

SHORT COMMUNICATIONS

MOLYBDENUM-CARBOHYDRATE
COMPLEXES

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Molybdenum as a trace element has been known to help the synthesis of haemoglobin and its use has been particularly championed by Dickmann,¹ a renowned American authority on pregnancy anemia. It is being used orally as trioxide, Mo_2O_3 , the insoluble compound, along with ferrous sulphate.² A step further would be to give it as a soluble compound, colloidal in nature, as an analogue of iron saccharate. Iron saccharate is painful when injected intramuscularly while it is usually administered intravenously. A preparation which is safe enough to be given intravenously is obviously safe orally. At first molybdenum saccharate would be used only orally.

When starting with molybdenum dioxide, MoO_2 , mere addition of sodium hydroxide results in sodium molybdate, Na_2MoO_4 , which is water soluble. Attempts to prepare molybdenum saccha-

rate require that the metal oxide should not dissolve apart from its complex formation. Next the trioxide, Mo_2O_3 , was tried. This oxide was not available here. Therefore its preparation was undertaken, starting with ammonium molybdate $(\text{NH}_4)_2\text{MoO}_4$ according to reactions given below, and a concentrated aqueous solution was prepared. It was reduced with zinc and hydrochloric acid to molybdic acid, H_2MoO_4 , according to reaction I, which is first precipitated and further redissolved. The solution is at first blue, then reddish brown and finally black, which colour completes the reaction. It is decanted and to the clear dark solution of molybdic acid, ammonia solution is added to precipitate it as molybdic trioxide, Mo_2O_3 . The precipitate is washed with dilute aqueous ammonia to remove traces of zinc oxide and of ammonium chloride, shown as side reaction IV below, that may be adhering to the wet molybdic trioxide. Lastly it is washed with water to free also the traces of ammonia. It is mechanically compressed to remove excess of water and afterwards heated from $50^\circ\text{--}60^\circ\text{C}$. under vacuum for 3-4 hours to complete dryness. The following equations serve to illustrate the reactions discussed above.



TABLE I.—MOLYBDENUM-SUCROSE COMPLEX: SUGAR VARIABLE.

No.	Mo: Sugar: NaOH in g.	Percentage of Mo in the complex	Stability of the solution on long heating	pH of the solution on long heating	pH of the solution after boiling	Iso-electric point	Density	Viscosity in pois
1.	1:8:2.25	95%	Stable	7.8	7.7	2.5-2.8	1.1	.01016
2.	1:7:2.25	"	"	7.9	7.7	2.5-2.8	1.096	.0113
3.	1:6:2.25	"	"	8.6	8.3	2.8-3.0	1.091	.0118
4.	1:5:2.25	"	"	8.8	8.7	3.0	1.087	.0124
5.	1:4:2.25	60%	Unstable	8.9				
6.	1:3:2.25		No complex formation					

N.B.—In the experiments 1 and 2, time of heating was 3.5 hours.

TABLE 2.—MOLYBDENUM-SUCROSE COMPLEX: ALKALI VARIABLE.

No.	Mo: Sugar: NaOH in g.	Percentage of Mo in the complex	Stability of the solution on long heating	pH of the solution before boiling	pH of the solution after boiling	Isoelectric point	Density	Viscosity in pois
7.	1:5:2.25	95%	Stable	8.0	7.9	2.5-3.0	1.088	.0103
8.	1:5:1.8	„	„	7.8	7.7	2.5-3.0	1.084	.0106
9.	1:5:1.5	„	„	7.6	7.5	2.8-3.0	1.083	.0107
10.	1:5:1.2	„	„	7.4	7.2	2.8-3.0	1.081	.0112
11.	1:5:0.9	60	Unstable	7.3				

TABLE 3.—MOLYBDENUM-SUCROSE COMPLEX: TEMPERATURE VARIABLE.

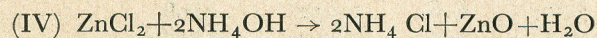
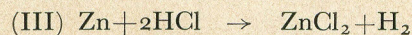
No.	Mo: Sugar: NaOH in g.	Percentage of Mo in the Complex	Temperature of heating	Time of heating in hrs.	pH. of solution before boiling	pH of the solution after boiling	Iso- electric point	Density	Viscosity
12.	1:5:1.8	90	180°C.	3.0	8.0	7.9	2.5	1.08	.0103
13.	1:5:1.5	90	140°C.	5	8.9	8.7	2.6	„	„
14.	1:5:1.2	95	170°C.	3.5	7.4	7.2	2.8-3.0	1.081	.0112
15.	1:5:0.9	90	200°C.	1.75	7.6	7.4	2.8	1.08	.0103

TABLE 4.—MOLYBDENUM-GLUCOSE COMPLEX.

No.	Ratio of Mo: carbohydrate: alkali in g.	Percentage in the complex	Stability after boiling	pH of the solution before boiling	pH of the solution after boiling	Iso- electric point	Density	Viscosity
16.	1:5:2.25	95%	Stable	9.0	9.2	2.5-3	1.09	.01
17.	1:5:1.8	„	„	8.0	7.8	2.8-3	1.08	.0103
18.	1:5:1.2	„	„	7.8	7.6	2.9-3	1.08	.0106
19.	1:5:0.99	60%	Unstable	7.4				

TABLE 5.—MOLYBDENUM-DEXTRIN COMPLEX.

No.	Ratio of Mo: carbohydrate: alkali	Percentage in the complex	Stability after boiling	pH of the solution before boiling	pH of the solution after boiling	Iso- electric point	Density	Viscosity
20.	1:8:1.5	95%	Stable	8.9	9.6	3.0	1.08	.01
21.	1:7:1.5	"	"	10.0	9.8	3.0	1.086	.0102
22.	1:6:1.5	"	"	10.1	9.9	3.0	1.084	.0104
23.	1:5:1.5	60%	Unstable	10.6				

Side Reactions

To the dry and weighed quantities of molybdenum trioxide different ratios of carbohydrates and sodium hydroxide were added. The oven was first set at a constant temperature of 170°C. and the mixtures were heated for different durations. The findings are given in the accompanying tables. In order to appreciate the contents the following facts are further indicated.

(1) Temperature was varied but 170°C. proved to be the best as shown in Table 3.

(2) Sodium hydroxide was used as 15% solution but has been indicated as solid, in terms of grams. Its presence along with molybdenum complex has been indicated in ratios as NaOH: Mo where Mo is the pure element.

(3) The test for stability was a clear aqueous solution containing 2% metal which remained as such even on boiling in an ampoule for one hour. The solution should neither form a gel nor reveal any precipitation.

(4) The ratios in which carbohydrates were added indicate 1 g. of metal to the quantity of the carbohydrate in grams.

(5) In all experiments a round porcelain dish, 5" broad and 1.5" high, was used, but the depth of the solution was 0.6 inch.

(6) The density, viscosity and pH refer to a solution with 2% of the metal.

Conclusions

(1) The best complex contained molybdenum, sucrose and sodium hydroxide in the ratios, 1:5:1.2.

(2) The best isoelectric point was 2.5-3.5 pH.

References

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MANGANESE-CARBOHYDRATE COMPLEXES

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Manganese sulphate was used as the starting material for the preparation of manganese saccharate. 4.2 grams of (manganese sulphate) $\text{MnSO}_4 \cdot (5\text{H}_2\text{O})$ equivalent to 1 g. elemental manganese was dissolved in about 40 ml. of distilled water in a beaker of 1-litre capacity and warmed to 30-40°C. to dissolve it quickly. To this solution 30-40 ml. 5% sodium hydroxide solution was added slowly while stirring until a white precipitate of manganous hydroxide was formed. Manganous hydroxide was then oxidized to manganic hydroxide (brown in colour) by

TABLE 1.—MANGANESE-SUCROSE COMPLEX (SUGAR VARIABLE).

No.	Mn: Sugar: NaOH in g.	Time in hours	Percentage of the Mn in the complex	Stability of the solution on long heating	Iso- electric point	Final pH before boiling	Density	Viscosity	Temper- ature of heating
<i>Sugar Variable</i>									
1.	1:12:3.0	3.0	95%	Stable	2.9	8.3	1.094	.0106	—
2.	1:10:3.0	3.0	95%	Stable	3.0	8.2	1.094	.0106	—
3.	1:8:3.0	3.0	95%	Stable	2.8	8.4	1.094	.0106	—
4.	1:6:3.0	3.0	95%	Stable	3.0	8.8	1.092	.0103	—
5.	1:5:3.0	3.0	80%	Stable	3.2	8.8	1.092	.0103	—

TABLE 2.—MANGANESE-SUCROSE COMPLEX (ALKALI AND TEMPERATURE VARIABLE).

<i>Alkali Variable</i>									
6.	1:6:2.7	3.0	95%	Stable	3.0	8.6	1.092	.0108	—
7.	1:6:2.4	3.0	95%	Stable	3.0	8.6	1.091	.0108	—
8.	1:6:2.1	3.0	95%	Stable	2.8	8.4	1.091	.0106	—
9.	1:6:1.5	3.0	95%	Stable	2.8	8.4	1.088	.0106	—
10.	1:6:1.2	3.0	80%	Stable	2.9	8.5	1.088	.0106	—
<i>Temperature Variable</i>									
1.	1:6:1.5	1.3	80%	Stable	3.2	9.2	1.092	0.0103	200°C.
2.	1:6:1.5	6.0	No complex formation		—	—	—	—	130°C.
3.	1:6:1.5	3.0	95%	Stable	3.0	9.1	1.092	0.0103	170°C.

TABLE 3.—MANGANESE-GLUCOSE COMPLEX.

No.	Mn: Sugar: NaOH in g.	Time in hours	Percentage of the Mn in the complex	Stability of the solution on long heating	Iso- electric point	Final pH before boiling	Density	Viscosity
1.	1:12:10	3.0	95%	Stable	3.0	7.8	1.088	0.0104
2.	1:10:10	3.0	95%	Stable	3.0	7.9	1.088	0.0104
3.	1:8:10	3.0	95%	Stable	2.8	8.4	"	9.0104
4.	1:6:10	3.0	95%	Stable	2.8	8.4	1.086	0.01049

TABLE 4.—MANGANESE-DEXTRIN COMPLEX.

1.	1:10:10	2.5	—	Unstable	3.5	8.4	—	—
2.	1:8:10	2.5	—	"	3.5	8.4	—	—
3.	1:6:10	2.5	—	"	3.5	8.2	—	—

the addition of 15% H_2O_2 solution (5 to 10 ml.).
 Equation: $\text{MnSO}_4 + 2\text{NaOH} \rightarrow \text{Mn(OH)}_2 + \text{Na}_2\text{SO}_4$
 $2\text{Mn(OH)}_2 + \text{H}_2\text{O}_2 \rightarrow \text{MnOOH} + 2\text{H}_2\text{O}$.

Then ($\text{SO}_4^{''}$) was removed by washing manganic hydroxide with distilled water, being done as follows:—

To a beaker of 1-l. capacity containing manganic hydroxide about 800 ml. of distilled water was added, stirred and allowed to stand for a few minutes, stirred again and then allowed to resettle. Water was then decanted; some 700 ml. distilled water was added again and decanted. Such washings with distilled water were repeated, in all four to five times. Thus the wet mass of manganic hydroxide was obtained.

To the wet mass obtained as described above, sugar in different quantities was added, dissolved and then concentrated solution of sodium hydroxide in calculated amounts was finally added. Heating was then continued to 170°C . for different periods indicated in the table. After heating, the moisture-free product formed a cake-like mass, dark in colour. The findings are given in the accompanying tables. In order to understand them the following facts are indicated.

1. Temperature was varied, but 170°C . proved to be the best, so that the results of temperature variations have been altogether omitted.
2. Fifteen % solution of sodium hydroxide was used, but has been indicated as solid and in terms of grams. Its presence along with manganese complexes has been indicated as Mn: NaOH, where Mn is the pure element of the complex.
3. The test for stability was a clear solution containing 2% metal, heated in an ampoule for 1 hour in boiling water. The solution should neither form a gel nor reveal any precipitation.
4. The ratio in which carbohydrates were added indicate 1 g. of metal to the quantity of carbohydrate in g.
5. In all the experiments the porcelain dish used was round with the diameter of 5" and a height of 1.5" but the depth of the solution was kept constant at 1.5 cm.
6. Other carbohydrates like glucose and dextrin were also tried. The procedure for the preparation of the complex was the same. The results of glucose complexes are given in Table 4. With dextrin no stable complex could be obtained so far. Further work is in progress.

Conclusions

1. The best ratio of Mn, sucrose and sodium hydroxide in the complex was 1:6:1.5.
2. The best isoelectric point was 3.0 pH.

NICKEL-CARBOHYDRATE COMPLEXES

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Nickel sulphate, $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$, 4.7 g. equivalent to 1 g. elemental nickel, was dissolved in 40 ml. of water and treated with 40 ml. of 5% NaOH. Green coloured precipitate of nickelous hydroxide was obtained. It was washed completely free of electrolytes. The nickelous hydroxide was oxidised to black nickelic hydroxide by sodium hypochlorite or bromite. But the nickelic hydroxide was not stable and when sugar was added to it and heated, the precipitate changed to greenish nickelous hydroxide. Therefore nickelous hydroxide alone was used as the starting material for further experiments.

The wet nickel hydroxide was taken into an enamel tray, sugar as required was taken, dissolved in water, and then the concentrated solution of the calculated amount of sodium hydroxide was incorporated thoroughly. The mixture was heated at 200°C . and maintained at that temperature for periods indicated in the tables. Other temperatures were tried and found ineffective. The results are given in the tables.

Following particulars are meant to enhance the value of the data given there.

1. About 5% Ni was oxidised to green nickel mono-oxide, so that a complete utilization of nickel was never achieved. Such a result, for example, is indicated as 95% Ni in the complex. The moisture free product formed a cake-like mass, dark in colour. As a poor conductor of heat the nickel-carbohydrate complex was not uniformly heated, the product on the lower surface was over heated, being decomposed to nickel oxide, thus preventing a complete utilization of the metal.

TABLE 1.—NICKEL-SUCROSE: SUGAR VARIABLE.

No.	Ni:Sucrose in g.	Percentage of Ni in the complex	Iso-electric point	Final pH before boiling	Density of 1% solution	Viscosity of 1% solution in pois
1.	1:30	95	3.8-4.1	9.3	1.086	.03767
2.	1:28	95	3.8-4.1	9.4	1.086	.03767
3.	1:25	85	3.9-4.0	9.6	1.081	.03170

Note.—All the above experiments were made at 200°C. and the time of heating was 3.5 hours. All the above samples were stable even on long boiling. The ratio of metal to alkali used was in the ratio 1:6. The pH after boiling remains unchanged.

TABLE 2.—NICKEL-SUCROSE: ALKALI VARIABLE.

No.	Ni:NaOH in g.	Time in hours	Stability of cold solution	Stability of solution on boiling	Iso-electric point	Final pH before boiling	Viscosity of 1% solution in pois
1.	1:9	3.75	Stable	Stable	3.8	10.2	.02004
2.	1:6	3.5	Stable	Stable	3.8-4.0	9.2	.03767
3.	1:4.5	2.5	Unstable	Unstable	—	—	—

Note.—The ratio of metal to sugar was 1:28. The temperature was 200°C. The density was found to be 1.086 in experiments 1 and 2.

TABLE 3.—NICKEL-GLUCOSE COMPLEX: GLUCOSE VARIABLE.

No.	Ni: Glucose in g.	Ni: NaOH in g.	Time in hrs.	Percentage of Ni in the complex	Iso-electric point	Final pH before boiling	Density of 1% solution	Viscosity of 1% solution in pois
1.	1:3	1:6	1.75	95	4.0	8.9	1.095	.03059
2.	1:28	1:6	1.5	85	4.1	9.4	1.091	.02622

TABLE 4.—NICKEL-GLUCOSE COMPLEX: ALKALI VARIABLE.

3.	1:30	1:4.5	1.0	90%	4.1	7.9	1.079	.03137
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In all the three experiments the temperature was kept constant at 200°C. The solutions were stable on boiling.

2. The stability of the complex was tested by boiling a 1% solution in an ampoule for one hour at 100°C. or for 30 minutes at 115°C. The complex did not gel and remained clear.

3. Sodium hydroxide was used as a 15% solution, but has been calculated as pure, and its dry weight indicated in its ratio with nickel.

Other carbohydrates used were dextrin and glucose. The procedure of the preparation was the same.

Conclusions

1. Minimum ratio of sucrose to nickel is 28.
2. The minimum alkali per g. nickel is 6 g.
3. The best temperature is 200°C.

COBALT-CARBOHYDRATE COMPLEXES

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Cobalt plays an important role in animal body with vitamin B₁₂ as its best form containing 4% cobalt. Its function is to increase the percentage of red blood cells. The metal further helps in the metabolism of iron.¹ Usually cobalt is given as a sulphate or gluconate to supplement iron. But, whereas vitamin B₁₂ is given both orally and peritoneally, other forms of cobalt are given only orally. However, there are two preparations which appear to be complexes of this metal. Ferro kobalt of Pharm-Fabrik, Montavit, Vienna, declared to be "ferrous and cobaltous gluconate as amino acid complex" meant for intravenous as well as intramuscular use, and "Kobalt Ferrlecit" intravenous of Nattermann, Koeln. This we had occasion to test once and with remarkable effect. It does not seem to be a carbohydrate complex, but its real nature has not been studied further. The use of inorganic cobalt should certainly give way to any complex form even for oral purposes since this would be least irritable. Partly with this view we have tried to prepare cobalt complexes similar to that of iron-sucrose² which are being used intravenously.

Preparation

Four grams cobalt chloride, CoCl₂·6H₂O, equivalent to 1 g. elemental cobalt was dissolved in 20 ml. water in a beaker of 1-l. capacity. To this solution 5% sodium hydroxide solution was

added in small portions with vigorous stirring. A blue-green precipitate is first formed but when excess of alkali is added, the precipitate turns pink according to the equation, CoCl₂ + 2NaOH = Co(OH)₂ + NaCl. Weiser and Milligan² stated that the X-radiograms indicate that the blue and pink hydroxides have different crystalline forms. They designated the green blue form as α-cobaltous hydroxide, which is unstable, and the rose coloured, as β-cobaltous hydroxide, a stable form. About 10 ml. of 6% hydrogen peroxide was added to the precipitate slowly with continuous stirring. The rose-red hydroxide was oxidised to cobaltic hydroxide which was dark. It was boiled for 15 minutes to expel the excess of hydrogen peroxide. Sodium hypochlorite has also been used as oxidising agent with success. Winkelblech³ and others described the colour of cobaltic hydroxide as dark brown, while Herranschmidt and Capelle⁴ described it as light brown. Huettig and Kassler⁵ on the other hand, explain that cobaltic hydroxide is black, while the brown preparations contain cobaltous hydroxide. We can confirm this by further stating that cobaltic hydroxide is black because the brown precipitate (obtained by adding less quantity, i.e., 2 ml. of hydrogen peroxide instead of 10 ml.) does not form a complex with cane sugar. There is a parallel case to red hydroxide of iron which chelates while the yellow ferric hydroxide does not form a complex with cane sugar.² Probably cobalt in the divalent state does not form a complex with carbohydrates. When the quantity of carbohydrate was increased as in experiments 1 and 8, the complex was unstable. It clearly indicates that the excess of carbohydrate reduces the cobaltic hydroxide, and hence the complex formation did not take place. In experiment 7, Table 1, there was a slight sedimentation of cobaltous oxide after 12-24 hours since the temperature was 170°C. instead of 200°C. A possible explanation in this case would be that at the lower temperature cobalt turned into a divalent form. This was contrary to our experience with iron-carbohydrate complexes² where the minimum quantity of sugar was 3 g. for 1 g. iron but could be increased to 8 g. successfully, and even then there was no reduction of ferric to ferrous state, indicating that the excess of sugar had no effect on the resulting complex.

The black cobaltic hydroxide was washed free of electrolytes and transferred to a porcelain dish. Carbohydrate dissolved in the minimum quantity of water was mixed with the precipitate and the required quantity of alkali added. The contents were heated in an electric oven at different temperatures (only successful experiments have been included in the table) so that a darkish cake

TABLE I

Expt. No.	Ratio of Co. Carbohyd: NaOH in g.	Temperature	Time in hours	Stability on long boiling	Iso-electric point	Final pH before boiling	Density at 32 °C.	Viscosity in poises at 23°C.
COBALT-SUCROSE COMPLEX.								
<i>Sucrose Variable</i>								
1.	1:9:1.65	200 °C.	1.20	Unstable	5.9	7.8	1.1008	0.0203
2.	1:8:1.65	"	"	"	5.6	7.9	"	"
3.	1:7:1.65	"	"	Stable	5.3	7.9	"	"
<i>Alkali Variable</i>								
4.	1:7:1.30	200 °C.	1.20	Unstable	5.8	7.8	1.1006	0.0203
5.	1:7:1.5	"	"	Stable	5.6	7.8	1.1006	0.0203
<i>Temperature Variable</i>								
6.	1:7:1.65	185 °C.	2	Stable	5.1	8.9	1.1008	0.0203
7.	"	170 °C.	3	"	3.9	9.1	"	"
COBALT-GLUCOSE COMPLEX.								
<i>Glucose Variable</i>								
8.	1:10:2.1	170 °C.	2	Unstable	3.1	7.1	1.1005	0.0172
9.	1:8:2.1	"	"	Stable	2.9	7.6	1.1005	0.0172
<i>Alkali Variable</i>								
10.	1:8:1.8	170 °C.	2	Unstable	3.5	7.1	1.1003	0.0172
11.	1:8:1.5	170 °C.	2	"	"	"	"	"
<i>Temperature</i>								
12.	1:8:1.8	130 °C.	4	Unstable	2.9	7.8	—	—
COBALT-DEXTRIN COMPLEX.								
<i>Dextrin Variable</i>								
13.	1:12:2.4	200 °C.	1.20	Stable	2.3	8.8	1.103	0.0296
14.	1:10:2.1	"	"	Unstable	3.0	8.8	—	—
<i>Alkali Variable</i>								
15.	1:12:2.1	200 °C.	1.20	Stable	2.3	8.7	1.103	0.0296
16.	1:12:1.8	"	"	Unstable	2.9	8.5	—	—
<i>Temperature</i>								
17.	1:12:2.1	170 °C.	2	No formation				

was obtained. The cake should give a clear solution when dissolved in water and remain so on boiling. The following particulars supplement the information given in the tables.

1. Sodium hydroxide was used as 15% solution.

2. The porcelain dish was of 5.0" in diameter and 1.5" deep to give a complex incorporating 1 g. elemental cobalt from a liquid mixture 0.5" deep.

3. Stability of the complex was tested by boiling its solution in a sealed ampoule for 1 hour at 100°C. or at 115°C. for 30 minutes. The solution should remain clear.

4. The dextrin used was yellowish in colour, chemically pure of Merck's.

5. About 5% cobalt was left unreacted in each case, so that a complete utilization of cobalt was never achieved.

6. Density, viscosity, pH and isoelectric point also refer to a solution containing 2% cobalt.

7. The isoelectric point was estimated with decinormal hydrochloric acid.

Conclusions

1. The minimum ratio of sucrose, glucose and dextrin per g. cobalt is 7, 8 and 12 respectively.

2. Minimum ratio of alkali per g. cobalt in case of sucrose is 1.65 g., in case of glucose 2.1 g. and in case of dextrin 2.4 g.

3. The best isoelectric point ranges between pH 4 and 5.6.

4. The time of heating and isoelectric point are related factors, longer the time the more acidic is the final product. When the time of heating was increased, the isoelectric point pH 5.3 of a stable complex (Experiment 3) was decreased to pH 3.9 (Experiment 5). There was, however a slight sedimentation after 12-24 hours due to the reduction of trivalent cobalt to divalent state.

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PREPARATION OF CHAKSINE CHLORIDE FROM CHAKSINE SULPHATE USING ION-EXCHANGE RESIN

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Chaksine is isolated as the easily crystallizable and sparingly soluble chaksine sulphate from the seeds of *Cassia absus* Linn. Chaksine chloride was needed for pharmacological studies and had to be completely free from traces of inorganic impurities. The procedure of conversion should also necessarily avoid treatment of chaksine salt solutions to alkaline medium since at neutral or alkaline pH, the base has been found to isomerise or decompose evolving ammonia.¹ Advantage was therefore taken of replacing SO₄²⁻ by Cl⁻ by passing the hot aqueous solution of chaksine sulphate through a column of Amberlite IRA-400 (Cl⁻). This method has been used for the conversion of streptomycin sulphate to the corresponding chloride.²

The resin freed of 'fines' by decantation was packed into a column 32 × 1.7 cm. long and washed thrice with distilled water. The column was heated with water at 40-50°C. to safeguard against the chaksine sulphate crystallizing in the column. The hot aqueous solution of chaksine sulphate was passed through the column which was further eluted with hot distilled water until the eluate ceased to give alkaloidal test with Dragendorff's reagent. The total eluate was evaporated to dryness in vacuo and the residue finally crystallized from alcohol and ether mixture. The crystalline product melted at 145-146°C. (yield 78%).

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A REVISED METHOD OF PREPARING RENNET

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Introduction

Rennet, the cheese making enzyme, has been prepared by many workers. Fenger¹ extracted the previously dried mucosa of suckling calves with hydrochloric acid and precipitated the enzyme by neutralizing it with alkali. Tauber and Kleiner² avoiding the process of drying proceeded to extract the enzyme from the fresh mucosa in the same way. Even dilute hydrochloric acid is a strong reagent. Berridge³ therefore extracted the dried and salted stomachs with sodium chloride. All the above-mentioned workers used stomachs of pigs or of suckling calves as their raw material. None of them being available here in sufficient quantities, the authors⁴ have employed the stomachs of adult sheep and goats instead. In our previous method the stomachs were salted and dried and the rennet extracted with 10% NaCl solution.

The method in order to be economic should not be lengthy as it increases the cost. Keeping this in mind the authors tried to extract the fresh stomachs with 10% glacial acetic acid and avoided the method of precipitation. The extract was concentrated by distilling it in vacuum at a low temperature further using glycerine as a vehicle for the effective preservation of the enzyme.

Experimental

Fresh stomachs of goats and sheep obtained from the slaughter house were cleaned and the adhering fat was removed. The stomachs were then cut into pieces of convenient size and placed

in a conical flask; 10% glacial acetic acid was added and kept for 24 hours at room temperature (30°C.) to give an extract. The extract was first filtered through cotton wool and finally through coarse filter paper which gave a clear solution. It was tested for its activity in the following way. The pH of the extract was found to be 2.5 pH. It was adjusted with 0.1N sodium hydroxide to 5.4. Ten ml. of milk (prepared by dissolving 12 g. of skimmed milk powder in 100 ml. of 0.2N calcium chloride solution) was warmed to 36°C. for 6 minutes; 1 ml. of the extract was then added when the milk set to a solid mass. The residue, or the stomach pieces, after the first extraction was subjected to a similar treatment, in all five times. The sixth was poorly active. All the active extracts were mixed together and the liquid distilled at about 40°C. under reduced pressure. It was observed that during distillation temperature should not exceed 50°C. as at higher temperatures it becomes inactive.

A sample of the extract was kept in incubator adjusted at 50°C. and tested for its activity first, with intervals of one to 24 hours. It was found that the activity remains for several days at 50°C. But when the temperature was raised to 60°C., even after 1½ hours the activity was totally destroyed.

After concentration the liquid extract was reduced to 1/20th of its original volume. This liquid is now practically free from acetic acid and its pH is 4.5, while traces of the acetic acid still remain along with the rennet, which does not harm the enzyme. It was thus clear that no loss of activity has occurred during the process of concentration. The data of an average sample are given below:

Fresh stomachs	100 g.
Acetic acid, 10%, for 5 extracts			2500 ml.
Total extracts obtained	..		2000 ml.
Concentrated to	..		20 ml.
Coagulates	10 litres of milk.

Standardization of the Rennet.—One ml. of concentrated extract (of 20 ml.) was diluted to 50 ml. as solution "A".

One ml. of "A" coagulates 10 ml. of milk in 6 minutes. Hence 1 ml. (concentrated extract, 20 ml.) coagulates 500 ml. of milk.

Discussion

No doubt other proteinaceous substances are

also extracted along with rennet while using acetic acid, but they do not in any way interfere with its activity; hence their removal was not considered. The present method avoids the process of drying and the use of sodium chloride. Secondly reagents like sodium biphosphate and potash alum, are not to be used. The only disadvantage of this method is that the product could not be dried to a powder. But this is rather an advantage for it is generally recognized that dry preparations of rennet have a poor storage life.

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MEDICINAL PLANTS OF WEST PAKISTAN: ACACIA SENEGAL WILLD

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Acacia senegal Willd which is a source of true gum arabic is known locally as 'Khor' and the gum is called 'Gund Kiteera'. It is one of the oldest commercial products of the world. Brought from the Gulf of Aden to Egypt in the seventeenth century B. C., in the works of Theophrastus it is spoken of as a product of upper Egypt. The name gum arabic is a misnomer in the sense that not much was ever produced in Arabia. The possible origin of this name is either because it was extensively used by the early Arab physicians or because it was mostly traded by the Arabs during the early periods. At present Sudan and Senegal are the main countries supplying the world requirements of gum arabic. It is one of the main export trade commodities of Sudan. The gum is extensively used in industry and medicine. In medicine it is mainly used as an emulsifying agent and as a demulcent.

Description of the Plant

It is a small thorny tree belonging to the family *Leguminosae* and sub-family *Mimosoideae*. It reaches a height of 20 feet and girth of 2 feet.



Fig. 1.—Gum arabic tears formed along the upper and lower ends of the cut.

The bark is smooth pale greenish grey in colour. It peels off in thin flakes. The young twigs are slightly puberulous, about 0.2 inch long and curved. Prickles are found in trees at the base of the petioles. The rachis varies from 1 to 2 inches in length and have 3 to 5 pairs of pinnae. Leaflets on the pinnae vary from 8 to 15 pairs. Leaflets are 0.1-0.2 inch long, linear, obtuse, subsessile and rigidly subcoriaceous. Leaf fall takes place in April to June before the onset of summer monsoon rains. The flowering spike is 2 to 3 inches long and is pendulous. The flowers are white and fragrant. These appear from September to November. The pods are flat, thin, indehiscent containing 5 to 6 seeds. The size of pods varies from 2 to 3 inches in length and 0.7 to 1.0 inch in breadth.

Ecology

Acacia senegal Willd is found in sub-tropical regions from Delhi eastward to West Africa. It is particularly found in great abundance in Sudan, Central Africa and Senegal. In West Pakistan it is found in Thar desert, colonizing the slopes of high sand dunes and the coastal low calcareous rocks bordering the Arabian Sea. It is a common tree of the calcareous rocks and the slopes of the valley in Kohistan, Karachi, Lasbela, Kharzan and Mekran. The average annual rain in these areas ranges from 5.0 to 24.0 inches. This rainfall in the above areas is mainly received during the summer monsoon period from the middle of June to September. For rest of the 8 months the plant is faced with very arid conditions. The vegetative growth takes place only during the summer rainy period. Flowering takes place from August to November and fruit matures from November to March. Due to very arid conditions from April to middle of June the plant becomes leafless.

Acacia senegal Willd is found on the slopes of calcareous hills along the Arabian Sea coast or

on the slopes of sand dunes in Thar desert which are rich in calcium. It constitutes edaphic climax vegetation in these regions. The common associates of this in this region are *Commiphora mukul* Engl, *Grewia populifolia* Vahl and *Euphorbia caudicifolia* Haines. Seedlings of *Acacia senegal* Willd, generally appear and survive in the clump of *Euphorbia caudicifolia* Haines. Thus in most cases there is association between the *Acacia senegal* Willd, and *Euphorbia caudicifolia* Haines which act as a nurse plant. Germination of seeds takes place in June with the commencement of summer monsoon rains. Due to the arid climate in which this plant is found, the growth of tree is necessarily very slow.

In the areas of its distribution maximum shade temperature during summer is often over 120°F and minimum daily temperature goes below zero during winter months in some areas. Thus it is a very hardy species being able to survive under very adverse conditions. According to Parker¹ it was tried in the Pabbi hills as well as in the Changa Manga Forest plantation. In the first instance it grew well for a short period but was soon lost sight of.

Collection of Drug

In Africa trees older than 5 years are tapped in February to May when the fruits are ripe. A transverse incision in the trunk of tree is given with a small axe. The twist of the axe loosens the bark which is pulled below and above the cut with the help of the hand. The portion of the trunk so bared to the cambium measures about 2 to 3 feet in length and 2 to 3 inches in breadth. In about 20 to 30 days the tears of the gum which have been formed on the surface may be picked. The gum is occasionally exposed to the sun to bleach it. Numerous minute cracks often appear on the outer portion during this bleaching process, thus giving them a semi-opaque appearance. In Sudan *Acacia senegal* plantations have been raised to obtain the gum. The quality of the gum from the cultivated tree is better as it is lighter in colour.

There are no gum canals or gumcells in *Acacia senegal* Willd. The gum is formed as a result of the disintegration of the internal tissue, mostly cellulose, through the process of gummosis. The possible role of bacteria in this process has not yet been established in this plant so far. The gum consists principally of arabin which is formed by arabic acid in combination with calcium and traces of potassium and magnesium. It is completely soluble in an equal weight of water. The resulting solution is slightly acidic. It should yield up to 1% of water-insoluble residue, not more than

4% of total ash and up to 15 % of moisture. The addition of 0.2 ml. of diluted lead acetate solution to 10 ml. of 2% cold aqueous solution of gum does not produce a flocculent curdy white precipitate which helps to distinguish it from its adulterants.

There are more than 27 types of gums in the market and one type is often substituted for another. Starch after being treated with sulphuric acid under pressure and dried resembles gum arabic and can be used as adulterant. *Acacia senegal* Willd resembles in appearance to *Acacia modesta* Wall, but it can be distinguished by its smooth pale bark, its stipular spines in trees and its larger pods. Gum from *Acacia arabica* Willd, and *Acacia jacquemontii* Benth, are likely to be other common substitutes of this.

Concluding Remarks

At present in West Pakistan gum is not being extracted from this tree. The main reason for this seems to have been the ignorance of people and lack of transport facilities till recently in the areas where *Acacia senegal* Willd, is found. In spite of extensive forests of *Acacia senegal* Willd, in West Pakistan every year gum arabic is being imported from India and Iran to the extent of about 2,250 maunds. The market price of this ranges from Rs. 180 to 210 per maund. According to Watt,² Captain M. A. Tighe, Political Agent of Southern Baluchistan was the first man who explored the possibilities of gum production in Pakistan. Since then there has been almost no effort in developing the gum production from this plant. In view of this, research work has been initiated in this field by the authors to find out the best season and best method of gum production in *Acacia senegal* Willd. There are immense possibilities of development in this field. *Acacia senegal* Willd is one of the few useful plants of the arid regions of West Pakistan and it can be grown without irrigation in rocky exposed places. In fact this tree is the only hope of clothing the highly eroded arid barren hill slopes of the southern parts of West Pakistan. The gum can be easily gathered by women, children and shepherds. At present one of the main exports from Sudan is gum arabic and millions of rupees in foreign exchange is being earned by that country. The same thing can be done in Pakistan by proper planning.

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