RESIDUAL PIGMENT ASSOCIATED WITH INTRAVENOUS FAT ALIMENTATION

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In 1957, Meyer, Fancher, Schurr and Webster [1] reported the observation of a brown pigment in the reticuloendothelial system of dogs and human beings receiving multiple infusions of an intravenous fat emulsion. In 1958, Thompson, Johnson and Forbes [2] described the staining characteristics of a pigment-lipoid complex which they designated "intravenous (i.v.) fat pigment." This pigment was deposited in the reticuloendothelial cells of the spleen and the Kupffer cells of the liver after multiple intravenous infusions of the fat emulsion. Recently, Thompson, Fox, Harrison and Forbes [3] reported the influence of fixatives, solvents and bleaches on the same pigment. They were able to demonstrate that i.v. fat pigment was an entity and not an artifact incident to methods of tissue processing and fixation.

The original investigators cited above dealt with the occurrence and the chemical and physical nature of i.v. fat pigment. The long-term effects of the presence of this pigment in the body have not been reported. To determine the fate and possible pathologic significance of the pigment, multiple infusions of the fat emulsion were administered to 25 adult albino rabbits. At periodic intervals subsequent to the last day of infusion, groups of animals were sacrificed and their tissues examined for histologic alterations. Since our previous experience had been chiefly with rabbits, this was the animal of our choice.

MATERIAL AND METHODS

Selected tissues were obtained at necropsy from the 25 rabbits. The intravenously administered fat emulsion was composed of a sterile aqueous preparation containing 15 gm. of cottonseed oil, 4 gm. of glucose, 1.2 gm. of soybean phosphatide, and 0.3 gm. of Pluronic F-68 (a polyoxyethylene-propylene polymer manufactured by Wyandotte Chemicals

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Corporation, Wyandotte, Michigan) per 100 ml. The emulsion (15 ml. per kg. of body weight) was administered daily for 28 days. Rabbits were sacrificed at 1 and 25 days, and at 3, 6, 12 and 18 months after the last day of infusion. There were no spontaneous deaths and no evident clinical illnesses. The livers and spleens from the animals killed up to and including 6 months after infusion were harvested and fixed in 10 per cent formalin. The livers, spleens, mesenteric lymph nodes and femoral bone marrow were obtained from the animals sacrificed 6 months after infusion. Replicate tissue blocks from each location were fixed in 10 per cent neutral buffered formalin and in absolute alcohol.

In addition to the experimental animals, a control group of 10 rabbits of similar age was also examined. Each of these rabbits received daily intravenous infusions of a solution containing 5 per cent dextrose in water for 28 days; again, the dose was 15 ml. per kg. of body weight. Necropsies were performed on these animals 1, 25 and 62 days, and 3 and 8 months after the last day of infusion. Here, too, there were no spontaneous deaths and no evident clinical illnesses. Blocks of liver and spleen were procured from all animals and fixed in 10 per cent neutral buffered formalin and in absolute alcohol.

Replicate sections, 6 to 8 μ in thickness were prepared from paraffin-embedded tissues in all 35 rabbits. Sections from each specimen in the test animals were subjected to the same physical and histochemical tests originally used in the characterization of the i.v. fat pigment. These included fluorescent microscopy with the use of exciting light in the wave length range of 360 to 400 mμ.[5] In the control rabbits, sections were stained with Harris' hematoxylin and aqueous eosin,[4] and for iron by Gomori's method.

Results

In the Kupffer cells of the liver and in reticuloendothelial cells of the spleen of all rabbits killed one day after infusion, there were deposits of a brown granular pigment, surrounded by lipoid substances. This pigment-lipoid complex exhibited reactions to staining techniques and to histochemical and physical tests which were characteristic of the i.v. fat pigment.[2, 3]

In the spleens of all test rabbits sacrificed 25 days after the last infusion, numerous reticuloendothelial cells were heavily laden with the complex identifiable as i.v. fat pigment (Fig. 1). In the Kupffer cells of the livers of these rabbits, variable quantities of a similar substance were observed. In addition, focal accumulations of lymphocytes and monocytes were encountered within sinusoids and portal areas. In all but one of the rabbits, circumscribed collections of macrophages were observed

in sinusoids, most frequently in the zones adjacent to portal areas. These cells were invariably heavily laden with i.v. fat pigment, and in some instances they appeared to have fused to form giant cells of the Langhans type (Fig. 2).

In the rabbits sacrificed 3 months after the last infusion, considerable quantities of i.v. fat pigment were observed in the cytoplasm of many reticuloendothelial cells of the spleen. The pigment was not as diffusely distributed as in the groups of animals sacrificed earlier (Fig. 3), and there was a definite tendency toward clumping of the macrophages, thereby concentrating the pigment in focal areas of the pulp. In sections stained with osmium tetroxide, the pigment granules frequently stained more darkly than in the animals sacrificed at the termination of the infusion period. In sections stained by the acid-fast technique, the pigment and associated lipoid substances stained an intense violet rather than faint red. The complex was partially extracted on 24-hour exposure to hot methanol chloroform. This reaction differed decidedly from that experienced in the animals sacrificed 1 and 25 days after infusion. When treated with anhydrous pyridine (acetylation) and subsequently stained by the periodic acid-Schiff (PAS) method, considerable portions of the pigment-lipoid complex remained in the tissues as amorphous smudges of golden-brown, PAS-negative substance, whereas in rabbits killed 1 and 25 days after infusion, this material was completely extracted by similar procedures. On deacetylation and subsequent PAS staining, this substance regained a faint PAS positivity. Hemosiderin was plentiful in each spleen examined, and was frequently mixed with the i.v. fat pigment.

Most of the Kupffer cells in rabbits sacrificed 3 months after infusion contained no i.v. fat pigment. However, those Kupffer cells which did contain it were heavily laden. Circumscribed collections of macrophages, some forming giant cells of the Langhans type, were observed in the sinusoids adjacent to the portal tracts in every liver (Fig. 4). The pigment-lipoid complex in Kupffer cells, macrophages, and giant cells exhibited identical staining reactions. Hemosiderin was not observed in these cells. The staining characteristics of the complex were similar to those observed in the spleen, with the exception that there were more intense reactions to PAS and phosphomolybdic acid (PMA), and more pronounced acid-fast staining. The focal lymphocytic infiltration observed 25 days after infusion was not encountered at 3 months.

At 6 months, i.v. fat pigment was present regularly in the cytoplasm of aggregates of reticuloendothelial cells in the spleen. The pigment was similar in appearance, sites of deposition, and histochemical characteristics to that observed at 3 months (Fig. 5). In the livers of these animals, there was no i.v. fat pigment demonstrable in most of the Kupffer cells.

Some of these cells did contain considerable quantities of hemosiderin, however. In all of the livers, many clumps of macrophages and giant cells appeared in the sinusoids adjacent to portal tracts (Fig. 6). These were laden with the lipoid substance which was occasionally mixed with hemosiderin. The pigment-lipoid complex had histochemical characteristics similar to those encountered at 3 months.

In the spleens of rabbits sacrificed 12 and 18 months after the last infusion of fat emulsion, large quantities of hemosiderin were observed, but there was no i.v. fat pigment. In the livers the pigment was present in only a few Kupffer cells; however, many heavily laden macrophages and giant cells were observed in the sinusoids adjacent to portal tracts (Figs. 7 and 8). The granular portion of the pigment complex was very dark brown in color, and fewer of the particles were birefringent than in the cases examined earlier. Considerable quantities of hemosiderin were also mixed with the i.v. fat pigment in almost every cell in which the latter was observed. It was also evident that although the pigment was present in tissues fixed in both 10 per cent neutral buffered formalin and absolute alcohol, less of it was manifest in the alcohol-fixed sections.

The fat pigment was present in the cytoplasm of reticuloendothelial cells of the femoral bone marrow in the animals sacrificed 12 and 18 months after infusion (Fig. 9). A brown, smudgy substance was also observed in phagocytic elements in the mesenteric lymph nodes, appearing in clumps of cells in the cell-poor parenchyma. It was less granular than that observed in the liver or bone marrow, contained fewer birefringent particles, but was otherwise similar to the pigment-lipoid complex seen in the other tissues (Fig. 10).

Variable quantities of iron-positive pigment, considered to be hemosiderin, were present in the spleen, liver and mesenteric lymph nodes of the control animals. Neither i.v. fat pigment nor other significant lesions were observed in any of these rabbits.

Discussion

It is evident that the deposition of the pigment-lipoid complex in tissues of animals receiving intravenous infusions of a cottonseed fat emulsion was related to the emulsion, since a similar substance was not found in any of the control animals. The presence of the complex in the tissues of animals sacrificed 18 months after infusion would indicate that the substance was not readily eliminated from the body.

The disappearance of the complex from the spleen at some time between the sixth and twelfth month after infusion is of interest, since the quantity present in the mesenteric lymph nodes increased during this period. In some of our previous studies, negligible quantities of pigment

were observed in the mesenteric lymph nodes of animals sacrificed soon after termination of the infusions. In contrast, heavy depositions were noted in the spleen at such times. This would appear to indicate that the complex, as seen in the spleen, was being handled much as one might expect, by transport to the regional lymph nodes.

The formation of pigment-laden giant cells in the liver as early as 25 days after infusion is noteworthy. The persistence of the giant cells over a period of 18 months, with no other associated inflammatory reaction, is difficult to explain. However, it is felt that the phenomenon represents a host response to a foreign substance. The pigment-lipoid complex in the bone marrow was essentially unaltered during the period of the experiment. Its histochemical characteristics 18 months after infusion were identical to those observed at one day.

The changes noted in the histochemical characteristics of i.v. fat pigment during the period it remained in body tissues are thought to be correlated with its initial concentration in various types of cells. The apparent intensification of its acid-fast, PAS, PMA, osmium tetroxide and oil red O staining qualities could easily be related to an increasing concentration. Likewise, the alteration of its solubility in anhydrous pyridine and hot methanol chloroform probably have a similar relationship to its concentration in heavily laden cells. The increased solubility in absolute alcohol incident to alcohol fixation, a feature noted 12 and 18 months after infusion, is not unexpected. It was reported earlier [3] that prolonged storage of alcohol-fixed tissues in 70 per cent alcohol gradually removed the i.v. fat pigment content.

We have examined liver biopsy specimens from a few dogs which had received multiple intravenous infusions of various cottonseed oil emulsions. These specimens had been procured over a period of a year. I.v. fat pigment-laden giant cells were frequently observed in the specimens collected approximately one year after infusion. Thus, the phenomenon is not limited to rabbits alone, and it is not unlikely that a similar situation may occur in human beings. A pigment-lipoid complex with identical histochemical characteristics has been demonstrated in human subjects following fat infusion.[2, 3]

In order to determine the ultimate effect upon the host of such residual pigment, life-span animal studies are indicated. Our present data do not indicate the potential harm that the pigment might evoke.

Summary

The pathologic effect of i.v. fat pigment in animal tissues over prolonged periods of time has been investigated. Twenty-five rabbits were given daily intravenous infusions of a cottonseed oil emulsion for 28

days. Representative groups of these animals and suitable control animals were sacrificed at intervals up to 18 months after the infusions. Tissue sections prepared from these animals were examined by a variety of histochemical procedures for the identification of the fat pigment. The substance was observed in representative tissues throughout this 18 months' period. It was accompanied by the formation of giant cells in the livers of all rabbits from the 25th day after infusion to the termination of the experiment. The pigment remained in body tissues as a foreign substance which was not metabolized.

References

1. Meyer, C. E.; Fancher, J. A.; Schurr, P. E., and Webster, H. D. Composition, preparation and testing of an intravenous fat emulsion. *Metabolism*, 1957, **6**, 591–596.
2. Thompson, S. W.; Johnson, F. B., and Forbes, A. L. Staining characteristics of a pigment associated with intravenous fat alimentation. *Lab. Invest.*, 1958, **7**, 533–543.
3. Thompson, S. W.; Fox, M.; Harrison, M., and Forbes, A. L. Influence of fixatives, solvents, and bleaches on intravenous fat pigment. *Lab. Invest.* (In press)
4. Gridley, M. F. Manual of Histologic and Special Staining Technics. Armed Forces Institute of Pathology, Washington, D.C., 1957, p. 30.
5. Mallory, F. B. Pathological Technique. W. B. Saunders, Philadelphia, 1938, p. 137.

All illustrations are U.S. Army photographs taken by Mr. William Hummel of the Pathology Service, Fitzsimons Army Hospital, Denver, Colo. The authors appreciate the technical assistance of Sergeant First Class Harry Hoey, United States Army.

Legends for Figures

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

Fig. 1. I.v. fat pigment in the reticuloendothelial cells of the spleen. Rabbit, 25 days after termination of intravenous fat infusions. × 850.

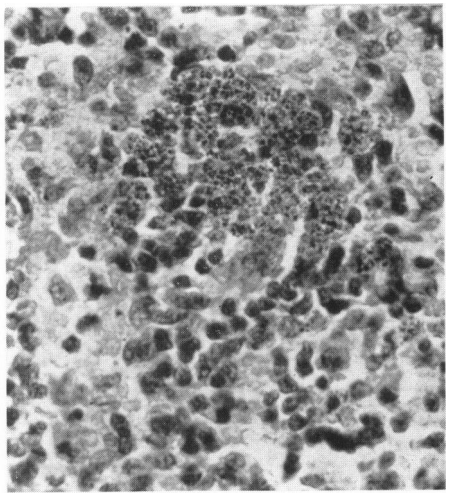
Fig. 2. An i.v. fat pigment-laden giant cell in the liver of the same rabbit photographed in Figure 1. × 850.

Fig. 3. I.v. fat pigment in the reticuloendothelial cells of the spleen. Rabbit, 3 months after termination of intravenous fat infusions. × 850.

Fig. 4. Pigment-laden giant cell in the liver. Rabbit, 3 months after termination of intravenous fat infusions. × 850.

Fig. 5. I.v. fat pigment mixed with hemosiderin in the reticuloendothelial cells of the spleen. Rabbit, 6 months after termination of intravenous fat infusions. × 850.

Fig. 6. I.v. fat pigment-laden giant cells in the liver. Rabbit, 6 months after termination of intravenous fat infusions. × 1000.



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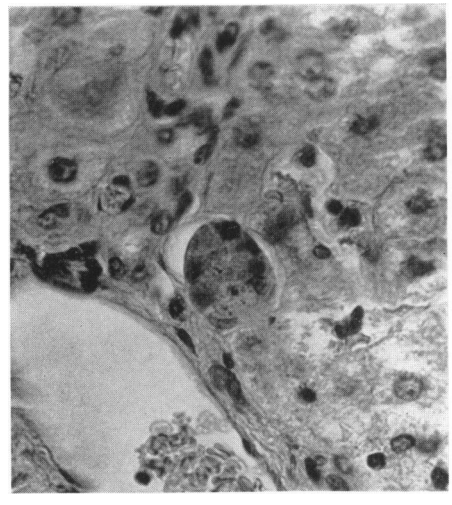

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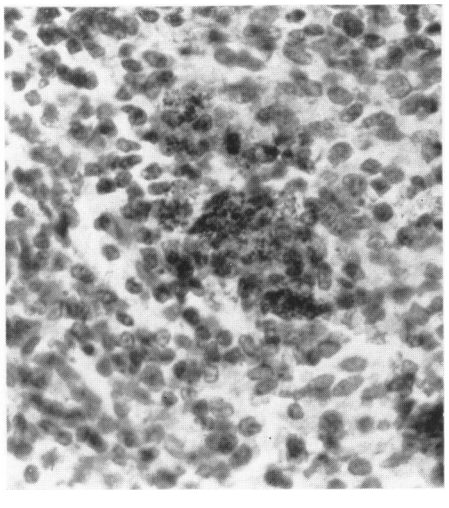

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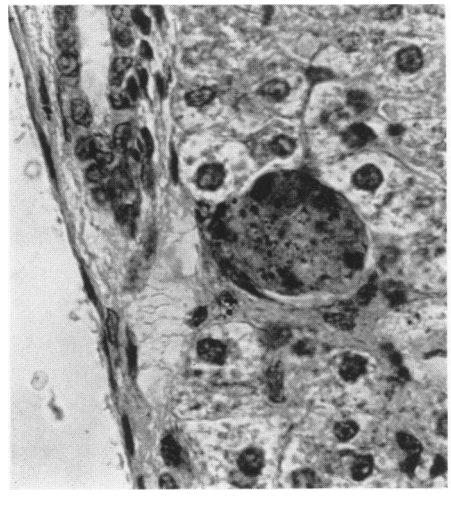

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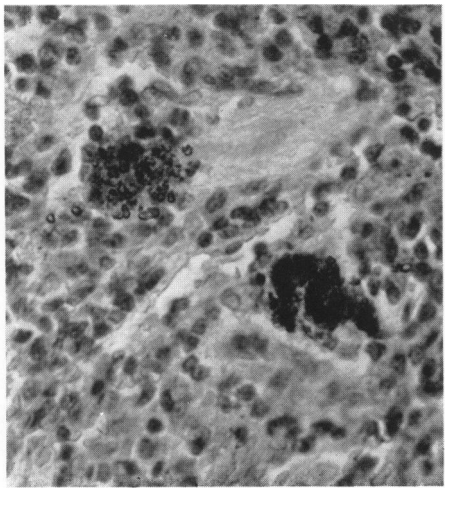

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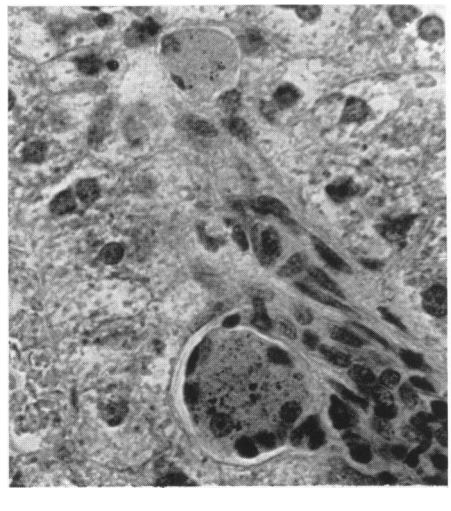

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FIG. 7. Pigment-laden giant cell in the liver. Rabbit, 12 months after termination of intravenous fat infusions. × 850.

FIG. 8. I.v. fat pigment mixed with hemosiderin in a giant cell in the liver. Rabbit, 18 months after termination of intravenous fat infusions. × 850.

FIG. 9. I.v. fat pigment in the femoral bone marrow. Rabbit, 12 months after termination of intravenous fat infusions. × 1000.

FIG. 10. Pigment in the mesenteric lymph node. Rabbit, 18 months after termination of intravenous fat infusions. × 850.

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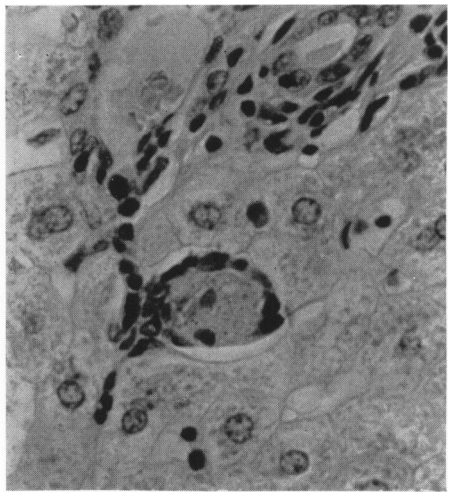

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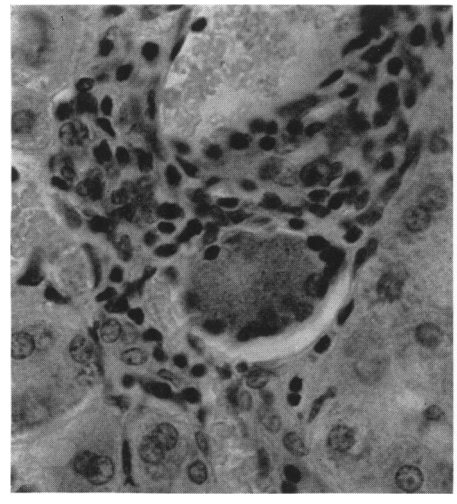

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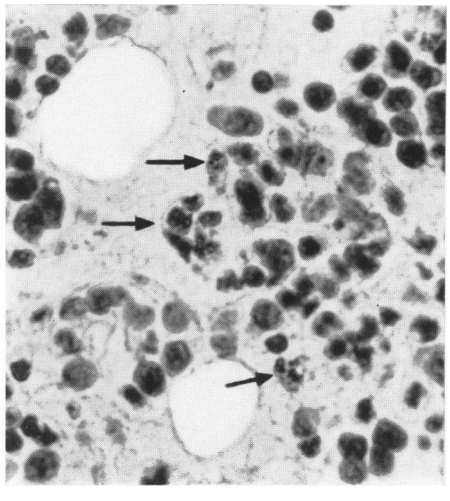

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