though undoubtedly fragments of pancreatic tissue may have caused part of the high levels. Diffuse and disseminated nature of fish pancreas makes it impossible to separate completely the pancreas from whole liver.

Amylase levels of intestinal tract (including pyloric caeca) were well above those of other organs and indicated that the pancreas provides considerable amylase even though the diet of these fish contains very little starch. Amylase *is* found in the gastro-intestinal tract and other visceral organs of the black bass despite previous statements(3) to the contrary.

In studies on isolated liver cells, livers were taken from 35 bluegill. In 7 of these fish, livers were quite pale, almost a light tan color, and extractions with ether indicated that they contained much larger amounts of lipid than normal livers. Since these might represent some pathological condition, these livers were not used in preparation of isolated cells but were pooled and analyzed separately. Their amylase content was 3610 units/100 g tissue compared to 7980 for normal livers. The isolated liver cell amylase (Table II) was much lower than whole liver level but still appreciable, and well above serum amylase, indicating that the liver had some amylase that could not be attributed either to pancreas or blood remaining in liver.

TABLE II. Bluegill Liver Amylase.

Condition of liver	Amylase units/100 g tissue
Whole livers (28)	7980
Isolated liver cells from 28 pooled livers	1390

Amylase in the hepato-pancreas of a carp was 550,000 units/100 g and bile amylase was 23,000.

Summary. Amylase levels of the gastro-intestinal tract and other visceral organs of bluegill and largemouth black bass were relatively high, ranging from 1000-8000 units/100 g, considerably above serum levels. Amylase levels of gills, heart, kidney and muscle were relatively low. Amylase was found in isolated liver cells from the bluegill.

1. Bodansky, M., Rose, W. C., *Am. J. Physiol.*, 1922, v62, 482.
2. Chesley, L. C., *Biol. Bull.*, 1934, v66, 133.
3. Sarbahi, D. S., *ibid.*, 1951, v100, 244.
4. McGeachin, R. L., Gleason, J. R., Adams, M. R., *Arch. Biochem. Biophys.*, 1958, v75, 403.
5. McGeachin, R. L., Ford, N. K., *Am. J. Physiol.*, 1959, v196, 972.
6. Van Loon, E. J., Likins, M. R., Seger, A. J., *Am. J. Clin. Path.*, 1952, v22, 1134.
7. Dallam, R. D., *Arch. Biochem. Biophys.*, 1955, v54, 24.
8. Lata, G. F., Reinertson, J., *Nature*, 1957, v179, 47.
9. Andreev, A. K., *Biokhimiya*, 1958, v23, 899.

Received January 11, 1960. P.S.E.B.M., 1960, v103.

Urinary Excretion of Patent Blue V after Intradermal Injection in Man.* (25681)

SAM A. THREEFOOT
Dept. of Medicine, Tulane University School of Medicine, and Research Lab., Touro Infirmary, New Orleans, La.

Patent Blue V† has been used to visualize the lymphatics of man in attempts to understand the role of these vessels in formation of edema of congestive heart failure and other disease states. During these studies, it appeared in the urine(1,2,3) suggesting use of urinary excretion as indicator of lymphatic uptake from interstitial spaces and transport to the venous circulation. Subsequent observations revealed, however, that PBV was absorbed by venous capillaries as well as lym-

* Supported by grants from Nat. Inst. Health, U. S. Public Health Service, John A. Hartford Fn. and J. Aron Research Fund.

† Nat. Aniline Division, Allied Chemical and Dye Corp.

TABLE I. Clinical Data of Subjects Studied.

Subject No.	Age	Sex	Diagnosis and status	Edema	Extremity studied
			Control		
1B	24	♀	Control	Trace-leg	Rt arm
3A (6/23/54)	28	♂	"	0	Lt arm
3B (6/ 6/57)	30	♂	"	0	Rt arm
8	26	♂	"	0	"
10A (12/ 7/54)	34	♂	"	0	Lt arm
10C (5/14/57)	36	♂	"	0	"
12	26	♀	Control—inguinal hernia	0	Rt arm
13	23	♂	Control	0	Lt leg
14	22	♀	"	0	Lt arm
15	32	♀	"	Trace	Rt leg
18	28	♀	"	0-Trace	"
21	22	♂	"	0	"
29	33	♂	"	0	Rt arm
30	22	♀	"	0	"
32	19	♀	"	0	"
34	31	♂	"	0	"
43	26	♀	"	0	"
48	54	♂	Intracranial tumor	0	"
71	22	♂	Control	0	Lt arm
			Congestive heart failure		
9A (11/30/54)	70	♀	Early CHF; untreated; gaining edema	Trace	Rt leg
9B (5/ 8/56)	72	♀	CHF; treated; gradually losing edema	1+	"
16	64	♀	CHF; rapidly losing edema	1+	Lt leg
17	45	♂	Early CHF; untreated	1+	Rt leg
23A (10/ 4/55)	57	♀	Severe CHF; no response to treatment	4+ legs; ascites	"
23B (12/13/55)	57	♀	CHF; slight response to treatment	0 arms	Rt arm
24	64	♀	" no treatment	1-2+	Rt leg
36A (5/29/56)	52	♂	" rapidly losing edema	4+ leg	"
36B (6/ 5/56)	52	♂	" marked improvement	0 arm	Rt arm
45	60	♂	" rapidly losing edema	3+	Rt leg
46	63	♂	" almost compensated	0	"
47	56	♂	Chronic renal disease developing CHF; gaining edema	Trace	Rt arm
49	76	♂	Myocardial infarction; CHF dev'l'ping	Trace	"
			Lymphedema		
7	67	♀	Bilateral inguinal hernia & venous dis.	Trace	Rt leg
19	70	♀	Obstructive lymphedema following radical mastectomy	4+	Lt arm
20	74	♀	Chronic lymphedema of legs; hypochromic microcytic anemia	3+ bilateral non-pitting	Lt leg
31	37	♂	Possible diverticulitis; G.I. neoplasm; edema of undetermined etiology	Trace	Rt leg
39A (6/18/56)	49	♀	Venous disease; immediately after removal of elastic stocking	0	Lt leg
39B (6/25/56)	49	♀	Venous disease; with edema of leg 1 wk after removal of elastic stocking	2+	"
42A (8/13/56)	37	♀	Lymphedema of legs; obesity; varicosities	Non-pitting; 2+ pitting superimposed	"
42B (3/11/58)	38	♀	Lymphedema of legs	Non-pitting; 1+ pitting superimposed	"
54	44	♂	Edema of undetermined etiology of ankles and hands	Slight pitting legs and hands	Rt leg
63A (12/10/57)	22	♀	Unilateral edema of undetermined etiology of right leg	2+ right leg pitting and hard	"
63B (1/ 7/58)	22	♀	Edema of right leg	0 left leg	Lt leg
66	13	♀	Milroy's disease; unilateral left leg	1-2+ unilateral	"

TABLE I (continued)

Subject No.	Age	Sex	Diagnosis and status	Edema	Extremity studied
67	54	♀	Edema of undetermined etiology	2+ pitting bilateral	Rt leg
70	39	♀	Hodgkin's disease; compression of inferior vena cava	1+ pitting	"
74	24	♀	Edema of undetermined etiology; obesity	0	"
77	50	♀	Venous disease; obesity	1+ pitting	"

phatics and thus excretory studies were not indicative of lymphatic function alone but of capillary uptake and transport as well(4).

Materials and methods. Since basic color of each urine specimen differed, it was necessary to develop a spectrophotometric blank by removing the dye from an aliquot of each sample of urine. Addition of 3 ml 5% sodium hypochlorite, 1 ml 20% trichloroacetic acid and 1 ml pH 12.5 buffer to 5 ml urine provided such a "blank," pH 10-11, the spectral characteristics of which were essentially the same as those of urine of the unknown to which buffer only was added. Addition of buffer was necessary for studies which included determination of simultaneous excretion of Patent Blue V and phenolsulfonphthalein. Maximum color of PSP develops at pH 10 and PBV fades rapidly above pH 11. At concentrations greater than 0.5 μg/ml, for determination of PBV or for simultaneous determination of concentration of PSP, excreted rapidly at high concentrations, the error was less than 5%. After intradermal injection of 0.1 ml 11% PBV (11 mg) in arm or leg, all urine was collected as voided in 58 experiments (22 control subjects, 13 with congestive heart failure, 16 with idiopathic or obstructive lymphedema, and 7 with miscellaneous disorders). Collection was continued until dye concentration was too low for measurement. The 48 used in this study are presented in Table I.

Results. Time of excretion of PBV in urine for 24 hours after intradermal injection is presented in Fig. 1, A, B, C, D. Range of variation in rate of excretion by control subjects was small. Dye appeared in urine in measurable concentration for approximately 24 hours, when approximately 10% of injected dose had been recovered. Patterns of excretion for subjetcs with congestive heart failure varied with stage of formation or loss of edema from the extremity studied (Fig. 1A and B). In subjects with early congestive heart failure developing edema, uptake and excretion of dye are slow and delayed, but in subjects receiving diuretics and losing edema fluid, excretory rates exceeded those of control group, especially after a few hours. In these latter subjects, measurable concentration appeared in urine for several days with as much as 30% of injected dose recovered. The curve of subject 9B (Fig. 1B) responding to treatment and losing edema, fell below the range of control subjects but after some delay exhibited increased output of dye and exceeded that of controls. In general, subjects with lymphedema manifested slower excretion, presumably because of delayed uptake or transport from site of injection. The curve of subject 42 (Fig. 1C) was within control limits for most of its length on 2 separate occasions. Although this subject exhibited some pitting edema (Table I), it was speculated that her disproportionately large legs were due to distribution of fat with no edema producing disease. Subject 74 (Fig. 1D) had large legs with no clinically detectable edema. While the excretory pattern of subject 63B (Fig. 1D) is lower than that of control subjects, it is significantly greater than that obtained when the subject was studied in the edematous extremity (63A, Fig. 1C). This suggests that although there was no detectable edema at the time of study, the patient was beginning to retain fluid and develop edema in that extremity. If the cumulative excretion is plotted for arbitrary time of 150 minutes after injection (indicated by arrow on base line of Fig. 1), differences in these groups are more distinct (Fig. 2A). All subjects studied in an edematous extremity, except those rapidly

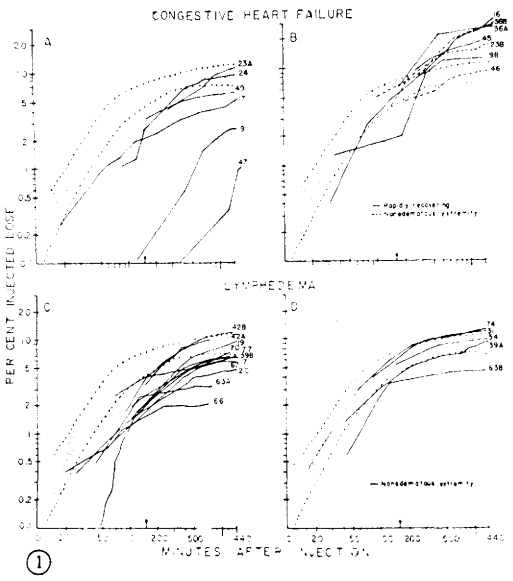
losing fluid under treatment, had less urinary output of PBV at 150 minutes than did controls.

Since collection of urine was by *ad lib.* voiding only, no accurate estimate could be made of time of appearance of dye in the urine. There was dye in urine of control subjects who voided as early as 10 minutes, but in some subjects with congestive heart failure or lymphedema, urine was collected for 2 hours before dye was evident.

In control subjects, there was no consistent difference between rates of excretion by those subjects injected in arm or leg. In subjects with edema from one cause or another, however, except for subjects diuresing, when a subject was restudied by injection in a nonedematous extremity, there was greater and more rapid excretion.

In consideration of decreased renal function as the cause for decrease in PBV excretion, 1 ml 0.6% PSP (6 mg) was administered simultaneously intravenously and excretion measured as a guide to renal function in 28 subjects. In subjects demonstrating decrease in dye excretion, decrease in PBV excretion was relatively greater than decrease in PSP excretion (Fig. 2B). This decreased PBV: PSP ratio indicates that diminished renal function alone does not explain the delay and decrease in PBV excretion. When equal quantities of PSP and PBV were injected in the same syringe intravenously in a dog, during first 2 hours ratio of PBV to PSP in urine was 0.7 and constant. This indicated that PBV and PSP are probably excreted in similar fashion.

Discussion. One of the original objectives of this study was to evaluate urinary excretion of PBV as indicator of uptake and transport of interstitial fluid by lymphatics. Kin-



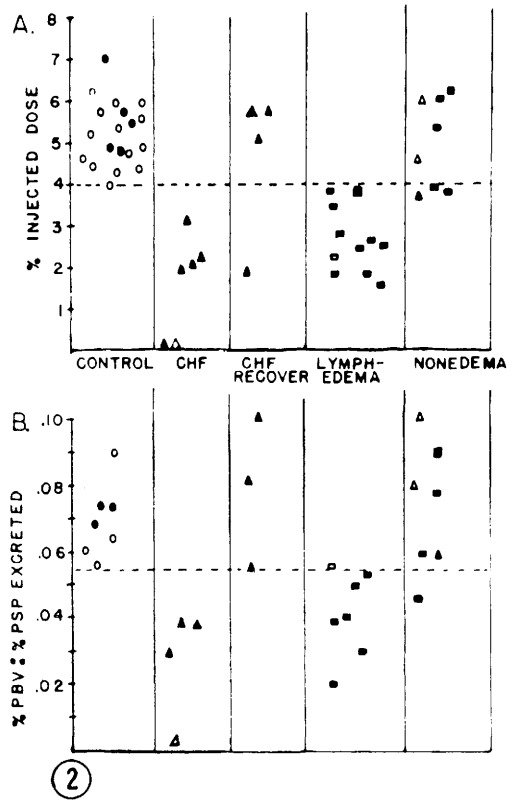


FIG. 1 A-D. Logarithmic plots of time-course of urinary excretion of PBV for first 24 hr after intradermal inj. Large dotted lines demarcate extremes of variation for 19 control subjects. Beginning of each line is at time of collection of first sample. Subjects in 1A and 1C were studied in edematous extremities. Status of subjects presented in Table I. Subject No. at right of each curve. Arrows on base line at 150 min. signify values plotted in Fig. 2A.

FIG. 2. A. % of inj. dose of PBV excreted by 150 min. after inj. B. Ratio of PBV excretion to PSP excretion at end of 150 min. In 2A and 2B: Circles, controls; triangles, congestive heart failure; squares, lymphedema. Open symbols = inj. in arm; closed symbols = inj. in leg. Dotted line is lower limit for control subjects. Group indicated as NONEDEMA, contains patients with congestive heart failure or lymphedema, as designated by symbols, who were repeated in a nonedematous extremity.

month indicated that this might not be feasible since PBV appeared to be absorbed and transported by other vessels as well(2). In studies of local spread of dye(4) and cannulation of the lymphatics(3), the dyes appeared in blood from venous capillaries and urinary excretion of PBV was not indicative of lymphatic transport alone but of total vascular uptake from an area. There was an inverse relationship between rate and area of spread of the dye at site of injection and rate of urinary excretion. The rates and areas of local spread were greatest in those subjects with edema while urinary excretion was slowest except when they were rapidly losing fluid under treatment. This relationship suggests that decrease in urinary output of dye by these subjects was due, in large measure, to disturbed uptake by either lymphatics or capillaries. A lower PBV:PSP ratio further demonstrated that decreased renal function was not a prime factor in differences observed between subjects with edema and controls, and that diminished uptake by local vessels and/or slower transport to the general circulation were the most reasonable explanations. No correlation between sluggish uptake of PBV and other substances in edema fluid has been attempted, but the implications are apparent.

Summary. Patent Blue V, used to visualize lymphatics is excreted in urine in measurable amounts after intradermal injection of 0.1 ml 11% solution. Differences in rates of excretion between edematous and nonedematous subjects suggested that this may be useful in studying rates of uptake of interstitial substances. Control subjects excreted approximately 10% of injected dose in 24 hours, ½ of which was excreted in the first 2½ hours. Except for patients who were rapidly losing edema under treatment, rates of excretion were less for subjects with edema of various causes. Correlation of urinary excretion of PBV with that of PSP administered simultaneously but intravenously, and correlation of urinary excretion with rates and areas of local spread of intradermally injected dye, indicate that the principal factor in differences in urinary excretion is decreased uptake and transport by vessels at site of injection.

1. Hudack, S. S., McMaster, P. D., *J. Exp. Med.*, 1933, v57, 751.
2. Kinmonth, J. B., *Clin. Sci.*, 1952, v2, 13.
3. Threefoot, S. A., *J. Lab. and Clin. Med.*, 1957, v50, 720.
4. ———, *ibid.*, 1960, v55, 250.

Received January 18, 1960. P.S.E.B.M., 1960, v103.

Effect of Carbon Tetrachloride Upon Glucuronide Formation by Guinea Pig Liver.* (25682)

KURT J. ISSELBACHER AND ELIZABETH A. MCCARTHY
Dept. of Medicine, Harvard Medical School and Mass. General Hospital, Boston

Glucuronide conjugation is a detoxification mechanism important in metabolism of many endogenous and exogenous compounds possessing alcoholic, phenolic or carboxyl radicals (1). This conjugation occurs primarily in liver and involves a microsomal enzyme (glucuronyl transferase) and uridine diphosphate glucuronic acid (UDPGA)(2,3). Although it seems likely that derangements in glucuronide formation might occur in hepatocellular disease, clinical and laboratory investigations have been contradictory. Both Englert *et al.*(4), and Peterson and associates(5) found no delay in clearance of tetrahydrocortisone (THE) from plasma of patients with liver disease and concluded THE-glucuronide formation was not impaired. In contrast, Cohn and Bondy(6) measured plasma levels of THE-glucuronide in patients with hepatic disease and found only trace quantities of these conjugates, while free THE was present in plasma in significant amounts. Barniville and Misk(7) found a lower urinary glucuronic acid excretion after oral salicylamide adminis-

* Supported in part by grant from Nat. Inst. Health.