



FIG. 1.

Blood film of white-tailed deer (heart blood) showing sickling phenomenon of red blood cells (Giemsa's stain).

autopsy, the animals were thin, emaciated and the blood appeared thin and watery. Atrophy of the spleen was noted in practically all of the affected deer. A similar condition has been noted in man.<sup>2</sup> Coincidental infestation with the deer louse fly, *Liptotena depressa*, or with lungworms was noted in some specimens. The abnormal frequency of broken leg bones in animals found dead in regions where sickling was observed suggests an analogy to possible bone changes (thinning of the cortex) noted in some human patients.<sup>3</sup>

It is possible that sickle cell anemia may be a factor, in addition to malnutrition, starvation and lungworm pneumonia, in the cause of the winter losses of deer in Michigan.

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#### Reaction of Some Rabbit Tissues to Graded Quantities of Trypan Blue.

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During the past 65 years a tremendous amount of effort has been directed toward the investigation of the nature and function of a large group of tissue cells designated collectively as the "reticulo-endothelial system". The principal tissues considered in these

<sup>2</sup> Steinberg, B., *Arch. Path.*, 1930, **9**, 876.

<sup>3</sup> Grinnan, Andrew G., *Am. J. Roentg. and Rad. Therap.*, 1935, **34**, 297.

studies have been the liver, spleen, lymph nodes, bone marrow and the lining structures of the serous cavities.

Ponfick<sup>1</sup> seems to have initiated the method of study most widely used; namely, the intravenous injection of particulate material. He employed cinnabar (red mercuric sulphide) and demonstrated its disposition in certain non-parenchymatous cells of the liver. It remained for von Kupffer,<sup>2, 3</sup> however, to identify these cells as phagocytes and to publish his classic descriptions. Since then many workers have contributed further to this study, notably Evans,<sup>4</sup> Evans and Scott,<sup>5</sup> Downey<sup>6</sup> and Evans and Schulemann.<sup>7, 8, 9</sup>

It is evident from all these investigations that a vast number of phagocytic cells, which accumulate and store particulate dyes to an extraordinary degree, exists within the animal body. Only after large quantities of dyes have been injected do fibroblasts and certain other tissue cells take up these substances in any great quantity.

It was thought that a study of the reactions of rabbit tissue to graded doses of trypan blue might show a differential storage of dye in certain types of tissue cells which do not belong to the histiocytic system.

*Methods.* Eight litter-mate Chinchilla rabbits in sound health were selected, housed and fed under constant conditions. They received by ear vein a sterile, 1% watery solution of trypan blue in 1.0 c.cm. quantities every 24 hours for the first 2 days. Thereafter the dosage was slowly increased until 5 c.cm. were given daily. The total dosages for rabbits numbered 1 to 8 were 5, 10, 20, 40, 50, 70, 85 and 100 c.cm.

The animals were killed and the tissues studied 24 hours after each had received its last injection of dye. Fresh tissue spreads were observed and material was also taken for fixation in 10% neutral formalin and in a modified Carnoy Lebrun's<sup>10</sup> solution. Sections were stained in carmalum.

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<sup>1</sup> Ponfick, Emil, *Arch. f. path. Anat.*, 1869, **48**, 1.

<sup>2</sup> von Kupffer, C., *Arch. f. mikr. Anat.*, 1876, **12**, 353.

<sup>3</sup> von Kupffer, C., *Arch. f. mikr. Anat.*, 1899, **54**, 254.

<sup>4</sup> Evans, Herbert M., *Am. J. Physiol.*, 1915, **37**, 243.

<sup>5</sup> Evans, Herbert M., and Scott, Katherine J., *Contrib. Embryol., Carnegie Inst.*, 1921, **10**, 3.

<sup>6</sup> Downey, Hal., *Anat. Rec.*, 1917, **12**, 429.

<sup>7</sup> Evans, Herbert M., and Schulemann, W., *Science, N. S.*, 1914, **39**, 443.

<sup>8</sup> Evans, Herbert M., and Schulemann, W., *Deutsche med. Wchnschr.*, 1914, **40**, 1508.

<sup>9</sup> Evans, Herbert M., and Schulemann, W., *Folia haemat.*, 1915, **19**, 207.

<sup>10</sup> Hetherington, Duncan C., and Tompkins, Edna H., *Stain Technology*, 1933, **8**, 31.

Kidney, liver, lung, adrenal and spinal cord were studied in particular for their reactions to the trypan blue. Other organs and tissues were also fixed, sectioned and studied largely as a check on the storage of dye in the non-parenchymatous cells.

*Results.* It was obvious from gross inspection of the animals that their tissues became progressively bluer with increasing doses of the dye. Sections of the tissues, as well as fresh spreads, showed that there was a differential storage of dye in certain cells other than those of the histiocytic system.

Dye became visible in the cells of the proximal convoluted tubules of the kidney after administration of only 5 c.cm. of trypan blue. The deposits were few and small and were found near the nucleus at the base of the cell. Progressively, as the dye was increased in quantity, the size and number of granular deposits increased in the tubular epithelium. In the animal receiving 100 c.cm. of trypan blue, not only was the dye present in greatest quantity in the cells of the proximal convoluted tubules but it was also visible in lesser amounts in the cells of the distal convoluted tubules and least of all in the loops of Henle. None could be detected in the epithelium of the collecting ducts. In the heavily dyed animals the epithelium of the proximal convoluted tubules was so crowded with dye accumulations that the outlines of the cells were obscured and in some cases the cells seemed to have become desquamated.

The livers of the lightly stained rabbits showed no evidence of storage except in the cells of von Kupffer. However, after 20 or more c.cm. of dye had been administered, there was a steady increase in accumulation of dye within the hepatic epithelium. At first the numbers of granules were few; later the dye was taken up in amazing quantities but not quite to such an extent as by the epithelium of the kidney. The Kupffer cells were not only enlarged remarkably within the sinusoids but they were increased in numbers and were so loaded with dye that it appeared to be in concentrated masses rather than in discrete granules. The mesothelial cells of the peritoneal surface of the heavily dyed livers also contained a few blue granules.

The lung tissues of the rabbits which had received small quantities of dye were free of colored pigment save that confined to the histiocytes which were not at all numerous. As the dosage of trypan blue was increased these cells became very noticeable not only by their numbers but also by the large quantities of dye stored in them. In some sections numerous dye filled histiocytes were found in the blood vessels, suggesting that many of them were carried to the lungs by way of blood coming from the liver.

The alveolar epithelium contained some blue granules in those animals which had received 40 or more c.cm. of dye. Only in the most heavily stained rabbits did the dye appear in the bronchiolar epithelium and then it did so to such a very slight extent that it was found only after careful search.

The epithelium of the adrenal cortex began to accumulate trypan blue in very small quantities only after 50 c.cm. of dye had been administered. In the highly stained rabbits blue granules, small in size and diffusely scattered, appeared particularly in the cells of the glomerular zone; the cells of the fascicular and reticular zones showed in comparison very few colored granules. Even fewer and finer dye accumulations could be seen in the cells of the medulla. Also very small, yet definite, amounts of dye were present in the endothelial cells of the medullary sinusoids.

In the spinal cord, dye was stored within the relatively rare histiocytes of the meninges but in no case could any trace of blue pigment be found in the neurons or glial cells. In spite of the large amounts of trypan blue in the other tissues of the heavily stained rabbits, the sections of spinal cord were singularly pallid.

Three of the rabbits studied were females. Sections of the ovaries showed that trypan blue was taken up in marked quantities by the low columnar cells composing the germinal epithelium. Other dye filled cells belonged to the macrophage group.

*Conclusions.* Following the administration of total graded doses of 5 to 100 c.cm. of 1% trypan blue to rabbits, the epithelium of the convoluted renal tubules, of the liver, of the pulmonary alveoli and of the adrenal cortex stored dye in decreasing amounts in the order named. The quantity of dye stored in a given tissue varied with the total quantity of dye administered.

In highly stained rabbits minute amounts of dye were observed also in the bronchiolar epithelium, in the endothelium of the medullary sinusoids of the adrenal and in the mesothelium of the liver, while larger amounts were present in the germinal epithelium of the ovary.