost unprecedented changes found in the liver, the question arises whether this degree of hepatic injury results from the anoxia consequent upon congestion and stasis of blood or whether a direct hepatotoxic action must be attributed to the gossypol. Also advocated has been the theory that increased blood pressure in the intralobular capillaries destroys the hepatic cells by pressure necrosis. That severe chronic passive congestion is quite capable of bringing about centrilobular necrosis of this nature is generally accepted, and is supported by the work of such investigators as Lambert and Allison,[5] who studied 112 human cases of hepatic congestion in 1916. The loss of liver cells in such disorders as cardiac insufficiency and their replacement by overflow of blood from the capillary sinuses usually were limited to the central parts of the lobules. In comparing such changes with the almost complete involvement of lobules, seen in our cases, it presumably could be argued either that gossypol poisoning represents an exaggeration of what those authors found or that a different mechanism is involved. Those who hold that a direct toxic action from something imported by the blood is essential to cause such extensive death of hepatic cells have only to point to the multitude of poisons and toxic products of metabolism which are known to have this effect, usually without any notable alteration in the rate or character of the hepatic blood flow. Such morphologic evidence on this question as is afforded by the tissue sections themselves is equivocal. Against the concept of a direct toxic action upon the liver was the nearly complete absence of cloudy swelling or other toxic degenerative changes in the peripheral hepatic cells which survived. Fat stains made on the liver in certain pigs were uniformly negative. Possibly favoring a direct toxic action was the prevalence of cloudy swelling in the epithelial cells of the renal tubules. Narrowing of the hepatic cell cords which remained was observed in several of these livers, a feature upon which earlier pathologists relied to support the theory of pressure necrosis but which is probably

directly incident to the developing necrosis and not the result of pressure.

For purposes of comparison, as well as from the standpoint of practical differential diagnosis, three other disorders of animals producing a similar picture of hepatic destruction may well be considered. The first of these, poisoning by mouldy corn, has been studied especially by Sippel *et al.*[6] While widespread hemorrhages, as well as an entirely different clinical history, readily distinguish that condition from gossypol poisoning, the changes in the liver appear in some instances to be almost identical. The second comparable disorder is poisoning by the coal-tar pitch of the "clay pigeons" used as targets by trapshooters. Pigs sometimes get this material from the ground where the shattered targets have fallen and develop an acute poisoning characterized by a spectacular centrilobular necrosis and filling of the central part of the lobule with blood. However, the hepatic damage probably never involves the whole lobule; destruction of about half of it, as measured along the radius, is usual. No cardiac insufficiency has been noted in either of these poisonings.

The third comparable disorder is of dietetic nature and is the most intriguing, as well as the most perplexing. Hove and Seibold[7] fed a diet low in protein (soybean meal), markedly deficient in vitamin E and containing cod-liver oil, of which highly unsaturated fatty acids are characteristic constituents. After from 1 to 4 months on this diet the animals usually died and, at necropsy, showed a "hemorrhagic necrosis" of the liver similar in most respects to the hepatic condition ascribed to gossypol. In pigs that lived for 6 months, cirrhosis developed. The authors considered the livers to be comparable to those produced by Obel,[8] which will be described presently. Their pigs showed numerous hemorrhages in various organs, including lymph nodes, endocardium, epiglottis, lungs, and gastro-intestinal mucosa. They mentioned the absence of lesions in the skeletal muscles. Other organs apparently failed to attract attention.

Obel[8] described a porcine disease known as hepatosis diaetetica which occurs naturally in Sweden and Northern Europe and which was susceptible of experimental reproduction by the combined deleterious effects of (1) inadequate dietary protein (brewer's yeast, which is deficient in the sulfur-containing amino acids), (2) deficiency of vitamin E, and (3) presence in the diet of considerable amounts (6 per cent) of cod-liver oil with its highly unsaturated fatty acids. The similarities between the lesions arising from excessive gossypol and those described for natural or experimental cases of hepatosis diae-

tetica are seen not only in the necrotic and blood-filled hepatic lobules but also in the concomitant presence of extensive edema in practically identical situations, and in the presence of retrograde changes in skeletal and cardiac musculature which, while called by different names, appear microscopically to have at least the degenerative features in common.

On the other hand, certain notable differences between the cases of gossypol poisoning and those which Obel[8] and Hove and Seibold[7] found to result from dietetic imbalances are readily apparent both in clinical and post-mortem aspects. While Obel's pigs were reported as dying "quickly without the owner noticing any illness" (although occasional dyspnea is mentioned in exceptional cases), the gossypol-poisoned pigs suffered, usually for several days, from respiratory and cardiac difficulties which no one could have overlooked. In the liver, typically affected lobules were very similar in both conditions, all but the most peripheral hepatic cells being replaced by blood, but the distribution of such lobules was very patchy in the "deficiency" pigs, while in our "gossypol" pigs it was almost universal. The result was livers of entirely different gross appearance, spotted and patchy in the "deficiency" animals but evenly discolored in the "gossypol" pigs. Gastric ulceration, hemorrhages in various organs and tissues, and degenerative and necrotizing changes in arteries ("fibrinoid degeneration," "periarteritis nodosa") were accorded considerable prominence in Obel's descriptions but were absent or negligible in the gossypol syndrome. Obel's pigs sometimes developed the condition of yellow fat and ceroid pigment which has assumed prominence in connection with deficiency of vitamin E in company with an excess of unsaturated fatty acids in the diets of mink, as well as swine,[9] and which is well known in experimental rats. Changes of this sort were not a part of the gossypol picture.

In trying to understand the relationship, if any, among these several destroyers of hepatic cells we find that very similar hepatic changes result from such widely varying disorders as (1) stasis of the hepatic blood flow, (2) poisoning by products of the growth of moulds in decomposing corn, (3) poisoning by coal-tar pitch, a mixture of complex hydrocarbons, (4) poisoning by gossypol (and to a lesser degree by numerous other substances), and (5) by a diet unusually rich in unsaturated fats and at the same time deficient in vitamin E and certain proteins, probably those providing the sulfur-containing amino acids. Is there any common factor? For the first of the series, local anoxia seems an obvious result of failure of replenishment by means

of new blood. With respect to the dietetic causes, the chemistry is still obscure. Vitamin E is frequently called an "anti-oxidant" although little is said as to just what oxidations are inhibited. Unsaturated fats or fatty acids are substances that are easily oxidized. (Note their action in "drying oils.") Of the chemical mechanisms by which the several poisons exert their toxic effects, nothing is really known. It is doubtless an over-simplification to suggest that, in the absence of the moderating "anti-oxidant" action of vitamin E, the easily oxidizable fats of cod-liver or other fish oils rob the hepatic cells of an adequate amount of oxygen, but there is a strong probability that all these hepatotoxic disorders cause similar hepatic changes because they institute similar types of interference with local oxidative processes, with or without the added factor of inadequate blood supply.

Summary

The principal lesions of gossypol poisoning are congestion and edema resulting from myocardial injury. The edema is especially prominent in the lungs and body cavities. The congestion is of great severity in the liver, where it is accompanied by destruction of all but the most peripheral parts of the hepatic cords. This striking change in the liver is comparable to what is seen in two other poisonings, in anoxia of circulatory origin and in dietetic imbalance involving an excess of unsaturated fatty acids coupled with deficiency of vitamin E and of certain proteins. Interference with oxidative processes may possibly be the fundamental mechanism in all cases.

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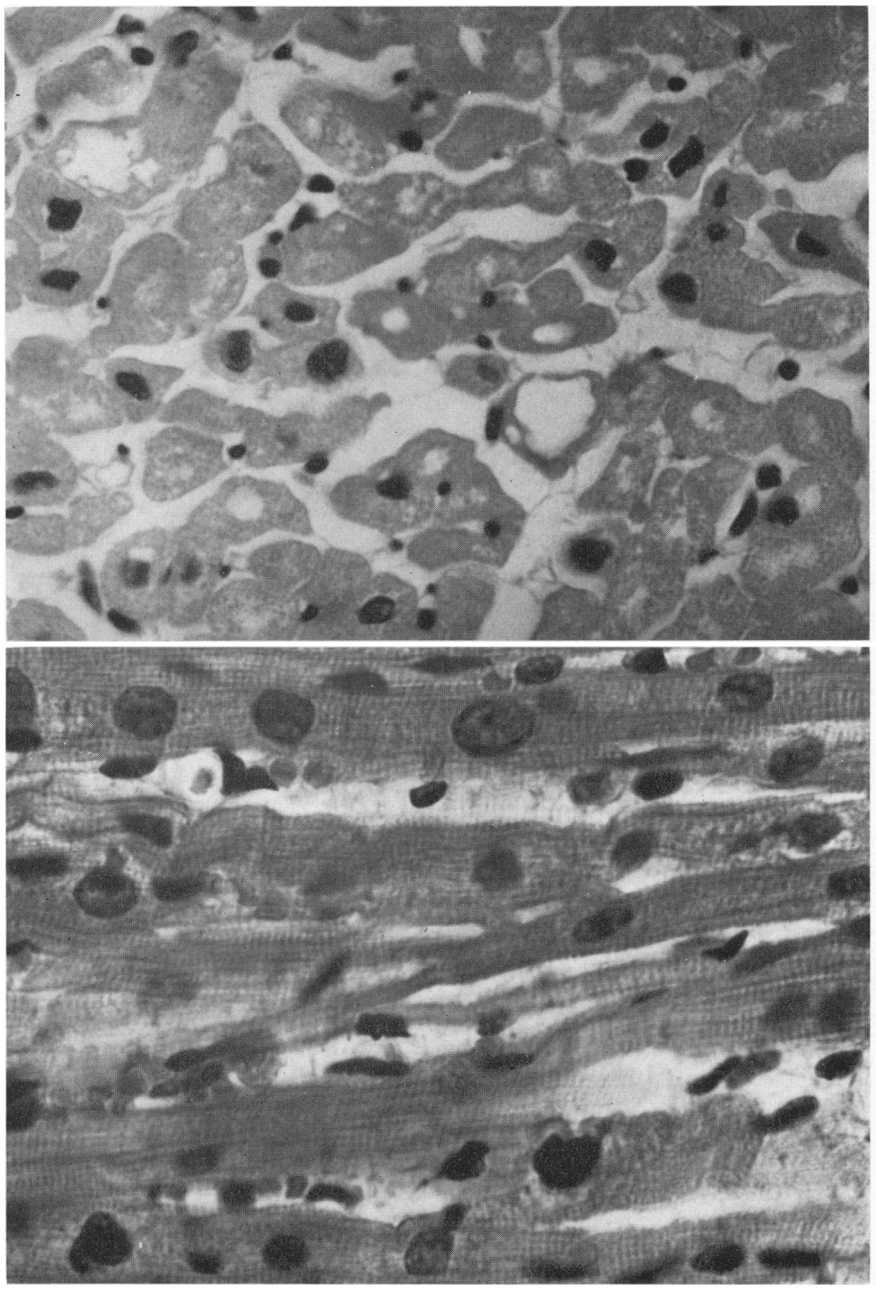


LEGENDS FOR FIGURES

FIG. 1. Vacuolization and other degenerative changes in myocardial fibers. Hematoxylin and eosin stain. × 400.

FIG. 2. Increased number of nuclei in myocardium. Hematoxylin and eosin stain. × 400.

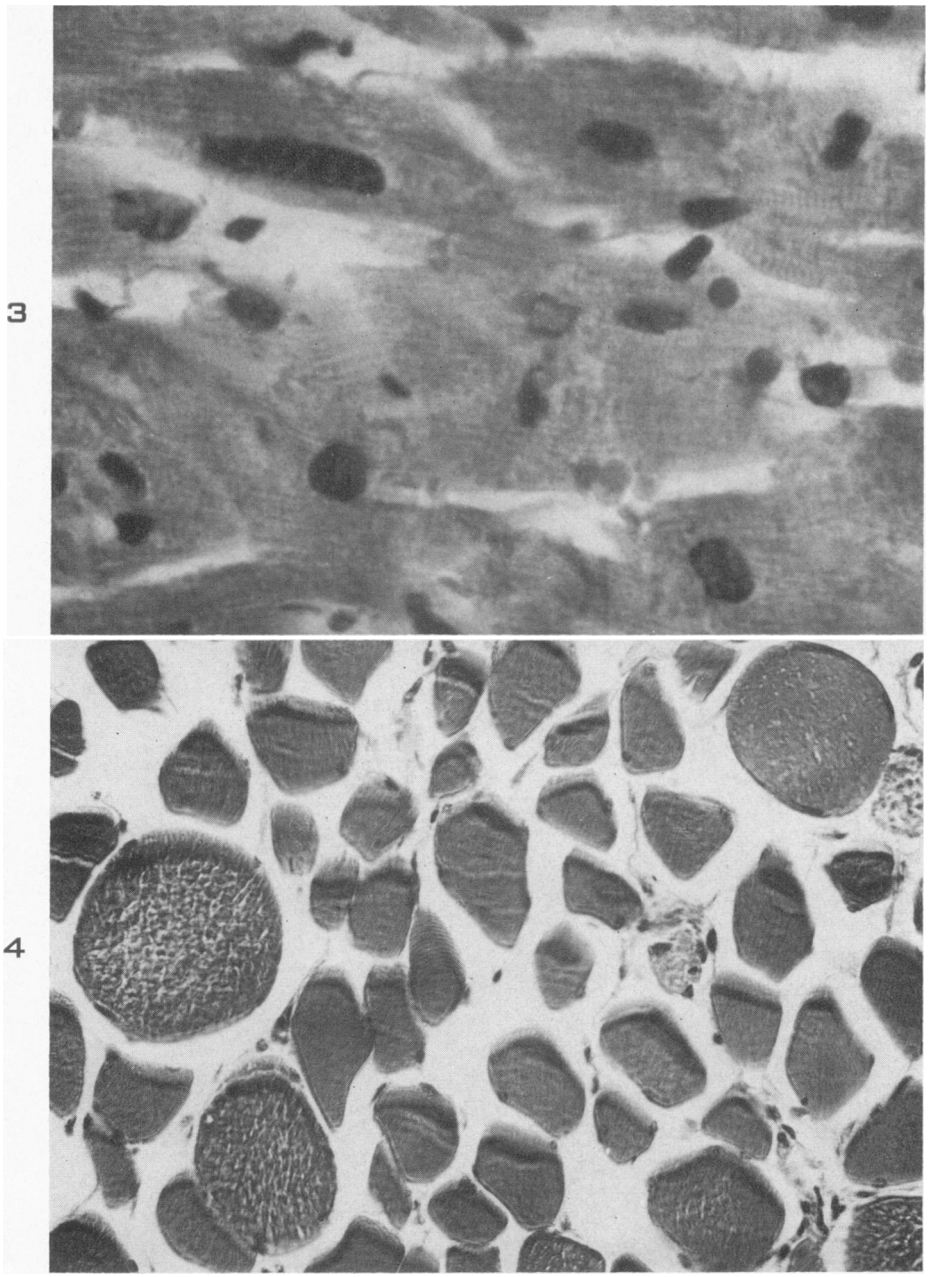
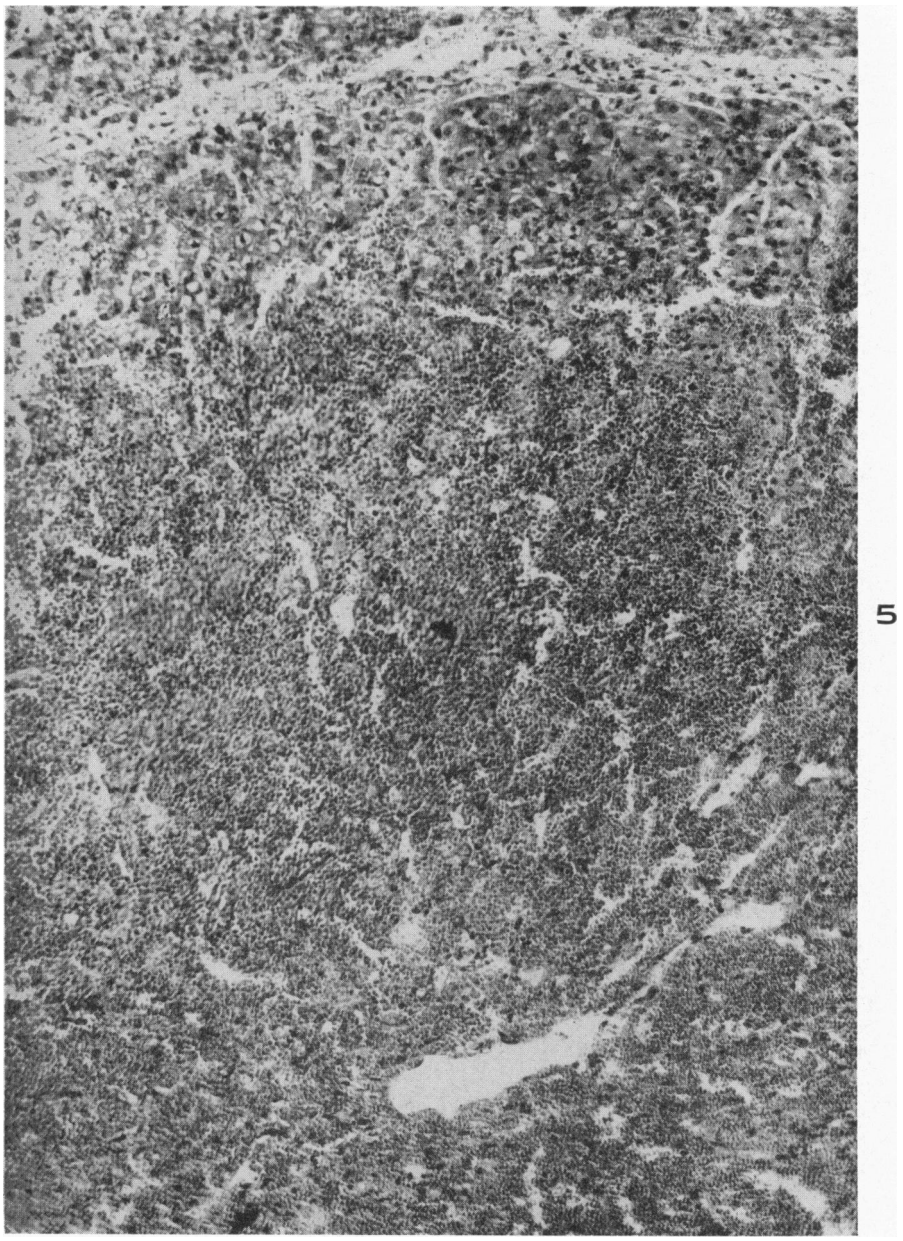


FIG. 3. Hypertrophic nucleus in midst of degenerative myocardium. Hematoxylin and eosin stain. × 400.

FIG. 4. Degenerative changes in skeletal muscle. Hematoxylin and eosin stain. × 600.

FIG. 5. Sector of a hepatic lobule, filled with blood except near the interlobular septum. Hematoxylin and eosin stain. × 175.



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