THE DIAZO REACTION OF EHRLICH

Its Value as a Factor in Clinical Diagnosis.

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I propose to divide the consideration of this subject into three headings:-

(1) History and Nature of the Reaction.

(2) Clinical applications of the Reaction by various observers.

(3) General conclusions.

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(1) History and Nature of the Reaction.

The researches of various chemists in the early and middle parts of the nineteenth century had established the fact that substances of the diazo group - that is, substances of the Benzene group containing two atoms of nitrogen (the French term "azote"-nitrogen being used) - in the presence of certain bodies of the aromatic series occasionally found in the urine, gave rise to aniline colours which became apparent under the influence of the alkalies.

It was found that such a diazoic substance (diazo-sulpho-benzol) could be conveniently produced

by acting upon an amido-sulpho-benzol (Sulphanilic Acid) with nitric acid.

Ehrlich,[1] in 1882, first conceived the idea of utilising this property, which diazo-sulpho-benzol possessed of producing aniline colours with certain aromatic substances occasionally found in the urine, as a means of clinical diagnosis.

In his first researches he employed a solution of sulphanilic acid, nitric acid and nitrite of sodium but later on, owing to the instability of the diazo-sulpho-benzol, he found it better to divide his reagents into two solutions, one containing sulphanilic acid and the other nitrite of sodium.

He was early struck with the imperative importance of a standardised formula for the two solutions and the proportions in which they were to be mixed - without which the proper comparison of results by various observers would be impossible and thus, in 1884, he published the following:-

Solution A.

Acid. Sulphanilic, ...5 parts.
Acid. Hydrochloric, ...50 parts.
Aqu. Distill., ...1000 parts.

Solution B.

Sodium Nitrite, ... 0·50 parts.
Aqu. Distill, ... 100 parts

50 c.c. of A. were to be mixed with 1 c.c. of B. to form the reagent.

The nitrous acid given off by the action of the hydrochloric acid on sodium nitrite changed the sulphanilic acid into diazo-sulpho-benzol.

This reagent mixed in equal parts with a urine containing the aromatic body or bodies requisite gives rise to the formation of aniline colours in the presence of an alkali - Ehrlich prefers ammonia.

The chemical method of using the test as usually employed in England is as follows:-

Two solutions (A.), a saturated solution of sulphanilic acid in 5 per cent hydrochloric acid:

(B.), a ½ per cent solution of sodium nitrite:

Both solutions should be as fresh as possible.

Add to some urine in a test tube an equal quantity of A. Then add two drops of B. Render alkaline with a few drops of Liquor Ammon: Fort: and shake until a froth appears. If the urine becomes of a red colour while the froth is also red the reaction is a positive one.

This red may be of varied tints - port-wine colour, cherry-colour, salmon-colour. Ehrlich has formulated four different shades which he calls:-

R.3 scarlet)
R.2 cherry)- all positive.
R.1 vermillion)
R.m which has a little orange in it - doubtful.

In the present state of our knowledge it is better to reject as negative all the orange reactions.

It is often very difficult to distinguish between the deeper shades of orange and the fainter reds - here the colour of the froth is of valuable significance and unless it be of a distinct red tinge the reaction should not be accepted as a positive one. This may often require a somewhat fine colour-sense to discriminate and in a very doubtful case it is better to allow the test tube to stand for twenty-four hours until a precipitate is formed. This precipitate is of a green colour if the reaction be positive (according to Escherich. Deut. Med.Woch. 1883, Mehlenfeldt, Wratch, 1886: and Spiethoff, Inaugural Dissertation, Berlin, 1884).

Proescher (Deut. Med. Woch: Dec. 3rd, 1903), states that the urine contains a substance which gives a reaction in normal as well as pathological conditions, but that the red colour is always pathological.

In the clinical performance of Ehrlich's test it is of very great importance for the observer to maintain a rigid technique - since varying proportions of reagents produce varying tints and proper comparison of results and even reliance on the evidence of the test thus become impossible.

Many researches have been made on the interesting question as to what is the nature of the body or bodies in the urine which gives the reaction with

diazo-sulpho-benzol, but unfortunately with no definite result up to the present time beyond eliciting the fact that it is almost certainly a member of the aromatic series which is the causal factor in the reaction.

Papenheim considers this causal substance to be Urobiline but Proescher (Deut. Med. Woch: Dec. 3rd, 1903), contends that he has proved that the reaction does not take place with any of the known constituents of urine. He considers that the substance causing the reaction is probably an acetyl-glucosamine. He finds that the pento-acetyl-glucosamine, which can be obtained by acting on glucosamine with acetic acid does not give the colour change; but it drives off some of the acetyl by crystallisation and then the remaining fluid gives the reaction typically. Whether this remaining fluid is a mono- or di-acetyl-glucosamine is still undecided.

Ehrlich also asserts that none of the known constituents of urine give the reaction. Experiments with urea, uric acid, creatin, creatinin, taurin, glycocholic and oxalic acids, the fatty acids, sugar and albumen have all given negative results.

MM. Loeper and Oppenheim (Gazette des Hôpitaux 25th May, 1901), have made systematic examinations for indican in urines giving positive diazo reactions but have found no apparent correlation.

The nature of the coloured product of the reaction is still unknown. The green-coloured precipitate of the positive reaction is held by Ehrlich and the majority of investigators of the subject, to be an oxidation product of the unknown coloured substance.

The source of the unknown aromatic substance producing the reaction has been the subject of many theories:-

(a) It has been advanced by several authorities that, since the presence of indican - sure indication of intestinal decomposition - and the diazo reaction are so often co-existent in the urines of those suffering from tuberculosis and enteric fever, the unknown substance, which combines with diazo-benzo-napthol to produce the reaction, is possibly a product of decomposition of intestinal contents.

MM. Loeper and Oppenheim oppose this view of its origin. They examined a series of nearly 300 cases of intestinal fermentation - copraemias, gastric and intestinal catarrhs and the like, in most of which indican was present in the urine but in none did they obtain the diazo reaction.

(b) It has been suggested that it is a product of the breaking down of caseous material or pus, but this is negatived by the absence of the reaction in the urines of cases of empyema, abscess of lung, osteomyelitis and like suppurative processes.

(c) As to whether the substance may be a toxin produced by bacteria various experiments to produce the reaction with cultures of Eberth's and other bacilli have given negative results. Moreover it has been shown that the pleuritic and ascitic fluids which are tubercular in origin do not give any more positive reaction with diazo-benzo-napthol than the ascitic and pleuritic fluids due to backward pressure in cardiac and allied conditions.

The view of MM. Loeper and Oppenheim is that the unknown substance is a product of some morbid metastasis of the albuminoid constituents of the cells of the human body produced by the action of certain pathogenic bacteria.

A very important consideration in connection with the diazo reaction is the possibility of substances, taken medicinally by the mouth, on being excreted by the urine, combining with diazo-sulphobenzol to give a positive result and thus occasioning fallacies in the clinical employment of the test.

Dr. Beringer (Boston Med. & Surg. Journal, July 13th, 1893), found the following drugs give a positive reaction:-

Creosote and Phenol (even in most minute quantities), the sulpho-carbolates of soda and zinc, Betanapthol, Thymol, and Tannic acid.

Dr. Hewlett (British Med. Journal, Aug. 24th, 1895), states that morphia, even in as dilute a solution as 1 in 5000, produced a positive reaction.

MM. Loeper and Oppenheim obtained a positive reaction from the urine of patients to whom they administered experimentally creosote, guiacol, or anti-pyrin.

As many of the above drugs are given in just those cases in which the Ehrlich's test is most frequently employed it is most important to exclude the possibility of their presence before relying on the evidence of a positive reaction.

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(2) Clinical Applications of the Reaction by various Observers.

A positive diazo reaction scarcely ever appears except in disease of an infectious and febrile nature.

(A) From normal urines it is consistently absent, (Ehrlich, Georgiewsky, Nissen).

(B) In Apyretic morbid conditions,

Hèze (Arch. prov. de Med., 1900), has the following results:-

90	cases	of diseases of alimentary canal, ...	1	positive	reaction
31	"	" hepatic affection, ...	2	"	"
102	"	" nephritis, etc., ...	7	"	"
165	"	" syphilis, ...	1	"	"
198	"	" various surgical affections, ...	0	"	"

MM. Loeper and Oppenheim's series:-

39	cases	of chronic affections of nervous system, ..	0	positive	reaction
40	"	" affections of circulatory system, ...	0	"	"
55	"	" chronic bronchitis and emphysema, ...	0	"	"
42	"	" chronic nephritis, ...	0	"	"
17	"	" chlorosis, ...	0	"	"
10	"	" lead poisoning, ...	0	"	"
7	"	" diabetes, ...	0	"	"
15	"	" cirrhosis of Liver, ..	0	"	"
13	"	" malignant disease, ...	2	"	"

These two positive reactions were in cases in the last stages of malignant cachexia, a condition in which many observers have found the reaction to occur.

(C). The reaction in certain Febrile conditions.

In Pneumonia it is fairly common.

Hèze cites:-	260	cases with	80	positive	reactions.
Georgiewsky[2]:-	221	" "	39	"	"
Loeper and Oppenheim:-	21	" "	2	"	"

In acute Bronchitis:

Nissen[3] finds it consistently absent.

In acute Rheumatism:

Rivier[4] cites	50	cases	with	1	positive	reaction
Loeper and Oppenheim,	48	"	"	1	"	"

In Epidemic Influenza:

Rivier has	7	cases	with	0	positive	reaction.
Heze "	24	"	"	1	"	"
Loeper and Oppenheim:-	53	"	"	6	"	"

In Malarial Fever:

A positive reaction frequently occurs (Osler).

(D) The Reaction in Enteric Fever:

Ehrlich and the first observers of the Diazo reaction have stated that it is in this affection that it is present in greatest frequency, intensity and duration.

Rivier in 550 cases obtained 534 positive reactions. He states that the reaction appears between the second and the sixth day of the disease, and continues intensifying progressively in colour until it reaches its highest development in the second week, then gradually declines and in cases of moderate severity disappears during the third week more or less following the temperature curve.

According to this observer if the reaction reappears after having entirely disappeared, it means that a relapse is occurring or about to occur.

If, on the contrary, the temperature remains high after the disappearance of the reaction, it is evidence that the condition is due to a complication caused by some secondary infection.

Dr. A. R. Edwards of Chicago (Med. News, April 2nd, 1902), in sixty-three cases in which the exact appearance and disappearance of the reaction was noted, found that the average life of the test was 13 days, although in some cases it persisted until the fiftieth day. He found that the intensity and duration of the reaction bore no relation to the severity of the disease.

Osler mentions 776 cases of Enteric with 543 positive reactions.

MM. Loeper and Oppenheim's results.

30 cases first examined between	3rd & 8th day	28	positive reactions.
28 " " " "	8th & 15th "	26	

From the results of many cases which they have been enabled to watch during the whole course of the disease they have come to the conclusion that the reaction becomes apparent very early on the third day or possibly earlier: that it gradually declines about the end of the second week - its persistence to the fifteenth day indicating, in their opinion, a severe form of the disease.

I, myself, in the Edinburgh City Hospital, examined the reaction systematically during the course of the disease in 40 cases - these cases all having been confirmed by Widal's reaction.

In these 40 cases -

Of reactions	taken	in	1st	week	35	were	positive.
"	"	"	2nd	"	37	"	"
"	"	"	3rd	"	6	"	"
"	"	"	4th	"	0	"	"
"	"	"	5th	"	1	"	"

In the one case in which the reaction was found in the fifth week, after having been absent for more than a week, a relapse occurred two days after the re-appearance of the reaction.

I could not find that the brilliancy and duration of the reaction bore any relation to the severity of the disease. I was also enabled, through the kindness of Dr. Ker, to examine the charts of enteric cases for several years back and from them to tabulate the following rough result:-

Of 111 cases examined at some period within the first 14 days of the disease 96 gave positive diazo reactions. All these cases gave Widal's reaction.

(E). The Reaction in Tubercular Diseases.

In acute Tuberculosis, especially in acute general Tuberculosis, the reaction is almost always present.

In acute general Tuberculosis:-

Rivier in 57 cases obtained 57 positive reactions.

Clemens[5] " 73 " " 73 " "

Loeper & Oppenheim 6 " " 6 " "

I have tried the test myself in two cases both of which gave marked positive reactions.

MM. Loeper and Oppenheim in 16 cases of tubercular pleurisies obtained 11 positive reactions.

In the more chronic forms of Tuberculosis the presence of the reaction is much more uncertain. It is not infrequently entirely absent during the whole course of the disease and when it does occur it is usually only in the advanced and final stages of the illness.

(F). The Reaction in Typhus Fever.

In this condition it is agreed by all observers that a positive reaction can always be obtained.

(G). The Reaction in the Exanthemata.

In Scarlet Fever: The reaction is frequently seen but its presence is by no means constant (Loeper and Oppenheim). It does not make its appearance until the rash is fully developed. Rivier, in 29 cases, obtained 12 positive reactions and, as a rule, not well marked and of short duration.

Loeper and Oppenheim in three cases had two positive reactions. I, myself, in 52 cases obtained 23 positive reactions. Most of these positive reactions in my series were in severe cases with either high temperature or complications such as adenitis, but not all. And there were one or two severe cases, with high temperature and with complications, such as adenitis and nephritis, which failed to give the reaction throughout their course.

In all the cases in which the reaction was positive it appeared during the first week, usually the second or third day, and generally gradually disappeared during the second week. But in one case, and that of no special severity and without complications, I could still obtain a positive result during the third week. And in another with adenitis and a persisting temperature the reaction could be elicited occasionally as late as the fifth week.

In Small Pox.

Sergent (Bull. Med., Feb., 1901), cites 40 cases with 40 positive reactions, 9 of these at the period of eruption, 31 at the period of pustulation. The reaction disappeared entirely when the scabs dried up.

In Measles.

The Diazo reaction is stated by all observers to be almost constantly present, appearing before or with the rash and increasing in intensity until the third day, disappearing with the fading of the rash.

In the eight cases in which I had the opportunity of testing the reaction I did not by any means find it constantly present. On the contrary, in these eight cases I only obtained three positive reactions. It must be said, however, that most of these cases were of a very mild type with scarcely any temperature, though one rather severe case, complicated with suppurative ademitis, failed to give the reaction throughout.

In Rötheln. The reaction is stated to be consistently absent.

In four cases in which I tried it I obtained no positive reaction. They were all cases of a simple type with practically no temperature. I confess that I should be glad to have the opportunity to thoroughly assure myself by personal observation that the reaction is consistently absent from those occasional cases of Rötheln of a severe type with a high temperature - those forms of the disease, in fact, in which the differential diagnosis of Rötheln usually becomes most difficult.

(H). The Reaction in Various Infectious Diseases.

IN Diphtheria. In 32 cases of this disease I tested the diazo reaction most carefully during every stage and never obtained a positive reaction. Several of the cases were of great severity and terminated fatally.

This bears out the dictum of the majority of observers that it is extremely rare to get a positive reaction in Diphtheria.

In Antitoxin Rashes.

In two rashes occuring during the course of diptheria and presumably due to antitoxin, I applied the diazo test without result. One of the rashes was of urticarial type, occurring fourteen days after the injection of antitoxin, profuse and covering the body but unaccompanied by pyrexia. The rash lasted three days, on none of which could a reaction be obtained.

The second rash was a general, profuse, scarlatiniform one, occurring seven days after antitoxin, and accompanied by a high temperature (103°-104°). On the second day of the rash suppression of urine occurred and the patient died. On neither of the two days of the duration of the rash could a positive reaction be obtained. This rash may possibly have been of a septicaemic nature but I am inclined to

think that it was antitoxic in origin as I have often observed similar rashes accompanied by pyrexia which were undoubtedly due to this cause, though, fortunately, they did not possess the serious characteristics of this one.

M. Lobligeois, in a paper at the Société de Pediatrie, Paris, May, 1901, states that the reaction is never found in eruptions due to serum medication, whether scarlatiniform or otherwise.

In Erysipelas.

I have personal experience of the reaction in twelve cases and obtained a positive result in three. These three were of a severe type. In one of them, after the positive reaction had been present for two or three days, while the erysipelatous eruption and temperature were at their height, it gradually disappeared when the redness faded and the temperature came down and then, after a day or two's complete absence, it suddenly reappeared again, the temperature being subnormal at the time and there being no sign of eruption. The next day a recrudescence of the disease occurred.

Coste (Th. de Paris, 1899), states that a positive reaction is exceptional in mild cases of erysipelas, but is frequently present in severe forms of the disease. He lays great stress on the sudden disappearance of the reaction after having been markedly present as indicating the gravest consequences.

(3) General Conclusions.

Having reviewed the various morbid conditions in which the Diazo-reaction has been tested by the principal observers and the results attained, it remains to consider the clinical value these results possess and to attempt to estimate the place the reaction occupies among the many new aids to accurate diagnosis at the disposal of the modern physician.

This may be examined under two principal heads:

(a) The value of the reaction as a means of Diagnosis.

(b) The value of the reaction as a means of Prognosis.

(a) As a Means of Diagnosis.

(1) In Typhoid Fever the condition in which the reaction has hitherto been most frequently made use of.

It is very unfortunate that in this disease - the one above most others in which we should welcome a valuable addition to our means of diagnosis - a positive diazo-reaction cannot be held to have much value as a confirmatory test. It fails where it is most keenly wanted because, although, as we have seen, it occurs so consistently in enteric fever, yet it also occurs as frequently in acute general Tuberculosis, a disease from which the differential

diagnosis of enteric is commonly most difficult, - in Typhus Fever, - and is by no means uncommon in Pneumonia which, in its early stages, often closely simulates enteric. In many other febrile conditions which are liable to be confounded with Typhoid Fever, such as septicaemia, uraemia, and malaria a positive reaction is often found.

Dr. Edwards of Chicago (Med. News, April 2, 1902) ranks the value of a positive diazo reaction in Typhoid Fever on a par with iliac gurgling and tenderness, but below the temperature, roseola, and splenic enlargement.

On the other hand, a negative reaction in Enteric Fever may be held to have a distinct diagnostic value. As the positive reaction is so consistently present in this disease in the first two weeks, "if a negative reaction be continually obtained from the fifth to the tenth day of a febrile disease it may be definitely considered that the case is not one of enteric," (Masbrenier, Th. de Paris, 1900).

Personally, from the experience of my forty cases in which the positive reaction was absent in five cases in the first week and three in the second, I consider this dictum somewhat too positive; at the same time I agree that the consistent absence of a positive reaction during the first two weeks of a disease constitutes strong presumptive evidence against its being enteric.

(2) In Tuberculosis. MM. Loeper and Oppenheim sum up the diagnostic value of the reaction as follows:- In a subacute or chronic affection in which the presence of tuberculosis is doubtful (cavity in the lung, pleural or peritoneal effusion, meningitis), the absence of the reaction is without precise significance: its presence, on the other hand, constitutes strong evidence in favour of tuberculosis.

Nissen considers that the sudden appearance of the reaction in a case of acute bronchitis which has passed into broncho-pneumonia is very suspicious of tuberculosis.

(3) In Diphtheria.

A positive reaction is very consistently absent in this condition. The failure to obtain a positive reaction in an affection of the throat would have no special significance: the presence of a positive reaction, on the other hand, would be strong evidence against its being diphtheria.

A research as to the consistent presence or absence of the reaction in a series of cases of the various acute affections of the throat, other than diphtheria, would be a most useful contribution to our knowledge of this subject and might definitely increase the value of the diazo reaction in the differential diagnosis of diphtheria.

(4) In Antitoxin Rashes. In those forms of serum-caused eruptions in which there is often a profuse scarlatiniform rash, frequently a high temperature, and occasionally an anginous condition of the throat - forms which it is very difficult, and, in a fever hospital, of the greatest importance - to distinguish from the onset of Scarlatina, I consider that we have in the Diazo reaction an agent of great diagnostic value. The reaction is consistently absent in these rashes and therefore the presence of a positive result in such cases as the above would be sufficient to prove that the rash was not due to antitoxin and to justify the instant removal of the patient to an isolation ward.

(5) In Rötheln. As the reaction is stated to be consistently absent in this condition, in the differential diagnosis between Rötheln and Measles or Scarlatina a positive reaction would negative Rötheln; a negative reaction would have no significance.

(6) In Measles and Scarlatina. In the differential diagnosis between either of them, and the various skin eruptions (erythemata, drug-rashes, etc.), simulating them, the presence of a positive reaction may be held to exclude the latter.

(b) As a means of Prognosis.

(1) In Typhoid Fever. The intensity and duration of the reaction is no evidence of the severity of the disease.

The reappearance of the reaction during the course of the disease after an absence of some time is generally a sign that a relapse is occurring or about to occur. Great care, however, must be taken not to confound a relapse with complications, such as lobar pneumonia, tuberculosis, etc., which also might be the cause of the recrudescence of the reaction.

(2) In Erysipelas. A positive reaction signifies that the disease is of a severe form. The reappearance of the reaction, after having disappeared and been absent for some days, would be a probable prognostic of a recrudescence of the disease or of the advent of some complication.

According to Coste the sudden disappearance of the reaction after having been present for some days, is of the very gravest import and is significant of a fatal termination.

(3) In Malignant Disease. The reaction is commonly absent during the course of the disease and its appearance is a sign of the last stages and a herald of approaching death.

(4) In Tuberculosis. The great majority of observers agree that the presence of the reaction in the urine of tubercular patients, especially if at all constantly manifested, signifies either a very acute form of the disease, or, in the more chronic and apyretic forms, the advent of cachexia and the near presence of the end.

I have endeavoured, in this summary of clinical observations on the Diazo-reaction of Ehrlich and their results, to show to some extent the position which it may be allowed to occupy as a factor in diagnosis.

It must be admitted that in many respects the test is a disappointing one. Its presence in so many different morbid conditions, many of them just those conditions which we so often desire to be able to distinguish one from another, and also the irregularity of its presence in so many of the diseases in which it does occur, greatly damage its value as a positive confirmatory piece of diagnostic evidence.

But, in spite of this, from the mass of evidence upon the subject, certain valuable properties of the test, it appears to me, are found to emerge:-

(a) The diagnostic value of a negative reaction in excluding the probability of enteric fever.

(b) In the same disease the power the test gives us of forecasting (taking care always to exclude the presence of complications), the advent of a relapse.

(c) In the distinction of Antitoxin rashes from Scarlatina or other exanthemata, of such vital importance in a Fever Hospital.

(d) If the consistency of its absence in cases of Rötheln, as vouched for by so many observers, can be relied on, the test should be of great value in the differential diagnosis in this often puzzling disease.

(e) As a prognostic of grave danger in Erysipelas.

(f) Its prognostic significance in chronic tuberculosis and malignant disease.

For these properties alone the test should be worthy to be retained as one of the important means of diagnosis at our disposal.

It is so easy to apply and so marked in characteristics that it seems worthy of still more extended systematic application, which might elicit data greatly enhancing its value. It might be found that the lesser degrees of the reaction - the orange

tints, which are so marked, but which are at present discarded as being of no positive significance, also possessed their value as diagnostic or prognostic adjuncts.

I have already made mention of two directions in which it appears to me further systematic research might be made and possibly increase the value of the test. One is the determining of the consistent absence of a positive reaction from the severer forms of Rötheln. The other a systematic application of the reaction to the various affections of the throat which are liable to be confused with diphtheria.

A routine application in hospitals of the reaction to every class of disease, over a certain length of time, would, in all probability, produce a few, at least, of data enhancing the value of the Diazo reaction and leading to its being employed as a means of clinical diagnosis considerably more widely than it is at present.

I take here the opportunity of expressing my very sincere thanks to Dr. C. B. Ker of the City Hospital, Edinburgh, to whose kindness I owe access to the major part of my clinical material.

Additional

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