AMINO ACID CONTENT OF FISH MUSCLE PROTEINS

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Introduction

The biological value of a protein is apparently related to a proportional relationship among its constituents of essential amino acids. So a knowledge of the amino acid content of a protein, containing material would serve as an indication of its biological value.

Some experiments were carried out mainly for comparing the nutritional value of animal meat with that of fish muscle. The previouly published results concerning the amino acid content of the fish muscle protein had been done on the whole muscle protein except those of Sharpenak and Eremin [1] who determined the amino acid content of fish albumin. Bailey [2] showed also that the myosin obtained from fish contained methionine, cystine, tyrosine and tryptophane.

Although the absence of glycine was reported by Wakamatu [3] in the hydrolysate of the muscle protein of herring, its abundance was emphasized by Umemura [4] in the Silver carp muscle.

The presence of all essential amino acids in fish muscle protein was emphasized by Agren, [5] Matas and Fellers, [6] Master and Magar, [7] Sugimura *et al.* [8] and Konosu *et al.* [9]

Amano and Bito, [10] determining the free amino acids in fish muscle protein found that, muscle of red meat fish (Tunny fish and Sardine) contained large amount of free histidine while white meat fish was characterised by an absence or small amount of this amino acid.

The present work was undertaken in order to study the amino acid content of the muscle protein of two Egyptian fish species, Karmout (*Clarias (angiullaris)* and Denis (*Chrysophrys auratus*).

Materials and Methods

The two fish species used were Karmout (*Clarias angiullaris*) and Denis (*Chrysophrys auratus*).

Paper Chromatography.—The analysis were performed on the whole fish meat or on the protein fractions obtained after separating them in a pure form.

One and two dimentional chromatograms were run. All the chromatograms were run by the descending technique. The technique used was that of Dent. [11]

Paper Electropheresis.—The fish tissue was ground finely in a mortar with a small amount of quartz sand, then treated with borate buffer (boric acid 0.075M+borax 0.044M) pH 7.7. The mixture was kept for two hours at 4°C. with occasional stirring, then centrifuged at 3500 r.p.m. for ten minutes. The resultant supernatant, containing the soluble proteins was referred to as "total extract". The apparatus and all solutions were cooled at 4°C. Durrum method [12] was used in the run, and phosphate buffer (potassium dihydrogen phosphate+sodium monohydrogen phosphate) ionic strength=0.076 and pH 6.2 was successfully used.

Protein Fractionation.—Paper electrophoretic analysis of the Denis muscle protein showed 7 protein components as shown in Fig. 1 These

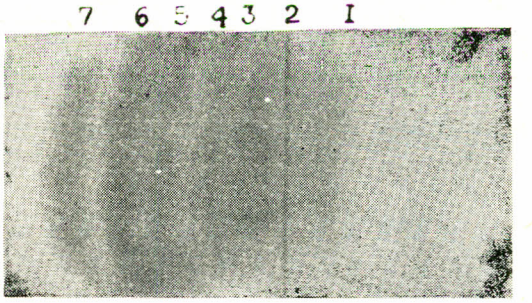

Fig. 1.—(1. Myoglobin; 2. Myosin; 3. Myogen; 4. Myogen; 5. Myoglobin; 6. Band 6; 7. Band 7)

components (numbered 1 to 7) were fractionated by the following methods:

(1) Myogen fractions were separated by using a modification of Henerotte's method. [13]

(2) Myglobin fractions were separated by using a modification of Hamoirs method. [14]

(3) Myosin fraction was separated by the method used by Reay and Kuchel. [15]

(4) The protein components of the electrophoretic bands 6 and 7 were separated lonely by Nikkila and Linko method. [16]

The analyses were performed on the alkali and acid hydrolysates of the samples. Samples from the hydrolysates were run chromatographically, the solvents used were: (1) Butanol-water-acetic acid 4:5:1 one way; (2) Phenol-water 80:20 one way; (3) Butanol-water-acetic acid 4:5:1 followed by phenol water 80:20 (two dimensions); (4) Phenol-water 80:20 followed by Butanol-water-acetic acid 4:5:1 in the second dimensions; (5) The micro chromatographic method of Bolling *et al.*[17] was used for detecting the amino acids tyrosine and histidine.

Different colour reagents were used in spraying the paper after drying it as follows, Dawson *et al.*:[18] (1) Pauly's reagent for tyrosine, histidine; (2) Nitroprusside-acetaldehyde reagent for proline; (3) Phenol-hypochlorite reagent for arginine; (4) Nitroprusside-alkaline ferricyanide reagent for arginine; (5) Phosphotungestic acid (Folin's uric acid reagent) for cystine; (6) Nitroprusside reagent for arginine; (7) Alkaline potassium permanganate reagent for tryptophane, tyrosine prolin and histidine; (8) Bromine reagent for histidine; (9) Phosphomolybdetungstic acid (Folin's phenol reagent) for tyrosine and tryptophane; (10) Ninhydrin colour reagent for the remaining amino acids.

Results

Fractionation of the different component of muscle proteins were made only on Dennis species muscle extract, while Karmout whole extract was used as it is without fractionation for amino acids hydrolysis. The results obtained are shown in Table 1.

Discussion

The above results indicate that the whole fish muscle protein contained all the essential amino acids. This finding is in agreement with the results of Agren,[5] Matas and Fellers,[6] Master and

TABLE 1.—AMINO ACIDS OF FISH MUSCLE PROTEINS.

Total muscle protein of Karmout	Amino acids detected on the chromatograms of the hydrolyzate of protein fractions of Dennis						
	Myosin band 2	Myogin of band 3	Myogin of band 4	Myoglobin of band 1	Myoglobin of band 5	Band 6	Band 7
Tyrosine	Tyrosine	Tyrosine	Tyrosine	Tyrosine	Trace	Tyrosine	Tyrosine
Valine	Valine	Valine	Valine	Valine	Valine	Valine	Valine
Leucine	Leucine	Leucine	Leucine	Leucine	Leucine	Leucine	Leucine
Serine	Serine	Serine	Serine	Serine	Serine	Serine	Serine
Isoleucine ..	Isoleu-cine	Isoleu-cine	Isoleu-cine	Isoleu-cine	Isoleu-cine	Isoleu-cine	Isoleu-cine
Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine
Phenyl-alanine ..	Phenyl-alanine	Phenyl-alanine	Phenyl-alanine	Phenyl-alanine	Phenyl-alanine	Phenyl-Alanine	Phenyl-Alanine
Proline	—	—	—	—	—	Proline	Proline
Alanine	Alanine	Alanine	Alanine	Alanine	Alanine	Alanine	Alanine
Arginine	Arginine	Arginine	Arginine	—	—	Arginine	Arginine
Glutamic acid ..	Glutamic	Glutamic	Glutamic	Glutamic	Glutamic	Glutamic	Glutamic
Histidine	—	Histidine	—	—	—	Histidine	Histidine
Methionine ..	Methio-nine	Methio-nine	Methio-nine	Trace	Trace	Methio-nine	Methio-nine
Cystine	Cystine	Cystine	Cystine	—	—	Cystine	Cystine
Glycine	Glycine	Glycine	Glycine	Glycine	Glycine	Glycine	Glycine
Aspartic acid ..	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid	Aspartic acid
Threonine ..	Threonine	Threonine	Threonine	—	Threonine	Threonine	Threonine
Tryptophan ..	Trypto-phan	Trypto-phan	Trypto-phan	Trypto-phan	Trypto-phan	Trypto-phan	Trypto-phan

Magar,[7] Sugimura *et al.*[8] and Konosu *et al.*[9] In the present work glycine was found to be present in all fractions of the Denis fish muscle protein, the hydrolysate of the whole muscle of Karmout. This supports the results of Umemura,[4] who reported its abundance in the silver carp muscle.

While methionine and cystine were found to be present in the whole muscle protein by Master and Magar, [7] and in myosin by Bailey, [2] the present work revealed the presence of methionine in all fractions and the absence of cystine in the myoglobins fractions in spite of its presence in the other fractions.

The absence of basic amino acids in myoglobin fractions had attracted authors' attention and it was extraordinary, as mammalian myoglobin is rich in basic amino acids. The different function between fish and mammal myoglobin can surely not be so profound as to allow the elimination of the basic amino acids. Similarly it is difficult to believe that these acids are absent from such a wide range of muscle protein, especially as the results of other authors showed that basic amino acids are present in mammalian muscles.

So the experiments were repeated and the authors obtained the same results. Further studies are being undertaken on this point to throw more light on the problem.

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STUDIES ON THE IDENTIFICATION OF SUGARS IN FRUIT JUICES BY PAPER PARTITION CHROMATOGRAPHY

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Introduction

Paper partition chromatography has been widely applied to the identification and estimation of various constituents in fruit juices. Lewis *et al.*[1] identified acids and sugars present in Java Plum. Organic acids in Peaches were identified by David, Luh and Marsh.[2] Sugars in some fruits were identified by Srivastava.[3] Extensive studies have been made paper-chromatographically on the identification and estimation of free amino acids in 32 fruits by Silber *et al.*[4] But few fruits have been examined to identify sugars in their juices. Studies have, therefore, been carried out on the identification of sugars in juices of: Sweet orange, Sour orange, Keno orange, Peach, Apricot, Plum (three varieties), Melon, Watermelon, Banana, Pomegranate, Loquat, Guava, Mango, Fig, Falsa, Mulberry both wild and cultivated.

Experimental

Materials.—The fruits were purchased from local market. They were ripe, but were not over mature.

1. *Solvents* (V/V): (i) n-Butanol 40: Ethanol 11: Water 19; (ii) n-Butanol 4: Acetic acid (Glacial) 1: Water 5: (iii) n-Butanol 6: Pyridine 4: Water 3

2. *Paper:* Whatman Paper No. 1.

3. *Spray Reagents:* Acetone Silver nitrate/$^N/_2$ Alcoholic NaOH.

4. *Sample Application Device:* Micropipette.

Procedure

The fruits after skinning were macerated and then filtered through Whatman filter Paper No. 1. The filtered liquid was diluted to double the original volume and then applied to previously marked spot by using micropipette. One percent reference sugars mixture having Maltose, Sucrose, Glucose and Fructose as well as individual pure sugars, were also applied by micropipette on the same paper for comparison. All the three solvents enlisted above were used for the development of the chromatogram.

Results and Discussion

Figure 1 gives the chromatogram of Sour orange, Sweet orange, Keno orange, Guava, Banana, Pomegranate, Peach, Apricot, Plum (three varieties), Mulberry wild, Mango, Mulberry cultivated, Loquat, Melon, Watermelon, alongwith those of synthetic individual sugars and their mixture also. On examining the chromatogram with reference to synthetic known sugars, it was seen that Glucose and Fructose were the common sugars present in all the fruit juices. Sucrose, Glucose, and Fructose were identified in Banana and Loquat. Maltose, Sucrose, Glucose and Fructose were indicated in Sour orange, Sweet orange, Keno orange, Plums, Peach, Apricot, Melon, Watermelon, Mulberry cultivated, Mango and Fig. Maltose, Glucose and Fructose were identified in Falsa fruit. On comparing the density of the spots of sugars on the chromatogram, it was observed that Glucose and Fructose are in abundance in all the fruit juices. The chromatogram of Falsa and Fig is not shown in the Fig. 1. R_f values of all the sugars identified in the fruit juices, are given in Table 1. All the three solvents given above, were used in developing the chromatogram. Solvents (i) and (iii) did not resolve the sugars in juices completely and tailing was quite conspicous. Solvent No. (ii) was found to be the best, here the resolution was complete and tailing was absent. Due to temperature changes and other environmental factors there are always variations in R_f values. With each fruit juice sample, a mixture of sugars was always applied to each chromatogram. So, under the same conditions of synthetic and fruit juice sugars, identification of individual sugars in the juices was confirmed and R_f values of both were recorded and found identical.

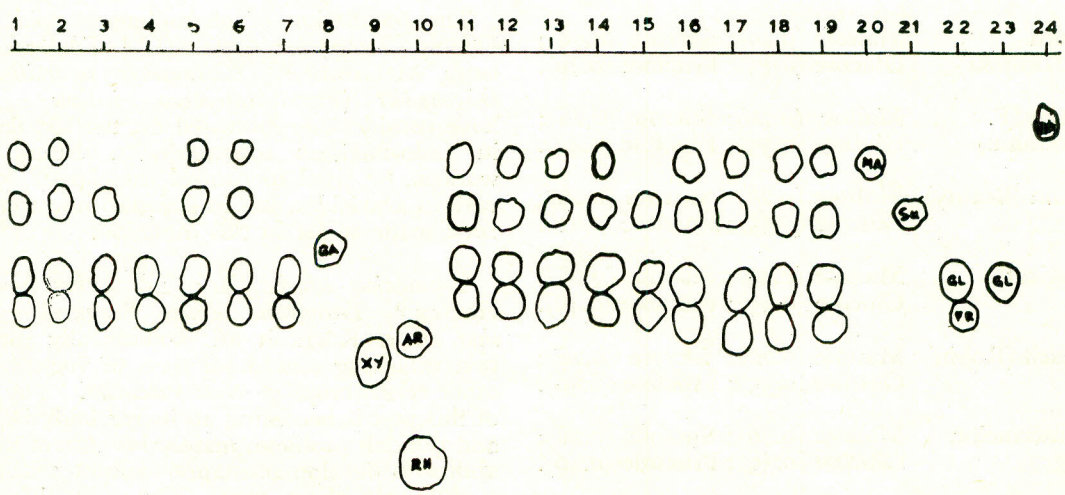

Fig. 1.—Showing a chromatogram of fruit juices and synthetic known sugars.

[1, keno orange; 2, Swest orange; 3, Banana; 4, Mulberry; 5, Sour orange; 6, Mango; 7; Pomegranate; 8, Galactose; 9, Xylose; 10, Arabinose and Rhamnose; 11, Peach (Robins); 12, Apricot; 13, Melon; 14, Watermelon; 15, Loquat; 16, Plum (Formusa); 17, Plum (Fazlimanani); 18, Plum (Beauty); 19, Mulberry (cultivated); 20, Maltose; 21, Sucrose; 22, Glucose and Fructose; 23, Fructose; 24, Raffinose].

TABLE 1.—R_f VALUES OF SUGARS IDENTIFIED IN FRUIT JUICES.

[Solvent (v/v): n Butanol 4: Acetic acid 1 (Glacial) 1: Water 5]

S. No.	Name of fruit	Sugars identified and their R_f values
1.	Keno orange	Maltose (0.16); Sucrose (0.21); Glucose (0.26); Fructose (0.30).
2.	Sour orange	Maltose (0.16); Sucrose (0.21); Glucose (0.26); Fructose (0.30).
3.	Sweet orange	Maltose (0.16); Sucrose (0.21); Glucose (0.27); Fructose (0.30).
4.	Guava	Maltose (0.16); Sucrose (0.21); Glucose (0.27); Fructose (0.31).
5.	Banana	———————; Sucrose (0.22); Glucose (0.27); Fructose (0.31).
6.	Pomerganate	———————————; Glucose (0.27); Fructose (0.31).
7.	Loquat	———————; Sucrose (0.15); Glucose (0.21); Fructose (0.25).
8.	Mango	Maltose (0.17); Sucrose (0.21); Glucose (0.30); Fructose (0.32).
9.	Melon	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
10.	Plum (Formusa)	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
11.	Plum (F. Manani)	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
12.	Plum (Beauty)	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.43).
13.	Apricot	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
14.	Peach (Robin)	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
15.	Watermelon	Maltose (0.20); Sucrose (0.26); Glucose (0.35); Fructose (0.40).
16.	Mulberry (wild)	———————————; Glucose (0.35); Fructose (0.40).
17.	Mulberry (cultivated)	Maltose (0.24); Sucrose (0.32); Glucose (0.37); Fructose (0.42).

* R_f values are given in brackets.

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ACARICIDAL ACTIVITY OF PETKOLIN AGAINST RED SPIDER MITES (ACARINA TETRANYCHIDAE)

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Introduction

The population of phytophagous spider mites namely, *Tetranychus telarius* (L),[1] *Tetranychus equatorius* McGregor,[2] *Tetranychus cucurbitae*[3] and *Oligonychus* (Paratetranychus) *indicus* (Hirst),[4] have considerably increased for the last few years in Indo-Pakistan sub-continent. Many Indian workers,[5-8] tried to control these pests by using various acaricides, but in Pakistan no attempt has been made so far in this direction.

Tetranychus telarius (L) = *Tetranychus bimaculatus* Harvey[9] = *Tetranychus altheae* Tragardh = *Tetranychus urticae* Koch, is an economically important pest attacking almost all sorts of vegetables and other cereal crops of West Pakistan. The control of this pest is becoming an increasingly important and difficult problem, mainly because of the mites ability to develop resistance to most acaricides in a relatively short time. The short life history coupled with high reproductive potentials, require an acaricide with prolong effects preferably over more than one generation. The various developmental stages of mites vary greatly in their susceptibilities to different chemicals. Most acaricides have been developed as adulticides because

that was the easiest stage to test. Some have proved effective against the immature forms, some are ovicides and some induce sterility in egg-laying females.

In the light of the above-mentioned facts, the present investigation was undertaken with a view to evaluate the acaricidal potentialities of the new pesticidal products [10,11] that have been obtained through the chlorination of indigenous and imported petroleum cuts by Qureshi.[12]

Materials and Methods

The red spider mites, *Tetranychus telarius* (L), were reared in the laboratories at a relative humidity of 75 ± 5% and a temperature of 32-36°C. The adult females were used exclusively for each test. To avoid possible age differences, the same size and colour pattern forms were taken for each experiment. The different concentrations of the test products, ranging from 1—7%, were prepared in acetone. A measured amount of 0.6 ml. were sprayed from each formulation. Ten adult female mites were placed on a cut leaf disc which in turn was kept on soaked cotton into a round-shaped metallic dish of 12.3 cm. diameter. A ring of lanolin[13] was applied around the entire corner of leaf disc so as to prevent the mites from going out of the dish. The metallic dish alongwith mites was then placed on the spray table. Spraying was done by means of the S.T.-4 Laboratory Spray Tower, made by Burkard Manufacturing Company Limited. The distribution of the spray was calculated by weighing the amount falling on microscope coverslips that were distributed over a control area of 9 cm. diameter on the spray table. The required deposit per unit area was first obtained by spraying onto a dish or plate of known area and weighing, then adjusting until the correct deposit was obtained. Constant pressure was obtained by using Handiair No. 2. Air Compressor and large reservoir supplied with the apparatus. Satisfactory results were obtained with the working pressure of about 2 lbs./sq. in. After spraying the whole amount at constant pressure and distance, (65 cm.) the sprayed material alongwith mites was removed from the spray table. The time for 100% mortality was noted. The mortality counts were made under the binocular microscope. Mites were considered dead if they failed to crawl forward when prodded with a needle.[14,15] The percent mortality was corrected by using Abbott's[16] formula. Each experiment was repeated five times. Controls were also run for each experiment and the readings were taken after 24 hours.

Results

The lowest percentage concentration (1%) of Petkolins 'M',† 'A'* and 'S'* gave 100% mortality in 58, 73 and 94 minutes, respectively. No revival was noted after 24 hours. Similarly 7% solution of Petkolin 'M', gave 100% mortality in less than 10 minutes, whereas Petkolins 'A', and 'S' took 12 and 20 minutes, respectively. Results with various concentrations are represented in histograms (Figs. 1, 2 and 3) Seven percent solution of Ovex did not give 100% mortality even after 24 hours. In controls no mortality was observed.

As a result of the present studies, it is concluded that Petkolins 'M', 'A' and 'S' can be used effectively for the control of phytophagous red spider mites. These new chlorinated petroleum hydrocarbons with specific gravity varying from 1.4 to 1.5 are definitely better and more potent than Ovex, a commercial imported acaricide.

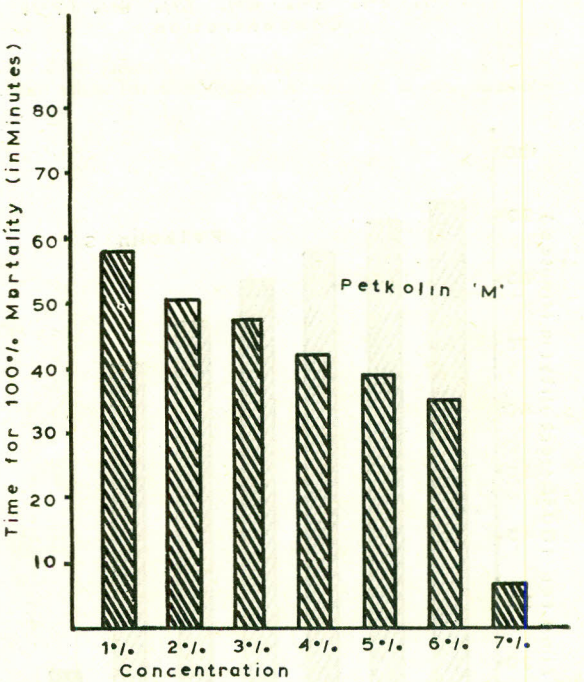

Fig 1.—Histogram showing 100% mortality with various concentrations of Petkolin 'M' against adult red spider mites.

† A modified Petkolin that has been obtained by inclusion of a certain additive in the chlorination process.

* The chlorinated petroleum products which have been obtained through the chlorination of petroleum cuts in the boiling range of 35- 155°C.

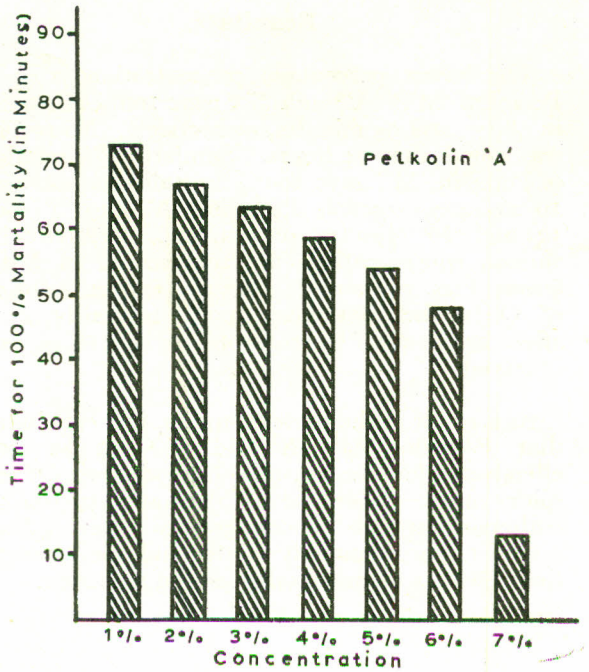

Fig. 2.—Histogram showing 100% mortality with various concentrations of Petkolin 'A' against adult red spider mites.

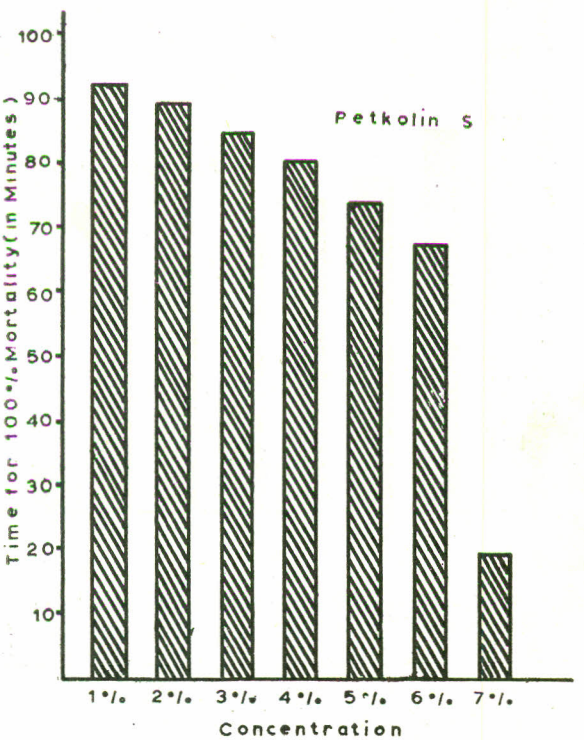

Fig. 3.—Histogram showing 100% mortality with various concentrations of Petkolin 'S' against adult red spider mites.

Investigations were also carried out to evaluate the ovicidal and larvicidal potentialities of these new chlorinated acaricides and their comparison with other commercial acaricides. Results of these experiments will be published at a later date.

Acknowledgement.—Authors are greatly indebted to Dr. Salimuzzaman Siddiqui, F.R.S., Chairman, P.C.S.I.R., for providing necessary facilities to carry out this work. Thanks are also due to Dr. S.A. Qureshi for supplying the samples of Petkolin.

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