cone. Based on the similarity of $R_f$ values of our glycoside with those of 3-O-rhamnogluco-
sy-l-kaempferol as reported in the literature, our glycoside was considered to be the same as this glycoside. But different m.p.s. have been reported by different workers for what they consider to be kaempferol-3-rhamno-
glucoside; 156-58°C (hydrated), 3 191-93°C (an-
hydrous), 3 180-82°C with sintering at 148°C and
222-23°C. 4 Our own m.p. was 218-26°C. The dif-
fferences in m.p.s could be due to different
modes of linking between the sugar units. In
order to ascertain this feature in our glyco-
side, it was subjected to permethylation em-
ploying the method of Hakomori 5 and the per-
methylate hydrolysed using Kiliani's mixture.
The resulting partially methylated sugar frag-
ments were examined by paper chromatography
employing 2, 3, 4, 6-tetra-O-methyl-D-glucose as
reference and two different solvent systems for
irrigation. In the solvent system n-butanol : 
ethanol : water, 5:1:4, upper layer, two
spots having $R_f$ values 1.01 and 0.71 were
obtained. According to the literature, the for-
erm corresponds to 2, 3, 4-tri-O-methyl D-
rhamnose and the latter to 2, 3, 6-tri-O-methyl-
D-galactose. Literature 6 $R_f$ values of 2, 3, 4, 
2, 3, 6, and 2, 4, 6-tri-O-methyl-D-glucoses are
0.85, 0.83 and 0.76 respectively. The $R_f$
value for 3, 4, 6-tri-O-methyl-D-glucose in this
solvent system is not available in the literature
and was therefore determined now by the
hydrolysis of permethylated graveobioside B 7
and was found to be 0.84. In the other sol-
vent system, n-butanol : water : carbon tetra-
chloride, 4:4:3, lower layer, the $R_f$ values
obtained with our unknown sugar fragments
were 0.37 and 1.03. These correspond to 2, 3, 6-
tri-O-methyl-D-galactose 8 and 2, 3, 4-tri-O-
methyl-D-rhamnose 8 respectively. In this
system for all the four most probable tri-O-
methyl-D-glucoses $R_f$ values higher than 0.48
have been reported. 9 It therefore appeared that our earlier identification of one of the
sugars present in our glycoside as D-glucose
was not correct. This was quite plausible since
the $R_f$ values of D-glucose and D-galactose
are so close in most solvent systems that they do not undergo clear separation in circular paper chromatography or in descending or ascending paper chromato-
graphy of short runs. We have therefore re-
examined the sugars obtained by the hydro-
lysis of our glycoside by descending paper
chromatography using a system specially suit-
ed for distinguishing between D-glucose and
D-galactose, viz., n-butanol : pyridine : water,
6:4:3, running the chromatogram for 30 hr
and employing D-galactose and D-glucose for
direct comparison. The sugar present in the
glycoside in question was found to be only
D-galactose. These results, therefore, neces-
sitate a revision of the structure of our glyco-
side to L-rhamnopyranosyl (1→4) D-galacto-
pyranosyl (1→3)-O-kaempferol. The natural
occurrence of rhamnogalactoside of kaemp-
ferol has not been reported in the literature
so far.

The authors thank Professor T. R. Seshadri
for his kind interest.

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THE EFFECT OF PHENYLHYDRAZINE ON GLUCOSE-6-PHOSPHATE DEHYDRO-
GENASE (EC 1.1.1.49) LEVELS IN RICE MOTH LARVA
(CORCYRA CEPHALONICA ST.)

Studies on the effects of phenylhydrazine
observed in rats, mice and rabbits have indi-
cated that the activities of many enzymes like
lactic dehydrogenase, 1 succinic dehydrogenase, 2
catalase, 3,4 glutathione reductase, 5 acid phos-
phatase, 6 ATPase 7 were affected. Since phenyl-
hydrazine produces haemolytic anaemia, 8 it was
of interest to study its effect on the enzyme
glucose-6-phosphate dehydrogenase, (G-6-
PDH) an enzyme involved in some types of
hereditary and a few drug induced anaemias. 9
The rice moth larva has been chosen as a test
organism since it has a well-defined metamor-
phosis and its use as a biochemical tool in toxi-
cology and nutritional studies, as well as in
a number of enzymatic studies have been
reported. 10
15 g. lots of whole wheat flour were distributed in a number of petri-dishes. Phenylhydrazine solution in varying concentrations from 30 to 90 mg./dish were added to the diet, dried at 80°C, and uniformly mixed. About 30 larvae of 12 to 15 days old were picked out at random from the stock colony and were put in the prepared diets. At the end of 10th day, 10 larvae were picked out from each dish, made free from adhering diet and weighed. The larvae were then homogenized using physiological saline containing 6.6 x 10⁻⁴ M EDTA in the ratio of (0.05 mL/mg. larva) in a Potter-Elvehjem homogeniser for 1 min. at 0-2°C. The homogenate was centrifuged for 20 min., at 0°C, at 25,000 r.p.m. in an international PR-2 refrigerated centrifuge. The clear supernatant fraction was used for the enzyme assay. Protein in the supernatant fraction from the homogenate was measured using the method of Lowry with bovine serum albumin as the standard. The assay of G-6-PDH was carried out following the method of Ells and Kirkman. The activities were expressed in milli-international units/mg. protein, and the overall estimations were carried out within 2 hr. of the preparation of the homogenate.

The G-6-PDH level of larvae during growth, fed on a normal diet, is presented in Fig. 1. The results of G-6-PDH level at the peak activity time of the larvae fed on diet containing phenylhydrazine in varying concentrations, are given in Fig. 2. Table I gives the weight of the larvae and the percentage inhibition of G-6-PDH levels.

**Table I**

<table>
<thead>
<tr>
<th>Phenylhydrazine mg</th>
<th>Weight of larva in mg.</th>
<th>G-6-PDH activity in m.I.U./mgm. protein</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>66</td>
<td>..</td>
</tr>
<tr>
<td>30</td>
<td>18.7</td>
<td>55</td>
<td>16.7</td>
</tr>
<tr>
<td>60</td>
<td>20.0</td>
<td>42</td>
<td>36.3</td>
</tr>
<tr>
<td>90</td>
<td>19.2</td>
<td>38</td>
<td>42.4</td>
</tr>
</tbody>
</table>

From Fig. 1 it can be seen that the G-6-PDH level of larvae during growth increases progressively from the eighth day with a peak period of activity between the 12th and 13th days. The G-6-PDH activity of larvae reared in diet containing varying concentrations of phenylhydrazine was measured at the 12th day, since during that period only, maximum activity of the enzyme has been observed (Fig. 1). The growth rate of the larvae as well as the percentage inhibition of the enzyme given in Table I clearly indicate that as the concentration of phenylhydrazine is increased the G-6-PDH level decreases to about half the original level only at a concentration of 90 mg./dish. It is very interesting to note that phenylhydrazine up to a concentration of 50 mg., does not show any toxic symptoms, nor does it show any deleterious effect on the nutritional status of the larva as seen from the weight of the larva. The enzyme G-6-PDH is known to occur in multiple molecular forms. According to Fig. 1 drop of the enzyme activity to the basal level on the 14th day shows a general recognition of the existence of multiple molecular forms (isoenzymes). The effect of phenyl-
hydrazine with respect to G-6-PDH activity (Fig. 2) may be viewed on the basis of selective inhibition of some isoenzymes by phenylhydrazine. It is known that G-6-PDH is a key enzyme in the pentose phosphate pathway and a detailed study of which may throw more light on our investigations.

The authors wish to thank the Indian Council of Medical Research for financial assistance to one of us (N. S. R.).

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November 9, 1970.


LABORATORY REARING OF BALANUS AMPHITRITE COMMUNIS (D.)

In recent years considerable efforts are being made to rear barnacles under laboratory conditions to obtain their cyprids for the screening of antifouling chemical compounds. Laboratory methodology for the rearing of temperate species with the above objective in view has been developed by Wisley? in Australia, Freiberger and Cologer? in the U.S.A. and by Tighe-Ford and his colleagues® in the U.K. Cestlow and Bookhout® examined larval development of Balanus amphitrite denticulata (B.) reared in the laboratory. This note gives a short account of the technique developed for the rearing of a common ship-fouling species Balanus amphitrite communis (D.).

Amongst larvaceous species encountered in Indian waters, B. a. communis, B. a. variegatus (D.), Chthamalus malayensis (P.), Ch. withersi (P.) and Tertracilia sp. are the dominant ones and their breeding pattern in Bombay waters has been earlier examined.® Of these, the first three named species are considered suitable for obtaining cyprids under controlled laboratory conditions.

The sea-water for the maintenance of the gravid adult barnacles and for rearing of the larval young ones was pumped from the harbour area. Twenty-four hour standing sea-water was passed through coarse glass-wool filters before its use in the rearing jars. The temperature of the water was 25°C to 28°C. and the salinity was 25 to 30 ppt.

For the successful completion of the larval metamorphosis, an availability of the supplementary food at adequate concentration is an important consideration. The dietary requirement of each barnacle species being specific, it is necessary that most suitable diatom or flagellate food for each species is determined. Some of the diatom species reported to be helpful are Skeletonema costatum, Phaeodactylum tricornutum, Cyclotella nana, Coscinodiscus sp. and Biddulphia sp. In our investigation, we examined eight diatom and two flagellate species for their dietary value. It is observed that *Dunaliella primolecta* supports good larval development of B. a. communis and Chthamalus species reared in the laboratory. It is suggested that use of dried liver powder or invertebrate eggs or blood as a supplementary food may be avoided since it encourages bacterial growth in the culture jars.

Earlier reports have suggested that the metamorphosis in temperate species is influenced by the ambient water temperature. Barnes and Barnes® have also reported their views on the subject. In the present study the quantitative effect of temperature on the development of B. a. communis has not been examined. It is, however, observed that at 25°C., the metamorphosis under laboratory conditions progresses most satisfactorily.

Tighe-Ford et al.® have emphasised the importance of varying light intensity on the rate of growth of the larvae. They observed that under totally dark conditions, the metamorphosis was slower than that noted under condition of constant illumination. We have observed that larvae of B. a. communis under-