Readings.—The controls should show a uniform opalescence, with no granules. In reading the test proper three forms of nomenclature are used for each tube: Positive (P) where the fluid shows clearing with definite granules (giving a hailstorm appearance); Doubtful (D) when there is some granulation but no clearing of the fluid; and Negative (N) where the fluid shows no trace of any granulation. A reading is taken of each of the three tubes of each test and recorded as PPP, DDD, or NNN, etc. Where one tube differs from the other two the majority is accepted, thus: PDD is marked as doubtful, DNN as negative; whilst a reading such as PDN would be called "doubtful." The amount of precipito-agglutination does not depend on the amount of the antigen, since frequently the tube containing the smallest amount of antigen will show the most marked granulation.

In all cases the second reading is accepted as the decisive one. In fact, the preliminary reading may quite well be dispensed with, since it seems to serve no useful purpose.

The main modification introduced by the authors into Kahn's technique were as follows:

1. The use of a hand lens instead of the naked eye, which makes the reading easier and probably gives a more delicate result.

2. The use of artificial light and a specially prepared dark background.

3. The interpretation of results. It appeared to the authors that it would be more satisfactory to decide whether each tube were positive, doubtful, or negative, and take the results of the three together, than to attempt to estimate the degree of precipitation in each tube and record it with a varying number of + signs. For example, PPP, PPD, PPN would indicate that the test was positive; DDD, DDP, DDN, PDN that it was doubtful; NNN, NNP, NND that it was negative.

In this series of tests two antigens were used; both gave the same titrations, but with the second the readings were clearer and gave less "doubtful" reactions. This may be due to it having been more freshly prepared, the first one being three months old.

ANALYSIS OF 500 TESTS.

Groups in which there was complete agreement.	No. in Group.
1. Kahn positive, W.R. positive	151
2. Kahn negative, W.R. negative	230
3. Kahn doubtful, W.R. doubtful	8
Total	389
Groups in which there was some disagreement.	
4. Kahn positive, W.R. negative— In favour of Kahn's test	20
Apparently false positive Kahn	1
5. Kahn positive, W.R. doubtful— Syphilitic, under treatment	18
Probably syphilitic	1
6. Kahn doubtful, W.R. negative— Syphilitic, under treatment	39
History of syphilis	5
Probably not syphilitic	9
Chancreoid 12 days old	1
7. Kahn negative, W.R. doubtful— Syphilitic, under treatment	9
No clinical evidence of syphilis	2
8. Kahn doubtful, W.R. positive (all syphilitic)	3
9. Kahn negative, W.R. positive	1
10. Cases of anticomplementary blood in W.R.— Kahn test negative (non-syphilitic case)	1
Kahn test positive (case of treated syphilis)	1
Total	111

Note.—The 20 cases in Group 4 in favour of Kahn's test were either under treatment or gave a history of old syphilis; the patient with an apparently false positive Kahn defaulted, so that it was not possible to investigate his case further. Of the 9 cases in Group 6 which were probably not syphilitic 2, on repeating the Kahn test, showed a negative result. The patient in Group 9 was syphilitic; she was two months pregnant, and had been treated with stabilarisan.

From the above analysis it will be seen that there was complete agreement in 389 cases (77.8 per cent. of the total), and some disagreement in 111 (22.2 per cent.). Of the 111 showing disagreement, 24 tests (4.8 per cent. of total) were in favour of the Wassermann reaction, and 87 (17.4 per cent.) in favour of Kahn's test.

Conclusions.

It will be seen that the test is simple and easy and can be carried out after very little practice by anyone who has had a medical training. The apparatus and materials are inexpensive. The only difficulty is the preparation of the antigen. Only three reagents are required—physiological saline solution, antigen, and patient's serum. We have used racks holding four rows of twelve tubes each (one row being for the patient's serum), and find we can set up eleven tests with controls in ten minutes. Allowing five minutes for each reading and five minutes for the two periods of shaking, eleven tests can be carried out in twenty minutes plus one hour for incubation. The test is therefore particularly applicable where small numbers of tests have to be done or a corroborative test is required. By using larger racks (to hold 96 or even more tubes) more tests can be

carried out together, with a corresponding saving of time. The results (except for one possible "false positive"—see table, Group 4) compare very favourably with those of the Wassermann test. Of the 111 which disagreed, 87 (or 17.4 per cent. of total) were in favour of Kahn's test, whilst only 24 (or 4.8 per cent. of total) were in favour of the Wassermann test.

We would point out that whilst a "doubtful" Kahn's test is of little value in an untreated case of syphilis it would appear to be distinctly valuable in treated syphilis (see table, Group 6). It will be noticed (Group 10) that in two cases the Kahn test gave an unequivocal result where the serum was so anticomplementary that the Wassermann test failed.

A test which can be carried out by any medical practitioner in his surgery with so little apparatus deserves an extended trial. It does not seem to have attracted much attention in England, but in America it has been compared with the Wassermann test by a number of workers. For example, J. A. Kolmer⁵ found the Kahn test better on the whole in treated cases, and also in anticomplementary cases. Out of 1,000 cases there was agreement in 904 and disagreement in 96, the balance being in favour of Kahn's test. Litterer⁶ found the agreement with the Wassermann test surprisingly accurate. W. Levin,⁷ in a series of 258 serums, found complete agreement in 86.4 per cent., relative agreement in 12.8 per cent., and complete disagreement in 0.8 per cent. Sobei Ide and G. S. Smith⁸ think that the value of Kahn's test is almost as great as that of the Wassermann test. A series of 2,165 comparative tests gave the following results:

	Wassermann.	Kahn.
Positive	396	423
Negative	1,769	1,742

C. C. Young⁹ recommends that Kahn's test should be used in combination with the Wassermann.

Our thanks are due to Colonel L. W. Harrison, D.S.O., Director of the V.D. Department at St. Thomas's Hospital, for permission to publish the results of these tests.

Since writing the above some 500 more tests have been carried out. These confirm and emphasize the results given in the foregoing table.

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A COLORIMETRIC METHOD FOR THE ESTIMATION OF DIASTASE IN BODY FLUIDS.

BY

I. COHEN, B.Sc.LOND.,

ASSISTANT IN BIOCHEMISTRY;

AND

E. C. DODDS, M.B., B.S., B.Sc.LOND.,

CHEMICAL PATHOLOGIST TO AND LECTURER IN BIOCHEMISTRY,
MIDDLESEX HOSPITAL.*

(From the Biochemical Department of the Bland-Sutton Institute of Pathology, the Middlesex Hospital.)

THE estimation of urinary diastase has been of clinical interest from the time it was first determined by Wohlgemuth in 1908. Since the publication of this observer's original results a great deal of work has been carried out on the technique of diastase estimation, and on the clinical value of the results so obtained. It is with the former that this paper is concerned.

In all the methods brought forward, the diastatic action is measured by the starch-splitting power of the solution containing the ferment. Either the amount of diastase solution required to digest completely a known amount of starch is measured by means of an achromic point method (Wohlgemuth¹), or else the products of starch digestion (maltose) are determined (Lovatt Evans,² Fyfe³). The former methods have found the greater popularity, undoubtedly owing to their simplicity.

* On behalf of the Medical Research Council.

In the original technique and its subsequent modifications a series of tubes is put up containing constant quantities of starch solution and decreasing quantities of urine. The volume is then made constant throughout the series, and the whole incubated for a definite space of time. Iodine solution is then added to each tube, and the one in which the starch has just disappeared is noted, and from the quantity of urine contained in it the diastatic index is calculated. The Wohlgemuth index is defined as the number of cubic centimetres of 0.1 per cent. starch solution digested by 1 c.cm. of urine. It was not until quite recently that account was taken of the effect of varying hydrogen-ion concentration of the solution on the diastatic power; it has been shown that this factor may produce very marked variations.

In simultaneous and independent publications Sladden⁴ and one of us (E. C. D.⁵) described modifications of the original method designed to overcome this difficulty. Sladden brings the reaction of the urine to a constant figure by the addition of acid or alkali, whilst we dilute the urine with a phosphate buffer solution at a pH of 6.1—a figure arrived at by determining the optimum pH of urinary diastase phosphate compound.

By means of these two modifications the difficulty of varying hydrogen-ion concentration (of the diastase-containing urine) was overcome, but there still remained some very grave disadvantages to the method. As performed by any of the methods mentioned above the possible readings run:

6.6, 10, 20, 22, 25, 28, 33, 40, 50, 66, 100, 200 units.

If values intermediate between these are required, elaborate dilutions are necessary, and in any case it is impossible to obtain anything but an approximate idea of the diastatic value. Again, the colour reaction of iodine with the dextrans produced by the action of the diastase on the starch makes the reading difficult by masking the blue starch-iodine coloration. At present the diastase estimation is only considered of diagnostic value when either very low or very high readings are obtained, and for such cases it must be admitted that the values quoted above are sufficiently accurate, but for research purposes they are far from adequate, and it was not found possible to modify the existing methods to give accurate readings.

The second group of methods mentioned in the introductory paragraph were investigated, but without success. In the case of blood this type of method appears to be of doubtful value, owing to the possibility that other enzymes, such as maltase, may react with the end-product. The measurement of starch digestion by the estimation of maltose produced was employed by Lovatt Evans and others in conducting experiments on salivary diastase. Although satisfactory results can be obtained with saliva by these methods, they prove unsatisfactory when applied to urine and blood, owing to the very small amount of ferment present. Polarimetric methods were tried, but were given up on account of the many complications introduced by mixtures of intermediate compounds derived from starch each having a different rotating power.

After a long series of experiments with various new methods, it was decided that the most accurate, and yet a simple means of estimating diastase in small quantities, was by employing erythrodextrin as the substance to be acted on by the enzyme. Diastase converts this substance into maltose; now if it could be proved that the red colour given by the addition of iodine solutions to dextrin was moderately permanent and proportional to the latter, an easy and at the same time accurate colorimetric method of estimating diastase could be devised. For such a colorimetric method it was essential to get a substance which after being acted upon by diastase gave end-products which produced no colour with iodine; and such is the case with erythrodextrin.

It was found that the iodine-dextrin colour was sufficiently stable for colorimetric comparison in the presence of ammonium sulphate, and also that the colour varied directly with the quantity of erythrodextrin present. A technique was elaborated, the principle of which consisted in adding buffered urine to a definite volume of dextrin solution, incubating for a stated time, adding an iodine-ammonium

sulphate solution, and comparing the colour so produced with that of a standard solution of dextrin in a colorimeter. From the readings the percentage of dextrin can be calculated, and, by subtraction, the amount of dextrin used up in the time can be calculated. That this factor must bear a direct relation to the Wohlgemuth unit is obvious, although the exact method of converting the dextrin factor into units is very difficult. Some sort of mathematical relationship can be worked out, but this does not hold good in practice.

After many trials it was decided to calibrate the dextrin method against the Wohlgemuth system. A series of diastatic indices in urines was estimated by both methods, and a chart was drawn showing the relationship between the two readings. It is, of course, impossible to obtain a smooth curve in this manner, owing to the large possible errors in the Wohlgemuth method. Thus a urine giving a reading of 100 units by the existing method might have an actual diastatic value which was 90 per cent. above or 30 per cent. below this figure. By a series of experiments in which the urine was diluted a series of samples was obtained, the diastatic index of which was definitely proved to be 6.6, 10, 20 units, etc. Estimations were performed in these urines by the dextrin method, and the results were included in the curve. The curve reproduced represents the mean of a series of values.

Curve to show Relation between Units of Diastase and Grams of Dextrin used up.

Technique of the Method.

The following solutions are required:

1. *Buffer Solution.*—This is made by mixing 15 c.cm. of Sorensen's phosphate solution A with 85 c.cm. of solution B. The resulting solution should have a pH of 6.1.

Solution A: 11.876 grams of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ dissolved in 1 litre of boiled distilled water.

Solution B: 9.078 grams of KH_2PO_4 dissolved in 1 litre of boiled distilled water.

The solutions are kept in paraffin-coated bottles, and a drop of toluene is added to prevent the growth of moulds.

2. *Standard Dextrin Solution.*—0.75 gram of pure dextrin is dissolved in a small volume of boiled distilled water, and the volume is made up to 100 c.cm.

3. *Iodine Solution:*

N/10 iodine in potassium iodide solution	...	20 c.cm.
Saturated ammonium sulphate solution	...	500 c.cm.
Distilled water	...	to 1,000 c.cm.

Method.

1.5 c.cm. of urine are pipetted into a tube and 6 c.cm. of buffer solution are added. The mixture is well shaken. 0.5 c.cm. of this buffered mixture is pipetted into a test tube graduated at 30 c.cm., and 2 c.cm. of the standard dextrin solution are added. After thorough shaking the tube is incubated in a water-bath at 37° C. for half an hour. Whilst the incubation is proceeding the standards are prepared. If the approximate diastatic power of the urine is not known, it is as well to prepare three standards in order to ensure accurate comparison in the event of very high or low readings. These are made up by pipetting 1.5, 1.0, 0.5 c.cm. of standard dextrin respectively into tubes graduated at 30 c.cm.

When the incubation period is complete the tube is removed from the water-bath, and 15 c.cm. of the iodine solutions are added to it and to each of the three standards. In highly coloured urine it is perhaps as well to add 0.5 c.cm. of the buffered mixture to the standard immediately before comparison. The volume of each is then made up to the mark, mixed and compared in the colorimeter, the standard being set at 20 mm. The standard with the nearest depth of colour to the unknown is selected, thus avoiding errors due to too wide a difference in tint.

The amount of dextrin present in the unknown is calculated by the familiar colorimetric ratio—that is,

$$\frac{20}{x} \times Y;$$

where x is depth in millimetres of unknown, Y is the quantity of dextrin in the standard. Since 2 c.cm. of 0.75 per cent. dextrin solution—that is, 0.015 gram of dextrin—was present before incubation, the amount of dextrin used up can be calculated by subtracting from this the figure obtained in the above calculation. By reference to the curve this can be converted into Wohlgemuth units.

Observations on Blood.

Before applying this method to blood it was necessary to determine the optimum pH of the blood enzyme. It is not justifiable to assume that it is the same as the urinary one, although the possibility of anything different is unlikely. It was decided to adopt the dextrin method for this purpose; practically no alterations were necessary, except that it was found advisable to add an equal quantity of serum to the standard just before comparison, and also that 1 drop of 40 per cent. formalin rendered the colour sufficiently stable to permit of comparison.

A series of estimations were performed on serums, keeping all the conditions constant except the buffer mixture. The pH of this was varied, and its effect on the digestion of dextrin recorded. A series of blank controls was set up, with the serum omitted, since it was found that hydrogen-ion concentration alone affects the red colour, and such effects have to be allowed for when calculating the digestion of dextrin. The amount of dextrin used up at varying hydrogen-ion concentrations was calculated, and the alteration in colour (calculated as dextrin) produced by the buffer solution alone was either added or subtracted, according to whether it showed a decrease or an increase respectively. In the table will be seen the average of a series of estimations performed at various pH, together with the correction factors. After applying this it can be seen that the maximum digestion occurs at pH 6.1. It appears, therefore, that the optimum pH for the phosphate diastase of the blood is 6.1, as in the case of the urine.

pH of Blood Buffer Mixture:	5	6.1	6.5	7	7.5
Average of 20 experiments showing apparent amount of dextrin used up (in grams).	0.0015	0.035	0.033	0.036	0.034
Correction factor, expressed as dextrin, to allow for action of buffer alone on colour. Result of 20 experiments.	0.00021	0.00038	0.00026	0.00018	0.00018
Absolute amount of dextrin digested at the various pH, after application of the correction factor.	0.00275	0.0353	0.0033	0.0034	0.033

A series of estimations performed on serums by both methods, and checked by the curve, was found to give uniform results, and hence the method is suitable for the estimation of blood diastase. The methods possess the advantage of giving results to the nearest unit instead of a single number with a possible *plus* or *minus* error of considerable extent. Also the technique is very simple and does not need the measuring out of a series of small quantities of fluid, as is required on the Wohlgemuth method.

Another advantage of the method consists in the fact that only a small volume of the diastase-containing fluid is required—1.5 c.cm. This may, of course, be so reduced in cases of necessity to such a volume as to give 1 c.cm. of the final buffered mixture.

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CAUSES OF FAILURE IN THE OPERATION FOR REMOVAL OF TONSILS AND ADENOIDS AND THEIR AVOIDANCE.

BY

H. MORTIMER WHARRY, F.R.C.S.,

LARYNGOLOGIST TO THE MOUNT VERNON HOSPITAL FOR DISEASES OF THE CHEST; ASSISTANT TO THE THROAT, NOSE, AND EAR DEPARTMENT, UNIVERSITY COLLEGE HOSPITAL, AND ST. GEORGE'S HOSPITAL.

THE removal of tonsils and adenoids is one of the commonest operations performed, and is generally considered one of the easiest. But it so frequently happens that inattention to what may be called some of the finer points of the operation leads to the object of the operation not being achieved, that I feel justified in calling attention to them.

Briefly, the object of removing adenoids may be summed up as the relief of nasal obstruction, of nasal and post-nasal catarrh, or of middle-ear suppuration and catarrh. Provided that the condition named is caused by adenoids, then failure is usually due to their incomplete removal. There are three places where "remains" are commonly found:

A. Anteriorly, in the upper part of the choanae (see Fig. 4). Here a fringe is sometimes left; and though the bulk of the adenoid mass is removed, this fringe is enough to maintain an appreciable obstruction to breathing, and to originate numerous colds or chronic post-nasal catarrh.

B. Laterally, in the fossae of Rosenmüller just above and behind the Eustachian cushions (see Fig. 5). These remains are very typical of incomplete removal, and, lying where they do, keep the Eustachian cushion on that side continually bathed with a mucous or muco-purulent catarrh which effectually prevents the relief of a middle-ear catarrh or suppuration.

C. Posteriorly, just behind the soft palate on the anterior tubercle of the atlas vertebra. In this place large tags are sometimes left which, proportionately to their size, make for the failure of the operation. These tags sometimes project into the pharynx below the palate.

In order to ensure the greatest possible success, the removal of adenoids should be performed in three definite steps, each of which need only take a few seconds; but neglect of any of them may lead to failure.

I. *Preliminary Digital Examination of the Post-nasal Space.*—This is necessary to estimate the size of the pad of adenoids, and to feel if the anterior tubercle of the atlas vertebra is unduly prominent, or the Eustachian cushions unduly large. (Fig. 6.) If the anterior tubercle is prominent care should be taken to cut out sooner with the curette, and if the Eustachian cushions are large a small curette should be used; if they are lacerated, scarring and atresia of the Eustachian tube, and consequent deafness, are likely to result.

II. *Removal of the Pad of Adenoids with the Curette.*—The most obvious point is to see the uvula lying free after the curette is introduced ready for cutting. If this is neglected the uvula may be found to be caught up by the blade and may be cut off; when this happens the adenoids are generally missed. Again, in cutting out care should be taken to do it sharply or tags may be scraped down the posterior wall of the nasopharynx.

III. *After-examination of the Post-nasal Space with the Finger.*—This is all-important. The whole post-nasal space should be searched for adenoid remains, with special attention to the roof of the choanae, and the fossae of Rosenmüller, the Eustachian cushions, and the posterior wall. These remains are usually friable and can be gently crushed by the finger and scraped away. At the end every part of the space should be quite smooth and clean. It is not only unsafe, but impossible to effect this "nettoyage" with the curette, which should only be reintroduced if part of the main adenoid mass still remains.

It should be borne in mind that a large mass of adenoid vegetations may fill up every corner of the post-nasal space, and form adhesions to the Eustachian cushions. (Fig. 3.) When adenoids are removed in conjunction with tonsils, it should be from a dry field after all haemorrhage from the tonsils has been arrested. This will greatly facilitate the whole operation.