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THE DIAZO REACTION OF EHRLICH.*

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SINCE Ehrlich † first introduced his "new urinary test," the diazo reaction, into clinical medicine it has been the object of much interest, for it is simple and easily carried out and of great diagnostic value. Many objections were at first offered, but these were soon proved to depend upon errors in the performance of the test.

Year by year the reaction has gained new adherents, so that it is now considered a very efficient diagnostic measure in most of the clinics of Germany and of many other countries.

In recent years new objections have been raised, and it is the main object of this paper to show that these are unwarranted.

The diazo reaction depends upon the principle that if sulphanilic acid (amidosphobenzol) is acted on by HNO_2 ,

* Read before the Clinical Society of Maryland, November 17, 1893.

† Ehrlich. *Charité Annalen*, viii. Jahrgang, und Ueber eine neue Harnprobe, *Zeitschrift für klinische Medizin*, Bd. v, 1882.

diazosulphobenzol is formed, which unites with certain aromatic substances (of the urine) to form aniline colors.

To obtain diazosulphobenzol in a fresh condition, sulphanilic acid is kept in solution with hydrochloric acid; to this sodium nitrite is added, whereupon HNO_2 is liberated and diazosulphobenzol is formed.

The reaction is carried out in the following manner:

Two solutions are prepared:

(a) Two grammes of sulphanilic acid; fifty cubic centimetres of hydrochloric acid; one thousand cubic centimetres of distilled water.

(b) Five tenth-per-cent. solution of nitrite of sodium.

In order to perform the reaction, fifty parts of *a* and one part of *b* are mixed, and equal parts of this reagent and of urine placed in a test tube and saturated with ammonia. In those cases in which the reaction is positive the solution assumes a carmine-red color, which, on shaking, must also be visible in the foam. If the test tube is allowed to stand twenty four hours, a greenish precipitate is found.

Ehrlich* arrived at the following conclusions:

1. The reaction is most commonly found in typhoid fever from the middle of the first week; and that whenever the reaction is missing the diagnosis appears doubtful.

2. Cases of typhoid fever in which the reaction is slight and only found for a short time are usually very mild.

3. In phthisis pulmonalis the reaction is a grave prognostic sign. The reaction is sometimes, but rarely, found in measles, miliary tuberculosis, pyæmia, scarlet fever, and erysipelas.

4. In all diseases unaccompanied by fever—such as chlorosis, hydræmia, diabetes, brain, spinal, liver, and kidney diseases—the reaction is never obtained.

* Ehrlich. *Loc. cit.*

These postulates of Ehrlich's were verified by his pupils and others.

Fisher * obtained the reaction constantly in measles and very frequently in typhus fever. This proves that the substance causing the reaction can not bear any relation to the chemical changes going on in the typhoid intestine, as such are entirely absent in these diseases.

Brecht † corroborated the statements of Ehrlich. He found the reaction in seventeen cases of typhoid fever, two cases of measles, and rarely in pneumonia. The appearance of the reaction in pneumonia is to be regarded as of grave significance.

In other non-febrile diseases the reaction could not be obtained.

Löwinson, ‡ after making numerous investigations, especially in pulmonary tuberculosis, came to the following conclusions, which entirely accord with those of Ehrlich—namely:

The appearance of the reaction in tuberculosis is a *signum mali ominis*. It is not dependent upon the fever. Marked reactions lasting for a long time (one to two months) are only found in tuberculosis, and especially in the most rapid forms.

Cnopf # and Grundies || arrived at the same general conclusions concerning tuberculosis.

* Fisher. *Die Diazoreaction bei Pneumonie, Morbillen und Typhus exanthemat.* Dissert., Berlin, 1883.

† Brecht. *Die diagnostische Bedeutung der Diazoreaction.* Dissert., 1883.

‡ Löwinson. *Ueber die Ehrlich'sche Diazoreaction, in besonders bei Lungenphthisie.* Dissert., 1883.

Cnopf. *Diazoreaction und Lungenphthisie.* Dissert., 1887.

|| Grundies. *Mittheilungen über Diazoreact. bei Phthise pulm.* *Zeitschrift für klinische Med.*, 1884.

Escherich,* See, † and Dorendorff ‡ also corroborated Ehrlich's investigations.

Goldschmidt * found the reaction constantly in miliary tuberculosis and typhoid fever, and remarks that the reaction is of great value in the diagnosis between typhoid fever and gastro-intestinal catarrhs running their course with fever. He did not find the reaction in influenza.

Spiedhoff || made experiments to discover the cause of the red diazo reaction, and found that neither diacetic acid nor æthyldiaceticæther give the true reaction. In order to obtain a reaction with these substances, stronger solutions (NaNO_2) are required. He therefore warns against the use of too strong reagents in performing the ordinary diazo reaction. He considers the reaction of great prognostic and diagnostic importance in tuberculosis and typhoid fever. He states that true reactions show the green precipitate.

Loewe ^ finds the reaction to be one of the most constant of the signs of typhoid fever, and absence of the reaction he believes is sufficient to make the diagnosis doubtful. Of thirty-one cases of typhoid fever, he missed the reaction only in four.

Georgiewsky ◇ admits the high prognostic value of the reaction.

* Escherich. Zur diagnostische Bedeutung der Diazoreaction. *Deut. med. Woch.*, 1883, No. 48.

† See. *De la phthisie bacillaire des hommes*, Paris, 1884, pp. 327-330.

‡ Dorendorff. *Die diagnostische und prognost. Bedeutung der Diazoreaction*. Dissert., 1884.

* Goldschmidt. Ueber die diagnost. Werth der Diazoreaction. *Münch. med. Woch.*, 1886, No. 85.

|| Spiedhoff. *Ueber Ehrlich's Diazoreaction*. Dissert., 1884.

^ Loewe. *Ueber Auftreten der rothen Diazoreaction Ehrlich's*. Dissert., 1888.

◇ Georgiewsky. Die neue Harnprobe Ehrlich's. *Deut. med. Wochenschrift*, 1883, No. 45.

Brewing,* who made twenty-five hundred examinations, finds the reaction of great diagnostic and prognostic value in typhoid fever, phthisis, puerperal affections, and concealed septic processes.

Roessingh † and Piering ‡ likewise confirmed Ehrlich's statements.

Rütimeyer,§ after a series of exact experiments, came to the following conclusions :

1. The diazo reaction is of great diagnostic value in typhoid fever; next to the enlargement of the spleen and roseola, it is one of the most constant and earliest signs.

2. The diagnosis of typhoid fever gains much certainty if the diazo reaction be present; if, on the contrary, the reaction is absent during the first and second weeks of the illness, the disease is not typhoid, or only a very light form.

3. Gastro-intestinal catarrhs accompanied by fever never give the reaction.

4. The reaction bears no relation whatever to the height of the fever and is not influenced by various medicinal or therapeutic measures (baths, etc.). Morning urine and evening urine give reactions of equal intensity.

5. If the reaction terminates in the second or third weeks of the disease an early fall of the fever and a mild course of the disease may be expected, but long continuance of the reaction indicates a severe and lengthy course of the disease.

6. Marked and continuous reactions do not, however,

* Brewing. *Zeitschrift für klin. Medicin*, Bd. x, p. 561.

† Roessingh. Die Ehrlich'sche Reaction. *Deutsche med. Wochenschrift*, 1883, No. 33.

‡ Piering. Ueber die Ehrlich Harnprobe. *Prager Zeitschrift für Heilkunde*, No. 1, 1885.

§ Rütimeyer. Zur klinische Bedeutung der Diazoreaction. *Correspondenzblatt für schweiz. Aerzte*. Jahrg. xx (1890).

in any way indicate whether the disease will terminate fatally or favorably.

7. In relapses we almost always get a renewed reaction if the reaction has disappeared before the relapse occurs.

Simon * considers the appearance of the foam as characteristic of the reaction and finds the reaction only in typhoid fever and tuberculosis. Of thirty cases of typhoid fever, the reaction was present in twenty-six.

C. Gerhardt † states that in his clinic during five years, the reaction was absent in but one certain case of typhoid fever; on the other hand, he has found the reaction present in non-febrile cases of typhoid fever, numerous cases of which were at the Charité in the spring of 1891.

Von Norden's ‡ experience at Gerhardt's clinic coincides with the statements of Ehrlich. The rise of temperature as such has no marked influence on the appearance of the reaction, for even severe cases of phthisis, running their course without fever, show marked reactions for weeks. In phthisis a continuous reaction denotes special severity of the case and makes the prognosis unfavorable. In typhoid fever the reaction is so constantly present at the beginning and during the course of the disease that we must be doubtful of our diagnosis of typhoid when the reaction is absent.

It is not unlikely that in those rare cases of gastro-intestinal catarrh in which certain authors detected the reaction the disease was really non-febrile typhoid fever.

Pape # made observations regarding the diagnostic

* Simon. *Johns Hopkins Hospital Bulletin*, November, 1890.

† C. Gerhardt. Ueber fieberlos. verlauf. Darmtyphus. *Charité Annalen*, xvi, 213, 1891.

‡ Von Norden. *Lehrbuch der Pathologie des Stoffwechsel*, S. 217.

Pape. *Ueber die diagnostische Verwendbarkeit der Diazoreaction bei chirurgischen Affectionen*. Dissert., 1892.

value of the diazo reaction in surgical tuberculous affections, and found that after every operation the reaction which had previously been present disappeared—in some immediately after the operation, usually within three to five days.

Warthin * found the reaction in only a few diseases and arrives at conclusions fully in accord with those of Ehrlich. He points to the importance of relying only on the red color in the foam.

Notwithstanding the many observations showing the diagnostic and prognostic value of the diazo reaction, certain writers make statements quite contrary to those of Ehrlich.

Penzoldt † and Petri ‡ were the first to attack Ehrlich's conclusions. They deny that any marked difference existed in regard to the reaction between healthy persons and patients, even those having fever. It is not necessary to prove here the error into which these writers had fallen.

Ehrlich himself showed that these authors used solutions of sodium nitrite much too concentrated. Concentrated solutions produce a pseudo-reaction, which may even be so marked as to cover a true reaction. The reaction should always be made with weak solutions of sodium nitrite (one half per cent.).

It is remarkable, however, that, though these objections have been so thoroughly answered by Ehrlich and the reaction has been proved to be of great value, von Jaksch, § in the new edition of his *Clinical Diagnosis*, "disclaims for the test any clinical importance whatever, and especially

* Warthin. Additional Notes on the Diazo Reaction. *Medical News*, May 27, 1893.

† Penzoldt. Ueber den diagnostischen Werth der Diazoreaction. *Berl. klinische Wochenschrift*, 1883, 14 and 49.

‡ Petri. *Zeitschrift für klinische Medizin*, Bd. vi, S. 742.

§ Von Jaksch. *Klinische Diagnostik*. 3te Auflage, S. 400. English translation, 1893, p. 328.

enjoins the necessity of avoiding inferences based upon the appearance of the reaction."

He believes that the color when obtained is always due to the presence of acetone. He bases his conclusions on the old Penzoldt and Petri statements and on a discussion before a medical society* without bringing forward any personal investigations on this subject. His conclusions can not be accepted, for—

1. Von Jaksch does not perform the reaction correctly. After adding sulphanilic acid and sodium nitrite to the urine, he does not saturate with ammonia.

2. Because he states that Ehrlich recommends the addition of five to six volumes of absolute alcohol to the fluid to be tested, and then drop by drop the reagent. Ehrlich recommends this procedure only for his bile test, not for this urinary test.

3. It is impossible to demonstrate the presence of acetone by means of this reaction, performed according to the method of Ehrlich, as I have already shown in the first part of this paper. I have frequently convinced myself of this fact.

Of still less value is the article of Monson and Oertel,† who endeavor to identify the substance producing the reaction as diacetic acid and to show that the reaction is the same as that obtained by means of Gerhardt's iron test. If Monson and Oertel had read the experiments of Spiedhoff,‡ which were made under Ehrlich's direction, they would have learned that Ehrlich long ago pointed to the fact that diacetic acid and ethyl diacetate gave a reaction with the diazo reagent; that, however, a stronger solution of the reagent was needed (NaNO_2), and that the red color was

* Von Jaksch. *Prager med. Wochenschrift*, 16, 94, 1891.

† Monson and Oertel. *New York Med. Journal*, Feb. 4, 1893.

‡ Spiedhoff. *Ueber Ehrlich's Diazoreaction*. Dissert., 1884.

heightened with ammonia or even caustic potash. More characteristic, however, is its relation to mineral acids, for, while small quantities turn the red color into yellow, large quantities change the yellow into a violet.

Warthin* has also proved that the substance occasioning the reaction must be quite different from diacetic acid.

In answer to the statements of authors, such as Edwards,† who find the reaction accompanying all forms of diseases with or without fever, we assert that the reaction has been wrongly performed by these writers.

1. Very weak reagents (one half per cent. NaNO_2) must be used.

2. The alcohol test is not to be employed.

3. A positive reaction is only one in which the red reaction is present in the foam.

My own observations with the diazo reagent includes over three thousand reactions.

Twenty-one cases of typhoid fever were examined; the reaction was absent in but one case. I subjoin a synopsis of the typhoid-fever cases in which the reaction was used:

CASE I.—First examination was made on the fifth day of the disease: reaction was present daily until the twenty-second day. The case ended in recovery.

CASE II.—First examination on the fourth day: reaction present on the fifth day and continued until the twenty-sixth day.

CASE III.—First examination on the fifth day: reaction present and continued until the twentieth day.

CASE IV.—First examination on the eighth day: reaction present until the nineteenth day.

CASE V.—Only two examinations were made: reaction present on the tenth and twelfth days.

* Warthin. Additional Notes on the Diazo Reaction. *Med. News*, p. 569, 1895.

† Edwards. *Medical News*, April 2, 1892.

CASE VI.—First examination on the sixth day: although daily examinations were made, the reaction was not present until the eleventh day; it continued from then on until the fifteenth day. This was an extremely mild case.

CASE VII.—Only one examination made: reaction present on the eleventh day of the disease.

CASE VIII.—First examination on the fifth day: reaction present until the eighteenth day.

CASE IX.—First examination on the third day: reaction first present on the fifth day and continued until the fifteenth day.

CASE X.—First examination on the sixth day: reaction present from then on daily until the nineteenth day.

CASE XI.—First examination fifth day: reaction present and continued till the twelfth day, on which the patient died.

CASE XII.—First examination second day: the reaction was found on the fourth day and continued until the nineteenth.

CASE XIII.—First examination on the eighth day: the reaction was present, and continued until the fourteenth day.

CASE XIV.—First examination third day of the disease: reaction found on the fourth day and continued until the twenty-fourth day.

CASE XV.—First examination on the fifth day: reaction then present and continued until the twentieth day.

CASE XVI.—First examination fifth day of the disease: reaction present on the seventh day and continued until the twelfth day.

CASE XVII.—Only one examination made: reaction present on the seventh day of the disease.

CASE XVIII.—First examination eighth day of the disease: reaction not present; repeated examinations were made daily for ten days. The case was exceedingly mild, with a temperature never above 102.5° F.

CASE XIX.—First examination fifth day of the disease: reaction first present on the seventh day and continued daily until the twenty-second day.

CASE XX.—First examination on the fourth day of the disease: reaction present and continued until the fourteenth day.

CASE XXI.—First examination on the fourth day of the disease: reaction first observed on the fifth day and continued until the twentieth day.

Case.	Day of first examination.	Day of first appearance of reaction.	Day of last appearance of reaction.	Remarks.
1	5th day of disease.	5th	22d	
2	4th " "	5th	26th	
3	5th " "	5th	20th	
4	8th " "	8th	19th	
5	10th " "	10th	Twelve days also reaction; only two examinations made.
6	6th " "	11th	15th	
7	11th " "	11th	Only one reaction made.
8	5th " "	5th	18th	
9	3d " "	5th	15th	
10	6th " "	6th	19th	
11	5th " "	5th	12th	
12	2d " "	4th	19th	
13	8th " "	8th	14th	
14	3d " "	4th	24th	
15	5th " "	5th	20th	
16	5th " "	7th	12th	
17	7th " "	7th	Only one reaction made.
18	8th " "	Reaction not present.
19	5th " "	7th	22d	
20	4th " "	4th	14th	
21	4th " "	5th	20th	

From these observations in typhoid fever it may be concluded (1) that the reaction is very constant in this disease; (2) that it makes its appearance usually within the first week; and (3) that the reaction gradually disappears between the end of the second and third weeks.

Examinations were made in forty-three cases of pulmonary tuberculosis. Of these, twenty-nine were severe cases, with almost constant reaction; fourteen were light forms which did not show the reaction. Of the twenty-nine severe cases, twelve died while still under observation;

eight are still under observation in an unimproved condition.

The presence of the reaction in this disease, extending over long periods of time, may therefore be regarded as a grave sign.

The reaction was also found to be present in three cases of erysipelas (for several days), in one case of bone abscess (tuberculous) in one case of liver abscess, in one case of suppurative glandular disease of the neck, in one case of tubercular hip-joint disease, in one case of tubercular spinal disease, in one case of pneumonia (very grave case—died), in one case of carcinoma ventriculi, and in one case of septicæmia.

The reaction was never obtained in healthy individuals; it was not found in four cases of cerebral disease, eight cases of spinal-cord disease, five cases of lung disorder excluding tuberculosis (six cases of pneumonia), four cases of heart disease, two cases of abscess of the liver, ten cases of cirrhosis of the liver, nine cases of gastric disorder (seven cases of cancer of the stomach), twelve cases of gastro-intestinal catarrh with fever, thirty four cases of gastro-intestinal catarrh without fever, seven cases of dysentery, nine cases of nephritis, eight cases of bladder disorders, sixteen cases of other genito urinary disorders, ten cases of rheumatism, eleven cases of diabetes (four of which gave Gerhardt's diacetic-acid reaction), five cases of abscess, five cases of hysteria, two cases of epilepsy, two cases of neuritis, eight cases of syphilitic disorders, three cases of skin disease (one case of lupus), two cases of diphtheria, two cases of erysipelas, twenty-one cases of malaria, three cases of sarcoma, five cases of carcinoma, and nineteen cases of injuries of various kinds.

My own observations, therefore, entirely confirm those of Ehrlich. In conclusion, I shall again emphasize Ehrlich's statements :

1. The diazo reaction is of great diagnostic value in typhoid fever.

2. If the case shows a slight or no reaction between the fifth and eighth days, other appearances pointing to typhoid fever, it can be looked upon at once as an exceedingly light form and the prognosis made accordingly.

3. Gastro-intestinal catarrhs accompanied by fever always run their course without a reaction.

4. Very marked and constant reactions may accompany mild forms of typhoid fever and do not justify a bad prognosis.

5. Reactions appearing continuously for a long time in phthisis pulmonalis (two months) always indicate a grave prognosis.

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FRANK P. FOSTER, M.D.

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