analysis (calculation of standard error of mean and Chi square analysis.)

Summary. The results suggest that circulating cholesterol exchanges with available cholesterol pools of intima of human aorta. This exchange is slow. Cholesterol in the atheromatous plaques appears to exchange or accept circulatory cholesterol with the greatest difficulty. The highest exchange or depositions were observed for area of the arch of human aorta; abdominal part of aorta showed a smaller exchange. The media showed about the same cholesterol rate of exchange through length of aorta. A disk technic suitable for various types of quantitative assays of intima, media, etc. of the aorta was described.

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Uptake and Excretion of Radioactive Rose Bengal Dye in Normal Dogs.* (26069)

NORMAN B. ACKERMAN,† EARL G. YONEHIRO AND JAMES F. MARVIN
(Introduced by Owen H. Wangensteen)
Depts. of Surgery and Radiology, University of Minnesota Medical School, Minneapolis

Delprat demonstrated that rose bengal dye (tetra-iodo-tetra-chlorfluorescein) is rapidly concentrated by liver cells and subsequently excreted into the biliary tract(1). Since the initial studies on radioiodine^{131}-tagged rose bengal dye by Taplin and his associates(2), various investigators have utilized this substance for liver scintiscanning as a diagnostic procedure for detection of tumors, cysts and abscesses within the liver(3,4). Although initial studies on nonradioactive rose bengal dye suggested that little or no dye is concentrated by spleen, kidney or pancreas(1,5), the possibility remained that minute amounts might be taken up by these organs. Our studies were stimulated by a recent liver scan performed at this hospital in which a faint area of radioactivity was noted over the left upper quadrant of the patient's abdomen. Employing the radioactive form of the dye on rats, Glaser and co-workers recently concluded that "no large portion of the injected dye could be observed outside of the liver, blood stream, or intestinal tract(6)." To assess the role of various abdominal organs in concentration and excretion of even trace amounts of rose bengal dye, studies in normal dogs were undertaken. Our experimental findings indicate that in normal dogs uptake and excretion of the dye occur mainly through the hepatobiliary system and that pathways through other organ systems are negligible.

Method. Mongrel dogs, weighing from 5 to 10 kg, were anesthetized with 30 mg/kg of sodium pentothal. Using aseptic technic, the abdominal cavity was entered through a midline incision. During period of anesthesia, the dogs were maintained on a constant intravenous infusion of 5% dextrose in water. The animals were given 10% dextrose in water intravenously in experiments in which the urine was collected in order to assure an

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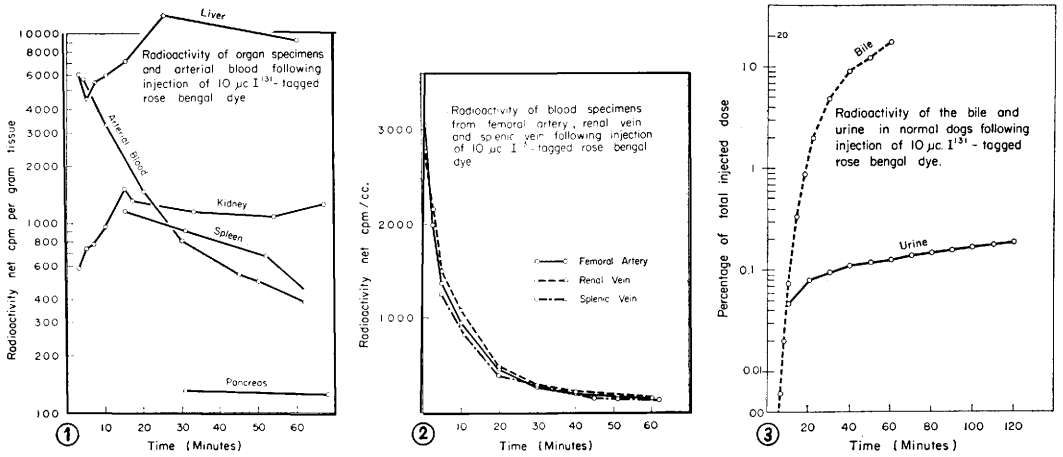

FIG. 1-3.

adequate urinary output during the study period. Ten μc of I^{131}-tagged rose bengal dye were then injected into a peripheral vein. In the initial study serial recordings on a rate meter were obtained for 60 minutes by holding a lead-shielded scintillation counter directly over various organs including liver, spleen, kidney, pancreas, duodenum, jejunum, and colon to determine gross changes in radioactivity during the acute experimental period. Using these recordings as a guide, subsequent experiments were performed, and serial biopsies of various organs obtained. Biopsies ranged in size from 125 to 825 mg in net weight and were obtained from liver, kidney, spleen, and pancreas in 5 animals. Splenic vein blood samples were obtained in 3 experiments *via* a small polyethylene catheter in one of the splenic veins near the hilus. Renal vein sampling was accomplished in 3 animals by threading a polyethylene catheter into the renal vein *via* the inferior vena cava thus minimizing any gross disturbance in renal hemodynamics. Serial arterial blood samples were collected in all animals with a catheter in the femoral artery. In 3 studies total urinary output was obtained by means of a bladder catheter. In addition, bile was collected in 2 animals through a polyethylene tube in the common duct. The duct was ligated distal to the catheter to divert totally the output of bile. Radioactivity of the various specimens, including the biopsy material, blood, urine and bile was determined immediately in a scintillation well counter for a minimum of 1,000 counts. Most specimens were counted for more than 10,000 counts.

Results. After injection of the dye, only small increases in radioactivity were observed over the kidney, spleen and jejunum when a scintillation counter was held over these organs. A slight increase in radioactivity was noted over the duodenum 40 minutes after radioactive rose bengal dye was administered. An equivocal increase in activity occurred at 55 minutes over the pancreas. It was felt that this represented duodenal radioactivity detected over the pancreas because of the proximity of the 2 organs and inadequate shielding of the counter to differentiate the two. A significant increase in radioactivity of liver was evident 4 minutes after injection of the dye and reached a peak in about 30 minutes.

Blood radioactivity after an initial elevation following injection rapidly decreased as the dye became concentrated by the liver (Fig. 1, 2). In 5 to 10 minutes, blood radioactivity fell precipitously to one half the concentration observed in the one minute sample.

Levels of radioactivity in splenic biopsies paralleled radioactivity of the blood stream, demonstrating the inability of the spleen to concentrate rose bengal dye (Fig. 1). Radioactivity of splenic vein blood appeared identical to that of femoral artery blood, also indicating lack of absorption of the dye by the

spleen (Fig. 2). In the relatively avascular pancreas, radioactive levels in biopsied specimens were consistently lower than levels of the blood or spleen (Fig. 1). Interestingly, concentrations of dye in renal tissue appeared slightly higher than corresponding blood levels obtained at the same time (Fig. 1). However, a comparison of renal vein and femoral artery blood values of radioactivity failed to demonstrate appreciable withdrawal of dye from the blood by the kidney (Fig. 2).

Studies on excised hepatic tissue demonstrated concentration of the dye within 3 minutes after injection (Fig. 1). Peak levels occurred approximately 30 minutes after administration of the dye, when radioactivity concentration per gram of liver tissue was 10 to 15 times that of 1 ml of blood. By 60 minutes, the blood was rapidly cleared of dye and the liver/blood ratio was over 30.

Detectable levels of radioactivity were present in the bile as early as 6 minutes after injection of dye (Fig. 3). Concentration of rose bengal dye rose significantly during the subsequent 60 minutes. The reddish tinge of the dye was grossly apparent in the bile by about 10 minutes. At one hour approximately 20% of the dye had been excreted through the biliary tract.

A small, but measurable quantity of radioactivity was present in urine collected during the first 10 minutes after administration of dye (Fig. 3). Peak output of dye in the urine occurred 10 to 20 minutes after injection of rose bengal dye. Only very small quantities of dye could be detected in the urine subsequently. Total urinary output of dye at 60 minutes approximated 0.1% of administered dye. At 120 minutes, less than 0.2% of dye had been excreted in the urine.

Discussion. The present studies clearly indicate that the liver and biliary tract are the major sites of uptake and excretion of I^{131}-tagged rose bengal dye in the normal dog. In an animal with hepatic disease or biliary obstruction other pathways may assume greater significance. In the normal dog renal concentration and excretion appear to be insignificant. The greatest urinary output of the dye occurred during the same period of time that blood levels were highest. Urinary excretion may be due to blood levels elevated above renal thresholds. If this is so, the kidney may play a greater role in handling the dye in animals with hepatic or biliary disease.

In dogs, the spleen and pancreas do not appear to play an important role in handling rose bengal dye. In a patient without biliary obstruction, the presence of radioactivity in the left upper side of the abdomen after administration of the dye would most probably be due to an enlarged left lobe of liver. Since duodenal levels of radioactivity begin to rise within one hour, it is preferable to complete lower and midabdominal scanning before this period of time. For this reason scans in this hospital are started at the lower edge of liver, working upwards. Study of uptake and excretion was limited in these experiments to 1 to 2 hours since the acute period after administration of dye is of prime importance in performance and interpretation of hepatic scintiscanning.

Summary. In normal adult mongrel dogs, radioactive rose bengal dye is rapidly removed from the blood stream with radioactivity reduced 50% in 5 to 10 minutes following intravenous injection of the dye. The liver takes up the dye rapidly, starting within 4 minutes, and excretes it quickly, as evidenced by appearance of dye in the bile 6 minutes after administration. Approximately 20% of the tagged dye passed through the bile in one hour. Urinary excretion is almost negligible, with approximately 0.1% of total dye present in the urine in 60 minutes. Renal, splenic, and pancreatic tissue levels of radioactivity are insignificant and probably represent amount of dye in the blood stream. Radioactivity over the duodenum is evident within the first hour and probably represents amount of dye excreted into the bile.

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Leukocytic Transfer of Immediate-Type Hypersensitiveness in Man. V. Transfer of Experimental Sensitivity to Ascaris Antigen. (26070)

MATTHEW WALZER AND KATHERINE L. BOWMAN
Department of Medicine, Allergy Division, Jewish Hospital of Brooklyn, N. Y.

Leukocytic transfer with peripheral blood leukocytes in an immediate type of hypersensitiveness was first demonstrated in man in the atopic form of sensitivity(1). The transfer reactions to pollens and danders in the recipients were specific but consistently small, never exceeding a slight, or one-plus (+), positive reaction. In more recent studies on experimental human sensitization with *Ascaris lumbricoides* antigen, marked transfer reactions of an immediate type were obtained in 2 noteworthy instances. These occurred in separate studies on donors who had been sensitized by repeated intracutaneous injections of *Ascaris* antigen and who exhibited, at time of transfer, delayed and immediate forms of hypersensitiveness to this antigen. In the first case, to be presented by Walzer, Bowman, and Coleman in another communication, both types of sensitivity were simultaneously and decisively transferred to a normal recipient with peripheral blood leukocytes or with extracts of these cells. In the second case described below, only the immediate type was carried over to a normal recipient by leukocytic transfer. The findings in this study represent the first instance of marked immediate-type transfer reactions elicited in man at sites prepared with peripheral blood leukocytes where only an immediate type of hypersensitiveness was transferred.

Materials and methods. The method of preparation and standardization of extracts of *Ascaris lumbricoides* worms removed from infested pigs has been described(5). Extract 11 prepared in 1954 and extract 12 prepared in 1959 were used in this study.

Donors, selected from members of the hospital medical staff, were young adults with a negative history of parasitic infestation. They were screened by an intracutaneous test with an *Ascaris* extract containing 0.01 mg N/ml and by tests with a few common allergens to rule out the presence of pronounced sensitivities. A positive family or personal history of atopy was not considered a basis for rejection, but individuals with active clinical symptoms of allergy were excluded.

The recipients in these experiments were young female student nurses and laboratory technicians with negative histories of parasitic infestation. They likewise failed to react to the screening test with *Ascaris* extract. Some were atopic, but none had active symptoms at time of this study.

To study the development of hypersensitiveness in the donor, intracutaneous tests were performed weekly with *Ascaris* extracts 11 and 12, using approximately 0.01 ml of the 0.01 mg N/ml dilutions for each test. Results were read in 15 minutes and graded according to accepted standards(6). At completion of these tests performed on the donor's right arm, a sensitizing injection of a mixture containing equal concentrations of extracts 11 and 12 was administered intracutaneously on his left arm. The delayed reaction produced by this injection was read in 24 hours. The horizontal and vertical diameters of the reaction were measured, and amount of induration was estimated by palpation and recorded in terms of 0 to ++++ (Table I).

In the successful experiment, the immediate-type sensitivity of Donor WT was transferred to Recipient BE. Donor WT is a physician, 26 years of age, who gave a posi-