PATHOGENIC BACTERIA OF THE DESERT LOCUST, SCHISTOCERCA GREGARIA

SHAHID H. ASHRAFI and MOHAMMAD GHAYASUDDIN
Drugs, Pharmaceuticals and Pesticides Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi
AND
SYED SADIQ HUSSAIN
Department of Plant Protection, Government of Pakistan, Karachi

(Received September 28, 1961)

Introduction

Desert locusts are a constant menace to our country. Almost every year large swarms of locusts invade and destroy the plant life of large areas. Our Government, in collaboration with other countries, is spending millions of rupees for fighting this menace. In spite of using insecticides and chemicals in large quantities every year, we have not been able to control the situation completely.

At present various micro-organisms are being used as destructive agents for different harmful insects without producing any adverse effect on the surroundings. With the object of finding a similar lethal micro-organism for the locusts, we undertook the isolation and identification of pathogenic bacteria from the desert locust, *Schistocerca gregaria.*

Material and Method

Living and dead locusts were brought to our laboratories from the Locust Research Centre of the Department of Plant Protection, Karachi, where large number of locusts were reported to be dying of some latent infection. The sick and dead locusts were examined bacteriologically for the presence of any pathogenic organism. The media used mainly was nutrient agar and nutrient broth. Besides these, other special media were also used for the screening of organisms in the initial stages.

The surface and abdominal washings of the dead locusts were obtained separately in sterile tubes with sterilized water. These washings were later plated out on different specific media. The inoculated differential plates were incubated at 37 °C. for 24 hours. The nutrient agar plates were incubated both at 37 °C. and at room temperature. The nutrient agar plates incubated at 37 °C. and room temperature showed similar growth of bacteria except that the pigmentation in the case of room temperature plates was more profuse. The differential media plates showed no growth. The organisms were isolated from the agar plates. Isolated pure colonies showing similar characters were transferred on nutrient agar slants. After checking the purity of the cultures, obtained by Grams' staining, they were transferred in nutrent broth. Then the pure cultures were identifiedi by studying their morphology, staining and biochemical reactions.

Results and Discussion

The identification of the micro-organisms was based on their study of cultural characteristics, staining properties, morphology and biochemical reactions. Bergey's *Manual of Determinative Bacteriology*[8] was followed for the confirmation of the results.

The petri dishes, inoculated with the surface washing suspension of the desert locust, were incubated at room temperature. *Serratia marcescens* (Gram negative), Burgey et al.,[1] Breed et al.;[2] *Bacillus subtilis* (Gram positive), Cohn;[3] and *Staphylococcus pyogenes* aureus (Gram positive), Rosenback[4] were identified. The same suspension was also incubated at 37°C., and the organisms identified were: *Serratia marcescens* (Gram negative) *Staphylococcus pyogenes* aureus (Gram positive) and *Bacillus cereus* (Gram positive) Frankland et al.[5]

The petri plates, which were inoculated with the washing suspension of the internal body of the desert locust and incubated at room temperature as well as at 37 °C., showed three different organisms. They were identified as *Serratia marcescens* (Gram negative), *Staphylococcus pyogenes* aureus and *Bacillus cereus* (both Gram positive).

From the results, it was found that the organisms *Serratia marcescens* (chromogenic) and *Staphylococcus pyogenes* aureus (chromogenic) were present externally and internally in the body of the desert locust. Along with these organisms the spore forming bacilli, *Bacillus subtilis* and *Bacillus cereus* were also present.

Earlier, Stevenson[6] had reported the isolation of a non-chromogenic strain of *Serratia marcescens* from dead desert locust; but in the present investigation this strain from the desert locust was found to be chromogenic. Both *Serratia marcescens* and *Staphylococcus pyogenes* aureus have been isolated repeatedly in our cultures. Stevenson attributed *Serratia marcescens* to be the etiological agent of the disease and Steinhaus[7] also reported that *Serratia marcescens* is highly pathogenic on inoculation to insects.

Acknowledgement.—The authors are highly indebted to Dr. Salimuzzaman Siddiqui for his encouragement during the progress of the work. We are thankful to Mr. Chowdhry Rashid Ahmad, Locust Entomologist, Locust Research Laboratory, Department of Plant Protection, Karachi, for giving the facilities to collect locusts.

References

1. Burgey, Breed and Murray, *Manual*, 5th edition, 1938 (October).
2. Breed and Breed, J. Bacteriol., **9**, 545 (1924).
3. Cohn, 1872, emend. Prazmowski, 1880.
4. Rosenback, *Mikroorganismen beiden Wundiufektionskrandkheiten des Menschen*, (1884).
5. Frankland and Frankland, Phil. Trans. Roy. Soc. London, **178B**, 187, 279 (1887).
6. J. P. Stevenson, J. Insect Pathol., **1**, 129-141 (1959).
7. E. A. Steinhaus, Hilgardia, **28**, 351-380 (1959)
8. Breed, Murray and Smith, *Bergey's Manual of Determinative Bacteriology* (1957), pp. 361, 465-466, 617-618, 620-621.

IRON COMPLEXES WITH LACTOSE, MALTOSE AND MALT

S. ALI HASNAIN ZAIDI, S. ASHFAQ HUSAIN AND S. MAHDIHASSAN

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

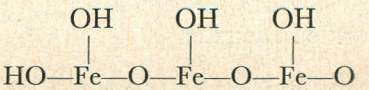
(Received August 17, 1961)

The iron-sucrose complex can be administered intravenously, but it is painful when given intramuscularly. For the latter purpose dextran-iron complex alone is most suited. We have studied the preparations of iron-sucrose, iron-glucose and iron-dextrin complexes previously.[1] The preparation of iron-lactose, iron-maltose and iron-malt (barley malt extract) complexes is undertaken in the present communication.

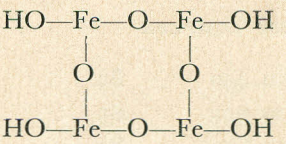
Method

$FeCl_3.6H_2O$ (pure)	4.8 g.
Na_2CO_3 anhydrous	3.2 ,,
Lactose	2 ,,
B-Maltose hydrate (B.D.H.)	2 ,,
Malt (barley malt extract)	6 ,,
NaOH (pure)	
in case of lactose and maltose	0.6 ,,
and in case of malt extract	0.9 ,,

Ferric chloride was taken in a beaker of 1 l. capacity dissolved in 20 ml. water and warmed to 40° C. Sodium carbonate was dissolved in 16 ml. and added to the ferric chloride solution in small portions while stirring vigorously. Red precipitate of γ-ferric hydroxide was formed.



When precipitation is effected in cold a yellow precipitate of α-ferric hydroxide is obtained.



The yellow hydroxide of iron does not react with any carbohydrate in forming a complex. The reddish ferric hydroxide was washed twice with tap water and five times with distilled water by decantation to get rid of electrolytes. The carbohydrate was taken in a porcelain dish, dissolved in minimum quantity of water and the wet ferric hydroxide was admixed thoroughly. The required quantity of alkali was then added, and the contents were heated at different temperatures for the periods indicated in Table 1. A dark brown cake was obtained which gave clear solutions when dissolved in water. Different ratios were tried to get the ideal complex, but only successful results have been recorded in the table. To understand the table some essential particulars are mentioned below.

(1) Sodium hydroxide is used as a 15% solution.

(2) About 5% iron is left unreacted in each case so that complete utilization of iron is never achieved.

(3) The porcelain dish was 5″ × 1.5″ with a product incorporating 1 g. elemental iron with a depth of 0.5″.

(4) Stability of the complex was tested by autoclaving the solution of the complex containing 2% iron in a sealed ampoule for 1 hour at 100°C. or for 30 minutes at 115°C. The solution should remain clear.

(5) Density, viscosity, pH and isoelectric point also refer to a solution containing 2% iron.

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

TABLE 1.—IRON COMPLEXES WITH LACTOSE, MALTOSE AND MALT.

Expt. No.	Ratio of Fe: carbohydrate: NaOH in g.	Temperature °C.	Time in hrs.	Stability on long boiling	Isoelectric point	Final pH before boiling	Density at 30°C.	Viscosity in poises at 30°C.
IRON-LACTOSE COMPLEX								
1	1:2:0.450	85	4	Unstable	2.5	7.0	1.05	0.0128
2	1:2:0.525	85	4	Unstable	2.5	7.5	1.05	0.0128
3	1:2:0.600	85	4	Stable	2.3	7.8	1.052	0.0129
IRON-MALTOSE COMPLEX								
4	1:2:0.450	85	4	Unstable	2.5	7.1	1.05	0.0128
5	1:2:0.525	85	4	Unstable	2.5	7.5	1.05	0.0128
6	1:2:0.600	85	4	Stable	2.3	7.8	1.052	0.0129
IRON-MALT COMPLEX								
	Malt variable							
7	1:8:1.5	130	4	Stable	3.5	7.2	1.085	0.014
8	1:6:1.5	130	4	Stable	3.5	7.8	1.082	0.0136
9	1:5:1.5	130	4	Stable	3.5	8.1	1.082	0.0136
10	1:4:1.5	130	4	Unstable	3.5	8.9	—	—
	Alkali variable							
11	1:5:1.2	130	4	Stable	3.5	7.9	1.081	0.0136
12	1:5:0.9	130	4	Stable	3.5	7.6	1.081	0.0136
13	1:5:0.75	130	4	Unstable	3.9	3.7	—	—

Conclusions

1. Minimum ratio of lactose, maltose per g. iron is 2 g. and of malt 5 g.

2. Minimum ratio of sodium hydroxide per g. iron in case of lactose and maltose complexes is 0.6 g. and in case of malt complex 0.9 g.

3. The best isoelectric point ranges between 2.5-3.5.

4. The time of heating and isoelectric point are related factors, and longer the time, the more acidic is the final product.

Reference

1. Naqvi et al., Arzeniemittel-Forsch., **9**, 720-721 (1959).

VANADIUM CARBOHYDRATE COMPLEXES

RASHEED BAKHSH QADRI AND S. MAHDIHASSAN

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received August 17, 1961)

Vanadium salts have been used as therapeutic agents in tuberculosis and anaemia as recommended by F. Laran, B. L. Lyonnet and co-workers.[1] The next improvement upon it would be a soluble compound, colloidal in nature and an analogue of iron saccharate. Vanadium saccharate can certainly be tried for oral administration.

In the preparation of the saccharates, only such metal oxides or hydroxides are taken which are

TABLE 1.—VANADIUM SUCROSE COMPLEX.

No.	V: sugar in g.	V: NaOH in g.	Time in hrs.	% of V in complex	Stability of solution on long heating	Iso-electric point	pH of complex	Density	Viscosity in poises	Temperature
Sugar Variable										
1	1:12	1:3.0	3.5	95	Stable	2.5	7.9	1.09	.0108	
2	1:10	1:3.0	3.5	95	,,	2.8	7.9	1.08	.0109	
3	1:8	1:3.0	3.5	95	,,	2.8	8.4	1.08	.0109	
4	1:6	1:3.0	3.5	65	Unstable	3.2	8.6	1.08	.0109	
5	1:4	1:3.0	No	complex	formation					
Alkali Variable										
6	1:8	1:3.0	3.5	95	Stable	2.8	8.1	1.08	.0107	
7	1:8	1:2.7	3.5	95	,,	2.7	7.9	1.08	.0107	
8	1:8	1:2.25	3.5	95	,,	2.6	7.9	1.08	.0108	
9	1:8	1:1.8	3.5	95	,,	2.6	7.7	1.08	.0108	
10	1:8	1:1.5	3.5	65	Unstable	3.0	7.3	1.08	.0108	
Temperature Variable										
11	1:8	1:1.8	3.0	95	Stable	2.5–3.0	7.9	1.08	.0103	180°C.
12	1:8	1:1.8	1.75	95	,,	2.5–3.0	7.6	1.08	.0103	200°C.
13	1:8	1:1.8	5	95	,,	2.5–3.0	8.7	1.08	.0103	140°C.

TABLE 2.—VANADIUM GLUCOSE COMPLEX.

No.	V: glucose in g.	V: NaOH in g.	Time in hrs.	% of V in complex	Stability of solution on long heating	Iso-electric point	pH of complex	Density	Viscosity in poises
1	1:15	3.0	3.0	95	Stable	2.8	8.2	1.08	.0104
2	1:13	3.0	3.0	95	,,	2.8	8.2	1.08	.0104
3	1:11	3.0	3.0	95	,,	3.0	8.4	1.08	.0104
4	1:9	3.0	3.0	95	,,	3.0	8.4	1.08	.0103
5	1:7	3.0	3.0	95	,,	3.0	8.6	1.08	.0104
6	1:5	3.0	3.0	60	,,	3.2	8.6	1.08	.0103

TABLE 3.—VANADIUM DEXTRIN COMPLEX.

1	1:15	3.0	3.0	95	Stable	3.0	8.6	1.09	.0106
2	1:10	2.7	3.0	95	,,	3.0	8.6	1.08	.0106
3	1:8	2.25	3.0	95	,,	3.5	8.2	1.08	.01067
4	1:5	1.5	3.0	60	Unstable	3.5	8.0	1.08	.0107

For the gift of two lbs. of ammonium vanadate we are thankful to Messrs. E. Merck, Darmstad, and their representatives Messrs. A. Sattar & Sons, Karachi.

insoluble in water. The only salt of vanadium which we could obtain was ammonium monovanadate. Vanadium oxide of required nature was procured from the above salt as follows:—

A known weight of ammonium monovanadate equivalant to a definite amount of elemental vanadium was dissolved in a small amount of water on gentle heating. It was then reduced to vanadic acid by means of zinc (granulated) and concentrated hydrochloric acid. Vanadic acid first formed appears as a reddish brown precipitate which becomes soluble on further addition of hydrochloric acid. The solution changes its colour from reddish brown in the beginning to blue and finally to dark brown, which shows that the reaction is complete. To this dark brown solution of vanadic acid ammonia solution was added, a precipitate of hydrated oxides of vanadium was obtained (dark brown or black in colour). The precipitate is washed with dilute aqueous ammonia to remove traces of zinc oxide and of ammonium chloride that may adhere to the oxide. Finally it is washed with water to remove all traces of ammonia. The precipitate thus obtained is used as a starting material for the preparation of the complexes.

The following equations serve to illustrate the reaction discussed above:—

$$(1)\ 2NH_4VO_3 + 4HCl \rightarrow 2H_2VO_3 + 2NH_4Cl + Cl_2 \uparrow$$

$$(2)\ 4H_2VO_3 + 4NH_4OH \rightarrow V_2O_3 + V_2O_5 + 4NH_3 + 8H_2O$$

Preparation of the Complex

The precipitate of vanadic oxide was then washed with distilled water to remove traces of SO_4'', or of Cl' present in it. To a known weight of vanadic oxide sugar in different quantities was added, dissolved, and then a concentrated solution of known amount of sodium hydroxide was admixed, and finally taken in a porcelain dish. Heating was then continued after setting the oven at 170°C. and in most cases, maintained at that temperature for periods as indicated in the table.

The following points are to be noted.

(1) The ratio between the oxides of vanadium and carbohydrate indicate 1 g. of the vanadium to carbohydrate in g.

(2) Sodium hydroxide was used as 15% solution but its presence in the complex has been indicated as V: NaOH, where V is the element in g. and NaOH also in g.

(3) When a solution with 1% metal was heated in an ampoule in boiling water for one hour, and did not show any sign of precipitation or of gel formation, it was considered stable.

(4) In all the experiments the porcelain dish used was round with the diameter of 5 inches and a height of 1.5 inches but the depth of the solution was kept at 1.0 cm.

(5) We also extended the technique to glucose and dextrin as carbohydrates, the method of preparation being the same. The findings are given in the accompanying tables.

(7) pH, isoelectric point, density, and viscosity refer to 1% solution of the metal.

Conclusions

(1) Minimum ratio to 1 g. vanadium to sugar is 8 g.

(2) Minimum alkali per g. vanadium is 1.8 g.

(3) The best temperature is 170 °C.

(4) The ideal isoelectric point ranges between 2.5 - 3.5 pH.

References

1. (*a*) F. Laran, La Presse Medical, **1**, 190 (1899); (*b*) B. L. Lyonnet, ibid., **1**, 199 (1899). from Mellor, *Comprehensive Treatise of Inorganic and Theoretical Chemistry*, Vol. IX, p. 735.

COPPER CARBOHYDRATE COMPLEXES

RASHEED BAKHSH QADRI AND S. MAHDIHASSAN

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

(Received August 17, 1961)

Preparation of Cupric Hydroxide

A blue colloidal hydrated form of cupric oxide $Cu(OH)_2$ is formed by adding a slight excess of dilute NaOH solution to a cold solution of cupric salt. Hence to get cupric hydroxide 4 g. of $CuSO_4$ was dissolved in 50 ml. of ice cold water and $Cu(OH)_2$ precipitated to completion by adding 5%

NaOH solution. The hydroxide is liable to turn black on keeping for a few hours in air or on slight heating. It is then repeatedly and rapidly washed with large quantities of water (1 l. of water per g. of hydroxide) until it is free from electrolytes.

The following equation serves to illustrate the reaction discussed above:—

$$Cu\,SO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4$$
(blue)

$$Cu(OH)_2 \longrightarrow CuO + H_2O$$
(black)

Preparation of the Complex

The precipitate as obtained above was used as the starting material for the preparation of the complex. To a definite amount of concentrated sugar solution cupric hydroxide and a solution of sodium hydroxide were added. It was then transferred into a large glass dish and heated for periods as indicated in the table after setting the oven at 170°C. The moisture free product became like a cake, dark in colour, with a shining surface.

The following points are to be noted.

(1) The ratio between cupric hydroxide and carbohydrate indicates 1 g. of the copper to carbohydrate in g.

(2) Sodium hydroxide was used as 15% solution but its presence in the complex has been indicated as Cu: NaOH, where Cu is the element in g. and NaOH also in g.

(3) In all the experiments glass vessel used was round flat bottomed with the diameter of 9″ and a height of 4″ but the depth of the solution was kept at 1.5 cms.

(4) When a solution with 1 % metal was heated in an ampoule in boiling water for one hour and did not show any sign of precipitation or of gel formation it was considered stable.

(5) pH, isoelectric point, density and viscosity refer to 1% solution of the metal.

When the cake-like product is dissolved in

TABLE 1.—COPPER SUCROSE COMPLEX.

No.	Cu: sucrose in g.	Cu: NaOH in g.	Time in hrs.	% of Cu in complex	Stability in the cold at pH 7	Stability on long heating	Iso-electric point	Density of 1% solution	Viscosity of 1% solution in poise
Sugar variable									
1	1:50	1:4.5	4	90	Stable	Unstable	3.6	1.086	.0377
2	1:45	1:4.5	4	90	,,	,,	3.8	1.086	.0377
3	1:40	1:4.5	4	90	,,	,,	3.8	1.086	.0377
4	1:35	1:4.5	4	90	,,	,,	3.8	1.086	.0377
5	1:30	1:4.5	4	90	,,	,,	3.8	1.086	.0367
6	1:25	1:4.5	4	90	,,	,,	3.8	1.084	.03170
Alkali variable									
7	1:25	1:4.05	4	90	Stable	Unstable	3.5	1.085	.03767
8	1:25	1:3.75	4	90	,,	,,	3.5	1.085	.03586
9	1:25	1:3.3	4	90	,,	,,	3.5	1.085	.03768
10	1:25	1:3.0	4	90	,,	,,	3.5	1.085	.03693
11	1:25	1:2.25	4	60	,,	,,	3.5	—	—

TABLE 2.—COPPER GLUCOSE COMPLEX.

12	1:35	1:3.75	3.5	90	Stable	Unstable	3.5	1.095	.03069
13	1:30	1:3.0	3.5	90	,,	,,	3.5	1.091	.02922
14	1:25	1:2.7	3.5	90	,,	,,	3.5	1.0903	.02911

water and centrifuged, a clear solution of Cu-sucrose complex is obtained. The pH of 1% metal solution is 9-10. It is unstable, and, when stored in a glass bottle, after a few hours reddish cupric oxide is deposited at the bottom of the container. ultimately after a few days the solution becomes free from copper, and all the copper settles at the bottom in the form of oxide. The following substances were tried in order to stabilize the complex.

Urea.—Urea forms some complexes with copper and in small quantities it is not harmful to the human body, hence 1% urea was added. The sample remained in good condition for 4 to 6 days; afterwards it deposited CuO.

Sodium thiosulphate and glucose.—Glucose and sodium thiosulphate were also tried but the results were unsatisfactory.

Citric acid.—Just after dissolving the final "cake", the pH of the complex was adjusted to 7 by the addition of 10% citric acid solution. Such sample can be kept for several days at room temperature, but, when heated above 40°C. for one hour, it showed precipitation and sometimes gel formation.

Conclusions

(1) Minimum ratio of sugar to 1 g. copper is 25 g.

(2) Minimum alkali per g. copper is 3 g.

(3) All the experiments were tried at 170°C. and proved successful.

(4) The best isoelectric point ranges between 3-4 pH.

(5) The copper complex is stable only at pH7 at room temperature. On heating above 40°C. it either shows precipitation or gel formation.

(6) The preparation can be used only orally, never intravenously.

RADIO-ACTIVITY OF GREEN SAND FROM CHICHALI PASS AREA OF KALABAGH DISTRICT (MIANWALI)

M. NABI BUKHSH

North Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Peshawar

(Received September 1961)

Large deposits of glauconite, commercially known as Green Sand, have been reported to be occurring in Kalabagh by the Geological Survey of Pakistan and several samples have been received for investigation through their courtesy.

While the mineral has been under investigation as a potential source of potassic fertilizer and as an ion-exchanger for softening water for industrial use, the material has incidentally been found to be radio-active. Four different samples have been tested and their activities are being reported in this paper.

Measurement of Radio-activity

All activity measurements have been done by a thin window (measuring both β and γ activity) G. M. tube attached to a scaler (decimal), made by Frieske and Hoepfner, Erlangen Bruck.

All measurements have been made under identical conditions. The samples and standards weighing one gram each had been placed uniformly at the bottom of a porcelain crucible and the distance of the window of the G. M. tube placesd at a fixed distance of about 4 cm. from the surface of the sample. Background activity has been checked daily during the course of the experiment.

In absence of a standard radiation source, a standard of activity was prepared from uranyl acetate by precipitating the hydroxide with ammonia and igniting the hydroxide to the oxide. One gram of the oxide U_3O_8 formed the standard activity.

Results

Average background activity 38.5 ± 0.5 counts per min.

Where 0.5 is the standard deviation which has been calculated from the formula $\sigma = \sqrt{N}$, where N is the total number of counts.

Activity of 1 g. of U_3O_8 – 13701 $\pm$ 47 c/m.

Activity of Green Sand including the background:

Sample 1 = 52.8 ± 1.6 c/m
Sample 2 = 45.3 ± 1.7 c/m
Sample 3 = 46.4 ± 1.7 c/m
Sample 4 = 42.5 ± 1.5 c/m

The net activities of the samples have been calculated after deducting the background activity and the standard deviation of the difference of the two counting rates obtained from the formula:

$$\sigma d = \sqrt{\sigma_1{}^2 + \sigma_2{}^2}$$

where σ_1 and σ_2 are the standard deviations of the sample and background, respectively.

Net activity of the samples:

Sample 1 = 14.3 ± 1.7 c/m
Sample 2 = 6.8 ± 1.8 c/m
Sample 3 = 7.9 ± 1.8 c/m
Sample 4 = 4.0 ± 1.6 c/m

The U_3O_8 equivalents of these samples when calculated from direct ratios of the activities are given below. The standard deviations of the ratio of two activities are calculated from the formula:

$$\sigma_Q = \frac{R_1}{R_2}\sqrt{\left(\frac{\sigma_1}{R_1}\right)^2 + \left(\frac{\sigma_2}{R_2}\right)^2}$$

U_3O_8 equivalents:

Sample 1 = 0.104 ± 0.012%
Sample 2 = 0.050 ± 0.013%
Sample 3 = 0.057 ± 0.012%
Sample 4 = 0.029 ± 0.012%

Conclusion

Although the samples of Green Sand are only slightly radio-active, yet in vew of the fact that all sources of radio-activity should be properly investigated, it is worthwhile to go into further details about the sources and cause of the radio-activity.

Acknowledgement.—The author is indebted to Dr. M. O. Ghani for his keen interest in the work.