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THE INTRANODAL DISTRIBUTION OF LYMPH-BORNE PARTICLES INJECTED INTRAVENOUSLY

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Summary.—The sequential distribution of lymph-borne, i.v. injected particles of tantalum in hepatic hilar lymph nodes was studied in rats in an attempt to determine which structural compartments of a node are responsible for mechanical filtration. The injected particles reached these nodes *via* liver lymph but the i.v. route of administration eliminated any possibility of disturbing either lymph flow or pressure. Particles began to enter hepatic hilar nodes only after an interval of 7–8 h. They were subsequently redistributed from marginal, trabecular and medullary sinuses to the paracortex and finally to medullary cords. Particles accumulated predominantly in the paracortex at 12–24 h and thereafter in medullary cords. This sequential pattern of distribution differed significantly from that observed previously in other lymph nodes after either intralymphatic or intratissue injection.

ACCORDING TO A PREVAILING VIEW, the ability of a lymph node to function as a simple mechanical filter is traceable to 2 specific compartments: the marginal sinus and the sinusoidal system (Yoffey and Courtice, 1970). This view is based on numerous studies of the intranodal distribution of marker particles injected either intralymphatically or into tissue.

During a study to determine whether lymph nodes draining the liver could be rendered radio-opaque by the i.v. administration of tantalum particles, these nodes were removed and sectioned at various intervals (Dumont and Martelli, 1969). When examined histologically, a pattern of particle distribution was noted which seemed to differ in some important respects from that described previously after intralymphatic or intratissue injection. Since the i.v. route of administration eliminated any possibility of disturbing either lymph flow or pressure and since information concerning the intranodal distribution of lymph-borne but i.v. injected particles was completely lacking, additional observations seemed warranted and these form the basis for this report.

METHODS

All experiments were performed in male Wistar rats (200–300 g). Fresh suspensions of tantalum powder, a physiologically inert, radio-opaque metal, easily visible under the light microscope, (particle size 1–2 μm) were prepared for each experiment as described in a previous report (Dumont and Martelli, 1969). Sixty-seven experimental animals received a single i.v. injection of 1 ml of this suspension containing 500 mg of tantalum and a minimum of 2 animals were killed at various intervals ranging from 6 h to 12 months after injection. At necropsy hepatic hilar nodes were removed, placed in formalin and prepared for histological study. The intranodal distribution of tantalum was examined by 2 observers without prior knowledge of the source of the specimen or the interval after injection. An arbitrary 0–4+ estimate was used to determine the amount or percentage distribution of tantalum in the various lymph-node compartments.

RESULTS

Lymph-borne free and intracellular particles first appeared in hepatic lymph nodes 7–8 h after i.v. injection. At 6 h

TABLE.—*Sequential distribution of tantalum particles in intranodal compartments*

Interval after injection	Marginal sinus	Trabecular and medullary sinus	Paracortex	Germinal centre	Medullary cords
6 h	0	0	0	0	0
8 h	1–2+	1–2+	1–2+	0	0
10 h	1–2+	1–2+	2–3+	0	0
12–24 h	1+	0–1+	4+	0	0
48–72 h	1+	0–1+	3–4+	0–1+	2–3+
1–4 weeks	0–1+	0–1+	3+	1–2+	3–4+
2–3 months	0	1+	2–3+	2+	4+
12 months	0	1+	1–2+	0–1+	4+

they were found only in the lumen of intranodal blood vessels. Their distribution during and after this initial period, shown in the Table and in Figs 1–4, can be summarized as follows: initially and for the next 2–3 h, particles accumulated predominantly in marginal and trabecular sinuses. The accumulation in the marginal sinus varied from focal aggregations to only an occasional particle. At 12–24 h after injection the paracortex appeared hyperplastic and contained the largest number of particles compared to marginal, trabecular and medullary sinuses. By 48–72 h particles had collected in increasing numbers in the medullary cords and in stainable body macrophages of germinal centres. At the same time accumulations in the paracortex began to decrease. During the next 1–4 weeks the accumulation of particles in medullary cords increased progressively and collections in the paracortex were mainly limited to a "cupping" pattern at the periphery of geminal centres. By 2–3 months particles were noted only in perivascular areas of the paracortex and the accumulation in medullary cords had increased further. One year after injection accumulations were predominantly in medullary cords, with only small amounts in perivascular areas of the cortex and paracortex.

DISCUSSION

Information concerning the passage of particulate material from circulating blood into liver lymph is still limited. Earlier studies disclosed that i.v. injected particles of titanium (0·2–0·4 μm) selectively enter liver lymph nodes 6–12 h after injection (Huggins and Froelich, 1966). Also, i.v. injected particles of carbon and colloidal gold were identified in macrophages in liver lymph within 24 h of injection (Smith, McIntosh and Morris, 1970). This observation suggests that, after uptake of circulating particles by Kupfer cells, the latter become detached from sinusoids and enter the space of Disse. Neither these cells nor the particles they contain appear to travel further than the regional nodes of the liver, a finding which suggests that these nodes serve a special function; while not screening circulating blood directly they serve as the final repository for a portion of circulating particulate material taken up from blood by hepatic RE cells. Huggins and Froehlich suggested that the strategic interposition of these nodes in a lymphatic outflow tract of the liver might account for their enlargement in murine leukaemia.

The sequential pattern of intranodal distribution of lymph-borne, i.v. injected particles differs significantly from that described after either intralymphatic or intratissue injection. Particles began to enter hepatic hilar lymph nodes only after an interval of 7–8 h. Their subsequent redistribution from marginal, trabecular and medullary sinuses to the paracortex and finally to medullary cords required approximately 3–12 months. After intralymphatic or intratissue (usually footpad) injection, in contrast, particles can be identified in the regional lymph node in 10–15 min (Drinker, Field and Ward, 1934; Fossum, 1980). During the next few hours they fill the

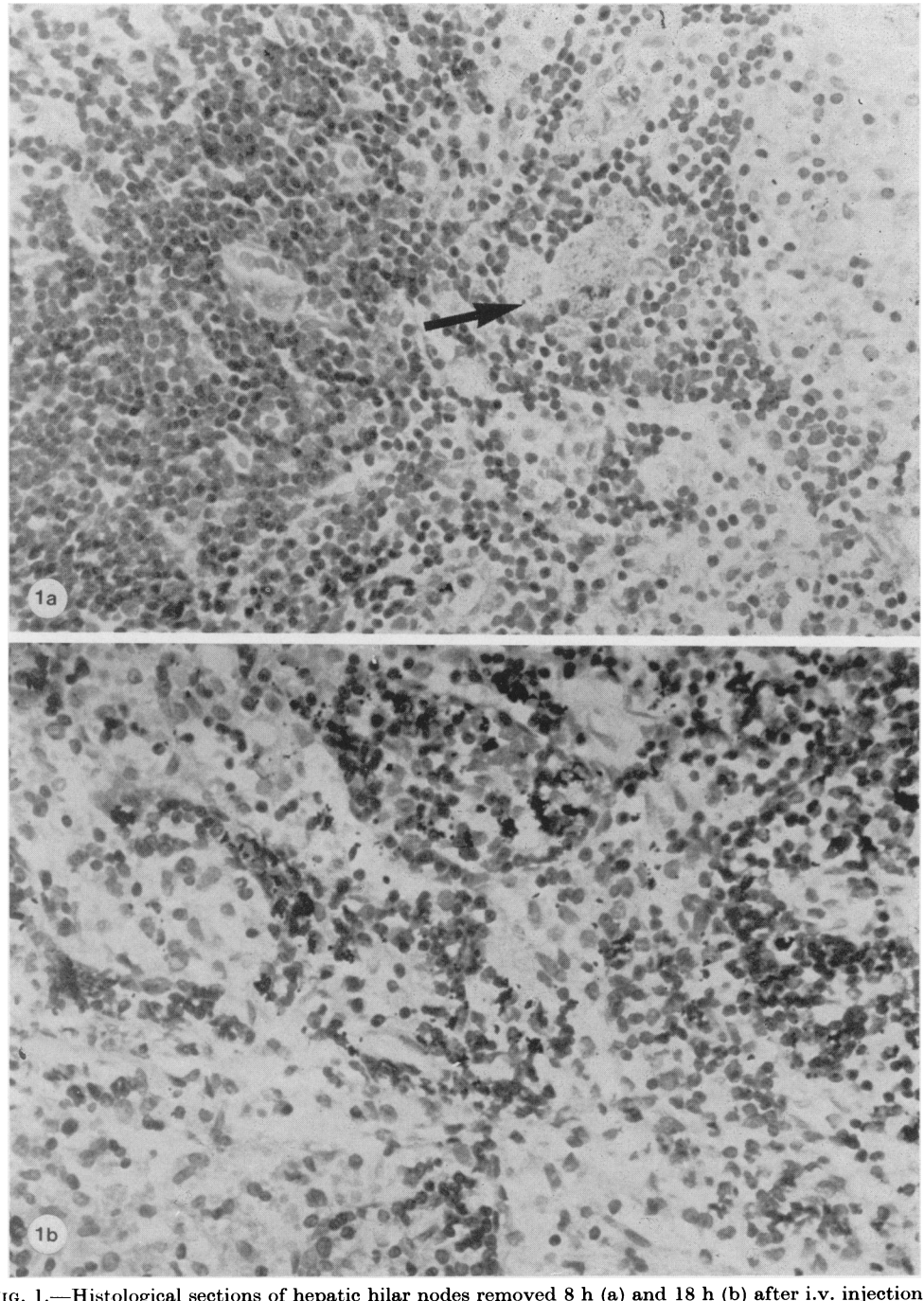
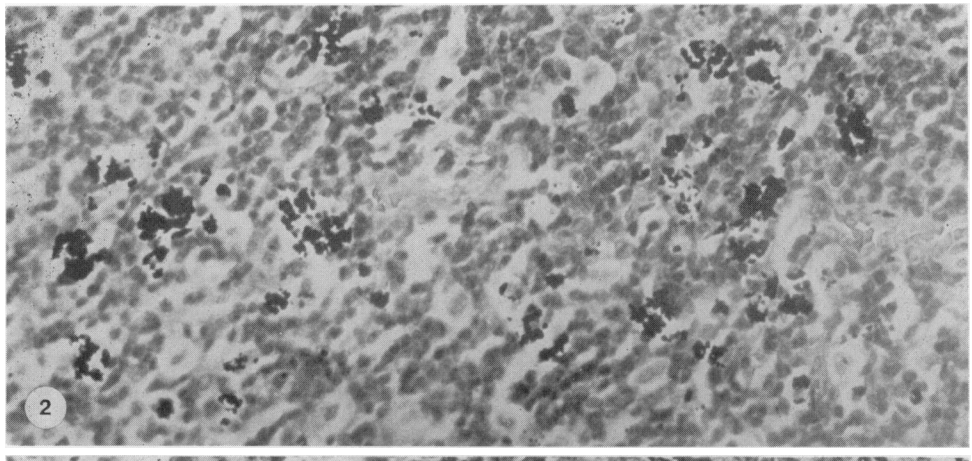
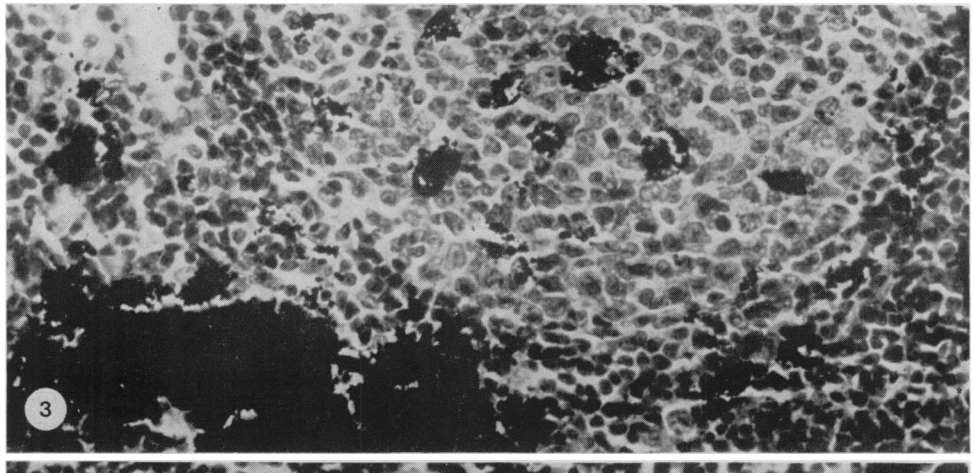


FIG. 1.—Histological sections of hepatic hilar nodes removed 8 h (a) and 18 h (b) after i.v. injection of tantalum particles. At 8 h the trabecular sinuses contain small amounts of tantalum within intrasinusoidal histiocytes (arrow) and almost none in the lymphoid tissue itself. By 18 h the amount of tantalum in trabecular sinusus has increased with accumulations predominating in lymph cords. H. & E. ×250.





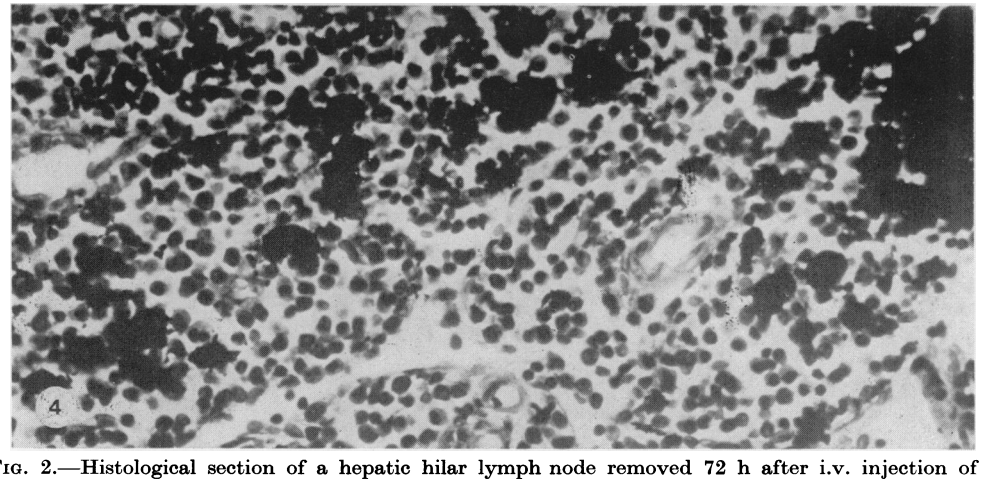


FIG. 2.—Histological section of a hepatic hilar lymph node removed 72 h after i.v. injection of tantalum. The paracortex still retains a considerable amount of tantalum. H. & E. × 250.

FIG. 3.—Histological section of a hepatic hilar node removed 30 days after i.v. injection of tantalum. Dense accumulations of tantalum are seen at the periphery of a germinal centre. Macrophages within the germinal centre also contain tantalum H. & E. × 250.

FIG. 4.—Histological section of a hepatic hilar node removed 3 weeks after i.v. injection of tantalum. Dense accumulations of tantalum are seen in medullary cords. H. & E. × 250.

subcapsular sinus and enter intermediary and medullary sinuses. By 24–28 h distribution throughout these spaces is practically complete, longer intervals (5–7 days) providing only for slightly deeper penetration into the interstitium of medullary and paracortical areas (Ludwig, 1971). Detailed observations beyond this period are not available.

On the basis of their observation of extensive filling of the marginal sinus with particles injected directly into a lymph vessel, Drinker *et al.* (1934) emphasized that the marginal sinus functions "not as a channel but a bowl-shaped lake" and that the flow of particles was thereby "instantly slowed". Subsequent descriptions of the distribution of particles in the marginal sinus after intralymphatic or intratissue injection support this view. Accordingly, particles or contrast materials are described as spreading "in all directions in the marginal sinus" (Tjernberg, 1962) "in a disc-like fashion through the subcapsular sinus" (Ludwig, 1971) and entering "the marginal sinus almost to the place where the efferent lymph comes out" (Kinmonth, 1967). Clearly none of these descriptions fits the pattern of distribution described here. Far from being filled with particles, this compartment contained at most a small number of poorly demarcated collections separated by an occasional particle. A similar distribution of marker particles in the marginal sinus of pulmonary hilar nodes of the dog was noted by Kubik (1967) 48 h after the introduction of coal dust into the lumen of a segmental bronchus.

The results of intralymphatic particle injection led Yoffey and Courtice (1970) to conclude that the sinusoidal system has a special role in filtration: "The entire arrangement from the point of view of mechanics, appears excellent for filtration. Lymph . . . finds itself in a huge space with an enormous number of wide and irregular paths leading to the hilus vessel . . . Not only are the sinuses in the node a perfect settling chamber, but the reticulum which they contain furnishes a number of baffles which again slow down lymph flow and make it easy for the phagocytic cells composing the reticulum to perform their function." The findings reported here seem incompatible with this conclusion. Although a small number of particles persisted in trabecular and medullary sinuses for 12 months, significant accumulations were observed only initially, and persisted for only 3–4 h. As the number of particles in these spaces decreased concomitantly with an increase in the paracortex, it would appear that the sinusoidal system functions more as a temporary conduit than as a "settling chamber". The rapid transfer of particles from sinuses into the parenchyma of a node probably occurs through intercellular gaps in the endothelium of marginal and trabecular sinuses (Folkert, Thliveris and Bertalanffy, 1977) and resembles the rapid transfer of labelled antigen between these compartments (Nossal *et al.*, 1968). Medullary cords seem to be the final destination of lymph-borne particles and it is to this compartment specifically that a general comment by Virchow (1860) seems particularly applicable: "The elements are crowded together like the particles in a charcoal filter so that lymph trickles out again on the other side in a more or less purified state".

Confident that a carefully performed intralymphatic injection does not alter lymph flow or pressure beyond physiological limits, Drinker *et al.* (1934) stated as follows: "The deposition of (intralymphatically) injected particles can be relied upon to fall in portions of the node normal for lymph travelling through the gland". Recent investigations suggest however that their confidence may have been misplaced. Under physiological circumstances, pressure in the interstitium and in initial and collecting lymph vessels appears to be maintained at or near atmospheric level (Aukland and Nicolaysen, 1981). That even the most careful intralymphatic or intratissue injection exceeds this pressure seems likely.

While it does not necessarily follow that this disturbance alters the intranodal distribution of particles, the observations described here suggest that this may prove to be the case.

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