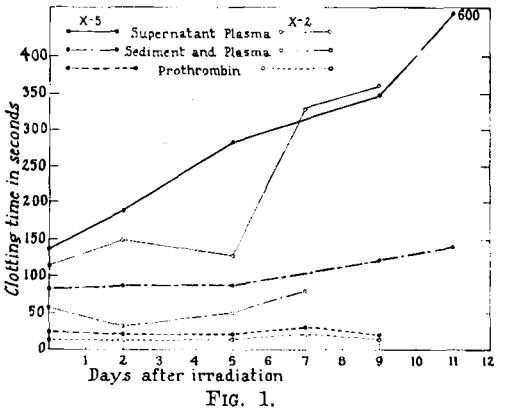

FIG. 1.

Graphic representation of the changes in supernatant plasma, sediment and plasma, and prothrombin in two experiments.

units of thrombin‡ to 0.1 ml of the supernatant plasma and 0.1 ml of 0.9% sodium chloride solution and obtaining satisfactory clots in less than 5 seconds.

Clotting times performed on whole blood throughout all experiments showed no significant changes. Determinations of the heparin cofactor likewise showed no change of importance. The failure to demonstrate any prolongation of the clotting time of the blood is at variance with the observations of Allen and his associates.[1,2] We have no satisfactory explanation of this. The determination of the clotting time of whole blood by any of the various test tube methods is notoriously difficult to perform and interpret. For this reason special precautions were taken. The same physician drew all the blood and performed all the clotting times under the same circumstances. We do not wish to deny that heparinemia may exist after irradiation of this character, but up to the present we have been unable to satisfy ourselves that it exists, whereas we have been able to demonstrate a hypothromboplastinemia and thrombocytopenia which explain the clinical manifestations exhibited by these animals.

Conclusions. 1. A hemorrhagic syndrome in dogs was produced by total body irradiation (450 r). Manifestations consisted of multiple areas of hemorrhage in the pulmonary, gastrointestinal, and urinary systems, anemia, leucopenia, thrombocytopenia, prolongation of clot retraction, and hypothromboplastinemia.

We wish to thank Mrs. Ruth Stavitsky for her technical assistance.

‡ Parke, Davis and Company.

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Fluorescence of Intravenously Administered Rose Bengal Appears Only in Hepatic Polygonal Cells.

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Although certain intravenously administered dyes have been employed in the study of hepatic function over the past 3 decades, the exact mechanisms by which they are removed from the blood are poorly understood. Earlier work indicated that the entire reticuloendothelial system participated in the removal of these substances. This concept is still widely prevalent, although more recently indirect evidence suggests that the normal liver alone is responsible for the uptake of such dyes as the disulfonate of phenoltetrabromphthalein (Bromsulfalein).[1] Now that hepatic blood flow in man is being estimated by a technique based on the extraction of Bromsulfalein by the liver,[2] it is essential to determine whether the removal of this dye, or of others similarly handled, measures the

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1 Ingelfinger, F. J., *Bull. New Eng. Med. Cen.*, 1947, **9**, 25.

activity of hepatic polygonal cells, or of Kupffer cells.

In order to study this problem, advantage has been taken of the fact that tetraiodotetrachlorfluorescein (Rose Bengal), a dye introduced for the study of liver function by Kerr *et al.*[3] exhibits a soft reddish-orange fluorescence in the long ultraviolet spectrum (3150-3650 Å). Tissues stained with 1-2% aqueous solutions of this dye emitted the same characteristic fluorescence, which was readily observed through a yellow eyepiece filter on an ordinary microscope by ultraviolet light (Source—General Electric H-4 medium pressure mercury vapor lamp) passed through glass condensers, Woods filter, 5% copper sulfate solution, glass substage condenser, and glass slides. The fluorescence was not intensified when observed in an all-quartz optical system, and remained unaffected by several hours' exposure to the illumination described above.

Aqueous solutions (1-2%) were prepared from standard bacteriological Rose Bengal powder;† these were autoclaved, and doses varying from 20 to 100 mg were injected into the ear veins of adult rabbits lightly anesthetized with ether. Liver and splenic biopsies in 20 such rabbits were obtained from 1 to 15 minutes after injection. The tissues were immediately placed in saline, then cut on the freezing microtome into sections 15 μ thick. These were floated on water, mounted on ordinary glass slides, and examined within 10 minutes after mounting. The characteristic sustained fluorescence due to Rose Bengal was noted constantly in the hepatic polygonal cells. In biopsies made within the first 3-4 minutes following injection of the dye, the polygonal cell fluorescence was somewhat less intense than in those biopsies made at 10 minutes after injection. Along the endothelial lining of the sinusoids, where Kupffer cells are normally found, no such fluorescence was observed. One constantly saw, however, in these locations as well as in the periphery of some polygonal cells, the greenish-white, rapidly fading fluorescence ascribed by Popper and Greenberg[4] to Vitamin A. Grossly, in ordinary white light, the livers were definitely reddish. In 16 rabbits, the color of the spleen differed in no way from that of uninjected controls, but in 4 rabbits it was slightly pink. Such faint orange fluorescence as was seen in splenic sections did not appear to be located in the parenchyma.

Two anesthetized human subjects undergoing laparotomy were subjected to liver biopsy just prior to and 10 minutes following intravenous injection of 150 mg of Rose Bengal (2% solution). These biopsy tissues were prepared exactly as described above. They showed the same picture as did the rabbit livers—the orange fluorescence due to Rose Bengal in the hepatic polygonal cells, but nowhere else.

These observations provide evidence that the dye Rose Bengal is taken up by, and stored in the hepatic polygonal cells rather than in the Kupffer cells of the liver. Whether the Kupffer cells participate in the removal of the dye from the blood, although unlikely, cannot with certainty be excluded by the technique of fluorescence microscopy. All one can say is that, during a period of 1 to 15 minutes after injection of the dye, the fluorescence due to Rose Bengal is not observed in those areas where Kupffer cells are commonly found. In this respect these findings are similar to those of Grafflin[5] on the handling of fluorescein by the frog liver. Other evidence obtained in this laboratory by hepatic venous catheterization of normal man[6] shows clearly that the hepatic uptake of Rose Bengal is slowed or blocked by simultaneously-administered Bromsulfalein. It is suggested, therefore, that the removal of both these dyes is normally a function of the hepatic parenchymal cells rather than of the Kupffer cells.

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[6] Mendeloff, A. I., and Ingelfinger, F. J., unpublished data.

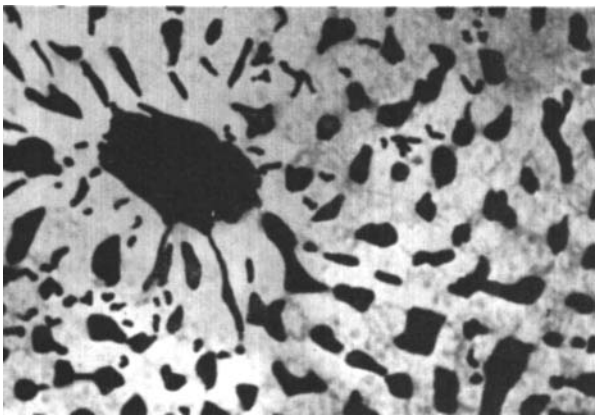

FIG. 1.
Low-power photomicrograph of rabbit liver (300 ×, enlarged 2 ×), fixed in formalin and examined unstained by ultraviolet light. The print has been retouched to emphasize the contrast between non-fluorescent sinusoids and fluorescent hepatic trabeculae, and to remove artifacts which displayed light yellow or green fluorescence.

Summary. Intravenously-administered Rose Bengal can be visualized by fluorescence microscopy in the livers of rabbits and humans. The fluorescence is seen in hepatic polygonal cells, but not in Kupffer cells. It is suggested that the polygonal cells of the normal liver are responsible for the storage of Rose Bengal, and, by analogy, Bromsulfalein.

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Effect of *in vivo* Addition of Selected Nitrofurans on Acid Production in Human Saliva.*

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Investigation of the relation between nutritional status and dental caries has been a major study at the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, since 1941. During the past year an extensive study was made of the effect of the *in vitro* addition of selected nitrofuran compounds on acid production in human saliva, and on the growth and acid production of a pure culture of a strain of the Lactobacillus acidophilus recovered from a carious lesion.[1] The antibacterial action of the nitrofurans has been reported by a number of investigators.[2-4] Our findings showed that 5-nitro-

* Northwestern University Studies in Nutrition at the Hillman Hospital, Birmingham, Ala.

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