THE PATHOGENESIS OF EXPERIMENTAL FAT EMBOLISM *

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The literature concerning fat embolism is abundant and has recently been surveyed comprehensively in the publications of Wilson,[1] Warren,[2] and Robb-Smith.[3] It is noteworthy, however, that among the various studies, whether clinical or experimental, the stress has been placed on the action of the fat upon the host. The condition of the patient and of the experimental animal has received incidental attention; it has been tacitly assumed that the host is biologically invariable and uniformly responsive, regardless of the previous history and treatment. That the response is remarkably variable is indicated by the results attained through the thorough search of routine autopsies for evidence of fat embolism. Such studies[4,5] reveal a very high incidence of fat embolism among cases of traumatic injury, especially with fractures, although the mortality due directly to fat embolism is low and unpredictable. Robb-Smith has reported similar findings and attributes much importance to the fat as a lethal factor. In view of this, the present study was planned to elucidate the behavior of the host toward fat embolism and to clarify the factors which might affect the response either favorably or adversely.

METHOD

Fat embolism was produced in albino rabbits, weighing 2,000 to 3,000 gm., of both sexes, by the injection of homologous liquid fat into the right ear vein. This fat was obtained from the perirenal fat pads of normal rabbits. It was finely minced, mixed with one and one-half volumes of 95 per cent ethanol, and homogenized in a Waring blender for 2 minutes. This homogenate was refluxed at 80° to 90° C. for 12 hours and subsequently filtered through a warm Buchner funnel to eliminate coarse, insoluble residue. The filtrate was allowed to stand in a separatory funnel at 38° C. for an additional 12 hours in order to allow separation of fat crystals insoluble at this temperature. Final exclusion of the insoluble fraction was achieved by rapid centrifugation. The extract was a clear, straw-colored liquid of low viscosity when the residual alcohol had evaporated.

Injections of this homologous fat were made into the ear vein at a

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rate approximating 0.4 cc. per minute, so calculated to permit formation of small, separate fat globules in the vein. The droplets could be visualized by transmitted light. After injections, the animals were returned to their cages and observed for respiratory, cerebral, and cardiac abnormalities. The animals that died were autopsied, the organs examined for gross alterations, and ample representative portions fixed in a 4 per cent solution of formaldehyde. Some of the surviving animals were sacrificed and similarly autopsied. The tissues were subsequently embedded in paraffin, sectioned at 8 μ, and stained with hematoxylin and eosin. From selected samples of the formalin-fixed lung and liver, frozen sections were taken and stained with sudan III.

Experimental Procedure

Effect of Dosage, Rate of Injection, and Repeated Injection

The amount of fat, injected at constant rate, was varied to include levels of 0.45, 0.55, 0.75, and 0.90 cc. of fat per kg. of body weight. In rabbits receiving doses of 0.90 cc. per kg., death occurred within 10 to 30 minutes in all animals, whereas in those receiving 0.75 cc. per kg., death was similarly rapid although the mortality was less. With the smaller doses the animals survived for at least 5 hours and often up to 24 hours. Such animals as survived over 24 hours recovered completely, regardless of the severity of the initial pulmonary signs, and in no instance was a late death encountered for as long as 120 days after injection. The mortality rate (Table I) varied directly with the dosage, 0.55

Table I

Effect of Dosage upon Mortality by Fat Embolism *

No. of animals	Dosage	Survivors	Fatalities	Mortality
	cc./kg.			%
18	0.45	15	3	16.6
12	0.55	6	6	50.0
6	0.75	2	4	66.0
6	0.90	0	6	100.0

* The rate of injection was constant for all animals.
Injections were made into the right ear vein, without anesthesia.

cc. per kg. giving a 50 per cent lethal result. Since the rate of injection was constant, it was excluded as a factor in varying mortality. As a further test of rate, a series of rabbits were injected with fat as rapidly as technically feasible without altering the mortality rate above the previous level.

In a group of animals which survived the infusion of 0.45 cc. per kg., repeated injections were administered at staggered intervals of 110 hours, distributed for a total of four injections. On the basis of this,

it was expected that adaptation to the fat might reveal itself by a lowered mortality with succeeding injections. It was found under the circumstances that the mortality rises slightly with the second and third fat injections until 75 per cent of the animals succumbed. No evidence of tolerance to repetitive fat embolism was observed.

Effect of Tourniquet Shock and Dehydration

Tourniquets were applied to the hind legs of rabbits as described elsewhere.[6] Subsequent to a period of 4 hours of complete ischemia, the tourniquet was released. Three hours after release, when swelling of the limb usually is maximal, 0.45 cc. of fat per kg. were injected. It is apparent (Table II) that the mortality rate is considerably elevated in the animals with tourniquet shock when compared with control groups which received only fat injection or which were subjected to ischemia alone. Although the mortality was thus increased, the time of survival was not altered appreciably; no death occurred before 5 or after 24 hours subsequent to injection.

In view of the considerable quantity of fluid which accumulated and pooled extravascularly in the ischemic limb, the effect of dehydration was studied in another group. The animals were allowed dried food but deprived of water for 72 hours. At the 48th hour each was injected intraperitoneally with 30 cc. of 25 per cent glucose to facilitate the dehydration. Fat was administered intravenously in a dose of 0.45 cc. per kg. at the end of 72 hours. In this series 50 per cent of the animals

TABLE II

Effect of Tourniquet Shock, Dehydration, and Oxygen upon Mortality Rate

No. of animals	Fat	Shock	Dehydration	O_2, 80%	Survivors	Fatalities	Mortality
	cc./kg.						%
18	0.45	−	−	−	15	3	16.6
12*	0.45	+	−	−	7	5	41.6
18	−	+	−	−	18	0	0.0
18†	0.45	−	+	−	9	9	50.0
12	0.55	−	−	−	6	6	50.0
12‡	0.55	−	−	+	12	0	0.0

* Three hours elapsed between the release of the tourniquet and the injection of fat.
† All of these animals were deprived of fluid for 3 days before injection of the fat.
‡ Oxygen percentage of effluent air was tested by the Beckman oximeter.

died following the injection (Table II) and in the majority of instances succumbed within 30 minutes in a manner similar to those injected with greater quantities of fat. Neither dehydration nor fat alone produced such a high mortality.

In a series of dehydrated animals, both loss of weight and alterations in hematocrit levels were followed. Hematocrit levels were estimated

by the capillary method.[7] The hematocrit readings and weight changes were taken at the start, and at the 48th and 72nd hour of dehydration. Variation was computed as a percentage alteration of the initial readings. From these studies it has become manifest that neither the magnitude nor direction of change in the hematocrit suggested the outcome of any particular experiment. The weight loss, presumably due to fluid depletion, similarly gave no indication of how the particular animal would respond. Loss of weight varied between 10 and 18 per cent of the original body weight. Dehydration, like tourniquet shock, altered the mortality in a striking manner, whether or not evidence of hemoconcentration was manifest. The mortality rate was identical in both sexes.

Effect of Oxygen upon Mortality

A group of animals which had received 0.55 cc. per kg. of fat were put into a sealed metal chamber with glass ports, immediately at the end of the injection. Oxygen was passed through the chamber under positive pressure from a pressure cylinder at a rate sufficient to maintain the percentage of the effluent gas at between 80 and 82 per cent. Hourly tests were made with a Beckman oximeter over a period of 30 hours. Both food and water were allowed *ad libitum*. Although these animals experienced marked tachypnea and in some instances severe dyspnea, within the period of the experiment none died. At the 30th hour all were killed and autopsied. The administration of oxygen in high volume altered the mortality rate from the 50 per cent seen in the controls to nil (Table II). The duration of 30 hours was set because among the controls fatalities occurred before the 24th hour. The control animals which survived over 24 hours were killed also to permit collateral estimation of visceral changes in the absence of oxygen among survivors.

Autopsy Findings

The only lesions observed in animals which died rapidly were scattered petechiae in the serosa and slight pulmonary congestion. In the remainder which survived for longer than 5 hours or were subsequently killed, extensive changes were observed in the viscera. The difference between the groups as related to period of survival is demonstrated in Table III.

Lungs. In all animals which survived longer than 5 hours the lungs were heavy, and contained gross areas of hemorrhage and scattered small areas of atelectasis. Congestion was extensive. In a few animals pleural effusions measuring 5 to 6 cc. were found. The weight of the lungs was greatly increased from the normal average of about 14.5 gm.

to an average of 35.1 gm., due mainly to congestion and edema, for the weights were only slightly increased, if at all, in those dying immediately after injection when congestion and edema were slight. In the animals receiving oxygen therapy the increase in weight of the lung was less conspicuous and averaged 25.5 gm.

TABLE III

Duration of Survival of Animals Dying of Fat Embolism

No. of animals	Dosage	Agent	Duration	Pulmonary lesions
	cc./kg.			
18	0.45	—	5–24 hours	+
12	0.55	—	5–24 hours	+
6	0.75	—	15–60 minutes	—
6	0.90	—	10–30 minutes	—
18	0.45	Dehydration	10–60 minutes*	Slight
12	0.45	Tourniquet	5–10 hours	+
12	0.55	Oxygen	At least 30 hours	+

* One animal lived for 7 hours, another for 24 hours.

Microscopic studies of the lungs revealed extensive hyperemia (Fig. 1), extensive pulmonary edema, and in most instances a moderate interstitial infiltration of polymorphonuclear leukocytes.* As previously indicated, the extent of these changes was dependent first upon the duration of survival (Table IV). Among both survivors and non-sur-

TABLE IV

Lesions of Various Viscera in Relation to Duration of Survival after Fat Injection

Lesions	10–60 minutes	5–24 hours	Sacrificed at 30 hours	80% oxygen	Dehydrated*
Pulmonary edema					
0	10	0	0	2	4
+	4	5	5	5	11
++	0	3	1	4	2
+++	0	7	3	1	0
Total	14	15	9	12	17
Hepatic necrosis	0	7	5	0	2
Myocardial necrosis	1	6	3	6	2

* Except for 2 animals, all died within 1 hour after injection of fat.

vivors severe grades of involvement were found in about the same proportion. Oxygen therapy, on the other hand, ameliorated the degree of edema and congestion considerably, although alterations were absent in only a few animals. All lungs contained a large number of fat globules, which lodged in both the arteries and capillaries.

* In a recent autopsied case of human pulmonary fat embolism, a similar interstitial leukocytic reaction was seen.

An interesting alteration occurred in the medium-sized arteries. These vessels (Fig. 2) were loose meshed, with separation of the medial cells from each other. In the subintimal connective tissue there was a marked vacuolization and ballooning. The effect of the alterations was a constriction of the caliber of the vessels by the edema of the walls. This ballooning of the intima was not due to imbibition of fat, since by frozen section none was demonstrable within the wall. In most instances the perivascular lymphatics were conspicuously filled with an eosinophilic fluid coagulum. These arterial lesions were absent or minimal in the animals dying rapidly and in those treated by oxygen.

Heart. Numerous petechial hemorrhages were scattered over the pericardium and seen frequently beneath the endocardium of the left ventricle. The right ventricle was in most instances dilated and contained grossly perceptible fat globules. Microscopically, there was extensive congestion and stasis of the myocardium. Many focal areas of necrosis (Fig. 3) were observed in which the muscle fibers had undergone lysis, and there were accumulated both monocytes and neutrophilic polymorphonuclear leukocytes. These necrotic foci were predominantly in the right ventricle, although also present elsewhere. They were rare in animals dying suddenly and most numerous in those which survived for some period of time. Oxygen did not preclude their formation or restrict their extent.

Liver. Grossly, the liver was intensely congested and enlarged. This was reflected in the usual microscopic picture of congested sinusoids and central veins. Parenchymatous degeneration was frequent, and in a few animals fatty metamorphosis was conspicuous. As is indicated in Table IV, the animals which survived over 5 hours or were sacrificed at 24 to 30 hours frequently suffered an extensive centrilobular necrosis, which destroyed up to three-fourths of the parenchyma in some instances (Fig. 4). There was concomitant leukocytic infiltration and some peripheral parenchymatous degeneration. The frequency of this type of necrosis was 25 per cent and appeared consistently in animals with more severe degrees of pulmonary involvement. Direct toxic action of the fat upon the liver was considered, but it was found that the necrosis occurred also after injection of paraffin oil, which is chemically bland. On the other hand, the animals treated with oxygen never developed necrosis; the livers of these animals were very congested, although the liver cords were normal.

Kidneys. There were no gross lesions in the kidneys. Microscopically, many glomeruli contained intracapillary fat globules; and as many as

40 to 60 per cent of the glomeruli might contain them. Signs of necrosis and inflammation were absent, even in glomeruli packed with fat. Fat was observed also within the lumina of the various segments of the nephron, particularly in animals which lived for some time. It appeared bland because, even though large globules filled cells occasionally, no evidence of necrosis was found throughout the nephron. It is pertinent to indicate the difference in the response of the renal and pulmonary tissues to the fat emboli: the former were not reactive, whereas the latter underwent profound inflammatory alterations.

Other Tissues. Among the other organs the brain was most closely studied. No gross lesions were detected in any instance. In a rare animal a few scattered petechiae were observed in the cerebellum without any concomitant perivascular necrosis. Lesions were consistently absent in the spleen, adrenals, ovaries, testes, skin, intestines, and aorta. In almost all animals, except those treated with oxygen, numerous petechiae were found in the thymus. A few animals suffered either pleural effusion or ascites.

Discussion

The mechanisms by which fat embolism is initiated are several and include such diverse conditions as fractures of long bones, orthopedic manipulations, crushing injuries, concussion, injection of oily fluids, infusion of ether, and the hyperlipemia of diabetes mellitus. Whatever the mode of origin, the crux of the problem lies in the method of disposal of the particulate fat. Fat particles below 8 μ in diameter are dealt with effectively by the organism, whereas those of larger size may behave as emboli. The response of an animal to the infusion of particulate fat into the circulation, regardless of route of entry, will depend considerably upon its ability to cope with the embolic manifestations. This presumes that the fat is chemically inert, and the present studies upon the relationship of dosage and mortality support the view that the animal initially reacts to the fat particles as to any inert, small emboli. Should the animal survive the initial insult, which depends mainly upon the quantity of fat, its subsequent behavior will be conditioned not only by action of the fat upon the tissues but also by the physiologic adaptability of the particular animal and its various viscera to the fat particles.

In the group of animals dying immediately after the fat infusion, death may be ascribed to massive plugging of the pulmonary vessels. Since no morphologic alterations were found in the lungs, chemical action by the fat may be excluded. Among animals which survived the injection by several hours, several viscera may manifest profound alterations.

It is requisite to determine whether the visceral lesions are due to direct action of the fat upon the tissues or are caused by systemic disturbance due to occlusive vascular obstruction by the fat. It might be presumed by some that, if the fat acts chemically, it will exert its effect indiscriminately in whatever tissue it comes to lodge. This is not so. For, although numerous fat emboli are caught up in the kidney and a few in the brain, these organs are singularly free of inflammatory reactions such as are common in the lungs. Furthermore, necrosis of the liver is encountered in the absence of demonstrable fat in that organ. Accordingly, it may be assumed that reaction of the tissues, and therefore of the animal, is decided by factors independent of the nature of the fat itself.

The first problem involves, therefore, the cause of the different reactions by the various organs to the fat. Although the quantity of fat contained in the pulmonary vessels exceeds that in any other organ, it might be expected that the type of reaction, if purely chemical and nonspecific, would vary quantitatively and not qualitatively. But the striking absence of renal lesions, although fat may be concentrated considerably in the kidneys, indicates that the tissues react qualitatively and individually. The absence of edema and inflammation in the lungs of animals injected with paraffin excludes anoxia and ischemia, and suggests that in the lungs the tissues may so act upon the fat as to release irritant substances, whereas such is not the case in other tissues. Lipase has been demonstrated histochemically[8] in the alveolar septa of the rabbit lung and may implement the inflammation by release of fatty acids which are known to cause severe pulmonary inflammation in minute quantity.[9] Furthermore, if the pulmonary lesions were due to anoxia or ischemia, it might be anticipated that they would be obviated by oxygen therapy. Yet, although oxygen ameliorates the changes, it does not exclude them.

On the other hand, the effect of oxygen in abolishing mortality supports the belief that the consequences of the pulmonary lesions, either anoxia or asphyxia, are directly responsible for death of the animals. The absence of hepatic necrosis in the oxygen-treated animal is consistent with this interpretation. Even with persistent severe pulmonary edema, oxygen prevents the occurrence of hepatic necrosis, affording proof that the hepatic lesions are due to anoxia, although those in the lungs are not. Moreover, it is interesting that injections of paraffin oil may cause hepatic necrosis, although the only pulmonary involvement is the plugging of the small vessels of the lung. The cardiac lesions are not affected by oxygen therapy and are, therefore, presumed to be due to emboli, which cause lesions known as Buchner's hypoxemic myo-

cardial necrosis.[10] The efficacy of oxygen therapy in experimental fat embolism indicates its specificity in this condition. It also emphasizes the few reports of dramatic results obtained clinically by the use of adequate, sustained oxygen therapy. Kolmert[11] was the first to discover the remarkable and, as he stated, surprising value of oxygen. Robb-Smith[3] and, more recently, Dunphy and Ilfeld[12] have substantiated the therapeutic value of persistent high oxygen concentration.

Thus it is apparent that the tissues react to the fat differentially and that the local effects may be separated from the systemic. It is clear also, from the behavior of animals either in tourniquet shock or dehydration, that the previous experience of the animal influences in considerable measure the response to fat. The mortality rate is significantly enhanced and the manner of death altered. From an acute variety of death the mechanism is converted to a peracute type by dehydration. Although the effect is striking, it cannot be correlated with any consistent change in either weight loss or hematocrit values. It may be presumed that when the hematocrit reading is elevated the extravascular fluid is depleted. Nevertheless, some animals with marked hemoconcentration and extravascular dehydration survive, whereas others with little or no detectable change succumb to the fat injection. This lack of correlation between manifest dehydration and fatality, and, conversely, the occurrence of fatality without hemoconcentration, are indications that the lethality of water deprivation may be operative in a manner more subtle than depletion of the fluid reserves alone. Whatever the underlying mechanism, it is indisputable that either rapid dehydration or tourniquet shock disadvantageously changes the animal's behavior toward fat embolism.

Summary and Conclusions

Injection of homologous fat into rabbits causes a mortality and mode of death correlated closely with the dosage given.

Mortality is increased by either dehydration or tourniquet shock.

Death is prevented by oxygen therapy, which also prevents hepatic necrosis.

Death by fat embolism is determined not only by the quantity and quality of fat entering the circulation, but it is influenced by the preconditioning of the animal, which alters its response to the injected fat.

REFERENCES

1. Wilson, J. V. The Pathology of Traumatic Injury. E. & S. Livingstone Ltd., Edinburgh, 1946, pp. 52–65.
2. Warren, S. Fat embolism. *Am. J. Path.*, 1946, **22**, 69–87.
3. Robb-Smith, A. H. T. Pulmonary fat-embolism. *Lancet*, 1941, **1**, 135–141.

4. Lehman, E. P., and McNattin, R. F. Fat embolism. II. Incidence at post-mortem. *Arch. Surg.*, 1928, **17**, 179–189.
5. Wright, R. B. Fat embolism. *Ann. Surg.*, 1932, **96**, 75–84.
6. Harman, J. W. The significance of local vascular phenomena in the production of ischemic necrosis in skeletal muscle. *Am. J. Path.*, 1948, **24**, 625–641.
7. Hamre, C. J. The capillary hematocrit method of determining blood cell volume. *J. Lab. & Clin. Med.*, 1939–40, **25**, 547–551.
8. Gomori, G. Distribution of lipase in the tissues under normal and under pathologic conditions. *Arch. Path.*, 1946, **41**, 121–129.
9. Harris, R. I., Perrett, T. S., and MacLachlin, A. Fat embolism. *Ann Surg.*, 1939, **110**, 1095–1114.
10. Mazzei Egidio, S., Taylor Gorostiaga, D., and Magalhaes, E. El embolismo pulmonar en clínica, obstetricia y cirugía. IV. El embolismo pulmonar experimental. *Prensa méd. argent.*, 1947, **34**, 307–311.
11. Kolmert, F. A few observations on fat embolism. *Acta chir. Scandinav.*, 1939–40, **83**, 263–268.
12. Dunphy, J. E., and Ilfeld, F. W. Fat embolism. *Am. J. Surg.*, 1949, **77**, 737–743.

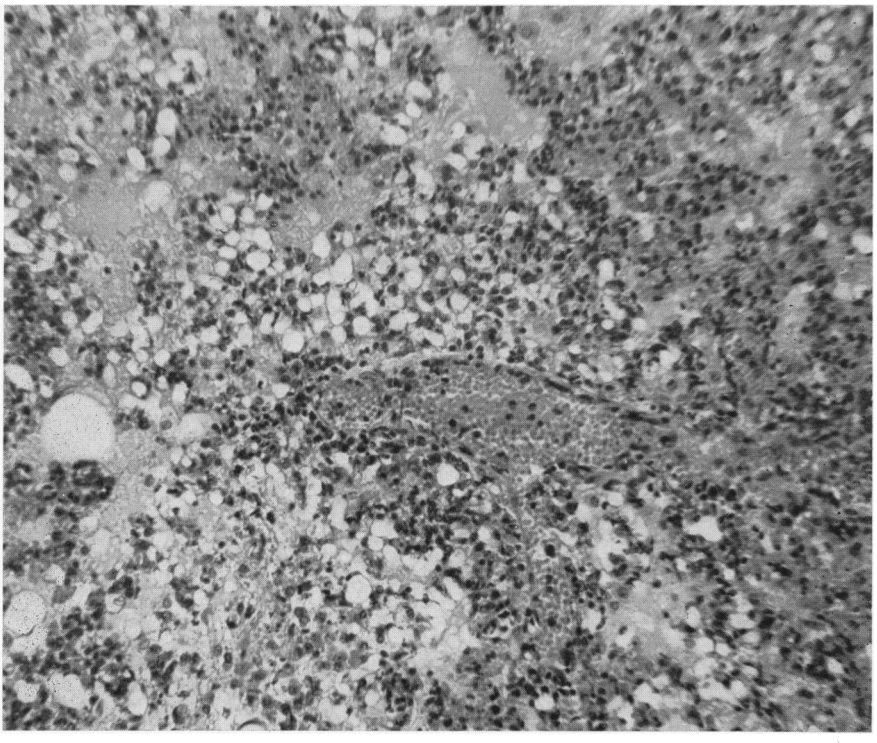
DESCRIPTION OF PLATES

PLATE 81

FIG. 1. Section of lung from a rabbit which was injected with 0.55 cc. of fat per kg. of body weight. The animal died 12 hours after injection. There are many capillaries filled with fat vacuoles and the alveoli are filled with coagulated edema fluid. The alveolar septa are congested and contain many interstitial polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 175.

FIG. 2. In the pulmonary artery of the same animal from which Figure 1 was made, there is extensive vacuolation of the subintimal tissue, which lifts off the lining endothelium of the artery. The muscle cells of the media are conspicuously separated by interstitial fluid. Hematoxylin and eosin stain. × 350.

1



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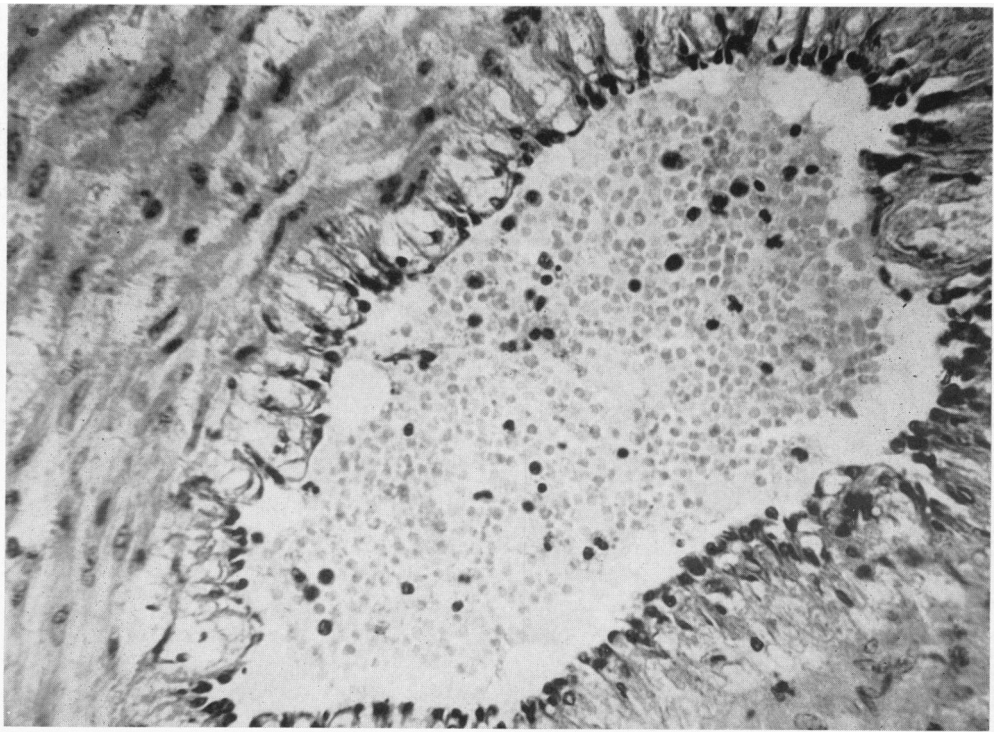

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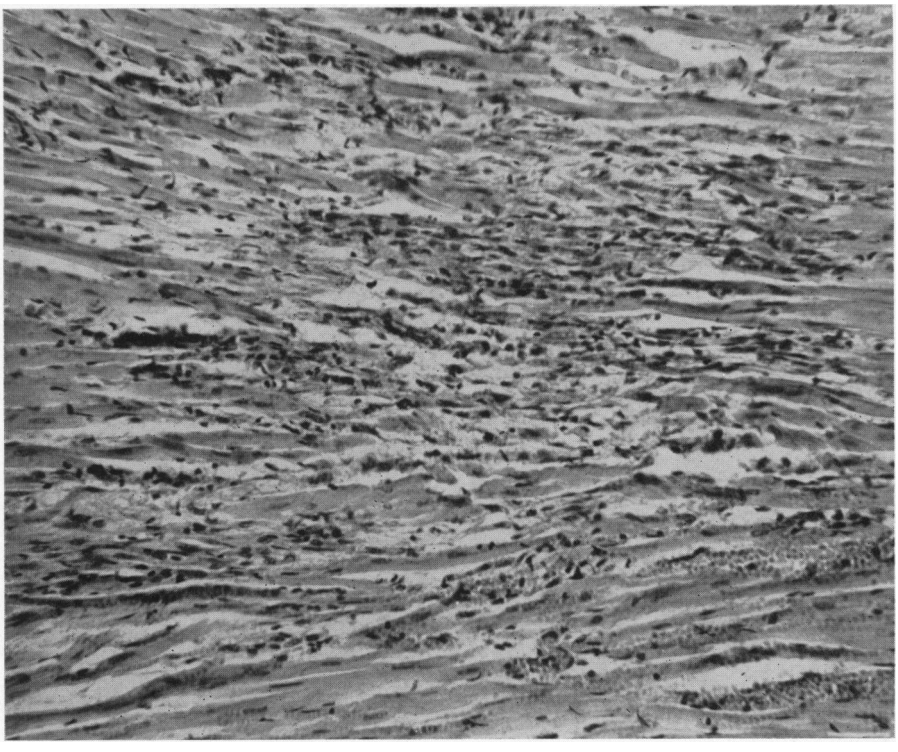
Experimental Fat Embolism

PLATE 82

FIG. 3. The cardiac muscle fibers are necrotic. and there is infiltration of polymorphonuclear leukocytes and monocytes. Many muscle fibers have undergone lysis and granular degeneration. This animal was injected with 0.55 cc. of fat per kg. of body weight. Hematoxylin and eosin stain. × 350.

FIG. 4. The liver of an animal which died 12 hours after injection of 0.55 cc. of fat per kg. of body weight shows extensive centrilobular necrosis with many infiltrated polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 65.

3



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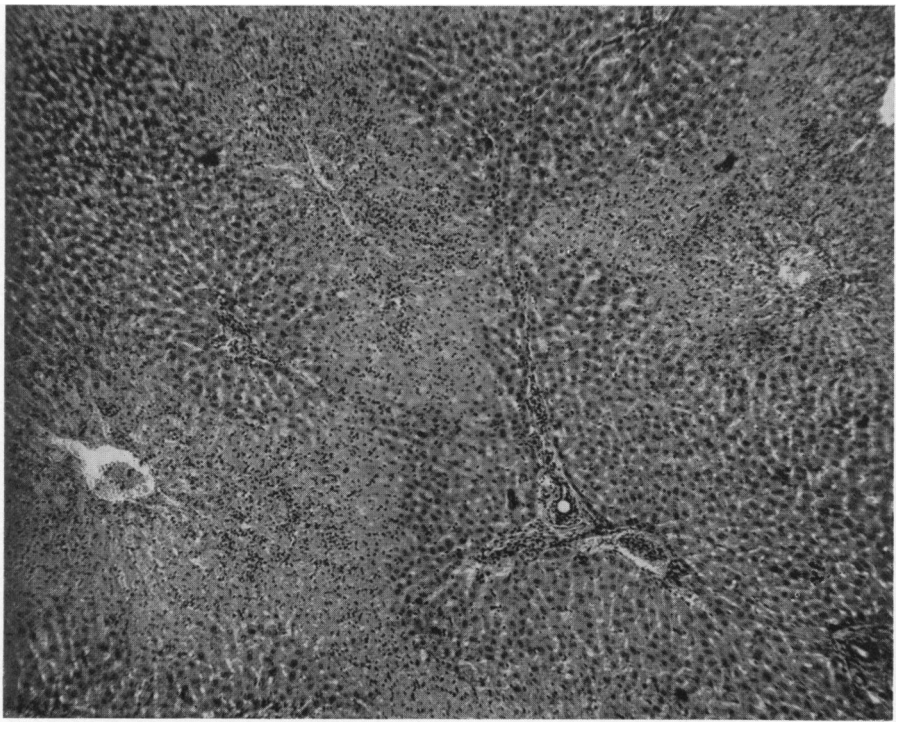

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