

Citation:

A.A. Hijmans van den Bergh & J.J. de la Fontaine Schluiter, The identification of traces of bilirubin in albuminous fluids, in:

KNAW, Proceedings, 17 II, 1914, pp. 807-809

Physiology. -- "*The identification of traces of bilirubin in albuminous fluids.*" By Prof. A. A. HIJMANS VAN DEN BERGH and J. J. DE LA FONTAINE SCHLUITER. (Communicated by Prof. H. J. HAMBURGER).

Several investigators have tried to demonstrate the presence of slight quantities of bilirubin in albuminous substances, for instance in normal human bloodserum. Most of them did this by adding various oxidizing substances, either directly to the serum or to an alcoholic extract of the latter. The first oxidation-stages of bilirubin having a green or a blue colour, the presence of bilirubin was regarded as established if an addition of these oxidizing substances gave rise to a green or a blue colour (OBERMAIJER and POPPER, STEIGER, GILBERT¹⁾ and others). AUCHÉ²⁾ employed a much more reliable method based on the fact that bilirubin, in alkalic solution in the presence of oxide of zinc, is changed, by careful oxidation with iodine, into a substance with a characteristic spectrum. This reaction had already been described by STOKVIS, but AUCHÉ, who mentions STOKVIS' work, owns the merit of having stated accurately the conditions required if the reaction is to take place with absolute certainty, so that it may be used to demonstrate the presence of bilirubin.

Undoubtedly the reaction of STOKVIS-AUCHÉ can be used with success. Only the spectrum-line is very slight in the case of the small amounts of bilirubin dealt with in this treatise: if the presence of bilirubin is to be demonstrated in normal human serum by means of this method, the layer of fluid intended for spectroscopic investigation is to have a thickness of ten centimetres. And even then the result is not always a positive one. For quantitative determinations this method cannot be used.

BIFFI extracted the serum at once with chloroform and carried out his reactions with this³⁾.

The reaction of EHRLICH has supplied us with an excellent means of tracing bilirubin in bloodserum and other albuminous fluids and of determining it quantitatively⁴⁾. The characteristic difference in colour between an alkaline and an acid medium increases its reliability, whilst the reaction is an extremely sensitive one. It must,

1) OBERMAIJER u. POPPER, Wiener Klin. Wochenschr. 1908.

STEIGER, Dissert. Zürich 1911.

GILBERT. See for his works the bibliography in: Clinique médicale 1910/1911.

2) AUCHÉ, Compt. rend. Acad. d. Sciences 1908.

3) BIFFI, Folia Haematolog. 1906 III. 189.

4) HIJMANS VAN DEN BERGH and SNAPPER, Deutsch. Arch. f. klin. Med. 1913.

however, be admitted that neither this nor any other colour-reaction enables us to identify the presence of bilirubin with absolute certainty. The possibility of other substances contained in the serum giving the same reaction with the diazo-body may be esteemed less probable, it cannot with absolute certainty be denied.

As far as we know it has hitherto been found impossible to isolate bilirubin from normal human serum, which would have afforded an incontestable proof of its presence. HAMMERSTEN has attempted it¹⁾. But though in a great majority of cases he obtained fine bilirubin-crystals from horse-serum, he never succeeded in obtaining them from normal human serum. From the terms used in the latest edition of his Handbook of Physiological Chemistry we gather that this investigator is not quite convinced yet of the presence of bilirubin in normal human serum.

With a view to researches on anhepatic bilirubin-formation we needed a method which would enable us to identify with absolute certainty the presence of small quantities of bilirubin in bloodserum, exsudates and transsudates, if possible by obtaining the pigment in the form of crystals. After some experiments we have succeeded in this, starting from the property of bilirubin — which we have not found mentioned anywhere — of dissolving readily in acetone.

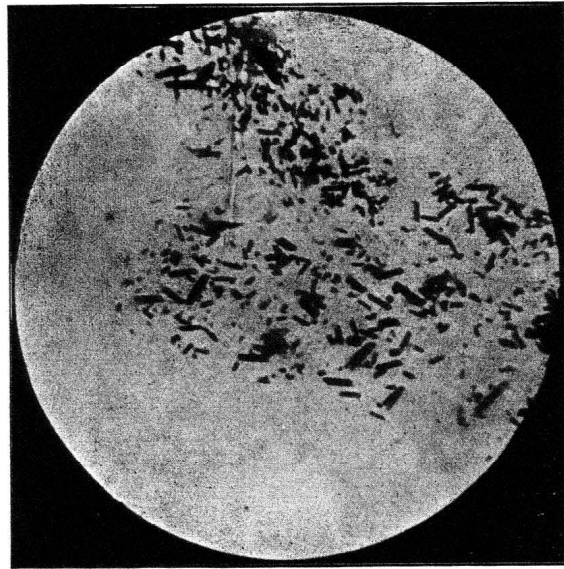
To 10 cm³. of bloodserum 20 cm³. of pure colourless acetone are added. An albumen precipitate is formed, which is centrifugalized. The fluid at the top, coloured more or less intensely yellow, contains all the bilirubin and only traces of albumen. This liquid is evaporated in vacuo at the ordinary laboratory temperature.

If one has a good vacuum-pump at one's disposal the liquid soon begins to boil; after some minutes the acetone is evaporated. A watery fluid remains in which, besides other serum-substances, all the bilirubin is dissolved. Then the fluid is shaken 2 times or more with aether to remove the fatty bodies as much as possible.

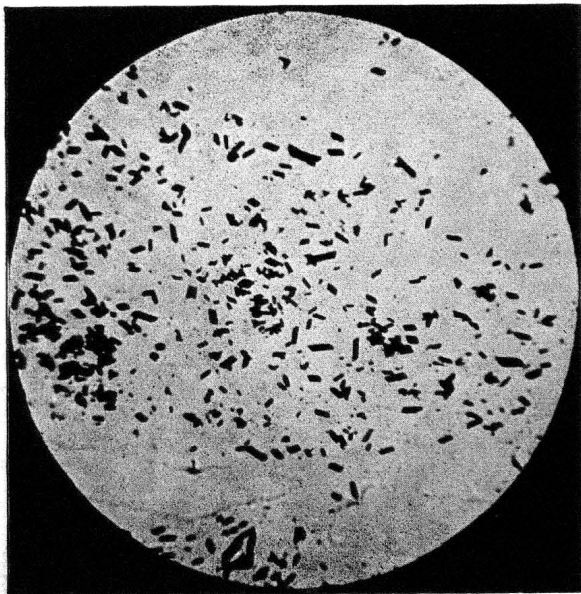
These pass into the aether which is pipetted. The last traces of aether are removed in vacuo. The aether may of course also be removed by means of a separatory. Then a certain amount of chloroform e.g. 2 cm³. are added, the fluid is slightly acidified with HCl and shaken. The bilirubin then passes into the chloroform. By centrifugalization the watery fluid can easily be separated from the chloroform. The chloroform is washed thoroughly with water to get rid of all the hydrochloric acid and centrifugalized once more, the water being removed by means of a separatory or by pipetting. Traces of water, however, remain mixed with the chloroform, which sometimes renders

¹⁾ HAMMARSTEN. Maly's Jahresber. 1878 II. 119.

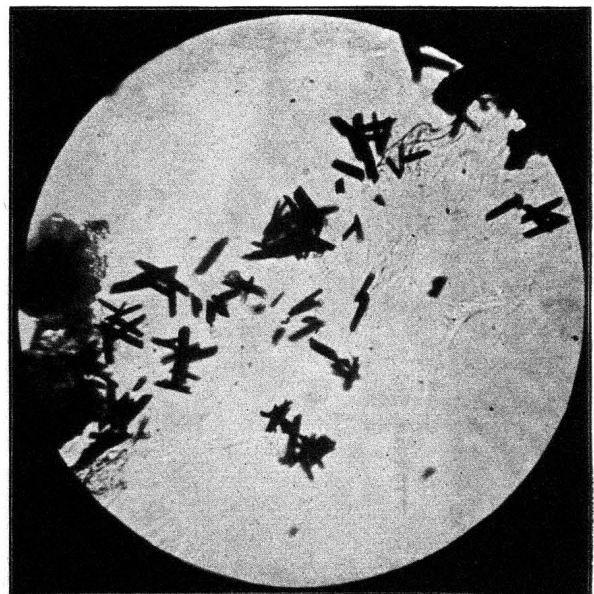
A. A HIJMANS VAN DEN BERGH and J. J. DE LA FONTAINE SCHLUITER. „The identification of traces of bilirubin in albuminous fluids.”



Bilirubin from human bloodserum (Chloroform-method).



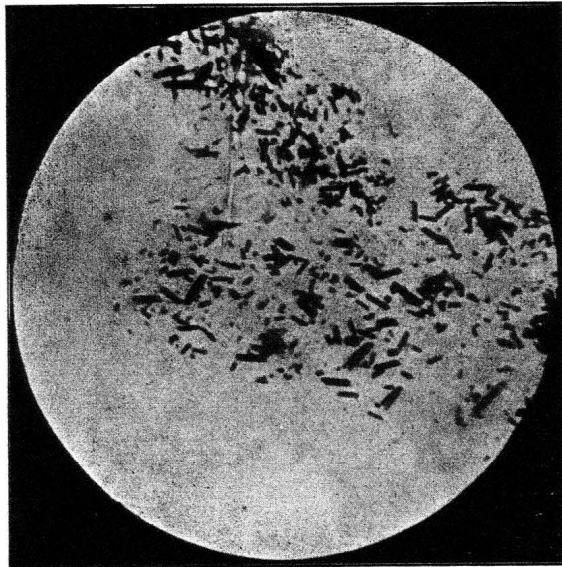
Bilirubin from human ascites fluid (Aether-method).



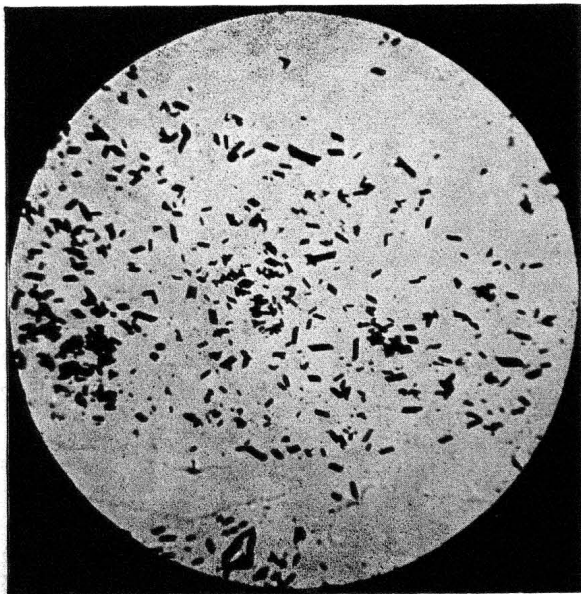
Bilirubin from human ascites fluid (Chloroform-method)

Proceedings Royal Acad. Amsterdam, Vol. XVII.

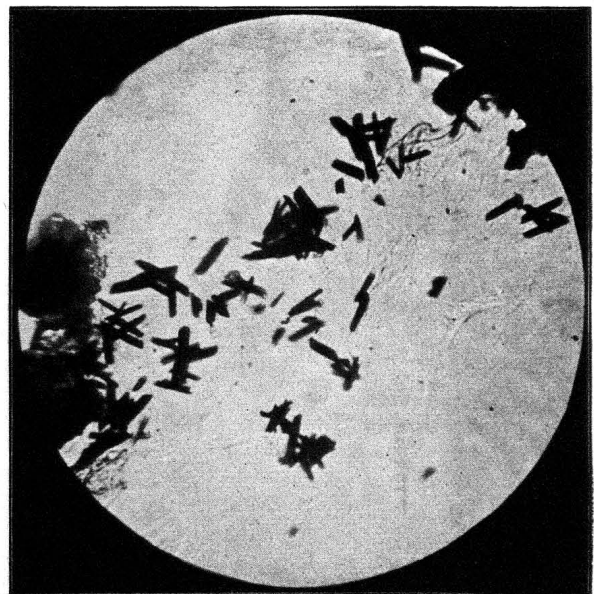
A. A HIJMANS VAN DEN BERGH and J. J. DE LA FONTAINE SCHLUITER. „The identification of traces of bilirubin in albuminous fluids.”



Bilirubin from human bloodserum (Chloroform-method).



Bilirubin from human ascites fluid
(Aether-method).



Bilirubin from human ascites fluid
(Chloroform-method)

Proceedings Royal Acad. Amsterdam, Vol. XVII.