THE RENAL ELIMINATION OF BILIRUBIN.

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The mode of escape through the kidneys of circulating blood and bile pigment has received but scanty attention in the past as compared with that of foreign dyestuffs. These last have been the subject of a multitude of experiments and of controversies that are not yet at an end. The reason for this is plain. The dyestuffs have been utilized for the better discovery of principles which would apply supposedly to the body pigments as well. But since these principles are still ill defined and their general application is unattested, direct studies on the renal elimination of hemoglobin and bilirubin would seem desirable—more especially since patients with these pigments in their urine continue to demand attention.

Authorities agree that the kidney is often severely injured by jaundice. Albuminuria and cylindruria (Nothnagel) appear early. Quincke[1] has comprehensively described the anatomical changes. At an early stage the cortex is diffusely stained with bilirubin. As time passes the pigment accumulates in granular form in the cells of the convoluted tubules and more markedly in those of the loop of Henle, and in the lumen of the latter many free granules may be seen, together with yellow, green, or brown casts. It is noteworthy that the glomeruli remain practically unstained. There is cloudy swelling of the tubular epithelium, with loss of the brush border, and even necrosis here and there. In Quincke's opinion, these severe changes cannot but result in a lessened renal activity, and thus may have serious consequences for the organism as a whole.

Significance of Jaundiced Cells in the Urine.

According to text-books on clinical microscopy a "bile-stained urinary sediment" is a regular accompaniment of marked jaundice. It has been tacitly assumed that the bile staining is a staining of dead

[1] Quincke, H., in Nothnagel, H., Specielle Pathologie und Therapie, Vienna, 1899, xviii, 63.

cells by the fluid in which they lie immersed, and is devoid, therefore, of clinical significance. Such is often the case. But there is another possibility, that some of the cells may be kidney cells impregnated with pigment prior to desquamation into the urine. Such cells would afford a direct index to the renal condition, like the cells containing granular hemosiderin that are to be found in the urine of patients with hemochromatosis and pernicious anemia.[2]

In the urine of icterus neonatorum, cells containing granular and crystalline bilirubin are almost regularly present, often indeed when no dissolved pigment can be demonstrated. An excellent paper on the theme is that of Cruse[3] whose technique for the microscopic Gmelin reaction we have found highly useful. No systematic observations have been reported on the urinary cells of jaundiced adults, or at least none carried out from the standpoint of kidney physiology.

We have studied the urinary sediment of many icteric human beings and of dogs with jaundice produced in several ways—by fasting, by blood destruction with toluylenediamine or a specific hemolytic serum, and by ligation of the common duct. Two types of cell staining with bilirubin have been discriminated, one consequent on sojourn of the elements in the urine, the other a direct expression of the renal condition. In the one the cells from all parts of the urinary tract are stained, whereas in the other, no matter how deep the jaundice, the leucocytes, squamous epithelium, and bladder epithelium are uncolored, whereas the cells of manifest renal origin may be deeply stained. Needless to say, both types of staining, or only that first mentioned, will be seen in urines that have stood for a long time. The specific pigmentation of the renal cells is most evident in specimens freshly voided; and some hours are required for the bilirubin to dissolve out of the more heavily impregnated cells. After long continued jaundice in man, the urinary sediment yields striking indications of the serious condition of the kidneys. Numerous cells, often disintegrating, will be observed, crowded with coarse, opaque, brown granules or irregular particles, and many such particles lie free. All give under the microscope an intense Gmelin reaction. The pigment cannot be confused with the yellowish lipoidal substances

[2] Rous, P., *J. Exp. Med.*, 1918, xxviii, 645.
[3] Cruse, P., *Arch. Kinderheilk.*, 1880, i, 353.

found in some pathological states.[4] Intracellular clumps of narrow brown needles are frequently present. The cytoplasm of the renal cells is usually stained deep yellow, though not always. The desquamated elements from the lower urinary tract are by contrast practically colorless, though lying in a dark, icteric fluid.

Special interest attaches to the findings in subicteric states and during slight and transient jaundice. Bile pigment is a threshold substance in human beings, one which readily passes into the tubules but is absorbed again in its course through them, and so rapidly that often none can be found in the urine when a considerable quantity exists in the blood. Will the cast off renal cells yield evidence of this absorption process when the plasma contains bile pigment but tests fail to disclose it in the urine? Such has not proved to be the case. In urines that fail to give the Gmelin reaction, cells stained with bilirubin are absent. Evidently there must be in human beings a special glomerular threshold for bilirubin as well as a more general, higher one for the kidney as a whole. For were this not so, the renal epithelium found in the urine should be tinted with bilirubin whenever the plasma is colored with it—and the plasma is so colored normally.

Human urine during slight or transient jaundice regularly contains renal cells tinted a diffuse yellow and yielding the Gmelin reaction, in contrast to the colorless and negatively reacting elements of the lower tract. Granular bilirubin is not seen. We are inclined to believe that Rosenbach's method of test,[5] whereby much urine is passed through a filter and this latter submitted to the Gmelin reaction owes its delicacy, in part at least, to the accumulation upon the paper of specifically stained cells.

In dogs the slightest and most transient jaundice may lead to an output of renal cells brilliantly stippled with bilirubin. The pigment occurs as small or coarse, rounded or oblong, granules of a bright mahogany-brown, scattered irregularly in the ground glass cytoplasm of large cells with a rather small, rounded nucleus. The cytoplasm itself is usually unstained save sometimes for a very distinct brownish red zone, or halo, around each granule. In kept specimens such

[4] Weicksel, J., *Deutsch. Arch. klin. Med.*, 1919, cxxx, 260.

[5] Rosenbach, O., *Centr. med. Wissensch.*, 1876, xiv, 5.

halos of dissolved bilirubin regularly develop. These and the ruddy tint of the pigment go far to differentiate the latter from hemosiderin in the absence of chemical tests. When the urine stands for 24 hours at room temperature, the brown granulation usually disappears but in the ice box it persists much longer. Severe jaundice is accompanied in both dogs and rabbits by heavily granulated cells and free particles of bilirubin in the urine, just as in the case of human beings.

It is a curious fact that many dogs with slight jaundice yield only diffusely tinted renal cells, whereas in others with no greater icterus, elements stippled with bilirubin are regularly encountered. This difference is consistently maintained over considerable periods of time. We have noted it day after day in catheterized specimens from animals possessing normal kidneys, and with "physiological icterus" induced by fasting. It is independent of the reaction of the urine or of diuresis. Quite possibly the difference is one in derivation of the cells, those from the stippled portions of the tubules failing to desquamate in some animals.

Effects of Flood Diuresis in Dogs.

The sources of damage to the kidneys during jaundice have never been precisely determined. The excretion of bile salts may be, and probably is, far more injurious than that of bile pigment, yet there is no doubt that the accumulation of the latter in the renal cortex, as in the system generally, should be avoided if possible. Diuresis has long been advocated for the purpose. We have tested out its efficacy upon dogs.

Animals were selected that during a period of several days showed no albuminuria or casts. They were kept in metabolism cages. Under ether, all of the large bile ducts were separately ligated, or the common duct was tied and cut and the neck of the gall bladder similarly obstructed to rule out any influence of this reservoir upon the course of the jaundice. Asepsis was maintained throughout, and the incision was closed in three layers. The accumulation of bilirubin in the blood, together with its output in the 24 hour urine, was carefully followed by means of the quantitative method of van der Bergh and Snapper[6] for the blood, and Hooper and Whipple's[7] modification of the Salkowski method for the urine.

[6] van der Bergh, A. A. H., and Snapper, J., *Deutsch. Arch. klin. Med.*, 1913, cx, 540.

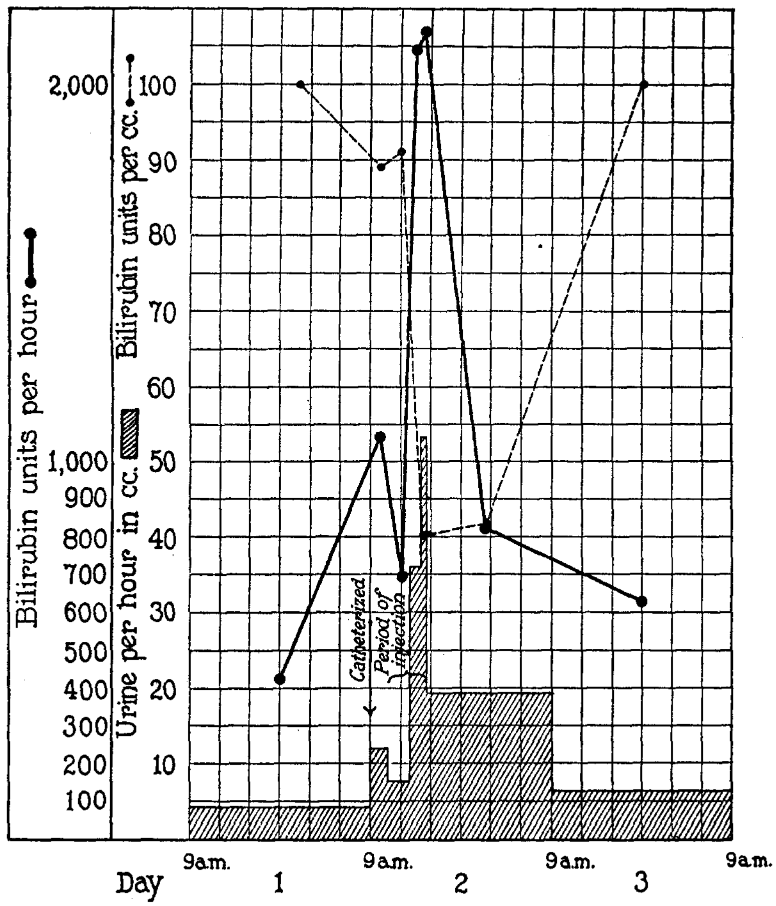
[7] Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1916, xl, 332.

TABLE I.

Effect of Flood Diuresis on Bilirubinuria.

Time of occurrence after operation.	Time.	Procedure.	Fluid injected.			Urine.			Bilirubin units.			Blood.	
			Total.	Rate per kilo per min.	Period.	Total.	Output per hr.	Color.	Output per cc.	Total.	Output per hr.	Hemoglobin.	Bilirubin content per 100 cc. of plasma.
days	*a.m.*		*cc.*	*cc.*	*hrs.*	*cc.*	*cc.*					*per cent*	*mg.*
18					24	100	4.17	Dark brown.	100	10,000	417		
19	9.00	Catheterized.											
	10.00											89	1.47
	11.15	Catheterized. Injection begun.		0 37	2¼	27	12.0	" "	89	2,400	1,067		
	11.45	Speed of injection altered.	100	0.17									
	p.m.												
	2.15	Catheterized. Speed of injection altered.	325	0.185	3	23	7.7	Orange.	91	2,090	698		
	3.00		400										
	3.30	Catheterized.	500	0.37	1¼	45	36.0	Yellow.	58	2,610	2,090		
	4.00	Injection stopped.	550	0.185									
	4.15	Catheterized.			¾	40	53.3	"	40	1,600	2,133	87	1.33
	a.m.												
20	9.00				∞16¾	325	19.4	"	42	13,630	814	87	1.72
21	9.00				24	150	6.25	Light brown.	100	15,000	625		

After 10 or more days of obstruction had elapsed and the icterus had reached a relatively constant level, the study of the effects of intravenous injections of fluid was begun. The animal was stretched out; the bladder was emptied with a catheter; and warmed 0.9 per cent sodium chloride solution was introduced into



TEXT-FIG. 1. Effect of flood diuresis on bilirubinuria.

the saphenous vein at a measured rate of from 0.17 to 0.7 cc. per kilo per minute. Dastre and Loye[8] have stated that even with the maximum rate here given no fluid accumulates in the tissues or blood; and we have assured ourselves that this holds true for the blood by taking its hemoglobin content from time to time during

[8] Dastre and Loye, P., *Arch. physiol. norm. et path.*, 1888, ii, series 4, 93.

the experiment. The injection was continued for 3 hours or more, during which time the urine was collected by catheter and its pigment content determined. The experiment was successfully carried out four times in all, on three dogs.

The findings were consistent. The diuresis consequent on the injection of salt solution regularly increased the output of bile pigment greatly. The amount per cubic centimeter of urine, on the other hand, was much lessened, unlike that of hemoglobin, which under similar circumstances[9] is notably augmented. The intensity of the bilirubinemia remained practically unaffected. A single protocol will suffice to show the findings.

Experiment 1.—A brown female collie weighing 9 kilos was operated upon 19 days before the diuresis experiment and all duct branches were tied, and, where possible, cut. The amount of bilirubin contained in the urine was not measured in milligrams but in terms of an arbitrary standard of units, so called. The findings for this reason are without quantitative significance in relation to the bilirubin content of the blood. Table I gives the course and results of the experiment and Text-fig. 1 illustrates it graphically.

Effects in Dogs of Diuresis from Water by Mouth.

The effects of large amounts of water administered by mouth to dogs with the jaundice of total obstruction were followed in several series of animals.

Dogs tolerate total biliary obstruction for many weeks but lose appetite, drink little, and gradually emaciate and become anemic. The kidneys undergo a progressive injury. For these reasons it was deemed best to begin the observations within a few days after the ducts had been obstructed at operation, and by alternating periods of forced fluid by mouth with those in which there was only the normal intake to render each animal its own control. Dogs with normal blood and urine were selected for the work. Since the character and amount of the food are supposed to influence the quantity of bile pigment formed[10] a constant ration of bread and meat was supplied each day and the amount taken was determined. Fortunately the animals ate about as much when water was forced as during the control periods.

Much difficulty was experienced in determining the precise amount of pigment eliminated in the urine from day to day, more especially during the periods of diuresis. The following modification of Hooper and Whipple's method was

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[9] Haessler, H., *J. Exp. Med.*, 1921, xxxv, 515.

[10] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916, xl, 349.

finally employed. 2 per cent of the 24 hour specimen was made up to 20 cc. with water, alkalinized with sodium carbonate, the bilirubin precipitated out with calcium chloride as usual, and the bluish green coloration obtained with acid alcohol was read in a colorimeter against the color got through the action of the same kind of alcohol on a chloroform solution of bilirubin (Schuchardt) containing 1 mg. in every 4 cc.[11]

The early results indicated that not only was the bilirubin output not increased during several successive days of diuresis, but that toward the end of the period it fell almost to zero. Since such a finding is contrary to all that is known of the effects of diuresis on renal elimination, control tests were undertaken.

Various mixtures of icteric and non-icteric urines, some of them concentrated, some dilute as the result of diuresis, showed that the difficulty did not lie with the method itself, which quantitated bilirubin equally well, no matter how dilute the urine.

Further controls, involving variations in the amount of sodium carbonate used in the alkalization of the urine, and in the concentration of acid in the acid alcohol used to redissolve the precipitate, proved that these factors had no essential influence to cause error. Incidentally, it may be remarked that precipitated calcium bilirubinate was found to keep well in the ice box, and the precipitate from our specimens was sometimes kept for 24 to 48 hours prior to quantitation.

At length, tests were made to determine the value of cage as compared with catheterized specimens. The observations indicating a decreased bilirubin output during diuresis had been made upon urine collected in a vessel placed beneath the metabolism cage in which the animal was kept. Such urine was only protected from fecal contamination by a coarse grating in the bottom of the cage. The animal during non-diuresis periods drank very little water and almost invariably passed a dry formed stool, so that during these periods the amount of fecal contamination of the urine was slight. During periods of forced diuresis, on the other hand, a loose watery stool was frequently passed, and the urine specimens then contained large amounts of fecal material. Our practice had been to filter the mixed total urine for the 24 hour period and make tests on the filtered specimen. Parallel tests now made on filtered and unfiltered specimens disclosed considerable differences in bilirubin content (Table II). What is more, it was found that the sediment of a centrifuged cage specimen, though twice washed with distilled water, still may contain as much as four times the amount of bilirubin present in the urine from which it had been separated.

Since the animals serving as our example (Table II) had complete biliary obstruction, as subsequently determined at autopsy, while, furthermore, repeated

[11] McMaster, P. D., and Rous, P., *J. Exp. Med.*, 1921, xxxiii, 731.

tests of their stools when unexposed to urine invariably gave negative reactions for bilirubin, the fecal matter can be excluded as the original source of the pigment.

We believe that these findings point to a precipitation of bilirubin out of the urine by some of the fecal elements. They have been set forth at length because it is the accepted practice to use cage urine for bilirubin estimations.

TABLE II.

Effect of Fecal Contamination on the Quantitation of Bilirubin in the Urine.

Dog.	Urine specimen.	Total 24 hr. output of bilirubin as calculated from the filtered and unfiltered specimens.		Remarks.
		Filtered.	Unfiltered.	
		mg.	*mg.*	
1 Male mongrel; weight 5 kg.	1	19	18	Practically no feces present.
	2	13	13	" " " "
	3	8	23	Marked fecal contamination.
	4	6	14	" " "
	5	11	19	" " "
	6	11	16	" " "
	7	6	13	" " "
2 Male mongrel; weight 6 kg.	1	9	13	Marked fecal contamination.
	2	4	5	" " "
3 Male mongrel; weight 8 kg.	1	5	6	Slight fecal contamination.
	2	9	8	" " "

It has been usually assumed that in the intestinal canal bilirubin is transformed to urobilin and related substances by the action of bacteria. We have observed that when bile-containing urine is mixed with bile-free fecal matter and incubated at 37°C. for 24 hours, a large proportion of its bilirubin is destroyed, while the remainder, no longer in solution, is to be found with the fecal sediment.

Following the recognition of these sources of error, some further carefully controlled experiments were made upon the effects of diuresis in jaundiced dogs.

TABLE III.

Effect on Bilirubin Excretion of Diuresis from Water by Mouth.

Period.	Day.	Duration of excretion.	Urine.					Blood.		Water by gavage.	Remarks.
			Amount.	Output per hr.	Type of specimen.	Bilirubin content.	Bilirubin output per hr.	Hemoglobin.	Bilirubin content per 100 cc. of plasma.		
		hrs.	*cc.*	*cc.*		*mg.*	*mg.*	*per cent*	*mg.*	*cc.*	
First non-diuresis period.	1	6	72	12.0	Catheterized.	5.61	0.93	69	4.04		
		18	115	6.4	" and voided.	16.56	0.92				
		Total or average.	187	7.8		22.17	0.92				
	2	6	35	5.8	Catheterized.	7.10	1.18	69	4.06		Weight 5.2 kg.
		18	91	5.0	" and voided.	21.20	1.18				
		Total or average.	126	5.2		28.30	1.18				
	3	6	42	7.0	Catheterized.	5.41	0.90	72	4.92		
		18	109	6.1	" and voided.	17.36	0.96				
		Total or average.	151	6.3		22.77	0.95				
	4	6	24	4.0	Catheterized.	4.83	0.80	72	4.78		
		18	70	3.9	" and voided.	15.80	0.88				
		Total or average.	94	3.9		20.63	0.86				

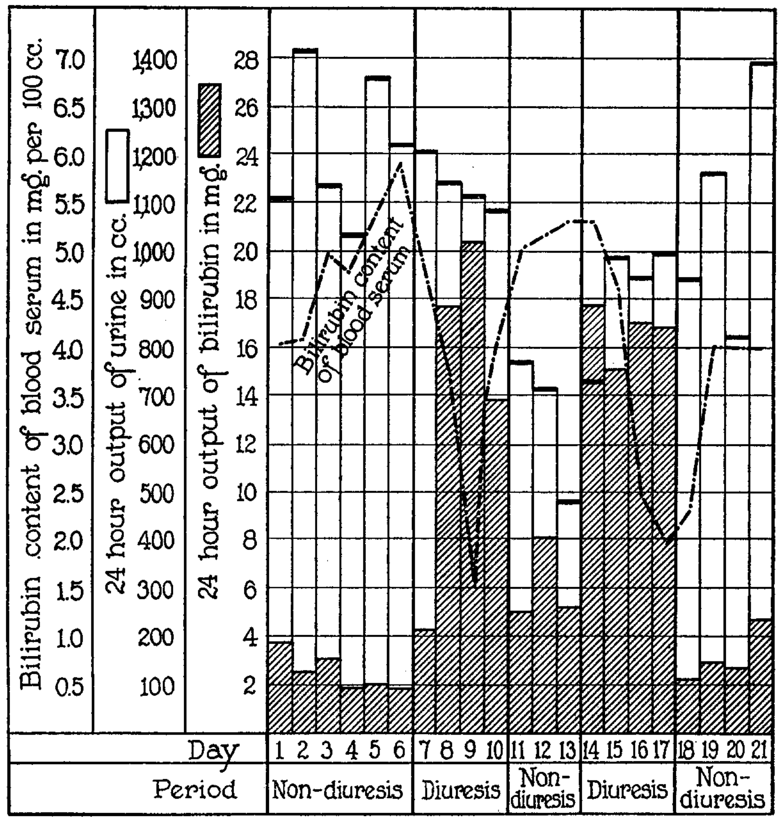
	5	24	100	4.2	Voided.	27.20	1.13				
	6	6	25	4.2	Catheterized.	5.00	0.83	72	5.90		
		18	67	3.7	" and voided.	19.40	1.08				
		Total or average.	92	3.8		24.40	1.02				
First diuresis period.	7	6	33	5.5	Catheterized.	6.43	1.07	66		500	
		18	178	9.9	" and voided.	17.71	0.99				
		Total or average.	211	8.8		24.14	1.00				
	8	6	750	125.0	Catheterized.	7.73	1.29	59	3.7	750	
		18	133	7.4	" and voided.	15.11	0.84				
		Total or average.	883	36.8		22.84	0.95				
	9	6	579	96.0	Catheterized.	7.10	1.18	72	1.5	750	
		18	439	24.4	" and voided.	15.12	0.84				
		Total or average.	1,018	42.5		22.22	0.93				
	10	6	495	82.5	Catheterized.	6.54	1.09	62	4.04	1,000	Weight 5 kg.
		18	195	10.8	" and voided.	15.22	0.85				
		Total or average.	690	28.7		21.76	0.91				

TABLE III—*Concluded.*

Period.	Day.	Duration of excretion.	Urine.					Blood.		Water by gavage.	Remarks.
			Amount.	Output per hr.	Type of specimen.	Bilirubin content.	Bilirubin output per hr.	Hemoglobin.	Bilirubin content per 100 cc. of plasma.		
		hrs.	*cc.*	*cc.*		*mg.*	*mg.*	*per cent*	*mg.*	*cc.*	
Second non-diuresis period.	11	24	250	10.4	Voided.	15.40	0.64	57	5.03		Weight 5 kg.
	12	24	405	16.9	Voided.	14.13	0.59				
	13	24	260	10.8	Voided.	9.60	0.40	56	5.30		
Second diuresis period.	14	24	885	36.8	Voided.	14.60	0.61	56	5.30	750	
	15	6	399	66.5	Catheterized.	6.27	1.04	49	4.60	750	
		18	352	19.5	" and voided.	13.50	0.75				
		Total or average.	751	31.3		19.77	0.82				
	16	6	320	53.4	Catheterized.	6.11	1.02	43	2.45	1,000	
		18	530	29.4	" and voided.	12.76	0.71				
		Total or average.	850	35.4		18.87	0.79				

	17	6 18	688 153	114.8 8.5	Catheterized. " and voided.	6.11 13.79	1.02 0.77	43	1.95	800	
		Total or average.	841	35.1		19.90	0.83				
Third non-diuresis period.	18	24	109	4.5	Voided.	18.80	0.78	43	2.3		
	19	24	144	6.0	Voided.	23.20	0.97	45	4.0		
	20	24	132	5.5	Voided.	16.40	0.68				
	21	24	232	9.7	Voided	27.8	1.16	45	4.0		Weight 4.2 kg.

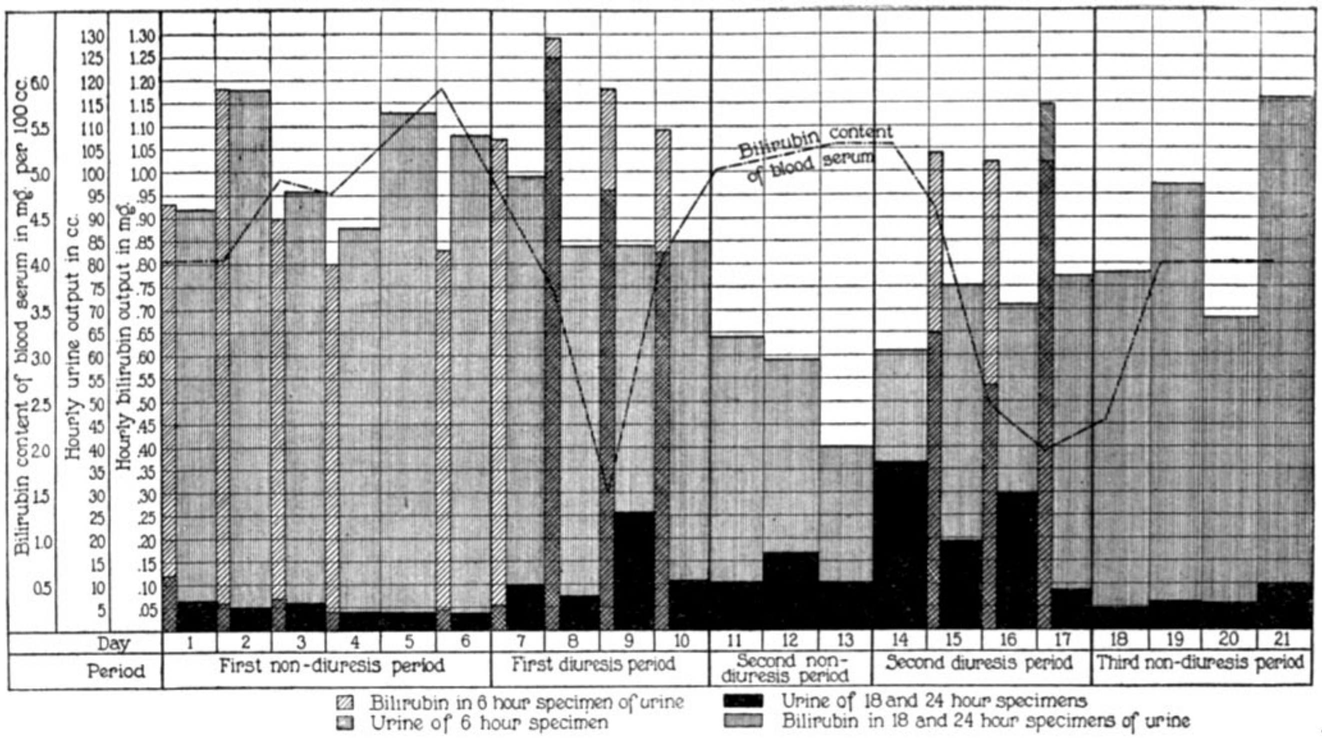
Thymol was added to the urines to check bacterial action; unfiltered specimens were employed, with their proportionate share of fecal sediment when this latter was present; and as much as possible of the 24 hour output was obtained by catheterization. Precipitation with calcium chloride was carried out on each specimen immediately after it had been procured. The bilirubin output was



TEXT-FIG. 2. Effect on bilirubin excretion in the urine of diuresis from water by mouth.

quantitated during alternate periods in one of which no effort was made to increase the water intake, which was invariably low, while in the other water was given by stomach tube, usually in 250 cc. amounts at 2 hour intervals three times each day.

A type instance of the results of the experiments is recorded in Table III and portrayed in Text-figs. 2 and 3. It will be noted that



TEXT-FIG. 3. The same as Text-fig. 2 except that the results are presented in greater detail.

on the 1st day when water was given by stomach tube, practically no diuresis ensued, owing probably to the fact that the animal had drunk but little for some days previously and fluid was needed by the tissues. A similar result was also obtained in later observations on diuresis in jaundiced human beings. On all subsequent days in which water was given there occurred marked diuresis, but as the greater part of the water was eliminated within 2 hours after each administration of it, there was only about 6 hours of diuresis altogether in each 24. The average urinary output for the remaining 18 hours was but slightly greater than that during the control, non-diuresis, periods. Cushny[12] has brought evidence that flood diuresis, such as follows upon intravenous injections, is practically never caused by fluid absorbed from the gastrointestinal canal, and we must assume that it had not occurred in our animals, despite the rapidity of the fluid elimination.

It will be seen that the bilirubin output per hour is fairly regular throughout each day of the first or non-diuresis period, the hourly average between 10 a.m. and 4 p.m. being in general somewhat lower than during the remaining 18 hours. Unfortunately no figures are available for the hourly output between 10 a.m. and 4 p.m. in the second and third non-diuresis periods. Only the average output per hour for the 24 hour period was recorded. In both of the diuresis periods, as already remarked, the increase in quantity of the urine was limited practically to the period between 10 a.m. and 4 p.m. The hourly output of bilirubin during this time is distinctly greater than during the remainder of the day. On the other hand, the output in the remaining 18 hours, when the urine was relatively scanty, is decreased to such extent that the output for the entire 24 hours averaged a little less than that during the first and third non-diuresis periods. True, the average 24 hour output for the second non-diuresis period is the lowest of the series, but there is no evidence to show that this was due to the administration of water during the preceding days. The main point sought after is clearly shown; diuresis from water by mouth has remarkably little effect on the day to day elimination of bilirubin.

[12] Cushny, A. R., The secretion of the urine, London, New York, Bombay, Calcutta, and Madras, 1917, 136.

The bilirubin of the blood plasma was followed by means of the diazo reaction, as has already been stated. There occurred a fall in the pigment concentration during the diuresis periods, but this can be explained in several ways other than by increased elimination of bilirubin from the body, notably by the accumulation of fluid in the tissues. Both in dogs and in human beings a fluid retention was indicated by changes in the weight. Quite possibly less bile pigment was produced in the organism during the periods of diuresis than in the intervals between.

Effects of Diuresis in Jaundiced Patients.

Observations were made on two men with catarrhal jaundice in whom periods of diuresis from forced fluid by mouth were alternated with periods of restricted water intake.[13] The icterus was marked when the observations were begun, but thereafter gradually and regularly diminished. The marked variations that were induced in the daily output of urine through forcing water by mouth had no evident effect on the rate at which the jaundice lessened from day to day as determined by the diazo reaction on the blood plasma. And the content of the 24 hour urine in bilirubin was no greater when the voidings amounted to several liters than when only half of this quantity had been passed. The findings, then, confirming those in dogs, show that diuresis by alimentary means fails, practically speaking, to increase bilirubin elimination.

DISCUSSION.

The foregoing experiments offer little support for the view advanced by some clinicians that forcing water by mouth has a direct effect to diminish the intensity of jaundice, by increasing the elimination of bilirubin in the urine. The flood diuresis which follows an intravenous injection of salt solution brings out, it is true, a relatively considerable quantity of bile pigment, but the method is clinically inapplicable, and, as has been shown, a copious diuresis from water, by mouth, yields no such consequence, so far as the 24 hour output is concerned. The fact is well recognized that "all the constituents

[13] These observations were made through the courtesy of Dr. E. F. Du Bois.

of the urine are increased in absolute amount per unit of time during diuresis."[12] In the present instance, the increase is more important theoretically than actually. Yet obviously this need not mean that diuresis is valueless as a means wherewith to combat the effects of bile retention. Its influence on the output of bile salts—substances more injurious to the organism than the pigments—remains to be determined when proper methods become available. Furthermore, diuresis may help to avert the accumulation of bilirubin in the kidneys with the disordered function consequent thereon.

Nonnenbruch[14] has shown that the acutely disordered kidney may fail to eliminate bile pigment during jaundice. It is probable that the extraordinarily pronounced icterus seen in some human beings during the later weeks of total biliary obstruction is due in part at least to a lessening of renal elimination. We have obtained data which would seem to bear significantly upon this point. In the attempt to increase jaundice in dogs, some injections of hemoglobin were given intravenously to animals already the subject of a long standing, total biliary obstruction and the mild general icterus that this entails. Hemoglobin for the purpose was obtained by the method of Sellards and Minot;[15] and eight to ten successive hourly injections were given, of amounts slightly less than that which should, on calculation, cause hemoglobinuria. In this way it proved possible to intensify the icterus markedly. At the end of a day of injections, the scleras of the dogs were pronouncedly more yellow than at the beginning. But the icterus was not maintained. By next morning only the previous, relatively pale, coloration characteristic of total obstruction was present. The urine during the transition period was heavily loaded with bilirubin. It is probable that in the case of bilirubin, as of urea, any increase in the circulating amount beyond a certain point is compensated for, when the kidneys are normal, by an increase in ease of elimination.

In man, as already stated, bilirubin is a threshold substance, in its renal relations, and it is normally present in appreciable amount in the blood. But in the dog, not only is the blood normally free from it, but whenever bilirubinemia can be detected there is bilirubinuria,

[14] Nonnenbruch, W., *Mitt. Grenzgeb. Med. u. Chir.*, 1918–19, xxxi, 470.

[15] Sellards, A. W., and Minot, G. R., *J. Med. Research*, 1917–18, xxxvii, 161.

while often the latter is to be found alone.[11] The question arises whether actually there is no threshold for bilirubin in this species, or whether the current tests for bilirubinemia are at fault. The presence in freshly voided urine, during even the slightest bilirubinuria, of renal cells specifically stained or stippled with the bile pigment constitutes evidence in this connection. According to the modern view enunciated by Cushny, all substances that pass into the urine leave the circulation by way of the glomerulus, and the so called threshold substances undergo resorption to a greater or less degree during their passage through the tubules. Thus it is that when the threshold substance is a dyestuff, the tubular epithelium becomes pigmented. Were all this quite certain, the existence in the urine of the dog of jaundiced kidney cells would be proof that in

TABLE IV.

Maximal Bilirubin Output during Total Obstruction.

Dog No.	Body weight.	Bilirubin.		Time of occurrence after operation.
		Expected 24 hr. output.	Greatest actual 24 hr. output.	
	kg.	*mg.*	*mg.*	*days*
1	4½	39.6	19.5	28
2	5⅓	45.8	28.3	28
3	9¼	81.4	24.0	10

this species bile pigment is a threshold substance. But as happens, there is recent work to show that some, at least, of the tubules have an excretory function.[16] Therefore, our cellular evidence is unconvincing.

The amount of bile pigment eliminated in the urine of the dog during complete and long continued biliary obstruction is never nearly so great as that formed during the same period by a normal liver. The bilirubin output of fistula animals in good condition amounts to about 8.8 mg. per kilo of body weight per day.[7] Table IV shows the observed pigment content of the urine of three dogs with persistent total obstruction. It will be seen that the maximum output of Dog 3 occurred on the 10th day of obstruction and that

[16] Oliver, J., *J. Exp. Med.*, 1921, xxxiii, 177.

of the other two animals on the 28th day. In all three instances the bilirubin content of the blood had already become fairly constant, and so too had the tissue icterus. The 24 hour output of pigment in the urine never approached the amount which a normal liver would have secreted in the same time. Several explanations suggest themselves for the discrepancy, more especially diminished liver function during jaundice, and destruction of pigment within the body.

SUMMARY.

The elimination of bile pigment during jaundice is, for practical purposes, unincreased by diuresis from water by mouth. Possibly, though, the flushing of the kidneys tends to lessen pigment accumulation within these organs and thus to diminish a serious potential source of trouble in long continued jaundice. Flood diuresis from intravenous injections of salt solution markedly increases the output of bile pigment. It is important to know the effect of variations in the urinary output on the elimination of bile salts, but methods for the purpose are not available at present.

The passage of bile pigment into the kidney cells during jaundice is attested by the presence in the freshly voided urine of desquamated renal elements specifically stained, stippled, or granulated with bilirubin. Pigmentation of this sort is readily to be distinguished from the indiscriminate staining of cellular debris that occurs in icteric urines on standing. It has clinical significance, furnishing direct evidence on the degree of renal change.