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ASM-68K (Analyse-System Manuell, Fa. Leitz) to determine the number and the amount of quartz induced reaction areas as percentage of slide area. For ultrastructural investigations tissue was plastic embedded (Durcupan-ACM, Fluka) and semithin sections (1µm) were stained with methylene-blue /AzurII. Ultrathin sections were post-fixed with osmium tetroxide and contrasted with uranyl acetate and lead citrate.

**Results**

*Histomorphology:* The liver of animals from groups I and IV reveals the normal hepatic structure. Silver impregnation shown a homogeneous black stained network. In the liver of all dust exposed animals numerous reaction areas of various size are obvious (Fig. 1). The smallest areas are clusters of cells. Phagocytosed quartz particles in the macrophages can be seen best in polarized light. Bigger roundish or oval areas predominantly consist of macrophages and fibroblasts, together with some polymorphonuclear leucocytes and lymphocytes. In silver impregnation, clearly visible nets of fibers can be seen. Some areas reveal many collagenous fibers. Liver cells surrounding these fibrotic granulomas show severe signs of degeneration.

*Electron microscopy:* TEM evaluation demonstrates quartz within the phagocytes, in sinuses as well as in granulomatous reaction areas. Furthermore in marginal zones of the reaction areas degenerative changes of the hepatocytes and proliferation of collagenous fibers are obvious.

*Morphometry:* Evident differences occur between the two dust exposed groups. The amount of granulomatous reaction areas in animals exposed to quartz dust only is 2.5 fold higher than after additional exposure to excess pressure.

**Discussion**

The morphology of liver granulomas reported here resembles hepatic lesions in man as well as in rats and mice, due to various kinds of occupational dust exposure (1, 6) and intravenous injection of silica (3, 4), respectively. Inhalation experiments did not reveal hepatic granulomas due to quartz of similar severity, yet. Additionally to lungs, lung associated lymph nodes and liver, quartz containing cells were detectable in the mesenterial lymph nodes and spleen (5). Different pathogenetic pathways have been discussed, but the lympho-hematogenous spread is the most probable route (6). The results of morphometric evaluation of hepatic lesions are comparable

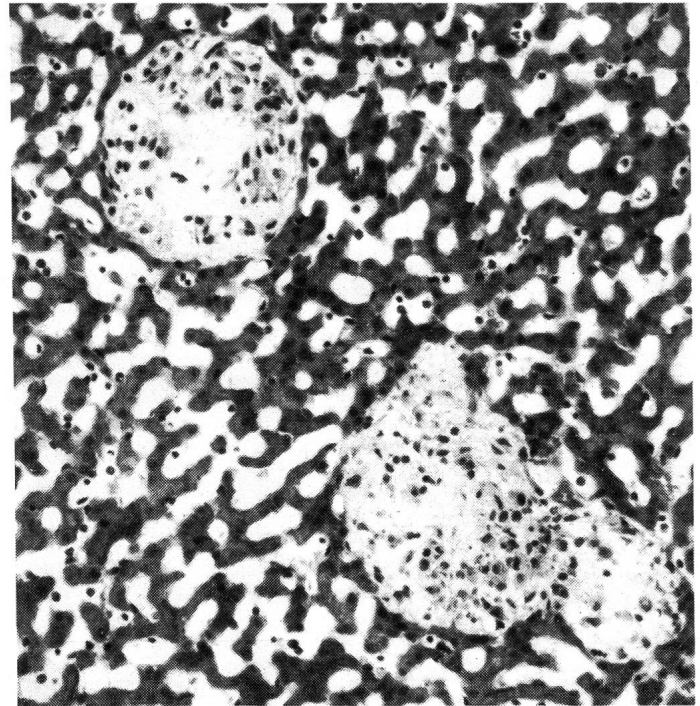


Fig. 1: Liver granulomas (H&E)

to those of lung granulomas (5). Finally, it has to be realized that due to the inhalation of fibrotic dusts, additionally to lung fibrosis, hepatic disorders may occur. These hepatic changes, including the development of granulomas, possibly cause structural as well as biochemical alterations, and may have a marked influence on the clinical symptoms.

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**IN VIVO NEUTROPHIL MOBILIZATION AFTER INTRADERMAL INJECTION OF COMPLEMENT FACTORS AND ENDOTOXIN: FUNCTIONAL DIFFERENCES BETWEEN NEWBORN CALVES AND ADULT CATTLE**

*D. Roth, R. Zwahlen*

The high mortality of neonates due to bacterial infections is partly attributed to abnormalities in the host defense system, particularly to incompetence of neonatal phagocytes. We demonstrated earlier *in vitro* that bovine neonatal neutrophils (N-PMN) have an enhanced migratory capacity in a chemotaxis assay, thus representing a major

functional difference to N-PMN of other species. This hyperirritability status of bovine N-PMN should function to enhance inflammatory defense mechanisms, although clinical findings suggest the contrary. We therefore conducted the following study to assess the mobilization of intradermal neutrophils by chemotaxins: 5 healthy crossbred newborn calves and 5 healthy adult crossbred cows wer-

injected intradermally with 100 µl of undiluted and 1:5 diluted zymosan-activated plasma (ZAP), bovine C5a, and 10<sup>-13</sup> M E. coli O 55 B:5 endotoxin (LPS) into the base of the tail. Pyrogen free saline (PFS) served as a control. 4 hrs later 6mm skin biopsies were punched out under local anesthesia and the tissue samples fixed in 4% formaldehyde, embedded in paraffin, processed routinely and stained with H&E and Lendrum's chromotrop 3R.

By using a set score (0 to 4) for randomized evaluation of the inflammatory infiltrate in the upper (from the epidermis to the deepest adnexal structures) and lower (from the deepest adnexal structures downwards) dermis, we semiquantified the granulocyte number in each section. The scores for the two areas were then added to provide a total score for the section (possible range 0 to 8). The relative amount of PMN and eosinophils was evaluated by counting 200 cells, 100 in the upper dermis and 100 in the lower dermis, which allowed to calculate the corrected score (PMN only).

While few extravascular granulocytes were found 4 hours after injection of PFS in both age groups, all chemotaxins used induced a significant inflammatory reaction at the same time point in neonates and adults. Undiluted ZAP was more active than diluted ZAP, and the total intradermal granulocyte score was comparable in both age groups. The total intradermal granulocyte score after LPS was slightly but nonsignificantly higher in newborn calves, whereas C5a induced a significantly (P < 0.05) stronger reaction in neonates. In newborn calves the inflammatory infiltrate was almost exclusively of neutrophilic (PMN) origin whereas in adults, a significant percentage of the cellular reaction consisted of eosinophils. The corrected score therefore provides a more differentiated pattern of reactivity. All three chemotactic agonists at all doses tested induced a significantly (P < 0.05) more intense neutrophil infiltration in neonatal calves whereas the difference in the score values was not different in the PFS treated sites (see Fig. 1).

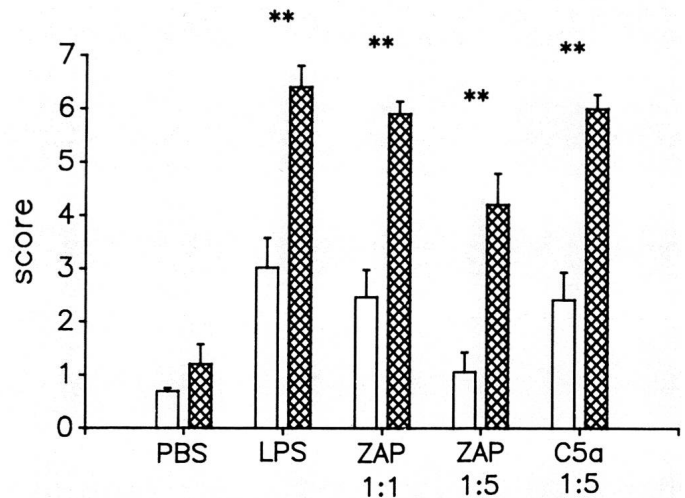


Fig. 1: Total score of neutrophil infiltrate in bovine skin 4 hours after injection of agonists or saline. The columns and the bars represent the mean ± SEM of experiments done in five animals. Open bars: adult bovines; hatched bars: newborn calves. (Adults vs. newborns: \*\*P < 0.01).

The presented data indicate that the enhanced motility of neonatal PMN encountered *in vitro* correlates with the finding of this *in vivo* study. The functional repertoire of neutrophils and eosinophils differs, but many aspects of it still remains to be analyzed. Especially the details of eosinophil functions within an inflammatory tissue are still not well known, although the participation of this cell in allergic and parasitic processes is well recognized. Further studies should help to elucidate the competence of host defense in newborn calves *in vivo*.

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**GRANULOMATOUS-LIKE REACTION IN RENAL LEPTOSPIROSIS OF SWINE**

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From January 1989 to March 1990 kidneys of 129 market weight pigs belonging to 25 different groups of animals were sampled at slaughterhouse and histologically examined. In these groups there was a high incidence of multifocal interstitial nephritis. Leptospiral infection was diagnosed on the basis of serology and/or bacteriological culture, and/or immunohistological staining.

Histologically, subacute to chronic interstitial nephritis was present in most of the kidneys. In mildly affected kidneys focal lymphocytic infiltration was present. In severely affected kidneys there were large foci consisting of lymphocytes and a variable number of neutrophils. Fibrosis, including scattered leukocytes, degenerating and regenerating tubules, was the main finding observed in chronic lesions.

In twelve cases granulomatous-like lesions were seen. Serial sections from these cases were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Ziehl-Neelsen and Gomori's Silver impregnation

and immunohistochemically tested with primary antisera against *Leptospira interrogans* serovar *pomona* (courtesy Dr. P. Mortarino) and vimentin (Dakopatts).

These granulomatous-like lesions were characterized by the presence of cells with large cytoplasm, some of them fused in small syncytia. Moreover, there were multinucleated giant cells which resembled those of the foreign body type (fig. 1). The number of giant cells varied greatly from one case to the next. Tubules partially disrupted and isolated tubular cells were commonly seen. Some giant cells were seen adhering to the basement membrane of tubules undergoing regressive changes. Lymphocytes were present both at the periphery of granulomas and in non granulomatous infiltrative foci. Ziehl-Neelsen stain consistently failed to detect acid-fast bacteria. PAS positive staining was observed in giant cells although this reaction varied greatly from one cell to the next. Immunoperoxidase for leptospiral antigen resulted positive in 10 out of 12 cases. The amount of leptospiral antigen was low; positive reaction was frequently detected