SHORT COMMUNICATIONS

CARBOHYDRATE COMPLEXES OF ZINC

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Zinc[1] is present in the human body up to the extent of 3.33 mg. per 100 g. of fat free body weight, liver and pancreas being its main depots. It is an important trace element forming part of the vital enzyme, carbonic anhydrase.[2] Dehydropeptidase,[3] a phosphatase,[4] and uricase[5] also appear to contain zinc. Novik[6] claims that he injected zinc sulphate at the rate of 0.5-1.0 mg./kg. wt. in some animals 22 days old and in consequence they gained 27.9% in wt. There was a remarkable increase in erythrocytes as well as in haemoglobin content. The quantity of 1.0-2.0 mg./kg. wt., however, caused a decrease in weight suggesting a toxic effect on higher dosage. Scouler[7] has reported that not less than 0.307 mg. of zinc per kilo should be included in the diet of a preschool child. Colloidal complexes of metals are always considered superior therapeutic agents than their ionic salts. We have therefore prepared zinc carbohydrate complexes which are reported here.

Ingredients

Zinc chloride pure (Merck's), (M. W. 136.29) 2.1 g., equivalent to 1 g. metallic zinc; sodium hydroxide 5% (for precipitation of $Zn(OH)_2$), 13 ml. containing 0.65 g. NaOH; tap water, 2 l.; distilled water, 10 l.; sucrose (refined cane sugar), 15 g.; glucose, 12 g.; maltose hydrate (B.D.H.), 11 g.; sodium hydroxide, 15% (for preparation of complex), in the case of sucrose complex =2.85 g.; in the case of glucose complex =2.25 g.; in the case of maltose complex=2.40 g.

Procedure

Zinc chloride was dissolved in 15 ml. of boiled water in a beaker of 1-l. capacity. Freshly prepared sodium hydroxide solution was added with vigorous stirring to get the white precipitate of zinc hydroxide. The hydroxide was washed twice with tap water and twelve times with distilled water. The last washings were found free from chloride ions when tested with silver nitrate.

Preparation of the Complex

Wet zinc hydroxide, carbohydrate dissolved in a minimum quantity of water, and sodium hydroxide all in requisite quantities were mixed thoroughly in a porcelain dish and heated in an electric oven at a fixed temperature and duration. When the reaction was completed a dark coloured cake was formed which gave a brownish clear solution. A turbid solution indicated incomplete heating or inappropriate ratios or both. The cake was dissolved in 60 ml. distilled and freshly boiled water, centrifuged and filtered. A few ml. of the clear solution were tested for metal content by E.D.T.A.[8] Metal was liberated as sulphate when the complex was heated to dryness with sulphuric acid.

Details of Preparation and Testing

1. The diameter and depth of the porcelain dish was 15 and 3 cms., respectively, while the layer of the heating mixture was 1 cm. deep.

2. About 20% of the metal was left unreacted. After the evaporation of water from the mass, the complex behaves like a bad conductor of heat. The upper and lower surfaces are differentially heated, leaving at the bottom the complex decomposed into zinc oxide. Possibly some zinc carbonate is also formed which can only give rise to zinc oxide.

3. Stability is tested by autoclaving the solution of the complex containing 1 g. metal at 100°C. for one hour. The solution remained clear.

4. The isoelectric point was estimated by decinormal hydrochloric acid.

Precautions

1. Measured amounts of water and sodium hydroxide should be used in the precipitation of zinc hydroxide, as excess of alkali redissolves the precipitate, forming sodium zincate.

2. Zinc hydroxide be washed with carbon dioxide free water. On keeping, the atmospheric carbon dioxide attacks the moist precipitate forming zinc carbonate.

TABLE 1.

Expt. No.	Ratio of Zn: carbohydrate	Ratio of Zn: NaOH	Temperature °C.	Time in hrs.	Metal in the complex	Stability on long boiling	Final pH before boiling	Iso-electric point
				ZINC: SUCROSE COMPLEX				
				(*Sucrose Variable*)				
1.	1:15	1:2.85	200	½	80%	Stable	9.0	3.9
2.	1:13	1:2.85	,,	½	75%	,,	8.6	3.6
3.	1:12	1:2.85	,,	½	75%	,,	8.6	3.6
				(*Alkali Variable*)				
4.	1:13	1:2.70	200	½	70%	Stable	8.3	4.5
5.	1:13	1:2.40	,,	½	70%	Unstable	—	—
6.	1:13	1:2.10	,,	½	70%	—	—	—
				(*Temperature Variable*)				
7.	1:13	1:2.85	170	formation not satisfactory				
				ZINC-GLUCOSE COMPLEX				
				(*Glucose Variable*)				
8.	1:12	1:2.40	200	½	80%	Stable	7.7	4.0
9.	1:11	1:2.4	,,	½	78%	,,	7.8	3.9
10.	1:10	1:2.4	,,	½	75%	,,	7.8	3.9
				(*Alkali Variable*)				
11.	1:11	1:2.25	200	½	75%	Stable	7.5	4.0
12.	1:11	1:2.10	,,	½	75%	Unstable	7.5	4.5
				(*Temperature Variable*)				
13.	1:12	1:2.4	185	1	80%	Stable	8.0	3.8
14.	1:12	1:2.4	170	2	formation not satisfactory			
				ZINC-MALTOSE COMPLEX				
				(*Maltose Variable*)				
15.	1:12	1:2.25	200	½	80%	Unstable	6.7	4.5
16.	1:10	1:2.25	,,	½	73%	Stable	7.9	3.9
17.	1:11	1:2.25	,,	½	80%	Stable	7.5	3.9
				(*Alkali Variable*)				
18.	1:11	1:2.1	200	½	75%	Stable	7.0	3.9
19.	1:11	1:1.80	,,	formation not satisfactory				
				(*Temperature Variable*)				
20.	1:12	1:2.25	170	1.30	80%	Unstable	7.0	4.5

3. Before heating the mixture of sodium hydroxide, carbohydrate and zinc hydroxide the ingredients should be thoroughly mixed.

Summary

1. Taking zinc as 1, the best ratio of sugar and alkali is 15:2.85, of glucose and alkali 12 : 2.4, and of maltose and alkali 11:2.25.

2. Best temperature for the formation of the complex is 200 °C.

3. Best isoelectric point is pH 3.9.

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CADMIUM CARBOHYDRATE COMPLEXES

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The importance of trace metals has been shown to consist in forming complexes with proteins which in turn constitute enzyme systems. The latest trace element which forms such an enzyme system has been proved to be cadmium.[1] The authors have therefore undertaken the study of carbohydrate complexes of this element hoping that the element in that form may be administered therapeutically, particularly along with iron[2] and cobalt[3] which have been reported previously.

Preparation of Cadmium Hydroxide using Cadmium Sulphate

Cadmium sulphate, $3CdSO_4 . 8H_2O$, (2.28 g.), equivalent to 1 g. elemental cadmium, taken in a beaker of 1-l. capacity, dissolved in 20 ml. of water and 13 ml. of 5% sodium hydroxide solution was added in small portions with vigorous stirring to get the white precipitate of cadmium hydroxide. This produces a yellow cadmium sulphide when treated with sodium sulphide. While the hydroxide, prepared by pouring a solution of cadmium salt into that of sodium hydroxide, gives an orange or red sulphide with an intermediate[4] dicadmium sulphohydroxide [$Cd_2S(OH)_2$]. There was, however, no difference in the formation of the complex when either of the two hydroxides were used. About 800 ml. of water was added to the precipitate and stirred well. After settling, it was stirred again so that sodium sulphate formed in the reaction may dissolve. Water was decanted after the precipitate had settled down. Similarly the hydroxide was washed 12 times with distilled water and last washings were tested for sulphate. Although the test was found to be negative it was more apparent than real. The hydroxide retained some amount of sulphate due to absorption. This was proved later when all the complexes prepared from the above hydroxide became unstable. When the precipitate was further washed with hot water, the washings did give the test for sulphate. Washing was further continued using hot water till no trace of sulphate was found. Even then the complexes formed with the hydroxide were unstable. Probably sulphate ions are adsorbed so tenaciously that even prolonged washings with hot water cannot remove them. The tenacity of sulphate ions has also been a hindrance in making other complexes, e.g. of iron, as previously reported.[2] The unstable complexes prepared from such cadmium hydroxide are recorded in Table 1.

Preparation of Cadmium Hydroxide using Cadmium Chloride

Cadmium chloride, $CdCl_2H_2O$ (1.79 g.) equivalent to 1 g. elemental cadmium was dissolved in 10 ml. of water in a beaker of 1-l. capacity and 13 ml. of 5% sodium hydroxide was

TABLE 1.

Expt. No.	Ratio of Cd sucrose	Ratio of Cd: NaOH	Temperature	Time in hrs.	Stability on long boiling	Isoelectric point	Final pH before boiling
1.	1:6	1:1.8	170 °C.	3	Unstable	5.5	9.1
2.	1:6	1:2.1	,,	,,	,,	,,	,,
3.	1:8	1:1.8	,,	,,	,,	,,	9.0
4.	1:6	1:1.8	200 °C.	1	,,	,,	9.2
5.	1:8	1:2.1	,,	,,	,,	,,	,,

TABLE 2.

Expt. No.	Ratio of Cd: Carbohydrate	Ratio of Cd: NaOH	Temperature	Time in hrs.	Stability of complex on long boiling	Isoelectric point	Final pH before boiling	Cadmium in the complex
			CADMIUM-SUCROSE COMPLEX					
			(*Sugar Variable*)					
1.	1:5	1:2.1	170 °C.	3	Stable	3.5	9.3	60%
2.	1:6	1:2.1	,,	,,	,,	,,	9.2	75%
3.	1:7	1:2.1	,,	,,	,,	,,	9.0	80%
			(*Alkali Variable*)					
4.	1:7	1:1.8	170 °C.	3	Unstable	4.0	9.0	70%
5.	1:7	1:1.5	,,	,,	,,	,,	9.0	70%
			CADMIUM-LACTOSE COMPLEX					
			(*Lactose Variable*)					
6.	1:8	1:2.1	170 °C.	3	Stable	3.1	9.1	60%
7.	1:10	1:2.1	,,	,,	,,	,,	9.0	80%
			(*Alkali Variable*)					
8.	1:10	1:1.8	170 °C.	3	Unstable	4.0	9.0	60%
9.	1:10	1:1.5	,,	,,	,,	,,	9.0	60%
			CADMIUM-MALTOSE COMPLEX					
			(*Maltose Variable*)					
10.	1:8	1:2.1	170 °C.	2	Stable	3.1	9.1	60%
11.	1:10	1:2.1	,,	,,	,,	,,	9.0	80%
			(*Alkali Variable*)					
12.	1:10	1:1.8	170 °C.	2	Unstable	4.0	9.2	60%
13.	1:10	1:1.5	,,	,,	,,	4.2	9.2	60%

added in small portions while stirring. It is worth noting that excess of alkali redissolves the precipitate of cadmium hydroxide. The solubility of cadmium hydroxide formed from cadmium sulphate, however, is comparatively lower than that of the hydroxide prepared from cadmium chloride. A similar phenomenon with zinc has been reported in the literature.[5,6]

Preparation of the Complex

A calculated quantity of the carbohydrate was taken in a porcelain dish and dissolved in a minimum quantity of boiled, carbondioxide-free water. Wet cadmium hydroxide was then admixed thoroughly. A known quantity of sodium hydroxide was added and the contents were heated at a fixed temperature in an electric oven for periods indicated in Tables 1 and 2. The whole mass, on the completion of the reaction, assumed a dark coloured cake which gives a clear solution, when dissolved in water. The solution of the complex prepared from cadmium hydroxide with traces of sulphate remains stable in the cold for a few hours, but in time or on long heating, forms a gel. On the contrary the solution of the complex prepared from the hydroxide derived from cadmium chloride remains stable even on long boiling. Following particulars further explain the data presented in the tables.

(1) Sodium hydroxide was used as a 15% solution but has been calculated as water-free NaOH and its weight indicated in its ratio with cadmium.

(2) About 20% of the metal was left unreacted. It is probably due to the fact that the complex being a poor conductor of heat was not uniformly heated. The product on the lower surface was overheated, being decomposed to cadmium oxide, thus preventing complete utilization of the metal.

(3) The porcelain dish was 5.0″ × 1.5″ with a product incorporating 1 g. elemental cadmium in a depth of 0.5″.

(4) The stability of the complex was tested by autoclaving its solution containing 2% metal for one hour at 100 °C. or for 30 minutes at 115 °C.

(5) The pH and isoelectric point also refer to a solution containing 2% cadmium.

(6) The isoelectric point was estimated by decinormal hydrochloric acid.

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ALUMINIUM CARBOHYDRATE COMPLEXES

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The importance of aluminium as a trace element is a recent finding. Randoin[1] tried a diet on rats containing most food elements including vitamin B, but lacking in several trace elements like iron, manganese, iodine and aluminium. It produced polyneuritis and malnutrition. The same workers then gave food containing iron, manganese, and iodine, but lacking in aluminium which nevertheless produced the same unhappy results. This showed for the first time that aluminium is essential to animal life. Later, in 1949, Voinar and Rusanov[2] found spectroscopically that aluminium along with other trace elements, like silver, bismuth, chromium and copper, are present in the human brain. Sections of the brain which differed in morphological structure and physiological function possessed a different chemical composition with regard to their trace elements. Wolff[3] found that aluminium is present in human blood. He analysed few samples of blood of females and males and found that the aluminium content was 55% in females and 53% in males. Aluminium complex will certainly prove to be superior than any of its salts when used as a trace element. Therefore we have prepared the complexes of aluminium with carbohydrates which are being reported here.

Preparation of Aluminium Hydroxide

(a) *From Aluminium Chloride (Anhydrous) and Caustic Soda.*—Aluminium chloride anhydrous (sublimated) (4. 8 g.) was cooled to 0-5 °C. Ten ml. of water was also cooled to 0-5 °C. The salt was then dissolved in water keeping the temperature as low as possible. The reaction is exothermic and some chloride sublimes while dissolving. In case it is dissolved at room temperature, almost all the aluminium chloride sublimes off as smoke. To the solution of aluminium chloride 30 ml. of 5% solution of NaOH was added slowly in portions and stirred; a glassy precipitate of aluminium hydroxide was obtained. Care should be taken not to add caustic soda in excess as the hydroxide is soluble with the formation of aluminate. The precipitate was then stirred thoroughly, 1 l. of tap water was added, allowed to settle and then decanted. It was decanted ten times, four times with tap water and finally six times with distilled water, when it was found free from electrolytes.

The following equations serve to illustrate the reaction discussed above:

$$AlCl_3 + 3NaOH \rightarrow Al(OH)_3 + 3NaCl.$$
$$Al(OH)_3 + NaOH \rightarrow NaAlO_2 + 2H_2O.$$

(*b*) *From $AlCl_3 . 6H_2O$ and NaOH.*—To avoid cooling the reaction mixture in the preparation of aluminium chloride solution from anhydrous aluminium chloride its hydrated form or $AlCl_3 . 6H_2O$ (Merck) instead was taken as the starting material. $AlCl_3 . 6H_2O$ (8.9 g.) was dissolved

TABLE 1.

Expt. No.	Ratio of Al: carbohydrate	Ratio of Al: NaOH	Temperature of heating	Time of heating in hrs.	Stability of the complex on long heating	Isoelectric point	pH of the complex	Metal in the complex	Density
				ALUMINIUM-SUCROSE COMPLEX					
				(*Sugar Variable*)					
1.	1:20	1:3.0	170°C.	2.75	Stable	2.9	8.6	88%	1.069
2.	1:15	1:3.0	,,	,,	,,	,,	8.6	80%	1.069
3.	1:10	,,	,,	,,	,,	,,	8.3	85%	1.069
4.	1:8	,,	,,	,,	,,	3.0	8.3	87%	1.068
5.	1.6	,,	,,	,,	Unstable	—	—	—	—
				(*Alkali Variable*)					
6.	1:10	1:3.0	170°C.	2.75	Stable	2.8-3.0	8.4	94%	1.06
7.	1:8	1:2.55	,,	,,	,,	,,	,,	90%	,,
8.	1:8	1:2.25	,,	,,	,,	,,	,,	,,	,,
9.	1:10	1:1.8	,,	,,	,,	,,	,,	,,	,,
10.	1:8	1:1.5	,,	,,	,,	,,	,,	85%	,,
11.	1:8	1:1.2	,,	,,	Unstable	—	—	—	—
				(*Temperature Variable*)					
12.	1:10	1:1.5	200°C.	1.25	Stable	2.8-3.0	8.6	91%	1.06
13.	1:10	1:1.5	180°C.	2.25	,,	,,	,,	,,	,,
14.	1:10	1:1.5	170°C.	2.25	,,	,,	,,	,,	,,
				ALUMINIUM-GLUCOSE COMPLEX					
				(*Glucose Variable*)					
1.	1:20	1:3.0	170°C.	2.5	Stable	2.6	7.8	90%	1.07
2.	1:17	1:3.0	,,	,,	,,	,,	,,	,,	,,
3.	1:15	,,	,,	,,	,,	,,	,,	,,	,,
4.	1:10	,,	,,	,,	,,	,,	,,	,,	,,
5.	1:8	,,	,,	,,	Unstable	—	—	—	—
				(*Alkali Variable*)					
6.	1:10	1:2.55	170°C.	2.5	Stable	2.5-2.8	7.8	85%	1.06
7.	,,	1:2.25	,,	,,	,,	,,	7.6	,,	,,
8.	,,	1:1.8	,,	,,	,,	,,	7.6	,,	,,
9.	,,	1:1.5	,,	,,	,,	,,	7.5	,,	,,
10.	,,	1:1.2	,,	,,	Unstable	—	—	—	
				ALUMINIUM-DEXTRIN COMPLEX					
1.	1:15	1:2.25	170°C.	2.75	Stable	2.8-3.2	8.2	78%	1.06
2.	1:10	1:2.25	,,	,,	,,	,,	7.9	75%	1.06
3.	1:8	1:1.5	,,	,,	,,	,,	7.6	70%	1.06
				ALUMINIUM-MALTOSE COMPLEX					
1.	1:20	1:3.0	170°C.	2.25	Stable	2.5-3.0	8.8	88%	1.07
2.	1:25	1:2.25	,,	,,	,,	,,	8.4	88%	1.07
3.	1:10	1:1.5	,,	,,	,,	,,	8.3	80%	1.07

in 10 ml. of water and 30 ml. 5% solution of sodium hydroxide in portions were added to precipitate the hydroxide. A precipitate of hydroxide was obtained, which is soluble even with a little excess of NaOH forming sodium aluminate.

$$AlCl_3.\,6H_2O + 3NaOH \rightarrow Al(OH)_3 + NaCl + 6H_2O.$$

$$Al(OH)_3 + NaOH \rightarrow NaAlO_2 + 2H_2O.$$

(*c*) *From $AlCl_3.\,6H_2O$ using Sodium Carbonate Solution Instead of NaOH Solution.*—Aluminium chloride, $AlCl_3.\,6H_2O$ (8.9 g.) was dissolved in about 10 ml. of water. To this solution 45 ml. of 20% Na_2CO_3 solution was added in portions while stirring, carbon dioxide being evolved. It was then boiled to remove carbon dioxide and one litre of tap water was added. The precipitate was allowed to settle down, water was then decanted. This process was repeated four times with tap water and six times with distilled water until it was free from electrolytes. Maximum amount of aluminium hydroxide is obtained by this method. The following equation serves to illustratre the reaction discussed above:

$$3Na_2CO_3 + 2AlCl_3.\,6H_2O = 2Al(OH)_3 + 6NaCl + 3H_2O + 3CO_2$$

Preparation of the Complex

The electrolyte-free aluminium hydroxide was used as starting material for preparing the complex. The carbohydrate was taken in a porcelain dish, dissolved in minimum quantity of distilled water and the wet hydroxide mixed with the required quantity of alkali. It was then heated at a constant temperature for durations indicated in Table 1. The final product after heating became a dark cake-like mass with a shining surface. The cake dissolved in water as a clear solution. Different ratios were tried to get the complex, but only the successful results have been recorded in Table 1. To elucidate the table, the following points are to be noted:

(1) A 15% solution of sodium hydroxide was employed.

(2) Some of the aluminium remained unchanged, and a complete utilization of aluminium was never achieved.

(3) The porcelain dish was ($7\frac{1}{4}''$ diameter), had a flat bottom and the product incorporated 1 g. elemental aluminium with the original mixture occupying a depth of 0.5″.

(4) Stability of the complex was tested by autoclaving a solution containing 1% aluminium in a sealed ampoule for one hour at 100°C.

(5) Density, pH, and isoelectric point all refer to a solution containing 1% aluminium.

(6) Isoelectric point was estimated by N/10 HCl.

Conclusions

(1) The best ratio of sugar to elemental aluminium is 8:1.

(2) The best ratio of alkali to elemental aluminium is 1.5:1.

(3) The best temperature is 170°C.

(4) The best isoelectric point ranges between 2.5-3.2 pH.

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CHROMIUM CARBOHYDRATE COMPLEXES

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In the human body seventy-four elements are present out of which sixty-five are trace elements, chromium[1] being one of them. Until recently nothing definitely was known about the biological importance of this metal although its presence was proved beyond doubt, being found in various parts of the body with the help of spectroscopic analysis. It is present in teeth, hair, urinary calculi, blood plasma and nervous tissue. Leonov[1] has shown the presence of chromium in cerebral and nervous tissues and its predominance over other trace elements in nucleus caudatus. According to

Monacelli and co-workers[2] its concentration in human blood plasma is 0.18 μg/ml. Wacker and others[3] have found that chromium is associated with ribonucleic acid (R.N.A.) in beef liver up to the extent of 1080 μg. Cr/g. dry weight, representing 20,000 fold aggregation of the metal over 0.05 μg./g. in the whole liver. Chromium has been found to affect both the structure and characteristic of chromosomes.[3] Schwarz[4] as well as Mertz[5] have shown trivalent chromium to be an active ingredient of glucose tolerance factor (G.T.F.) and that it increases the absorption of glucose in the fat tissue of rat by 67% in the presence of small amounts of insulin. It

TABLE 1.

No.	Cr: Carbohydrate	Cr: NaOH	Temperature	Time in hrs.	Metal content	Stability on boiling	Isoelectric point	Final pH	Density at 28°C.
				CHROMIUM SUCROSE COMPLEX					
				(*Sugar Variable*)					
1.	1:24	1:3.15	180°C.	2-30	75%	Unstable	3.1	8.2	1.148
2.	1:25	1:3.15	,,	,,	85%	Stable	,,	,,	1.151
3.	1:26	1:3.15	,,	,,	87%	Stable	,,	,,	1.154
				(*Alkali Variable*)					
4.	1:25	1:3.00	180°C.	2-30	80%	Unstable	2.8	8.0	1.150
5.	1:25	1:3.15	,,	,,	85%	Stable	3.1	8.2	1.151
6.	1:25	1:3.30	,,	,,	85%	Stable	3.1	8.5	1.151
				CHROMIUM MALTOSE COMPLEX					
				(*Maltose Variable*)					
1.	1:24	1:3.15	200°C.	1-45	75%	Unstable	1.5	5.6	1.079
2.	1:25	1:3.15	,,	,,	90%	Stable	,,	5.8	1.082
3.	1:26	1:3.15	,,	,,	90%	Stable	,,	5.8	1.082
				(*Alkali Variable*)					
4.	1:25	1:3.00	200°C.	1-45	85%	Unstable	1.4	5.4	1.080
5.	1:25	1:3.15	,,	,,	90%	Stable	1.5	5.8	1.082
6.	1:25	1:3.30	,,	,,	90%	Stable	1.5	6.1	1.082
				CHROMIUM LACTOSE COMPLEX					
				(*Lactose Variable*)					
1.	1:24	1:3.15	180°C.	2-0	80%	Unstable	1.5	5.8	1.097
2.	1:25	,,	,,	,,	90%	Stable	,,	,,	1.100
3.	1:26	,,	,,	,,	90%	Stable	,,	,,	1.100
				(*Alkali Variable*)					
4.	1:25	1:3.00	180°C.	2-0	85%	Unstable	1.8	5.4	1.100
5.	1:25	1:3.15	,,	,,	90%	Stable	1.5	5.8	1.100
6.	1:25	1:3.30	,,	,,	90%	Stable	1.5	6.2	1.100
				CHROMIUM GLUCOSE COMPLEX					
				(*Glucose Variable*)					
1.	1:24	1:3.15	200°C.	1-45	82%	Unstable	1.5	6.5	1.082
2.	1:25	1:3.15	,,	,,	85%	Stable	,,	6.6	1.085
3.	1:26	1:3.15	,,	,,	87%	Stable	,,	6.5	1.087
				(*Alkali Variable*)					
4.	1:25	1:3.00	200°C.	1-45	75%	Unstable	1.4	6.0	1.079
5.	1:25	1:3.15	,,	,,	85%	Stable	1.5	6.6	1.085
6.	1:25	1:3.30	,,	,,	87%	Stable	1.6	7.0	1.087

catalyses the phospho-glucomutase reaction,[6] and also stimulates other enzyme systems like succinate-cytochrome-*c*-reductase[7] and favourably influences many biological phenomena. Its role in various unclarified diabetic conditions is being further investigated by them. Considering its importance we have taken up the preparation of various chromium-carbohydrate complexes aiming at their therapeutic use in the future.

Preparation of the Hydroxide

The work was started with chromic sulphate $Cr_2(SO_4)_3$ but it was revealed that the complexes prepared later from the resulting hydroxide were not stable. The reason proved to be the adsorption of sulphate ions on the precipitated hydroxide and the difficulty of removal on subsequent washing. Chromic chloride hexahydrate (Merck's) was used for obtaining chromic hydroxide.

$$CrCl_3.\ 6H_2O + NaOH = Cr(OH)_3 + 3NaCl.$$

Chromic chloride (5.1 g.), equivalent to 1 g. elemental chromium, was taken in a beaker, dissolved in 50 ml. of distilled water and boiled for a few minutes. To this was added 45 ml. of 5% NaOH solution in small portions while stirring vigorously so that a granular hydroxide is formed which is easily washed by decantation. If the solution is not boiled or vigorously stirred it gives rise to a colloidal hydroxide which does not settle easily. This precipitate was then washed free of electrolytes, taking about 700 ml. of distilled water each time; last washings being tested for the absence of chloride. The process requires 10 washings, using in all about 7 litres of H_2O.

Preparation of the Complex

The hydroxide was taken up in a stainless steel dish with the help of a little distilled water and to this were added requisite amounts of carbohydrate and sodium hydroxide. The contents were heated in an electric oven at a definite temperature until the mass assumed a dark chocolate colour. A small portion was tested for the completion of reaction by dissolving in distilled water when a clear solution was obtained, leaving no residue. The complex was then dissolved in 100 ml. of distilled water and centrifuged to remove the unreacted material. Various data concerning the complex are given in the tables to which the following points may be supplemented:

(1) Best ratios for the complex were 1 g. chromium, 25 g. carbohydrate and 3.15 g. NaOH.

(2) Sodium hydroxide was used as a 15% solution but in tables it is given as grams.

(3) A stainless steel dish of 6″ diameter and 1½″ depth was used for the complex formation.

(4) Chromium complexes with glucose and maltose were best prepared at a temperature of 200°C. whereas with sucrose and lactose the best temperature was 180°C.

(5) During heating some of the hydroxide at the bottom gets decomposed into chromium oxide, therefore only 90% utilization of the metal was achieved.

(6) pH, isoelectric point, and density refer to a solution with 1% of the metal.

(7) 0.1 N. HCl was used to determine isoelectric points of solution.

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TIN CARBOHYDRATE COMPLEXES

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Work on trace elements was started a century ago but as therapeutic agents they have been introduced in recent years although they were being used much earlier in Unani medicine. Dutoit[1] and others reported that on spectrographic analysis of ash of human organs, tin was found in all organs, particularly in the brain, spleen and thyroid. The following data by Emile Misk[2] shows the distribution of tin in the body of an average person aged 42; one kg. of dry tissue

revealed the presence of tin as follows:

Lungs	0.6317 g.
Liver	0.4678 g.
Alimentry canal	0.3077 g.
Brain	0.0199 g.

The administration of tin in the diets of animals promoted growth, bone formation and increased fertility, as well as serum phosphorus.[3] Keeping in view the importance of tin we prepared complexes of this metal, since they are retained better than inorganic salts which are rapidly excreted.[4]

Preparation of Tin Hydroxide

One hundred ml. of stannic chloride (fuming was made up to one litre. On estimation as SnO_2 (gravimetric), it was found that 5 ml. of this solution contains 1.85 g. of $SnCl_4$, which is equivalent to 1 g. of metallic tin. Five ml. of tin chloride was taken in a beaker of 1-l. capacity and diluted to 30 ml. to which 30 ml. of 5% sodium hydroxide solution was added. A precipitate of hydrated oxide of tin was obtained at pH 3. This was then washed four times with tap water and finally six times with distilled water to free it from electrolytes. The following equation illustrates the reaction discussed above:

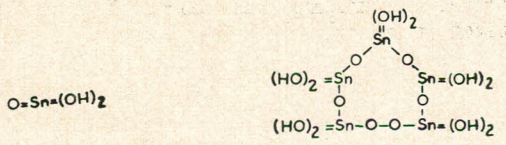
$$SnCl_4 + 4NaOH = H_2SnO_3 + 4NaCl + H_2O$$

Hydrated oxide of tin was also prepared by the hydrolysis of $SnCl_4$ solution as follows:

Five ml. of the $SnCl_4$ solution were taken in a litre flask and 200 ml. of water added. The solution was boiled for half an hour. A gelatinous precipitate of H_2SnO_3 is formed which settles on standing, according to the equatino:

$$SnCl_4 + 3H_2O = H_2SnO_3 + 4HCl$$

The beaker was filled with 600 ml. of tap water and was allowed to stand for ½ hour. The clear supernatant liquid was decanted. The beaker was again filled with 800 ml. of distilled water and the process repeated until the supernatant liquid did not give the test of any electrolyte. Complete washing required repeating it 5 to 6 times. This hydrated oxide of tin is called stannyl hydroxide, metastannic acid or α-stannic acid. It is acidic in character and reactive in complex formation. On keeping, it changes to β-stannic acid which is not so reactive due to its structural rearrangement. The structural formulae of the two acids[5] are given below:



α-stannic acid β-stannic acid

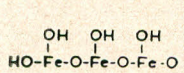
TABLE 1.

No.	Ratio of metal carbohydrate in g.	Ratio of metal to alkali in g.	Temperature of heating	Time of heating in hour	Stability of the solution in the cold	Stability of solution on long boiling	pH of the complex	Isoelectric point	Percentage of the metal
				TIN-SUCROSE COMPLEX (*Sugar Variable*)					
1.	1:25	1:3.45	200°C.	1	Unstable	—	—	—	—
2.	1:21	1:3.45	,,	,,	Stable	Stable	7.6	2.1	89%
3.	1:20	1:3.45	,,	,,	,,	,,	7.8	1.6	92%
4.	1:18	1:3.45	,,	,,	Unstable	—	—	—	—
				TIN-SUCROSE COMPLEX (*Alkali Variable*)					
1.	1:20	1:3.3	200°C.	1	Stable	Stable	6.8	2.1	90%
2.	1:20	1:3.15	,,	,,	,,	,,	6.0	2.8	86%
3.	1:20	1:3.0	,,	,,	Unstable	—	—	—	—
				TIN-SUCROSE COMPLEX (*Temperature Variable*)					
1.	1:20	1:3.45	200°C.	1	Stable	Stable	7.8	7.6	92%
2.	1:20	1:3.45	180°C.	1¼	,,	Unstable	—	—	—
3.	1:20	1:3.45	170°C.	1½	,,	,,	—	—	—

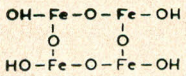
TABLE I (*contd.*).

No.	Ratio of metal carbohydrate in g.	Ratio of metal to alkali in g.	Temperature of heating	Time of heating in hour	Stability of the solution in the cold	Stability of solution on long boiling	pH of the complex	Isoelectric point	Percentage of the metal
				TIN-GLUCOSE COMPLEX					
				(*Glucose Variable*)					
1.	1:25	1:4.35	180°C.	1	Unstable	—	—	—	—
2.	1:21	1:4.35	,,	,,	Stable	Stable	7.7	3.0	88%
3.	1:20	1:4.35	,,	,,	,,	,,	7.6	2.9	89%
4.	1:19	1:4.35	,,	,,	Unstable	—	—	—	—
				TIN-GLUCOSE COMPLEX					
				(*Alkali Variable*)					
1.	1:20	1:4.2	180°C.	1	Stable	Stable	6.1	2.5	90%
2.	1:20	1:4.05	,,	,,	,,	,,	5.4	2.1	91%
3.	1:20	1:3.75	,,	,,	Unstable	—	—	—	—

There is likewise a reactive open-chain compound and a non-reactive ringed structure as two forms of iron hydroxide[6] one alkaline and chelating and the other non-chelating and acidic as shown below:



γ-Ferric hydroxide



α-Ferric hydroxide

Preparation of the Complex

The hydrated oxide of tin was taken as the starting material for the preparation of the complex. The carbohydrate was taken in a porcelain dish of $7\frac{1}{4}''$ diameter and dissolved in the minimum quantity of water and the wet stannic hydrated oxide was admixed thoroughly. The required quantity of alkali was then added and the contents heated at different temperatures and for periods indicated in Table 1. After heating, the product became a cake-like mass, dark in colour with a shining surface which gave a clear solution in water. Different ratios were tried to get the ideal complex but only the successful results are given in Table 1.

To further clarify the table, the following points have been added:

(1) The ratio of metal to carbohydrates are indicated in g. wt.

(2) 15% solution of NaOH was employed but its ratio to metal is also given in g. wt.

(3) About 10% of the metal was oxidized and as such did not form complex.

(4) The stability of complex was tested by autoclaving a solution containing 1% Sn in a sealed ampoule for one hour at 100°C. The solution remained clear.

(5) The porcelain dish was $7\frac{1}{4}''$ diameter but the depth of the heating mixture was kept constant $\frac{1}{2}''$.

(6) pH and isoelectric point also refer to a solution containing 1% Sn.

(7) The isoelectric point was estimated with N/10 HCl.

Conclusion

1. Minimum ratios of sucrose and glucose to 1 g. of elemental tin each are 20 g.

2. Minimum ratio of NaOH in case of sucrose is 3.45 g. and in case of glucose 4.05 g.

3. The best temperature is 200°C.

4. The best isoelectric point in the case of sucrose is pH 1.6 and in case of glucose pH 3.0.

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THE PREPARATION AND ESTIMATION OF IRON SACCHARATE

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The preparation of iron-complexes has been studied mainly with the veiw to using iron as a therapeutic agent. The best preparation has been accepted as iron saccharate. Several firms manufacture it showing minor differences. These have been subjected to critical studies and reported already.[1] The preparation requires observing some subtle features without which the end product does not prove to be ideal. We are, therefore, presenting details which should result in removing all difficulties in the preparation and estimation of iron saccharate.

Preparation of Ferric Chloride Solution

Iron pieces (scrap iron) (40 g.) and 150 ml. of commercial hydrochloric acid (200 ml. of sp. gr. 1.14 diluted to 300 ml.) were taken in a round bottomed flask of 300 ml. capacity, fitted with a reflux condenser. The contents were heated at 85-90 °C. for 3 hours until the evolution of hydrogen ceased. The reflux condenser prevents the escape of hydrogen chloride vapours and also the access of air which tends to oxidise some amount of the ferrous chloride to ferric oxide. The ferrous chloride solution (pH 2.-2.5) is filtered from the unreacted iron (about 13 g.) and the remaining 150 ml. of HCl is added to it. This iron was supplemented with another 27 g. making 40 g. for which another lot of about 300 ml. com. concentrated hydrochloric acid was required. Into another flask 30 ml. of commercial nitric acid (20 ml. of sp. gr. 1.36 diluted to 40 ml.) was taken and the above solution of ferrous chloride was added to it slowly. The contents were heated for 2 hours at 80-85 °C. cooled to room temperature and filtered. Solution of ferric chloride (pH 0.2) containing 10% iron was obtained.

Note: The solution should be tested for ferrous iron by potassium ferricyanide solution. If ferrous ion is suspected, add a few drops of the above-mentioned nitric acid and boil.

Preparation of Ferric Hydroxide

Ten ml. of ferric chloride solution (containing 1 g. iron or 4.8 g. $FeCl_3 . 6H_2O$ equivalent to 1 g. elemntal iron) was diluted to 50 ml. in a beaker of 1-l. capacity and heated to 40 °C. Sodium carbonate solution (3.2 g. carbonate dissolved in 16 ml. of water to make 20% solution) was added slowly with vigorous stirring to get the red ferric hydroxide. About 800 ml. tap water was added to the precipitate and stirred well, allowed to settle and stirred again. Water was decanted after a few minutes when iron hydroxide had settled down. Again 700 ml. tap water was added, stirred and decanted. In this way iron hydroxide was washed three times with tap water using about 2.5 l. and four times with distilled water using in all about 3 l. The last washing was tested for chlorides with silver nitrate, the test should be negative. The time for washing is about 4 hours.

Note: NaOH is not used for the precipitation of ferric hydroxide, because the electrolyte is very difficult to wash.

Preparation of Iron Saccharate

The wet ferric hydroxide (1.9 g. equivalent to 1 g. elemental iron) was taken in a dish, 3.5 g. sugar dissolved in 10 ml. of H_2O, 0.3 g. NaOH dissolved in 2 ml. of H_2O, and 30 ml. of water were added to it and mixed thoroughly. The contents were heated at 130°C. for 4 hours till a dark brown cake is formed, which gave a chocolate coloured clear solution when dissolved in water. The cake was dissolved in distilled water, centrifuged and tested for stability by boiling a few ml. of it in a sealed ampoule for 1 hour. The solution should remain clear. The isoelectric point with HCl would be 4.8-5.2 pH. The pH of the solution is lowered by cirtic acid from 10.0 to 7.5.

Estimation of Iron in Iron Saccharate

Reagents.—Potassium dichromate, 1.226 g.

$K_2Cr_2O_7$ in 1 l., 0.025 N; hydrochloric acid pure; stannous chloride solution, 1.5 g. $SnCl_2.H_2O$ + 10 ml. pure HCl made up to 100 ml.; mercuric chloride saturated solution, phosphoric acid solution 25 ml. *o*-phosphoric acid + 75 ml. pure H_2SO_4 made up to 500 ml., diphenylamine (internal indicator) 1 g. in 100 ml. pure H_2SO_4.

Procedure.—Five ml. of iron saccharate was taken in a small dish, 5 ml. of pure HCl added and heated on a sand bath at 70°C. for 2 hours, till the complex was charred completely. Ferric chloride formed in the reaction was dissolved in water; filtered into a 100 ml. volumetric flask and made up to the mark. Five ml. of this solution was taken in a conical flask of 250 ml. capacity; 5 drops of hydrochloric acid added, and heated to 80°C. Stannous chloride solution was dropwise added to the above solution while hot, avoiding excess, so that ferric iron was reduced to ferrous state. At the complete reduction the light yellow colour of the solution became colourless. The solution was diluted to 100 ml.; cooled to room temperature and unused stannous chloride was neutralized by adding 5 ml. mercuric chloride solution. The colour was changed to a whitish tinge. Twenty-five ml. phosphoric acid solution and 2 or 3 drops of diphenylamine were added and titrated against potassium dichromate to violet colour.

1 ml. $K_2Cr_2O_7$ (0.025 N) = 0.0014 g. iron.

The following reactions take place:

(1) Iron saccharate + $3HCl \rightarrow FeCl_3$ $[Fe_2O_3 (C_{12}H_{22}O_{11})x]$

(2) $2FeCl_3 + SnCl_2 = 2FeCl_2 + SnCl_4$

(3) $SnCl_2 + 2HgCl_2 = SnCl_4 + Hg_2Cl_2$

(4) $6FeCl_2 + K_2Cr_2O_7 + 14HCl = 6FeCl_3 + 2\ CrCl_2 + 2KCl + 7H_2O.$

Note: 1. Iron saccharate should not be heated at high temperature, as it causes spurting.

2. Long heating should be avoided as the ferric chloride formed is converted into oxide.

3. Sugar component should be burnt completely, otherwise its colour may interfere in the estimation.

4. If excess of stannous chloride is added, the solution becomes black when later mercuric chlroide is added, due to the formation of metallic mercury according to the reaction:

$$SnCl_2 + HgCl_2 = Hg + SnCl_4.$$

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STUDY OF MAKROLIN AS A FUNGICIDE

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Every year a large number of crops and vegetables are destroyed in the country due to fungus infection such as rust of wheat, the late blight of potatoes and the root rot infections. It was therefore decided to investigate the possibility of using an indigenous material either to destroy the pathogenic fungi completely or to inhibit their growth and reproduction. And as such the fungicidal activity of Makrolin, an indigenous product, was compared with that of the imported fungicide, copper naphthenate.[1,2]

Biological Material and Methods

The following three different strains of fungi were selected for testing the fungicidal activity of Makrolin in comparison with copper naphthenate: (a) *Aspergillus niger*, which causes a widely spread disease of onion bulbs, which is known as black mold,[3] and hence destroys the economic value of the onion to a great extent. (b) *Fusarium* species which is known to cause common root rot of vegetable and other plants, especially cotton. The disease is very common in the lower parts of West Pakistan and is causing great damage to the roots of the cotton plants. (c) *Alternaria solenii*, which causes black leaf spot disease of cabbage, and cauliflower.

The above-mentioned three strains of fungi were grown on petri dishes. The medium used was Czapeck's agar medium. The procedure adopted was that the medium was poured in the sterilized plates and after its solidification the pure culture of fungus was inoculated in the centre of each petri plate to obtain a circular growth. The fungi were allowed to grow for 72 hours at room temperature before using them for chemical treatment. The 5% solutions of Mak-

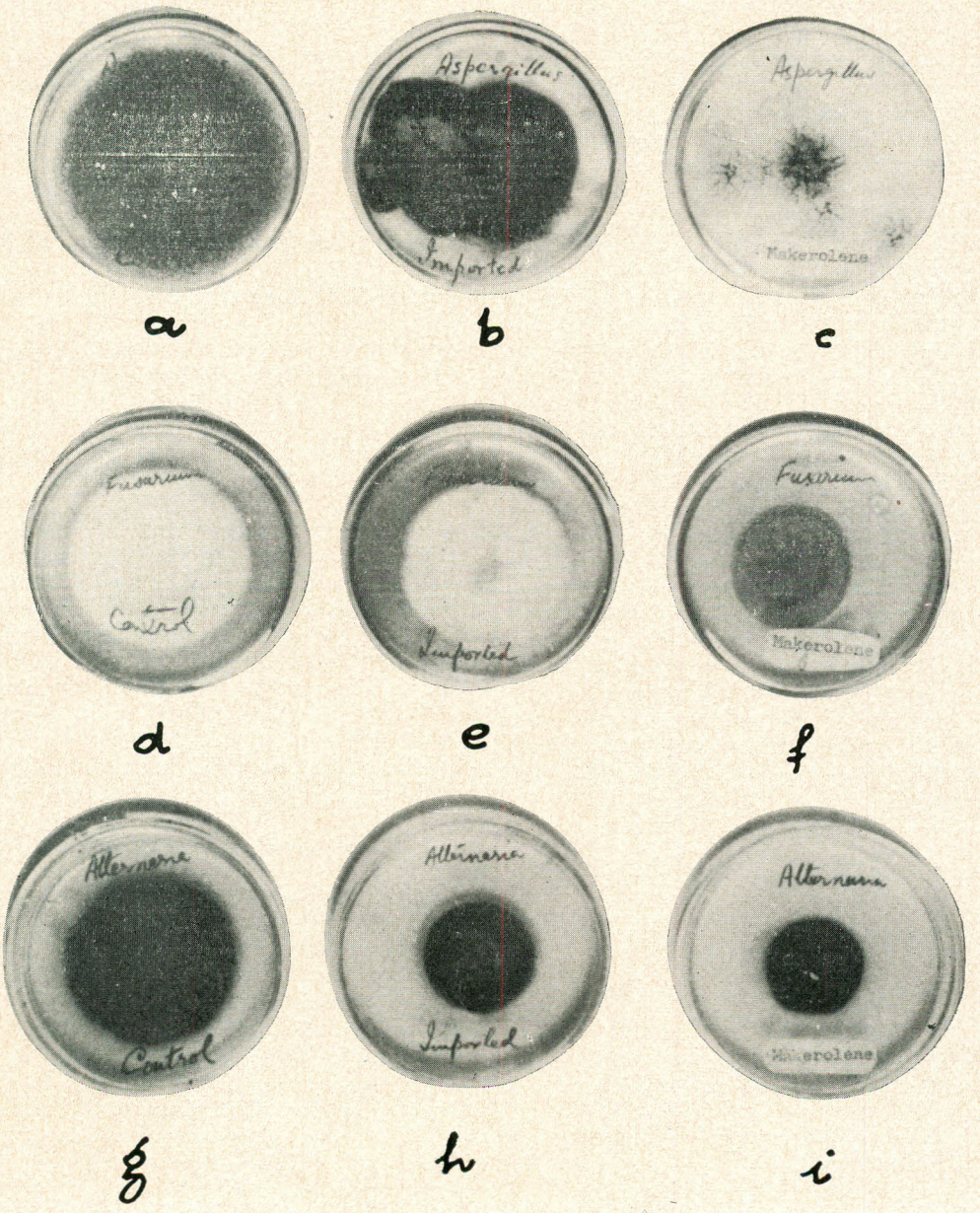

Fig. 2.—Fungicidal effect of Makrolin and copper naphthenate on *Aspergillus niger* (petri plates a,b, and c); *Fusarium* species (petri plates d,e, and f) and *Alternaria solenii* (petri plates g, h, and i).

rolin and copper naphthenate were prepared in acetone for experimentation. A sterile wooden box of one cubic foot, open on one side, was used as a spraying chamber. The inoculated plates were kept in the box, and 0.5 ml. solution of each chemical was sprayed on the circular fungal colonies from a distance of 14″. Care was taken in spraying the material uniformly by the help of devillibis automizer on the surface of the growing fungi. Controls were also run for each experiment and the readings were taken after 48 hours from the time of fungicide treatment. Twenty experiments were performed and each set of experiment was done in triplicate.

Results and Discussion

The results obtained were based on the mean values of 20 experiments and has been shown in Table 1. The photographic representation of one complete experiment is shown in Fig. 1.

It was found that the growth of *Aspergillus niger* was almost completely checked by Makrolin in

TABLE 1.—FUNGICIDAL EFFECT ON THE GROWTH RATE OF THE FUNGI DUE TO MAKROLIN.

Fungi	Diameter of the fungus colony in cm.			
	Before experiment	After 48 hrs. of spraying		
		Control	Makrolin	Copper naphthenate
Aspergillus niger	3.5	8.0	3.5	5.5
Fusarium species	4.0	6.8	5.0	6.0
Alternaria solenii	3.0	6.0	3.0	3.5

comparison with the control and whereas it was partially checked by the imported fungicide (Fig. 1, a,b,c,). In the case of *Fusarium* species the growth of the fungi was completely controlled by Makrolin where copper naphthenate was less effective (Fig. 1, d,e,f,). Both Makrolin and copper naphthenate checked the growth of *Alternaria solenii* effectively.

It was concluded that 0.5 ml. of 5% solution of Makrolin in acetone is effective in checking the growth of *Aspergillus niger*, *Fusarium* species and *Alternaria solenii*.

Acknowledgements.—The authors are highly indebted to Dr. Salimuzzaman Siddiqui, F.R.S., for his encouragement and valuable suggestions during the progress of the work. We are also thankful to Dr. Mustafa Kamal, Plant Pathologist, Agriculture College, Tandojam, for supplying the fungus strains.

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DETERMINATION OF VITAMINS BY NON-AQUEOUS TITRATION

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Titration in non-aqueous solvents is a simple and convenient method for the quantitative determination of many weakly basic or acidic substances which give a sharp end point which they fail to do if water is used as solvent. This method has already been applied for the estimation of pharmaceutical agents like alkaloids,[1-4] sulphonamides,[5] analgesics,[3] antihistaminics[6] and antibiotics.[7] Tablet excipients such as starch, lactose, talc and stearic acid do not react with acetous perchloric acid and it is possible to titrate these pharmaceutical preparations without performing a preliminary extraction process.

In the present investigation we have extended this method to the estimation of some vitamins.

Procedure

A glass electrode as indicator electrode and a saturated calomel electrode as reference electrode and a direct reading pH meter were used. The two electrodes were connected in series with a variable resistance.

Assay of Thiamine-HCl and Pyridoxine-HCl.—Exactly 10-30 mg. of the vitamin was dissolved in 5-10 ml. of acetous mercuric acetate. The solution was then titrated against 0.1N acetous perchloric acid both by potentiometry and by visual indicators. The difference between this and a blank reading gives the volume of acetous perchloric acid required to completely neutralise the vitamins.

Assay of p-Aminobenzoic acid.—Exactly 10-30 mg. of the acid was dissolved in known amount of 0.1N acetous perchloric acid and the excess acid was back titrated with 0.1N acetous sodium acetate, both potentiometrically and visually.

Assay of Nicotinic Acid and Ascorbic Acid.—Exactly

TABLE 1.—ASSAY OF VITAMINS IN GLACIAL ACETIC ACID.

Vitamins	Amount taken	Amount found
1. Thiamine hydrochloride	32.0 mg.	32.39 mg.
2. Thiamine hydrochloride tablets	25.0 ,,	25.81 ,,
3. Pyridoxine hydrochloride	31.0 ,,	31.1 ,,
4. Pyridoxine hydrochloride tablets	40.0 ,,	30.0 ,,
5. Nicotinic acid	21.0 ,,	20.9 ,,
6. *p*-Aminobenzoic acid	22.5 ,,	22.25 ,,
7. Ascorbic acid	48.5 ,,	48.43 ,,

10-30 mg. of the vitamin was completely dissolved in 10 ml. of dimethyl formamide. The solution was then titrated with 0.1N potassium methoxide potentiometrically and by visual indicators. Ascorbic acid gives correct results only by visual indicators.

The assay results (average of three titrations) are given in Table 1.

Acknowledgement.—The authors are indebted to Dr. S. A. Warsi, Director, North Regional Laboratories, for his interest and encouragement in the present work.

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CERCOSPORAE OF THE SIND REGION INCLUDING 35 NEW RECORDS FROM PAKISTAN

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The genus *Cercospora* Fres. is world-wide in distribution and in certain cases is responsible for causing heavy damage to host plants. Sultan Ahmad[1] listed 29 species from West Pakistan, and later a few additions to these were made by various workers.

The 41 species listed in the present paper are the first records from the Sind region which forms a distinct ecological zone. They have been collected from a wide variety of hosts occurring at the Agricultural Research Institute, Tandojam, and neighbouring areas. As far as known to the authors the first 35 are the first records from Pakistan and these include two new species.

Cercospora pentatropoidis Shakil
on *Pentatropis cynanchoides* R. Br.
Cercospora phyllanthicola Shakil & Kamal
on *Phyllanthus niruri* L.
Cercospora phyllanthi Chupp.
on *Phyllanthus niruri* L.
Cercospora macutensis Syd.
on *Corchorus trilocularis* L.
Cercospora corchori Swada
on *Corchorus trilocularis* L.
Cercospora cruenta Sacc.
on *Dolichos lablab* L.
on *Vigna catjang* Walp.
Cercospora cajani P. Henn.
on *Cajanus indicus* Spreng.
Cercospora atromarginalis Atk.
on *Solanum nigrum* L.
Cercospora jujubae Chowdhury
on *Zizyphus nummularia* W. & A.
Cercospora zebrina Passer
on *Trifolium resupinatum* L.
Cercospora citrullina Cooke
on *Lagenaria vulgaris* Ser.
on *Citrullus vulgaris* var. *fistulosus* Stocks.
Cercospora avicennae Chupp.
on *Abutilon indicum* G. Don
Cercospora ficina Tharp.
on *Ficus carica* L.
Cercospora papavericola Chupp.
on *Argemone mexicana* L.
Cercospora sesbaniae P. Henn.
on *Sesbania aegyptica* Poir.
Cercospora erythrinicola Tharp.
on *Erythrina suberosa* Roxb.
Cercospora malayensis Stev. & Solh
on *Hibiscus esculentus* L.
Cercospora furfurella Speg.
on *Boerhaavia diffusa* L.
Cercospora tabernaemontanae H. + P. Sydow
on *Tabernaemontana coronaria* Br.
Cercospora zizyphi Petch.
on *Zizyphus nummularia* W. & A.
Cercospora dalbergiae-latifoliae Chiddarwar
on *Dalbergia sissoo* Roxb.
Cercospora lepidagathidis Govindu & Thirum.
on *Lepidagathis hyalina* Nees.
Cercospora cocculi Syd.
on *Cocculus hirsuta* (L) Diels.
Cercospora achyranthina Thir. & Chupp.
on *Achyranthes aspera* L.

Cercospora canescens Ell. & Mart.
on *Cyamopsis psoraloides* DC.
on *Phaseolus aureus* Roxb.
on *Phaseolus mungo* L.
on *Rhynchosia minima* DC.
on *Rhynchosia aurea* DC.
on *Crotalaria juncea* L.
on *Bauhinia variegata* Linn.
on *Dolichos lablab* L.
on *Artocarpus integra* Merr.

Cercospora glycyrrhizae-echinatae (Savul. & Sandu) Chupp.
on *Glycyrrhiza glabra* L.

(Note: The collection shows shorter conidia and conidiophores. There is also a conspicuous thickened scar both on the conidiophore, and at the base of the conidium.)

Cercospora beticola Sacc.
on *Spinacia oleracea* Linn.
on *Beta vulgaris* L.
on *Chenopodium murale.*

Cercospora salvadorae Maire
on *Salvadora oleoides* Dcne.
on *Salvadora persica* Linn.

Cercospora gossypina Cooke
on *Gossypium hirsutum* L.
on *Gossypium arboreum* L.

Cerco. (*Passalora*) *personata* (B. & C.) E.E.
on *Arachis hypogae* L.

Cercospora calotropidis Ell. & Ev.
on *Calotropis procera* R. Br.

Cercospora subsessilis Syd.
on *Azadirachta indica* Juss.

Pseudocercospora vitis (Lev.) Speg Syn. *C. viticola* (Ces) Sacc.
on *Vitis vinifera* L.

Cercospora rosicola Thuem.
on *Rosa* sp.

Cercospora mali Ell. & Ev.
on *Pyrus malus* L.

Cercospora cichorii J. J. Davis
on *Cichorium intybus* L.

Cercospora sesami Zimm.
on *Sesamum indicum* L.

Cercospora snelliana Reichert
on *Morus alba* L.

Cercospora mitteriana Syd.
on *Dodonaea viscosa* L.

Cercospora ricinella Sacc. & Berl.
on *Ricinus communis* L.

Cercospora helianthicola Chupp. & Viegas
on *Helianthus annuus* L.

Cercospora traversiana Sacc.
on *Trigonella foenum-graecum* L.

The specimens are available in the Herbarium, Plant Pathological Section, A.R.I. Tandojam, and duplicates in the Herbarium, Commonwealth Mycological Institute, Kew, England.

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STABILITY OF VITAMIN A IN GHEE AND VITAMINISED VANASPATI

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Stability of vitamin A in ghee and 'vanaspati' has been studied by a number of workers.[1–8] Hattiangdi and Ketayan[9] have reported that the greatest loss of vitamin A occurs on shallow frying (220°C.), somewhat less on deep frying (200°C.), and minimum in the case of simmering (100°C.), the losses being considerably higher in ghee than in vanaspati. From the analytical constants given by them it appears that the ghee under examination contained high initial acidity (2.3%) and peroxide value (1.3), which they felt was responsible for the destruction of vitamin A. Since the acidity of an average Pakistani ghee, especially the one procured in the former Punjab province of West Pakistan, is generally less than 1.5%, it was considered of interest to study the heat stability of vitamin A in this ghee.

It has also been observed by Hattiangdi and Ketayun,[9] that vitamin A in ghee and vanaspati was also destroyed to the extent of 8-10% at

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100 °C. in half an hour. In view of the fact that meat and vegetable curries take a much longer time, experiments were also carried out to investigate the stability of vitamin A in both the fats by boiling them in air-oven at 100°C.

Materials and Methods

Ghee and Dalda vanaspati were obtained from the local market and their analytical constants determined (Table 1).

Frying.—About one pound of each type of fat was poured into a one-litre beaker. The temperature was quickly brought to 200 °C. and a portion of the fat was removed for analysis. A few gram-flour balls (locally called 'pakoras') were introduced and fried for about 2 minutes. A second charge of the gramflour balls was then added and the frying continued for 15 minutes. Samples of fats were removed just before frying and at 5, 10 and 15 minutes intervals.

Heating at 100°C.—(a) Boiling in water: About 600 ml. of water was boiled in a beaker and about 100 g. of each fat was added to it. The water was gently boiled and samples of fat were removed at 1/2, 1, and 2 hours intervals. To remove excess moisture, the fat was stirred while hot with a small quantity of anhydrous sodium sulphate and centrifuged.

(b) Heating in air-oven at 100 °C.—About 30 g. of each fat was taken in a shallow beaker and heated in an air-oven maintained at 100 °C. Samples of the fat were as usual withdrawn at 15, 30 and 60 minute intervals.

Determination of Vitamin A.—Vitamin A in the samples was determined according to the Carr Price method.[10] The blue colour developed was measured in a Hilger photoelectric colorimeter. A standard curve was prepared with pure vitamin A palmitate and the readings of the unknown were read against it.

Results and Discussion

It is evident from Tables 2 to 4 that heat treatment under varying conditions brings about almost the same loss of vitamin A in ghee as in vanaspati. The results thus suggest that vitamin A in ghee of low acidity is as stable to heat treatment as in vanaspati. Our observation that frying causes more than 70% loss of vitamin A after 15 minutes is in conformity with the findings of earlier workers.[9]

Boiling ghee or vanaspati in water for half an

TABLE 1.—ANALYTICAL CHARACTERISTICS OF GHEE AND DALDA VANASPATI.

	Ghee	Dalda vanaspati
Melting Point	37°C.	42°C.
Per cent of free fatty acid as oleic acid	0.8	0.1
Vitamin A, I.U./g.	41.5	22.0

TABLE 2.—LOSS OF VITAMIN A IN GHEE AND VANASPATI.

	Ghee		Dalda vanaspati	
	Vitamin A I.U./g.	Loss %	Vitamin A I.U./g.	Loss %
Control	41.5	—	22.0	—
Before putting the charge (100 °C.)	30.0	27.8	16.0	27.5
After 5 mins.	24.0	42.1	12.8	41.9
,, 10 ,,	16.5	60.2	9.0	59.2
,, 15 ,,	11.7	71.8	6.5	70.6

TABLE 3.—LOSS OF VITAMIN A ON BOILING GHEE AND VANASPATI IN WATER.

	Ghee		Dalda vanaspati	
	Vitamin A I.U./g.	Loss %	Vitamin A I.U./g.	Loss %
Control	43.0	—	22.0	—
After 30 mins.	36.0	16.3	18.4	16.2
,, 60 ,,	14.0	44.2	12.7	42.3
,, 120 ,,	11.5	73.3	6.2	71.8

TABLE 4.—LOSS OF VITAMIN A BY KEEPING GHEE AND VANASPATI IN AIR-OVEN AT 100 °C.

	Ghee		Dalda vanaspati	
	Vitamin A I.U./g.	Loss %	Vitamin A I.U./g.	Loss %
Control	41.2	—	22.0	—
After 15 mins.	41.0	Insignificant	21.8	Insignificant
,, 30 ,,	34.1	17.5	18.4	15.5
,, 60 ,,	33.0	19.4	17.9	17.8

hour causes a loss of 16% which is more than recorded by Hattiangdi and Ketayun.[9] This may be due to different set of conditions in our experiments. When boiling is continued for another half an hour, the loss shoots up to 44% and after two-hour boiling it is more than 70%. This shows that after an initial induction period of half an hour or so the decomposition of vitamin A is more progressive. The extent of loss is the same in both the fats throughout the heating period and hence no superiority can be claimed for vitaminised vanaspati in the retention of vitamin A. Heating at 100°C. in an oven causes practically no loss of vitamin A in 15 minutes. Even after one hour only 18% of the vitamin is lost which shows that at 100°C. the loss is comparatively less than in other types of heat treatments mentioned above.

These results have shown that there is an appreciable loss of vitamin A in ghee and vanaspati during cooking. It will, therefore, be a more healthy practice to consume butter or vitaminised fat, e.g. margarine, in the raw state and employ oil and fats with little or no vitamin for cooking purposes.

Boiling of vitamin-bearing fats under laboratory conditions does not exactly represent the conditions in our homes. More vitamin A may possibly be destroyed while cooking in ordinary cooking utensils. Shroff et al.[6] have noted that under normal conditions of clarifying ghee by heating at 125°C. for 30 minutes, no loss of vitamin A occurred, but all vitamin A in cow or buffalo ghee was destroyed by heating in aluminium containers for 240, 120, 15 and 10 minutes at 125°, 150°, 175° and 200°C., respectively. There are also chances of some preservation of vitamin A due to the antioxidant effect of spices and condiments so extensively used in our dietary. From the point of view of practical dietetics it will be more useful to determine the vitamin A content of the meals as actually eaten in our homes and compare it with that present in the raw foodstuffs. This will also give a measure of the carotene derived from vegetables which contribute substantially to our vitamin A requirements.

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CULTIVATION STUDIES ON SOME FOREIGN ECONOMIC PLANTS. PART I

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Cultivation studies were carried out on some foreign medicinal and economic plants in the Experimental Farm of the North Regional Laboratories, Peshawar. This was done with a view to introduce some economically important plants in this country. The cultivation of pharmacopoeial drug plants and other economic plants can bring substantial income and also help to develop export trade.

The soil of the Experimental Farm is stiff clayey and has been made workable with the addition of suitable quantity of sand and manure. The climate of Peshawar is very severe, extremely hot during summer and extremely cold during winter. The plants have to be protected from hot winds during summer and from frost during winter.

All the seeds cultivated in these experiments were obtained from Messrs. Thompson and Morgan Ltd., Ipswich, England.

Following plants have been successfully grown in the Experimental Farm:—

Origanum majorana.—Syn. *Majorana hortensis;* English name, majoram (sweet), family, *Labiatae.*

The plant is a native of Europe. It is an erect little bush with narrow petiolate leaves, white flowers and green bracts. Flower whorls in a thick clustered spikes.

It is commonly grown for the leaves to be used in the kitchen but is also a delightful fragrant plant for gardens.

2. *Rosmarinus officinalis.*—English name rosemary, garden rosemary; family, *Labiatae.* A single species native to the Mediterranean region, well known as a garden plant. Evergreen shrub; leaves narrow, entire with revolute margins; flowers blue, subsessile, in short axillary recemes, with minute bracts. The plant is common on the chalk hills of the south of France, Spain, Dalmatia, Tunisia and Morocco.

3. *Ricinus communis.*—English name, castor bean castor oil plant; family, *Euphorbiaceae.* Plant is probably native of tropical Africa but now widely distributed. Shrub or a small tree.

Following varieties have been successfully grown: (i) *R. communis.* var. *combodgensis*-stamens and leaves very dark, (ii) *R. communis.* var. *gibsonii*-small form, leaves dark with metallic lustre, and (iii) *R. communis.* var. *zanzibarensis*—leaves bright green with white veins.

4. *Santolina chamaecyparissus.*—English name, lavender cotton, dwarf cypruss; family, *Compositae.* The plant is native of Mediterranean region. Much branched evergreen sub-shrub. 1-1/2 to 2 ft. high.

Cultivation

SOWING AND GERMINATION

Seeds of all the plants were sown in earthenware basins which contained a mixture of equal parts of sand, clay and leaf mold manure. The basins were watered twice daily by shower. Dates of sowing and of germination and percentage of germination are given in Table 1.

TRANSPLANTATIONS

Seedlings of all the plants were later on transplanted to small pots, one in each, which contained 1:1:1 clay, sand and farmyard manure. Transplantations were done on the dates mentioned in the following:—

Origanum majorana. 20.9.1959.—At this stage the seedlings were 1″ high and had six leaves. This transplantation was 100% successful. Later these seedlings were transplanted in soil on 26.11.1959. These plants were 4″ high with about five branches and covered an area about 4 sq. in.

TABLE 1.

Plant	Date of sowing	Date of germination	Germination %
Origanum majorana	24.8.59	1.9.59	100
Rosmarinus officinalis	18.8.59	1.9.59	20
Ricinus communis var. *cambodgensis*	16.9.59	9.10.59	100
Ricinus communis-var. *gibsonii*	26.9.59	13.10.59	100
Ricinus communis var. *zanzibarensis*	18.8.59	25.8.59	100
Santolina chamaecyparissus	18.8.59	9.9.59	15

Rosmarinus officinalis. 19.12.1959.—At this stage the seedlings were 1″ high with 4-6 linear, lanceolate leaves. In April, 1960, the plants were again transplanted to bigger pots. In summer the plants were kept under complete shade. On December 12, 1960, the plants were transferred to soil beds. The plants were 7′-8″ high with 9-11 branches. The plants covered an area of one sq. foot.

Ricinus communis. Var. *cambodgensis.* 9.12.1959.—The plants were transplanted directly into the soil and were about 5″ high with five leaves. One plant dried in the end of December, 1959, probably due to frost.

Ricinus communis. Var. *gibsonii.* 4.11.1959.—The pots were exposed to sunshine. The seedlings were 2″ high with three leaves and 3″ long roots. In December, 1959, the plants were transferred to flat ground. The plantlets were 6″ high with 4 leaves. The petiole and lamina had each a gland near their bases. Due to heavy frost only one plant survived.

R. communis. Var. *Zanzibarensis.* 25.9.1959.—At this stage the seedlings were about 4″ high

with 4 leaves in two whorls, the second whorl consisted of elongated and narrow leaves. These plantlets were transferred to soil in December 1959.

Santolina chamaecyparissus. 21.12.1959.—The seedlings had 7-9 bipinnate leaves. On 14.4.1960, the plants were transferred to bigger pots which were kept under partial shade and watered by shower twice daily. The plants were 3″- 5″ high and covered an area of 4″- 5″. The plants were prostrate. The plants were finally transplanted in the soil on 18.12.1960. At this stage these were about 6″ high.

WORKING OF THE SOIL

The soil was thoroughly ploughed in each case and one maund of cowdung manure was added in a bed of 24′ × 10.′ The plants were transplanted on 6″ high and 2′ apart ridges in all cases except otherwise stated previously. The distance between each plant varied according to the size of the plants.

Growth Observations and Discussion

Origanum majorana.—The plants are perennial and are still growing well. First flowers appeared in April, 1960 and the fruits matured in June, 1960. A single plant is about 1-1/2 ft. high and covers an area of one sq. foot. Root cuttings were also tried for the propagation of the plants in October, 1961. All the root cuttings are growing well and this method of propagation seems more suitable. The seeds collected in June, 1961, were again planted in September, 1961, and these plants are also growing well. The germination in this case was 100%.

Rosamarinus officinalis.—The plant grows to a height of 3-4 feet covering an area of 4 feet and grows year after year. The flowering period starts in July. Seeds mature in September. Seeds obtained were again sown in September, 1961. The germination in this case is about 60%. Other methods of propagation, i.e., by cutting, by roots, and by layering, were also tried in September, 1961, which have proved successful. Propagation by stem cuttings is more convenient and suitable.

Ricinus communis. Var. *cambodgensis.*—The flowers appeared in February, 1960, and the fruits were collected in May, 1960. At the time of fruit collection the plants were 3′ high, covering an area of 4′. At present the plant is 8′ high covering an area 10 sq. ft. The seeds collected during June, 1961, were again sown in September, 1961. The germination in this case is again high. The seedlings have been transplanted in beds and are growing well.

Ricinus communis. Var. *gibsonii.*—The plant produced flowers in March, 1960, and the seeds were collected in July, 1960. At the time of fruit collection the plants were 4′ high covering an area of 6′. At present the plant is 7′ high covering an area of 9′. The seeds collected in July, 1961, were again sown in September, 1961. The percentage of germination is again high. All the seedlings have been transplanted in the soil and are growing well. The plants are protected from frost.

Ricinus communis Var. *zanzibarensis.*—The plant produced flowers in the end of October, 1959, but later the inflorescences withered. New flowers appeared on 26th March, 1960. Mature fruits were collected on June 29th, 1960. Seeds collected in June, 1960, were again sown in August, 1961. The germination was again successful and the plants are growing well.

Santolina chamaecyparissus.—The plants have attained a height of about one foot and cover an area of 1.5 sq. feet. Only few flowers were produced by the plant in April, 1960. New flowers again appeared in April, 1962 and matured in July, 1962. These seeds were sown in August, 1962 and the percentage of germination is considerably high as compared to the imported seeds. Other methods of propagation of the plants were tried in October, 1961. Stem cuttings were sown in basins. All the cuttings have sprouted. These cuttings are growing well. This method of propagation will be more economical and convenient as the percentage of germination of the seeds is very low.

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