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J Clin Invest. 1927;4(4):545-553. <https://doi.org/10.1172/JCI100140>.

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QUANTITATIVE PETTENKOFER VALUES IN BLOOD WITH SPECIAL REFERENCE TO HEPATIC DISEASE

A PRELIMINARY REPORT¹

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(Received for publication May 14, 1927)

So long as there was not a satisfactory method of determining quantitatively the level of the bile acids in the blood it was practically impossible to ascertain the part these substances play in disease. Since the bile acids have unusual properties and are physiologically important in the organism, it is highly desirable that something should be known of their quantitative distribution in the blood, tissues, and body fluids, and also of the changes accompanying their altered concentration. With this in mind one of us (Aldrich) undertook the development of a method for the quantitative determination of bile acids in the blood. The Pettenkofer reaction, although subject to several objections, appeared to offer the best basis for the method. The quantitative adaptation of this reaction, is, in our opinion, of value and is yielding data of considerable significance.

That bile acids may occur in the blood after biliary obstruction or intravenous injection was shown by the experiments of Kühne and Huppert. Various observers have reported qualitative colorimetric tests for bile acids in the blood. Moleschott, Lehmann, Blankenhorn, Gilbert, Chabrol and Benard, Pétren and others obtained positive Pettenkofer tests under appropriate conditions. Recently Tashiro, and Herzfeld and Haemmerli have reported partially successful attempts to adapt this test to quantitative determinations. Perlzweig and Barron report a new colorimetric test for bile acids, using acetic anhydride and sulphuric acid; Szilard precipitates the bile acids with

¹Read before the American Society of Clinical Investigation, Atlantic City, New Jersey, May 2, 1927.

ferric chloride and determines the iron colorimetrically; Rosenthal and Wislicki use a modification of the gasometric method for the determination of amino-acids in the bile. McNee has suggested a similar method in which the amino-acids are colorimetrically determined. Because of the difficulties in the application of chemical tests, Adler and others turned to physical methods and reported extensive studies on the surface tension of serum. Work which was carried on by Baldes in the laboratories of the Mayo Clinic over a period of several months led us to the conclusion that changes in the surface tension of the serum could not be used as a reliable index of the amount of bile acids present. These various methods were not sensitive or specific enough, or were not clinically applicable.

The quantitative Pettenkofer test which we have employed in this study is described in detail elsewhere (1). Although this test has been criticized for lack of specificity, its great sensitivity makes it particularly applicable to the analysis of blood in which the concentration of bile acids is low, and the amount of material for analysis necessarily limited. Substances interfering with the specificity of the test, have, so far as possible, been removed. The test requires only 5 cc. of oxalated blood. The bile salts are extracted from the blood with alcohol, interfering substances removed, and the Pettenkofer color developed under standard conditions, which with pure solutions of bile acids, yield results accurate within plus or minus 5 per cent. The color is compared in a colorimeter, of the Duboscq type, with that developed under similar conditions by pure glycocholic acid, and results reported in terms of glycocholic acid. By this method, between 90 and 100 per cent of added bile acids can be recovered.

Values equivalent to from 2.5 to 6 mg. of glycocholic acid have been found in normal blood, while increased values may be present in abnormal blood. Care must be taken in interpreting the results obtained by a reaction which is not specific and it is not possible to attach a definite identity to all of the Pettenkofer reacting material, especially in normal blood, although as far as possible interfering substances have been removed. If it is definitely accepted that bile acids are retained in the blood, as following intravenous injection, or in obstructive jaundice, the method is of value in determining the changes occurring in such conditions. Since bile acids may be recovered from

the blood by this method, a normal Pettenkofer value indicates the absence of any increase of bile acids in the blood. Keeping in mind such limits in interpretation of values, one may use the method to obtain information concerning the metabolism of bile acids, as shown by the changes in the content of the blood under certain experimental and clinical conditions.

PETTENKOFER VALUES IN EXPERIMENTAL STUDIES

Snell, Greene, and Rowntree have studied the level of the bile acids and bilirubin in dogs with experimental obstructive jaundice. Following ligation of the common duct, the Pettenkofer value of the blood increased gradually and, in cases in which cholecystectomy had been performed, very rapidly, often reaching the level of 30 mg. for each 100 cc. During experimental jaundice of long duration, both bilirubin and bile acids tended to return to normal levels, but the fluctuations in the level of the bile acids were greater than for serum bilirubin.

Greene and Snell have studied the rate of elimination of bile acids from the blood stream of dogs following the intravenous injection of bile acids. When the sodium salts were injected intravenously in normal dogs, the increase in the concentration in the blood was dependent on the dosage and on the rapidity of the injection. In all instances, the bile salts were eliminated with extreme rapidity. Even with the maximal dosage compatible with recovery of the animal (400 to 500 mg. for each kilogram of body weight) the excess was eliminated within two hours. In these experiments Pettenkofer values as high as 100 mg. for each 100 cc. of blood were attained.

In other studies with this method, we elicited evidence to confirm the theory of the entero-hepatic circulation of bile acids. In the fasting animal a significant difference was not found between the amounts of Pettenkofer-reacting material in the jugular and in the portal blood. Within fifteen minutes after the injection of bile acids into the duodenum, increased Pettenkofer values were demonstrated in the portal blood (from 10 to 20 mg.) while the values were not increased in the blood of the jugular vein. An increase in the rate of bile flow and in the amount of bile acids in the bile was noted shortly after the oral administration of bile salts.

Results of clinical studies. Pettenkofer values in normal subjects

vary from 2.5 to 6 mg. by this method. These figures are based on a study of forty normal subjects (laboratory workers) and seventy hospital patients who were without evidence of hepatic disease. In

TABLE 1
*Results of functional tests in various diseases of the liver**

	Cases	Bilirubin			Bromsulphthalein retention†			Bile acids		
		Minimum	Maximum	Positive	Minimum	Maximum	Positive	Minimum	Maximum	Positive
		mg. per cent			mg. per cent			mg. per cent		
Normals:										
Laboratory workers.....	40	0.2	1.0	0	0	2	0	2.6	5.1	0
Hospital patients.....	70	0.2	1.8	0	0	10	0	2.6	5.2	0
Chronic cholecystitis.....	40	0.2	1.9	0	0	30	8	3.0	5.7	0
Obstructive jaundice:										
Common duct stone.....	14	2.4	12.8	14	20	60	14	3.4	8.8	6
Stricture of duct.....	15	1.2	9.1	9	10	60	15	3.7	10.4	2
Tumor of pancreas.....	8	10.2	33.4	8	14	96	8	3.0	19.8	5
Carcinoma:										
No hepatic involvement.....	14	0.2	0.9	0	1	12	2	3.0	3.8	0
Metastasis, no jaundice.....	36	0.2	5.8	2	2	72	31	2.0	8.0	6
Metastasis and jaundice.....	6	3.0	39.6	6	40	64	6	4.1	16.5	3
Hemolytic jaundice.....	16	2.9	8.7		0	8		3.1	6.2	1
Pernicious anemia.....	8	0.8	4.6		0	8		5.3	6.2	1
Splenic anemia.....	20	0.2	2.8	2	0	60	15	3.4	6.2	1
Myocardial failure with passive congestion.....	30	0.2	4.5	2	8	64	25	2.7	5.4	0
Hypertension.....	16	0.2	1.7	0	0	8	0	3.2	5.7	0
Portal cirrhosis:										
Small liver.....	16	0.6	2.2	3	6	60	14	3.9	8.1	2
Large liver.....	20	0.6	3.1	7	5	64	18	3.8	7.2	3
Biliary cirrhosis:										
Obstructive type.....	11	1.2	7.3	7	20	44	11	2.7	14.3	4
Nonobstructive type.....	9	1.6	17.8	7	24	56	9	5.0	8.0	3

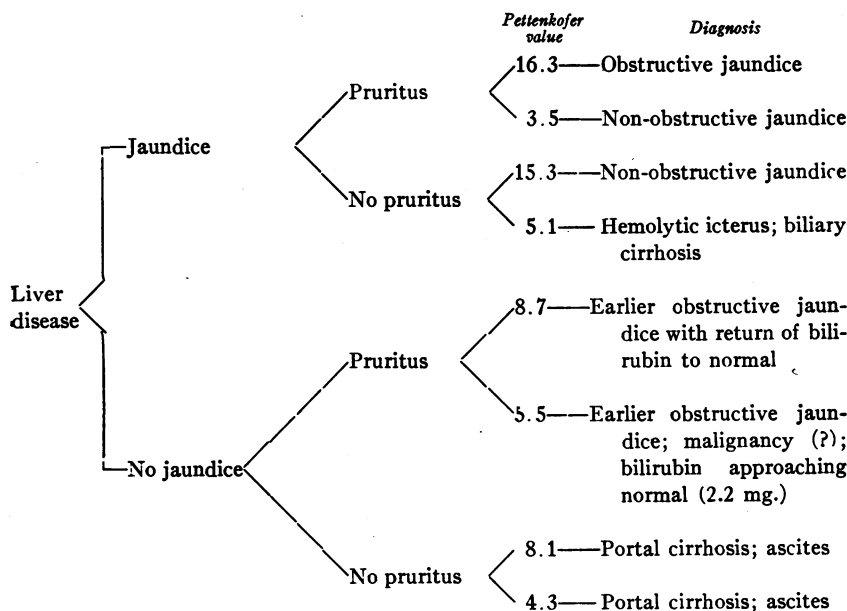
* Figures in the columns headed "positive" indicate the number of cases in the series yielding abnormal values.

† Grading of sample taken at one hour.

addition, between 250 and 300 patients suffering from various forms of hepatic disease were observed. The highest value was 27 mg., observed in a case of fatal nonobstructive jaundice of less than two

months' duration, in which bleeding was a prominent symptom. Table 1 shows the results in a series of cases; the various diseases, the minimal and maximal values for bilirubin, bromsulphalein and bile acids, together with the number of positive data with each of these tests are presented. As is shown, the Pettenkofer values are not so delicate an indication of hepatic disease as are the bilirubin and dye retention tests. High values are found most frequently in

CHART 1. PETTENKOFER VALUE IN RELATION TO PRURITUS AND JAUNDICE IN LIVER DISEASE



diseases associated with jaundice. In general, Pettenkofer values are high early rather than late in the course of obstructive jaundice.² As a rule practically normal values are encountered in hemolytic jaundice, pernicious anemia, splenic anemia, and in hypertension and myocardial failure with passive congestion. Increased values are found occasionally in the portal and biliary types of cirrhosis.

² This indicates that with the passage of time in cases of obstructive jaundice there is a decrease in synthesis of serum bilirubin and of bile acids. For the elucidation of this problem however further studies are essential.

According to the French school, bile acids are largely responsible for the pruritus and bradycardia occurring in hepatic disease, especially in jaundice. Our observations thus far do not entirely con-

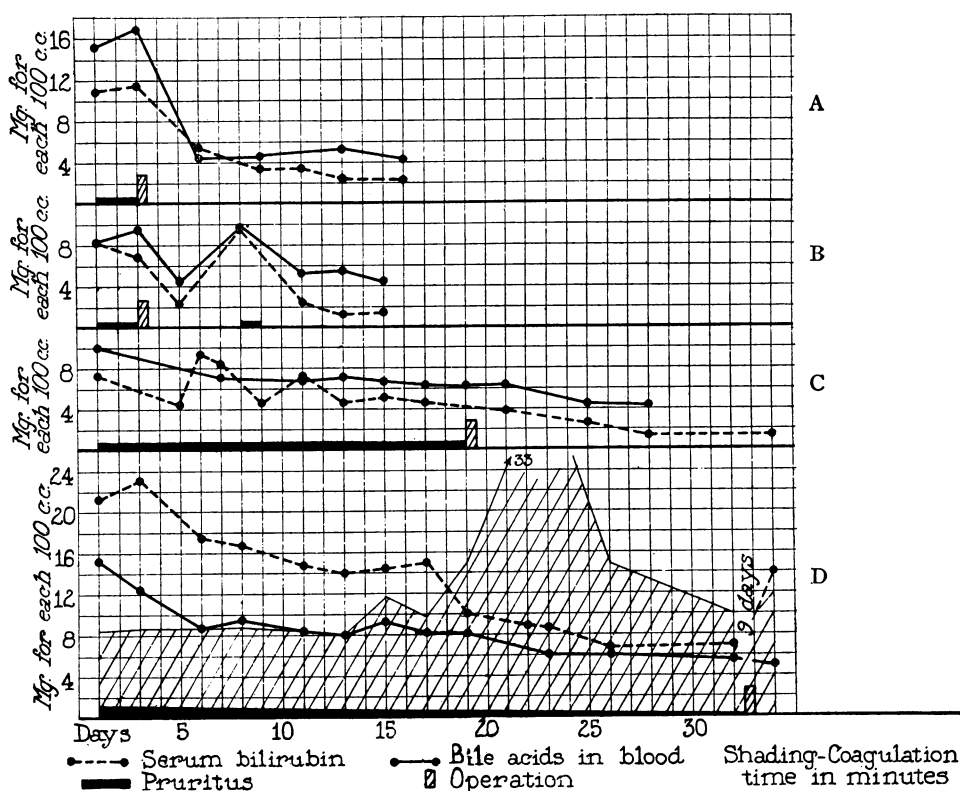


FIG. 1

A. Woman of 25 years. *Operative diagnosis:* Stricture of common duct following operation elsewhere. *Operative procedure:* Choledochoduodenostomy.

B. Woman of 62 years. *Operative diagnosis:* Chronic cholecystitis with cholelithiasis; pancreatitis. *Operative procedure:* Cholecystectomy.

C. Man of 70 years. *Operative diagnosis:* Biliary cirrhosis; suppurative cholangitis; chronic cholecystitis; pancreatitis. *Operative procedure:* Cholecystgastrostomy.

D. Woman of 43 years. *Operative diagnosis:* Stricture of common duct following operation elsewhere. *Operative procedure:* Insertion of drain.

firm this view. That pruritus is common in jaundice is well-known. Our figures indicate that while definitely increased Pettenkofer values tend to accompany pruritus, normal Pettenkofer values may also occur

with pruritus. Such observations exclude a direct causal relationship between the Pettenkofer value of the blood and pruritus. This lack of relationship is further shown in the diagrammatic representation

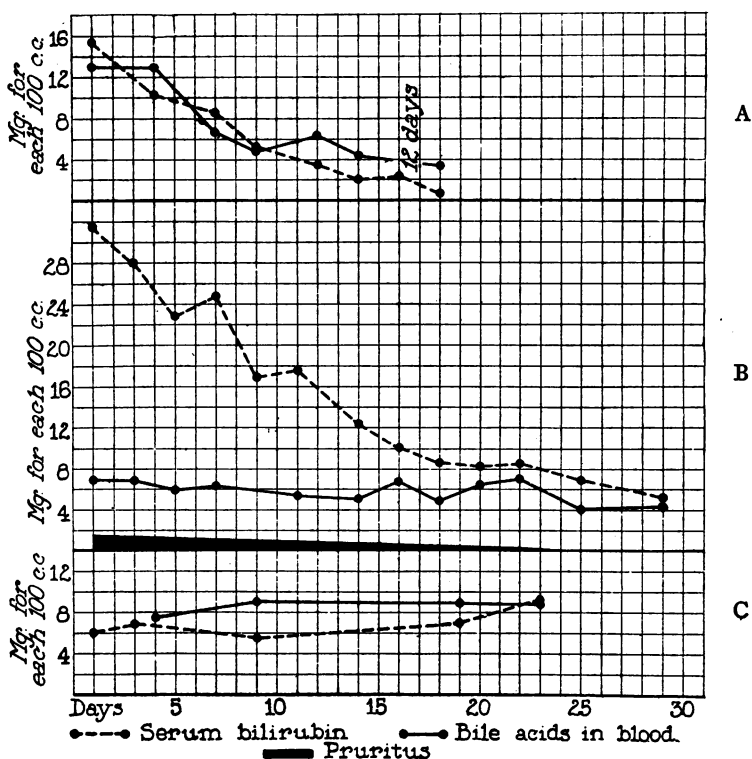


FIG. 2

A. *Catarrhal jaundice*. Man aged 30 years. Three weeks painless jaundice before admission to hospital. Little or no pruritus at any time. Recovery complete at time of dismissal.

B. *Catarrhal jaundice(?)*. Man aged 67 years. Six weeks of jaundice and one week pruritus before admission to hospital. Edema of lower extremities developed in hospital makes diagnosis uncertain.

C. *Cirrhosis of liver with splenomegaly*. Man aged 31 years. Two attacks of jaundice during ten weeks preceding admission to hospital. No pruritus at any time.

of the relation of some of the signs and symptoms of hepatic disease to the acutal Pettenkofer values found in the blood (chart 1).

Disease of the liver may or may not be associated with jaundice. Jaundice may be present with or without pruritus, and pruitus may

be present in the absence of jaundice. High or low Pettenkofer values may be found in the presence or absence of jaundice and in the presence or absence of pruritus. Neither has any relationship been established between increases in Pettenkofer values and bradycardia. Bradycardia is an infrequent accompaniment of jaundice except in the intrahepatic type or the so-called acute catarrhal jaundice. Our determinations do not appear to yield figures confirming any definite direct and causal relationship between pruritus, bradycardia and Pettenkofer values.

Single determinations of bile acids by the Pettenkofer test do not appear to yield information of great diagnostic, prognostic or therapeutic value. Our experience, however, is limited as yet, and more extensive study may reveal its greater significance.

In utilizing functional tests for the liver, the situation is analogous to that pertaining to the kidney. In the study of renal disease multiple tests are commonly employed. Multiple and repeated tests also yield information of the greatest significance in the study of hepatic disease. Figures 1 and 2 show in graphic form the data related to seven cases of disease of the liver. Special points of interest are: (1) the frequent simultaneous occurrence of high values for bile pigments and bile acids in jaundice; (2) a lack of quantitative parallelism in these two substances, although there is a decided tendency to simultaneous increase or decrease in their level in the blood; (3) striking dissociation at times in the level of bile pigments and bile acids; (4) tendency of pruritus, common in jaundice, to disappear with the relief of jaundice, and to reappear as jaundice recurs, and (5) the tendency of Pettenkofer values to increase and decrease with the appearance and disappearance of jaundice, although causal relationship between bile acid level and pruritus is not established (note curve fig. 2, especially).

In the last curve decreased coagulability of the blood and hemorrhage are indicated. Increased coagulation occurred during a period in which the level of the bile acids was falling and approaching normal. On the other hand, uncontrollable hemorrhage has been encountered in another fatal case of nonobstructive jaundice in which the Pettenkofer values on three occasions were between 20 and 26 mg. for each 100 cc. of blood. The addition *in vitro* of similar amounts of

bile salts to normal blood has only a minimal effect on its coagulation time. Such relationship of bile acids to the coagulability of the blood is being investigated.

SUMMARY AND CONCLUSIONS

Determinations of the quantitative Pettenkofer value in blood have been made in a study of hepatic disease. Studies have been made of animals, and several hundred determinations have been carried out on patients in the wards. The Pettenkofer value of normal blood varies from 2.5 to 6 mg. (in terms of glycocholic acid) for each 100 cc. Marked increases in these values are found in obstructive experimental jaundice, and after the injection of bile salts into the blood stream. Bile salts so administered leave the circulation rapidly. The administration of bile salts by mouth definitely increases their level in the portal vein but not in the peripheral circulation, and increased quantities of bile acids may be quickly recovered from the bile.

Increased Pettenkofer values are frequently encountered clinically in hepatic disease. High values are most common in the presence of jaundice and in the earlier rather than the later stages of obstructive jaundice. High values may be found in cirrhosis of the liver in the absence of jaundice. Pruritus is commonly encountered in jaundice and is frequently associated with high Pettenkofer values. However, a direct causal relationship is lacking, since high values may persist over periods of weeks without pruritus, and itching in chronic disease of the liver may be marked, when the Pettenkofer value is strictly normal. With high Pettenkofer values, tachycardia or normal pulse rate is encountered more frequently than is bradycardia. The level of the Pettenkofer value does not seem to bear a direct causal relationship to decreased coagulability of the blood or to hemorrhage in cases of jaundice. Further clinical and experimental studies relating to the amounts of bile acids in the blood and tissues, and the effects of their altered concentration on various physiologic functions, are in progress.

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