THE USE OF THE VAN DEN BERGH TEST IN THE DIFFERENTIATION OF OBSTRUCTIVE FROM OTHER TYPES OF JAUNDICE.

BY

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At a recent meeting of the Association of Physicians of Great Britain and Ireland, held in Oxford, Professor Hijmans van den Bergh of Groningen gave a short account of his important work on the presence of bile pigment (bilirubin) in the blood serum under normal and pathological circumstances. In doing so he made reference to the test for bilirubin in serum and other albuminous fluids which is now prominently associated with his name. I have made use of this test in the wards and in experimental work for some months, and the results obtained have up to the present fully realized expectations. It was intended to wait until a much larger series of observations had been carried out, but since none of the work of van den Bergh has so far been published in English the writer has been asked to make some of the main facts accessible at once, leaving the fuller account for subsequent publication. It must therefore be understood that the conclusions reached in this short paper cannot be regarded as final, unless confirmed elsewhere or by future work on the subject.

The first account of the work of Hijmans van den Bergh on the presence of bile pigment in serum appeared in 1913,¹ and a full description of his observations has been collected in a monograph entitled *Die Gallenfarbstoff im Blute*, published in 1918.² More recently (June, 1921) a short summary of the main methods and facts appeared in the *Presse Médicale*.³

Confirmation of some of the chief results claimed by van den Bergh has already been given in Germany by Lepelne (1921)⁴ and Rosenthal and Holzer (1921).⁵

The clinical application of the test in the differentiation of various types of jaundice will be dealt with here alone, although this is merely a small part of the ground which has been covered by van den Bergh. The important observations which are more concerned with experimental work on diseases of the liver, and with the occurrence of latent jaundice under conditions in which icterus has not hitherto been recognized to exist, must be omitted. It is already certain, however, that future research work on hepatic disorders must be greatly influenced by the application of the knowledge made available by van den Bergh's methods.

The chief clinical value of the test is that by its use jaundice due to obstruction of the main bile ducts by carcinoma, hepatic cirrhosis, obstruction in the portal fissure, or gall stone in the common bile duct, can be clearly differentiated from jaundice of haemolytic origin or due to functional derangement of the liver cells. In this latter category are now included the various forms of haemolytic and acholuric jaundice, and also functional jaundice, such as catarrhal jaundice, toxic jaundice in infective diseases (typhoid fever, pneumonia), icterus neonatorum, etc. It is perhaps not yet generally accepted, except by those who have followed closely the work on hepatic disorders published in recent years, that all modern work strengthens more and more the view that a true haemolytic icterus, apart from the liver, does occur, and also that "catarrhal jaundice" depends on a hepatitis with functional derangement of the liver and not on an obstruction to the bile ducts. The newer methods of van den Bergh throw further light on the question from a new angle, and entirely support both the occurrence of a functional icterus apart from biliary obstruction, and of a haemolytic icterus with which the liver itself is not concerned.

The value of van den Bergh's test in the differentiation of obstructive from what may be termed functional and haemolytic jaundice is illustrated by a few chosen reports of cases given at the end of this short communication.

MECHANISM AND TECHNIQUE OF THE TEST.

Hijmans van den Bergh began his work faced with a difficulty which has confronted all who have worked chemically or experimentally on the different forms of jaundice—namely, the want of a delicate and trustworthy test for small amounts of bile pigment in an albuminous fluid such as blood serum. The tests hitherto employed, such as the Gmelin and Huppert tests, with their various modifications, have many disad-

vantages (especially in albuminous fluids), and are, besides, far from delicate for quantitative estimations.

Van den Bergh has applied for his purpose the so-called "diaz reaction," first described by Ehrlich, who found that bilirubin, when dissolved in chloroform or alcohol, gives with diazonium salts a reddish colour in neutral solutions and a bluish colour in acid solutions. Making use of this reaction to detect the presence of bilirubin in blood serum, van den Bergh and Snapper found that it gives extraordinarily delicate and certain results. They observed, for example, that every normal serum contains bilirubin in a dilution of from 1 in 400,000 to 1 in 250,000. Such a dilution in human serum is readily detected by the diazo test. They found further, after much observation, that no other substance likely to be present will give the reaction, and they have never detected any other substance in a human serum, except bilirubin, which has given a positive result. Biliverdin does not react to the test. It is to be noted also that lutein, which in certain cases (diabetes, etc.) may deeply colour human blood serum and even give the appearance of jaundice to the skin, does not give the reaction.

Technique of the Test.

For the test, as ordinarily carried out, about 3 c.cm. of serum may be required, although less will suffice after some practice has been obtained. The blood is taken from a vein in the usual way into a dry test tube, allowed to clot, and the separated serum is then removed by a pipette. It is best to begin to practise the test on a case of fairly intense icterus.

Apparatus and Reagents Required.

1. A few test tubes of ordinary size.
2. Freshly prepared Ehrlich's diazo reagent. This consists of two solutions, each of which keeps well, but the mixture of the two must only be made immediately prior to the test. The two solutions are made up in the following proportions:

A. Sulphanilic acid	...	...	...	1 c.cm.
Concentrated HCl	...	...	...	15 c.cm.
Distilled water...	...	...	...	1,000 c.cm.
B. Sodium nitrite	...	...	...	0.5 gram
Distilled water...	...	...	...	100 c.cm.

The diazo reagent consists of a mixture of these two solutions in the proportion of 25 c.cm. of solution A to 0.75 c.cm. of solution B.

3. A graduated 1 c.cm. pipette.
4. Absolute alcohol (96 per cent.).
5. A centrifuge and centrifuge tubes.

The test is then carried out as follows: To 1 c.cm. of the serum, in a small test tube, van den Bergh adds 0.25 c.cm. of freshly prepared diazo reagent. (Lepelne, and the writer, have found that better results are frequently obtained by adding 1 c.cm. of the reagent.) One of three events may now occur:

1. *An Immediate (Direct) Reaction.*—This begins instantly and is maximal in ten to thirty seconds. The colour reaction obtained is a bluish-violet, of intensity depending on the amount of bilirubin present.

2. *A Delayed Reaction.*—This begins only after one to fifteen minutes, or even longer, and consists in the development of a reddish coloration, which gradually deepens and becomes more violet. (It will be seen later that this reaction is not made use of further, being replaced by the so-called indirect reaction or test—vide infra.)

3. *A Bi-phasic Reaction.*—In this a slight reddish colour appears immediately (ten to thirty seconds), which after a minute or much longer time is seen to deepen gradually and become more violet.

Interpretation.

If the reaction is immediate or direct an obstructive jaundice is indicated.

If a direct or immediate reaction is not obtained proceed as follows: To 1 c.cm. of serum add 2 c.cm. of 96 per cent. alcohol. The mixture is made in a centrifuge tube, which is then centrifuged until all the albuminous precipitate has sunk to the bottom to leave a clear yellowish supernatant fluid. To 1 c.cm. of this supernatant fluid add 0.5 c.cm. of alcohol and 0.25 c.cm. of Ehrlich's diazo reagent. (The reason for the addition of 0.5 c.cm. of alcohol is to get a proper dilution for the quantitative test, referred to below, and may be omitted where that test is not being carried out.) A violet-red colour is then obtained if bilirubin be present, which is of maximal intensity almost at once.

Where no direct reaction has been given, but a perfect indirect reaction after alcohol precipitation, then the jaundice

can be inferred to be either haemolytic in origin or dependent on some functional derangement of the liver cells without obstruction.

It should be mentioned that all serums which give a direct reaction will give in addition an indirect reaction, but the converse is not, of course, true.

By these two simple tests, therefore, a distinction can be drawn between icterus due to obstruction of the main bile ducts from gall stones, tumours, hepatic cirrhosis, etc., and an icterus of haemolytic, infective, or functional origin.

What is the mechanism of the test, and how is it that the bilirubin present in the serum in obstructive jaundice reacts to the diazo reagent quite differently from the bilirubin in haemolytic, catarrhal, and other forms of jaundice?

It appears to depend on the fact that the bilirubin differs molecularly in the two conditions, and, without going deeply into theoretical considerations here, van den Bergh suggests that in the case of bilirubin giving the indirect test only, the pigment has been in some way bound to albuminoid substances in the serum, and the union is only broken down by time or by alcoholic precipitation. The reader must be referred to the original papers for further consideration of this point.

Taking this short explanation for granted, the rationale of the *bi-phasic reaction* becomes intelligible. It would appear to depend on the presence of both types of bilirubin, in different proportions, in the same serum. If the first variety predominates, the reaction might be described as *bi-phasic direct*, and the other as *bi-phasic indirect*. The occurrence of the *bi-phasic reaction* is fortunately not very common, but until much further experience with the test has been gained it is best for the moment to draw no absolute conclusion in a case giving such a result. The writer is finding such cases of great interest at present, and it is already evident that in, for example, cardiac valvular disease with failure, back pressure, and hepatic enlargement, the icterus which sometimes occurs may be at first functional, and later obstructive in type as the hepatic enlargement increases, the bilirubin showing a gradual transition through a *bi-phasic stage* with the test.

Application of the Test for the Quantitative Estimation of Bile in Serum.

Although the simple test as described above will probably be the first to be commonly used clinically, it is an obvious advantage to be able to estimate the increase or decrease of bile pigment in the blood serum, especially in cases of obstructive jaundice. This may have importance, for example, in cases of suspected carcinoma of the liver, or in the recognition of the exit of a gall stone from the common bile duct. It is, of course, well known that the icteric tint of the skin changes comparatively slowly. It has also an importance in the study of various forms of "latent icterus," such as in cases of pernicious anaemia. These questions will be dealt with in a later communication.

The so-called *indirect test* of van den Bergh, which, as has been stated, is given by all forms of icterus whether obstructive or non-obstructive, lends itself easily to a quantitative estimation of bilirubin by a colorimetric method. For full details of the method the original papers should be consulted, but the main principles may be briefly given here. At first van den Bergh made use of chemically pure bilirubin to prepare a solution for comparison, but he was able soon to replace this with an artificial standard solution giving a colour suitable for comparison. This solution, moreover, is made up in a strength which can give a definite reading in "units" of bilirubin. The artificial solution consists of iron sulphocyanide dissolved in ether, in a concentration of 1 in 32,000 normal. This solution is of a colour which corresponds exactly with that of azo-bilirubin (as produced in the "indirect test") of 1 in 200,000, the quantity found to be the average amount in the serum of a healthy individual. An indirect reaction giving a colour exactly corresponding to this standard is taken as indicative of "1 unit" of bilirubin.

The Solution of Sulphocyanide.

The standard solution of iron sulphocyanide is prepared as follows:

Dissolve 0.1508 gram of ammonium iron-alum in 50 c.cm. concentrated HCl, and add water to 250 c.cm. This gives a dilution of 1 in 8,000 normal, which will keep for about six months.

To 3 c.cm. of this solution add an equal volume of 20 per cent. potassium sulphocyanide and 12 c.cm. of ether. Shake well, and when all the reddish colour has passed into the ether

transfer the ether carefully, either into a colorimeter, or other comparative tube. This solution is in a concentration of 1 in 32,000 normal, and must be prepared freshly each day a test (or tests) is made.

I have made use of the simple Autenrieth-Funk colorimeter for the quantitative estimation, but any form of colorimeter is, of course, applicable. For rough clinical use dilution of the fluid obtained in the indirect test may be made in test tubes of equal calibre, and reasonably accurate comparative results obtained. It should be pointed out that even with the complete technique of van den Bergh the results are of an accuracy which is adequate for clinical purposes only. There are various fallacies which prevent a completely accurate estimation of the whole of the bilirubin present in any serum. One of the chief of these depends on the fact that some bile pigment is always carried down in the albuminous precipitate when alcohol is added. The amount, however, is always small, and is greater in cases of obstructive than of non-obstructive icterus.

REPORT OF CASES.

Case 1.

Female. History of three attacks of jaundice, with vomiting and epigastric pain, within a period of a few months. Stools clay-coloured. X-ray examination showed two shadows at the level of the twelfth rib, to the right of the middle line, but deeply back in the body. There was doubt as to what these shadows were—biliary calculi, renal calculi, or calcified glands. On the clinical features of the case a diagnosis of gall stone blocking the common bile duct was made.

On applying the van den Bergh test the following result was obtained:

Direct test ... Negative.
Indirect test ... Positive (3 units of bilirubin).

Exploratory laparotomy was performed; the gall bladder and bile ducts were normal, and patent throughout. The liver was not enlarged, but icteric in colour. The diagnosis was changed to catarrhal jaundice, and the patient made a straightforward recovery.

Case 2.

Female. This patient, a nurse in a fever hospital, had been previously admitted for jaundice, which was said to have followed a febrile illness of some weeks' duration. Typhoid was at first suspected, but the Widal reactions for the enteric group of fevers were negative. The jaundice passed off, but the patient was readmitted for a second attack, accompanied by pain in the right hypochondrium. The stools were light coloured. A diagnosis of gall stones was made.

The result of the van den Bergh test was as follows:

Direct test ... Negative.
Indirect test ... Positive (4½ units of bilirubin).

The abdomen was opened, but the gall bladder and bile ducts were found to be normal. The patient quickly recovered, and the jaundice passed off.

Case 3.

Female. History of several attacks of severe pain in the right hypochondrium. Never jaundiced until one week before admission. Jaundice of moderate degree. Cholecystitis was the diagnosis regarded as probable.

The van den Bergh test gave the following result:

Direct test ... Positive.
Indirect test ... 4 units of bilirubin.

At operation the gall bladder was found to be much contracted and embedded in dense adhesions passing along the portal fissure. One large stone was removed from the gall bladder, but examination of the common bile duct was almost impossible owing to the dense adhesions. A leak unfortunately occurred from the septic gall bladder, and fatal peritonitis followed. At the necropsy the gall bladder was found to be greatly contracted and strictured. The common bile duct was embedded in dense adhesions, but no stones were found.

Case 4.

Male. History of jaundice of three weeks' duration; loss of weight; stools light coloured. Nothing could be made out by palpation of the abdomen.

The van den Bergh test resulted as follows:

Direct test ... Positive.
Indirect test ... 12 units of bilirubin.

At operation a carcinoma of the head of the pancreas was found, obstructing the ampulla of Vater.

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REFERENCES.

- Hijmans van den Bergh, A., and Snapper, I.: Die Farbstoffe des Serums, *Deut. Arch. f. klin. Med.*, 1913, cx, 540.
- Hijmans van den Bergh, A.: Monograph, *Die Gallenfarbstoffe im Blute*; Leiden, van Doesburgh, 1918; and Barth, Leipzig, 1918.
- Hijmans van den Bergh, A.: La recherche de la bilirubine dans le plasma sanguin par la méthode de la réaction diazoïque, *Presse Médicale*, 1921, No. xlv, 441.
- Lepehne, G.: Weitere Untersuchungen ueber Gallenfarbstoff im Blutserum des Menschen, *Deut. Arch. f. klin. Med.*, 1921, cxxxv, 79.
- Rosenthal, F., and Holzer, P.: Beiträge zur Lehre von den mechanischen und dynamischen Iktusformen, *Deut. Arch. f. klin. Med.*, 1921, cxxxv, 257.