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ADDITIONAL NOTES ON THE DIAZO-REACTION.

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THE fact that our use of chemical tests of the urine as important elements of diagnosis is practically limited to the recognition of albumin, sugar, and bile-pigment, has led many investigators to seek for new reactions that may be found in the urine of pathologic cases, in the hope of finding tests having definite diagnostic importance. So, when Ehrlich, about ten years ago, announced his diazo-reaction as a test having diagnostic significance in the early stages of typhoid fever, and of prognostic importance in pulmonary tuberculosis, it was hoped that a new and valuable reaction had been added to the meager list already in our possession. But, since that time, the many workers who have investigated the reaction have arrived at the most diverse results regarding its occurrence and value, and at this date the significance of the reaction cannot be said to have been finally determined. The matter has been brought to notice so many times that it may now seem trite; but when we consider

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our poverty in urine-reactions of diagnostic value, and the immense gain to be obtained by the discovery of any new test of a possible aid in diagnosis, the subject will bear constant revival, until the worth of the reaction has been settled beyond all doubt.

Reviewing briefly the literature, we find, first, Ehrlich's announcement of a new test, discovered by making use of the well-known property of the diazo-compounds to form color-bodies with aromatic substances, and the application of this principle to the testing of urines, by means of a reaction-mixture containing a small amount of nascent sulphodiazo-benzol. When equal parts of the reagent and urine were mixed, and ammonia added, there was obtained a yellow color in normal urine, but in many pathologic urines a red color, which by shaking showed most markedly in the foam, and after standing deposited a green pigment. From his experiments with this reaction, Ehrlich arrived at certain conclusions, the most important of which were, that the reaction is one of the most constant signs of typhoid, and of both diagnostic and prognostic importance in that disease, and a sign of bad omen in pulmonary tuberculosis.

These observations were confirmed by Fischer, Brecht, and Löwinson; but opposed on the other hand by Penzoldt and Petri, who stated that the reaction occurred in normal urine, and in many pathologic conditions, and hence did not have diagnostic value. Penzoldt believed that the color-changes were quantitative, and not qualitative; he did not consider the behavior of the foam, and concludes that

the reactions were caused by substances present in both normal and pathologic urine, but not appearing in typhoid in such characteristic manner as to have diagnostic worth. He also discovered that the reaction was unusually brilliant in the urine of diabetes.

These results were answered by Ehrlich, who claimed that they were due to a misapprehension of the reaction, and resulted from poor technique. He therefore stated more exactly the composition of the mixtures used, and described the technique. He further declared that milk or grape-sugar did not give the reaction.

He was then sustained by Escherich, who found the red foam reaction only in typhoid and morbilli, and a purple-red reaction in diabetic urine. Penzoldt, after working again on the more definite lines laid down by Ehrlich, came a second time to the conviction of the worthlessness of the reaction as an element of diagnosis, concluding that the bodies that produce the reaction are of a different kind, and are not yet known, and that the color-changes are not qualitative.

Georgiewsky agreed with Ehrlich, speaking of the reaction as carmine or purple-red, especially marked in the foam. Spiethoff also upheld the diagnostic value of the test, but limited the reaction to those cases only in which a green precipitate is observed. Petri again, following Ehrlich's method, investigated the significance of the reaction in tuberculosis, and found it to bear no relation to the course of the disease. The same conclusion was reached by

Brehmer, while Grundies constantly found it to be a bad sign in this disease.

Brewing found the reaction to be of diagnostic and prognostic importance in four conditions, typhoid fever, pulmonary tuberculosis, puerperal conditions, and concealed septic processes, such as liver-abscess; but that in typhoid it is of diagnostic value only in connection with the other symptoms. Rütimeyer considered the reaction diagnostic in typhoid. Bacnacci believed that the test had some relation to sugar and acetone, and stated that thymol, strychnine, and iodol will produce the reaction. Simon considered the appearance of the foam as a characteristic feature of the reaction, and found it only in typhoid and pulmonary tuberculosis.

Edwards considered only the color of the ring formed at the junction of the NH_4OH, and the mixture of urine and reagent, and found a red color to be independent of any disease or group of diseases, and untrustworthy as a diagnostic sign in typhoid. He concluded that the reaction must be caused by many different substances. Finally, Munson and Oertel have just announced that they have discovered diacetic acid to be the cause of the reaction, and that its diagnostic importance depends, therefore, upon the relation of this substance to disease, which has not been found to be of great importance.

From the widely varying results of different investigators, and the consequent difference of opinion regarding the clinical value of the test; and from the fact that several recent text-books have mentioned

the test as a probable sign of typhoid fever, I have been led, by a suggestion from Dr. Dock, to make, during the past year, systematic investigation as to the occurrence of the reaction. To that end, in the Laboratory of Clinical Medicine over 1500 diazo-tests were made, not counting many quantitative variations of the test in the same urine. These covered 400 cases, and included a great variety of chronic internal diseases, many acute diseases, numerous surgical conditions, and a small number in which the urine was normal. In certain cases the tests were made daily for several weeks. I was thus enabled to obtain a certain amount of definite clinical knowledge regarding the test, and to draw certain conclusions concerning its value and occurrence.

The method used was the prescribed one. Two solutions were prepared in separate bottles: Solution No. 1, consisting of hydrochloric acid, 50 c.c., distilled water to equal 1000 c.c., and saturated with sulphanilic acid; solution No. 2, consisting of a ½ per cent. solution of sodium nitrite. When desired for use, 40 c.c. of solution No. 1 is mixed with 1 c.c. of solution No. 2. Equal parts of this mixture and urine are then mixed in a test-tube, and ammonia added, so as to form a layer above the mixed urine and reagent. At the junction of the two layers the colored ring is formed, which, when varying from an eosin or carmine to a deep-garnet color, has been styled the diazo-reaction.

After noting the color of the junction-zone, I carried the test further by shaking up the mixture in the test-tube, and noting the appearance of the

foam. In this way I found early that urines giving a typical diazo-reaction in the color-ring could be divided into two distinct classes after the shaking-up process, viz. : Urines giving an eosin-pink foam of greater or less intensity, not quickly fading, and urines giving colorless foam, or a color quickly fading. This division of the reactions I found to be exceedingly important when considering the diagnostic value of the reaction.

Edwards gives a review of 600 tests. Acording to his article, the color-ring only was regarded, a hue from eosin to garnet being recognized as the Ehrlich reaction. No attention was paid to the characteristics of the foam, or to any division of the reactions, so far as any peculiar behavior of the color in the foam is concerned. In my work I quickly came to the conclusion that this very striking characteristic of the foam was the most important feature of the reaction ; and upon it alone could be based the claims of the reaction to any diagnostic significance.

In my tests, proceeding upon the same basis as Edwards, that is, calling a ring of color varying from eosin to garnet the Ehrlich reaction, I arrived not only at the same conclusions obtained by him as to its widespread occurrence in disease, but I also found it in the urine of healthy persons and in the urine of persons suffering from minor surgical conditions, but otherwise healthy. Obtaining this reaction in health, in chronic diseases of heart, lungs, kidneys, and stomach, in rheumatism, acute and chronic enteritis, febricula, tonsillitis, measles, scarlatina, etc., and in minor surgical conditions, I

could only confirm his observations, that it was a reaction of frequent occurrence, bearing no relation to any disease or group of diseases or to any peculiar condition, and hence of no diagnostic importance.

But the foam-color reaction was so striking when obtained as to leave no doubt as to its being a reaction of definite peculiarity and significance, and I came to consider it alone as the typical diazo-reaction, throwing out all other urines, in which the foam did not show a pink or eosin tinge, even though the ring showed the typical shades of color. The color of the ring and foam of the accepted reaction varied from rose-pink to deep cherry-red, never showing any violet hue, the foam always retaining the color until settled. In no case did I observe a green precipitate; this may be explained by the fact that the test-tubes were probably not left standing long enough for the pigment to settle.

Why, in the case of two urines giving precisely the same ring-color, the foam of one should present the color, and that of the other be colorless I cannot presume to answer. It was suggested that probably the presence of mucin played a part, but this was found not to be the case, for the removal of the mucin did not affect the behavior of the foam. The reaction is analogous to the behavior of two dark urines, one containing bile-pigment, the other colored by uric acid and urates. On shaking the bile-containing urine a permanent yellow foam results; the other gives a colorless foam.

I found that by accepting the foam-test as the reaction, I greatly limited its occurrence, finding it only in those conditions mentioned by Ehrlich and

others whose work tended to confirm his investigations. According to the standard mentioned, making the foam-test the reaction, I found positive results only in the following :

Typhoid fever. Case I. The reaction was present from the ninth day to the twenty-first. Relapse occurred in the fifth week. Reaction again present.

Case II. Reaction from sixth day to sixteenth.

Case III. Only one test, made in second week. Reaction present.

Case IV. Reaction present in second week.

Case V. Reaction present from the fourth day to the seventeenth.

Tuberculosis, advanced pulmonary. Eight cases. Reaction almost constantly present. One case of incipient pulmonary tuberculosis, without physical signs. Bacilli were found in minute pus-bits in the sputum. The patient had been treated for malarial fever. The reaction was brilliant, varying in relation to patient's symptoms.

One case of tuberculous iliac disease was complicated with empyema, and exhibited a typhoid state two weeks before death. Reaction was brilliant during this period. No intestinal lesions existed.

One case of enlarged axillary glands removed as new-growths, stated by Dr. Gibbes to be tuberculous. Reaction was constantly present.

Carcinoma. Two cases of carcinoma of stomach.

Sarcoma. One case of retro-peritoneal sarcoma. These three cases were in extreme stages of exhaustion, and in a condition of intoxication resembling the typhoid state. Death occurred soon after coming under obervation. The reaction was brilliant in all three cases, and constantly present.

Osteomalacia (case to be reported). An unexplained rise of temperature occurred, lasting several

days, during which time a brilliant reaction was present.

Nephritic abscess. One case. Reaction present for two days after removal of the diseased kidney.

Pleurisy and bronchiectasis. One case, showing a weak reaction.

Chronic ulcerative enteritis. One case, the patient very cachectic. Reaction existed at intervals.

Auto-intoxication (?) resembling the so called diabetic coma. Urinalysis gave no proofs of the existence of sugar, acetone, or diacetic acid. Marked typhoid condition was present from entrance on the eighth day of illness to death, four days after. Patient was comatose during the last two days. Brilliant reaction was present from entrance to death. This was the most intense reaction of any that I have seen. Post-mortem examination was absolutely negative as to lesions.

Acute tonsillitis. In a surgical case with pyelitis, and a temperature of 106°, brilliant reaction existed for three days. Other cases of uncomplicated tonsillitis as severe gave no reaction.

Erysipelas. One case. Moderate reaction existed for four days from the beginning of fever.

In five cases of febricula, one in which typhoid was suspected at the beginning, but did not develop, and in one case of perityphlitic abscess, resembling typhoid, the foam-reaction was not obtained. In three cases of diabetes no reaction could be obtained; and I have never been able to obtain a similar reaction with glucose, as Penzoldt claimed to have done.

This division of the test into two classes, according to the behavior of the foam and the importance of the foam-test, do not seem to have been recognized by all who have investigated the reaction;

and it is probable that from this arises in part the conflicting results obtained by so many workers. The experimenters who have considered the importance of the color in the foam would find the reaction limited in its occurrence very nearly to the conditions mentioned by Ehrlich and those who have confirmed his results, such as Simon in this country; while those, like Penzoldt, Edwards, etc., who have considered the ring-color chiefly, or alone, would find the reaction in the whole catalogue of disease —even in health—and in consequence would draw different conclusions as to the diagnostic worth of the reaction.

Faulty technique may also give rise to incorrect conclusions, for, as Penzoldt and Petri have shown, an increased strength of nitrite solution deepens the color of the mixture. The amount of this reagent used according to Ehrlich's method should be 1 c.c. of a ½ per cent. solution. In the recent paper of Munson and Oertel, a 5 per cent. solution of sodium nitrite was used. From my tests, urines giving a yellow ring with Ehrlich's solution would give a red color with the stronger solution—the foam, however, not being affected.

Since the appearance of Munson and Oertel's paper, claiming aceto-acetic acid to be the cause of the reaction, I have had three urines giving the diazo-reaction, and these did not give the reaction for diacetic acid with ferric chlorid, and two other urines giving a typical Ehrlich reaction had been tested with ferric chlorid, and did not give the Bordeaux-red color obtained when diacetic acid is present. One of these, as mentioned, gave a most

intense diazo-reaction. The reaction in these five urines was, therefore, not caused by diacetic acid.[1]

Further, in direct contradiction of the work of Munson and Oertel, it was impossible by the test, or by any modification of it, to obtain with aceto-acetic acid, made for my tests by Dr. Freer, of the University Chemical Laboratory, who has done important work in that field of organic chemistry, a reaction in any way resembling the diazo-reaction. Only a light-yellow color, with colorless foam, and, with strong nitrite, orange-color could be obtained. The potassium salt of the acid gave the same reaction. I cannot explain this contradiction, but from the fact that five diazo-urines gave no ferric-chorid reaction for diacetic acid, and that specimens of the acid itself, prepared by a well-known authority in organic chemistry, gave no diazo-reaction, I cannot agree with the conclusion of Munson and Oertel that aceto-acetic acid is the cause of the reaction, and must believe that the agent is as yet unknown. Thymol, iodol, strychnine, sugar, and acetone, do not give the reaction.

In conclusion, I should say that the reaction in which the foam presents the characteristic color is the only striking and important reaction obtained by the method. The cause of the reaction is as yet unknown, or it is produced by more than one substance. It bears a certain relation to a condition of disease. Typhoid fever is rare here, and from our five cases a general conclusion could not be drawn;

[1] Since the above was written, I have had two cases confirming the belief that diacetic acid plays no part in the production of the positive test.

but it occurred in every case, and in such a manner as to be looked upon as a sign. Moreover, the reaction was not found in cases in which, at first, the diagnosis from typhoid was doubtful.

The reaction was found in only one-fourth of our cases of tuberculosis, but in those patients in whom the constitutional symptoms were strongly marked.

The common feature of all of the cases in which the reaction occurred was a more or less pronounced typhoid state. The test, when positive, seemed to be a distinct feature of a condition of auto-intoxication, characterized by weakness, lassitude, headache, stupor, fever, etc., and varied in proportion to the condition, the most brilliant reaction being obtained in the fatal case of coma.

It is reasonable, therefore, to infer that the test bears some relation to an unknown chemical agent playing a part in the production of a state of intoxication similar to the typhoid state.

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