XXI. THE ACTION OF NORMAL AND BASIC LEAD ACETATE ON THE SUGARS, WITH REMARKS ON RUBNER'S TEST FOR DEXTROSE AND LACTOSE.

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During the course of some experiments on diabetic urines, Dr E. L. Kennaway observed the formation of a pink colouration in many cases when the urines had been treated with basic lead acetate and ammonia for the removal of dextrose. The colouration developed very gradually, and after two days' interval, a pink precipitate separated, leaving the solution almost colourless. As noted, the colouration was not obtained in every instance, and it was inferred either that the colouration was due to the so-called "Rubner reaction" indicating the incomplete removal of dextrose, or that some pathological substance might be present.

The author, at the suggestion of his colleague, conducted some experiments in order to elucidate this point and found that the colouration was due to dextrose.

It was deemed advisable to ascertain how other sugars behaved towards normal and basic lead acetate under different conditions, not only with and without ammonia but also other alkalies. The results obtained indicate that "Rubner's test" for dextrose and lactose is given equally well by many other sugars.

The normal lead acetate used was a saturated solution, and the basic solution was prepared according to the British Pharmacopoeia.

I (a). *Normal lead acetate alone at ordinary temperature.*

1 cc. sugar solution (10 %).

10 cc. saturated lead acetate.

Dextrose	No change in 12 days.
Laevulose	Yellow in 5 days, darker in 12 days.
Galactose	Faint yellow in 1 day, gradually darker to 12th day.
Mannose	No change in 12 days.
Lactose	" "
Sucrose	" "
Maltose	" "
Arabinose	Similar to galactose

The dextrose and galactose used in the above experiment had been soaked in alcohol for eight years, so there can be no doubt about the purity of these two sugars. If the above Table I(a) be compared with the Table I(b) it will be seen that the results depend almost entirely on the temperature. No precipitates were formed in any of the above cases.

I (b). *Solutions heated just to boiling.*

Dextrose	Yellow at 60°. Orange-yellow at 100°. No ppt.
Laevulose	" " Lighter orange-yellow. "
Galactose	" " Ppt.
Mannose	" " "
Lactose	" " Very slight ppt.
Sucrose	No colour. No ppt.
Maltose	Light yellow at 60°. Orange-yellow at 100°. Very slight ppt.
Arabinose	" " 80°. Darker yellow at 100°. No ppt.

The above results indicate that the reaction alone with normal lead acetate would make it somewhat difficult to ascertain the presence of any individual sugar. Sucrose is of interest because it does not show any colour either at the ordinary temperature or on boiling the solution. This is the case also when basic lead acetate is used; indeed, sucrose is the only sugar which did not give any results which could be noted.

II (a). *Basic lead acetate alone at ordinary temperature.*

Dextrose	Very faint yellow in $\frac{1}{2}$ hr., orange in 3 days, yellow after 18 days.
Laevulose	Light yellow " yellow " yellow "
Galactose	Orange-yellow " orange " orange "
Mannose	No change " no change (slight ppt.) " faint yellow "
Lactose	No change " faint yellow " yellow after "
Sucrose	No change " no change " no change "
Maltose	Opalescent " yellowish-pink " yellow "
Arabinose	Cf. galactose " yellow " yellow "

II (b). *Solutions heated just to boiling.*

Dextrose	Faint yellow at 50°. Dark yellow at 100°. No ppt.
Laevulose	" " " " Slight ppt. on cooling.
Galactose	Orange-yellow at 40°. Very dark at 70°. Lighter at 100°. Ppt.
Mannose	No colour before 100°. Yellow rapidly, then darker. Initial ppt.
Lactose	Same as mannose but no initial ppt.
Sucrose	No colour. No ppt.
Maltose	Faint yellow at 75°. Lemon-yellow at 100°, then darker.
Arabinose	Yellow at 30°. Then same as galactose.

If Tables I (b) and II (b) be compared it will be seen that the colourations obtained in the latter case are much more pronounced than those obtained in the former. The same results were observed also when the sugars were treated with the above reagents in the presence of alkalis.

III (a). *Normal lead acetate with ammonia.*

At the ordinary temperature, this was done with dextrose only, when the following result was obtained:

10 cc. dextrose (10 %) and 200 cc. lead acetate (7.5 %); added NH_4OH (0.880) until a slight precipitate formed which occurred after 38 cc. had been used.

After 75 minutes, a faint pink colouration was evident, which became darker until, after five days, the solution became cloudy and a copious pink precipitate separated, leaving the liquid almost colourless.

III (b). *Solutions heated just to boiling.*

1 cc. sugar (10 %).

1 cc. NH_4OH (0.880).

10 cc. saturated lead acetate.

Dextrose	Pink at 45°, orange-red at 100°. No immediate precipitate.
Laevulose	Yellow at 45°, darker and then lighter at 100°; again darker.
Galactose	" " then same as dextrose.
Mannose	No colour before boiling, then pink to dark yellow.
Lactose	Faint yellow at 55°, darker at 100°, then reddish-brown.
Maltose	" " 75°, orange " then opalescent.

Rubner's test for Dextrose and Lactose.

This test [Rubner, 1885] is carried out by adding some lead acetate (the ordinary laboratory reagent) to a dilute solution of dextrose, and then adding ammonia, drop by drop, until a permanent precipitate is formed.

On standing in the cold the liquid becomes coloured gradually, first yellow and then rose or flesh red; on heating the colouration takes place more quickly. It is also stated that the addition of the reagents must take place in the order named.

A series of tests was accordingly performed according to the directions given by Rubner, when the following effects were noted:

IV.

10 cc. sugar (1 %).

1 cc. normal lead acetate (about 10 %).

NH₄OH until permanent precipitate.

On warming, Dextrose gave flesh colour and precipitate.

Laevulose	„	„	„
Galactose	„	„	„
Mannose	„	yellow colour but no precipitate.	
Lactose	„	„	and precipitate.
Maltose	„	„	to buff colour and precipitate.

The observations, as noted in Table IV, clearly indicate the futility of adopting this reaction as a specific test for dextrose and lactose, and consequently it should be deleted from the literature.

It was deemed of interest to ascertain the effects of organic liquids on the coloured solutions and precipitates obtained in the above-described reactions. On shaking the solutions, in which a colouration had developed, with ether or chloroform the colour disappeared; but, on keeping, the colouration was gradually restored, and on renewed shaking did not entirely disappear.

The effect of using alcohol was then tried. A saturated 90 per cent. alcoholic solution of dextrose was treated with alcoholic solutions of lead acetate and ammonia, when a colouration ranging from pink to orange-red was obtained on standing at the ordinary temperature. The precipitate first formed dissolved in the excess of lead acetate solution added, but no precipitate was subsequently thrown down, and the colour was confined solely to the solution.

The behaviour of basic lead acetates towards the sugars is much more sensitive than that of the normal salt, and the experiments to be described have been extended to most of the sugars one would be likely to meet with in biochemical investigations. Other alkalis besides ammonia were tried, as well as acetates of other metals, but in the latter case no definite effects were observed.

V (a). *Dextrose, basic lead acetate and ammonia at the ordinary temperature.*

10 cc. dextrose (10 %).

5 cc. NH_4OH (0.880).

100 cc. basic lead acetate (B.P. fortis).

After 2 minutes	Yellow tint	Solution clear
1 hour	Salmon pink	" "
2 hours	Blood-red	" "
3.5 "	Darker red	" "
21.5 hours	Very dark red	" "
3 days	Lighter red	Pink precipitate
14 "	Dark yellow	Pink and buff precipitate

It will be seen from the above Table V (a) that the colour goes to a maximum before any precipitate forms; and it was also observed by using 10, 5, and 1 per cent. solutions that the amount of precipitate actually formed on mixing the solutions was proportional to the amount of sugar present, as was also the maximum tint developed. Therefore the colour is proportional to the sugar-strength of the solution. Other sugars and polyhydric alcohols gave the following results:

V (b).

Laeulose (m.p. 100)	Same as dextrose	Pink precipitate
Galactose (m.p. 144)	Same as dextrose	Pink "
Mannose	No colour	White cryst. precipitate
Lactose	Reddish-orange	Pink precipitate
Maltose	Yellow	Buff "
Sucrose	No colour	No "
Arabinose	Dark orange	Buff "
Xylose	Slight pink	Pink "
α -Methyl glucoside	No colour	White cryst. precipitate
Dulcitol	"	White amorph. "
Mannitol	"	No precipitate

The results shown in the above Table V (b) indicate that it would be almost impossible to identify any particular sugar tested under the above conditions at ordinary temperature. A full series of observations were then made with various sugars, the solutions being raised to boiling, and the temperature noted at which any colouration first appeared.

The following table shows the results obtained for dextrose.

V (c).

Experi- ment	Dextrose (10 %) in cc.	H ₂ O in cc.	NH ₄ ·OH (·880) in cc.	Basic lead acetate in cc.	Solutions before heating	Effect on heating
1.	1·0	0·0	1·0	10·0	Clear after few seconds	Pink at 45°. Then darker.
2.	0·5	0·5	1·0	10·0	Clear at once	„ 55° „ „
3.	0·1	0·9	1·0	10·0	„ „	Pink at 65°. Then yellow and white cryst. ppt. slowly forms.
4.	0·1	0·9	0·5	5·0	„ „	Pink at 63°. Darker yellow than (3).
5.	0·1	0·9	0·1	0·9	„ „	Pink at 62°. Darker than (3) or (4), but does not turn yellow.
	(1 %)					
6.	1·0	0·0	1·0	10·0	„ „	Pink at 65°. Then yellow (cf. 3).
7.	0·5	0·5	1·0	10·0	„ „	Pink at 75°. Yellow at 80°, white cryst. ppt. on standing.
8.	0·1	0·9	1·0	10·0	„ „	No colour. White cryst. ppt. on boiling. Almost solid.
9.	0·0	0·0	1·0	10·0	„ „	Same as 8.
10.	0·1	0·9	0·5	5·0	„ „	No colour. White cryst. ppt. on boiling, but not large.
11.	0·1	0·9	0·1	0·9	„ „	Yellow at 95°. Slight ppt.

The above results in Table V (c) clearly indicate the sensitiveness of the reaction. The white crystalline precipitate obtained in 8, 9, and 10 is of interest since this is known to be a basic lead acetate of definite composition, and the fact that it is only formed when the sugar is present only in very small amount shows that dextrose (and other sugars) exerts an inhibitive effect on its formation.

A corresponding series of experiments was performed with all the other sugars and the results obtained were much of the same order. Only one test with each is quoted in the following table, the quantities used being the same as in the first experiment in Table V (c).

VI.

Sugar	Solution before heating	Effect on heating
Laevulose	Solution not quite clear	Yellow, then pink, clear at 70°, then dark orange on boiling.
Galactose	Solution clears slowly	Yellow at 30°, orange-pink at 65°, pink ppt. separates.
Mannose	„ „ „	No colour before boiling, then yellow. No pink, orange or ppt.
Lactose	Considerable ppt.	Faint yellow at 60°, yellow on boiling. Rapid pink ppt. Solution orange.
Maltose	„ „	Opalescent on warming, yellow on boiling, then pink, and orange. Pink ppt.
Sucrose	Solution clears slowly	No colour and no ppt.

In describing his test, as previously noted, Rubner directs the reagents to be added in a definite order, viz., to the sugar solution add lead acetate and then ammonia. The above experiments, however, show that it is quite immaterial whether the lead solution or the ammonia is added first, the difference being that, if the ammonia is added first, the initial precipitate formed on adding the lead solution is much greater than if the solutions are mixed in the alternative order. In any case the result is the same.

Basic lead acetate and different alkalies with dextrose.

It was deemed of interest to see how other alkalies would behave, and the hydroxides of sodium and barium were accordingly tried with dextrose in the presence of basic lead acetate. The results, which were obtained at the ordinary temperature, may be thus summarised:

VII.

NaOH	After 15 minutes, white precipitate. After 50 minutes, slight pink colouration evident. After 15 hours, orange. No more precipitate.
Ba(OH) ₂	Fugitive yellow which became permanent after 7 hours.

The action of different acetates with dextrose and alkali.

Since lead acetate gives intense colourations with many sugars, it was decided to see if the acetates of other metals gave similar reactions. The acetates of the following metals were used: barium, zinc, mercury, manganese, cadmium and uranium. Of these, the only acetate which showed any colouration was that of barium, when the solution was at the ordinary temperature. In this case the following results were obtained:

VIII.

Ba(C ₂ H ₃ O ₂) ₂ and Ba(OH) ₂	After 1 day, yellow colour, no precipitate.
„ „ NH ₄ OH	„ 4 days, faint yellow, no precipitate.

The action of alkalies alone on the sugars.

One of the characteristic tests for dextrose is the action of sodium or potassium hydroxide (Moore's reaction), and barium hydroxide behaves similarly. Therefore in performing the tests described above where NaOH and Ba(OH)₂ were used, the effects observed at the ordinary temperature have been noted. Moreover, the present author has observed that if solutions of various sugars are boiled with ammonia for a short time, colourations

varying from pink to yellow are formed. The following results were obtained with 10 per cent. solutions, and boiling for a few minutes with about an equal volume of ammonia (0.880).

IX.

Dextrose	Faint yellow in 1 minute. Distinct in 3 minutes.
Laevulose	" " " " "
Galactose	" " " " "
Mannose	Very faint yellow after 3 minutes.
Lactose	Cf. dextrose. Yellowish-pink on standing.
Maltose	Faint yellow in 1 minute. Orange in 3 minutes.
Arabinose	No change.

It will be apparent from the above observations that the combined action of the acetates of lead and ammonia is somewhat complex, and it is impossible to correlate the effects observed with any particular sugar in order to identify it. No quantitative experiments have been carried out up to the present, but there is little doubt that the precipitates obtained in many cases are complex derivatives of the sugar and the acetates of lead.

REFERENCE.

Rubner (1885). *Zeitsch. anal. Chem.*, 24, 447, 603.