and organic matter content were 7·5 and 0·36% respectively. The plot size was 5 × 2 sq. m. The field was basal dressed with urea and superphosphate @ 20 kg N and 21·8 kg –P/ha respectively

Azotobacter chroococcum B_4 was isolated from B.H.U. farm soil. Strains H_{44} and H_{45} of gram *Rhizobium* and J_3 of *Beijerinckia indica* were obtained from J.N.A.U. culture collection, Jabalpur. *Rhizobium* was grown on yeast extract mannitol agar medium slants. *Azotobacter* and *Beijerinckia* were grown on Burk's medium. After an incubation period of 10 days the bacterial growth from a slant was dislodged into 100 ml sterile water for single culture treatment, but for mixed culture treatment, bacterial growth of *Rhizobium* and *Azotobacter*, or *Rhizobium* and *Beijerinckia* from two different slants was transferred to the same amount of liquid.

For seed treatment, 2 ml culture suspension and 5 ml sticker solution (2·5% sugar + 1% gum acacia) were added to 40 g seeds in a beaker and thoroughly shaken. Bacterial treatment was done just before sowing and seeds of gram (*Cicer arietinum*) var. type-1 were sown @ 40 kg/ha. After 73 days of sowing, the plants were uprooted for nodule study. At harvest the grain yield was recorded and subsequently crude protein of seeds was determined.

Results and Discussion

Data on grain yield, nodule weight, and per cent crude protein of seeds are given in Table I. Gram *Rhizobium* H_{44} caused significant increase in the nodular mass, and an increase of 23% in the grain yield which was statistically insignificant. But the same strain along with *A. chroococcum* B_4 increased the nodule weight as well as grain yield significantly over the uninoculated control. The grain yield was increased by 45%. The protein content of the seeds was not affected by bacterial inoculation.

A. chroococcum B_4 being an isolate from the same locality might have established itself when used as seed inoculant in comparison to the *Beij. indica* J_3 which was an isolate from medium black soil of Jabalpur (Sanoria, unpublished work). The beneficial effect of *Azotobacter* to *Rhizobium* and the plant might be attributed to the synthesis of growth promoting substances (Jones and Greaves, 1943; Gebgardt and Kovalchuk, 1958). Moreover growth substance like β-indole acetic acid, possibly concerned in the nodulation process (Alexander, 1961) has been reported to be synthesized by *Azotobacter* (Smaly, 1954; Vancura and Macura, 1960). No information is available regarding the production of growth substances by *Beijerinckia*. Possibly *Beijerinckia* which produces abundant slime, excretes little of the growth substances. Both the strains H_{44} and H_{45} were reported to be effective under the medium black soil of Jabalpur (Sanoria and Dube, 1972) but in the Indo-gangetic alluvial soil of Varanasi only H_{44} is found to be effective. Local strain B_4 is found to exert synergistic effect on H_{44}.

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RHYTHMIC VARIATIONS IN THE PHOSPHORYLASE ACTIVITY IN THE SCORPION *HETEROMETRUS FULVIPES* (KOCH)

DIURNAL rhythms in various activities like locomotion, poison secretion[1], neurosecretion[2], rate of heart beat, cholinesterase activity in the heart muscle[3], spontaneous electrical activity in the ventral nerve cord and segmental nerves[4], have been reported to occur in the scorpion *Heterometrus fulvipes*. Similar rhythms have also been shown to occur in the levels of metabolites like blood glucose and hepatopancreatic glycogen in the same species[5]. Carbohydrate distribution and synthesis were studied in different tissues of scorpion[6]. The present report concerns the study of phosphorylase activity in the pedipalpal muscle and hepatopancreas of the scorpion *H. fulvipes* as a function of time of the day. The pattern of activity of this enzyme which plays a vital role in glycogen breakdown should reveal the pattern of utilization of carbohydrate energy sources for various activities during the course of a 24 h period.

The details of collection, maintenance of scorpions and sampling of tissues were described earlier[5,6]. The activities of phosphorylase '*a*' (active) and '*ab*' (total) have been estimated in the absence and

presence of AMP respectively according to the method described by Cori, Illingworth and Keller (1955)[7]. A 5% homogenate was prepared in aqueous medium containing 0·037 M ethylene diamine tetra acetic acid (EDTA), pH 6·5 and 0·1 M sodium fluoride, pH 6·5, as recommended by Guillory and Mommaerts[8]. The inorganic phosphate was estimated by the method of Taussaky and Shorr[9] and protein concentration was estimated by the method of Lowry *et al.*[10].

The results presented in Fig. 1 show that the total phosphorylase (*'ab'*) ranges from 25·73 to 31·0 μ moles of Pi/mg protein/h in the pedipalpal muscle and 23·1 to 32·7 μ moles of Pi/mg protein/h in hepatopancreas. The range is essentially similar even though statistical treatment shows that at 04·00 h and 08·00 h the total phosphorylase activity in muscle is significantly slightly higher ($P < 0·05$) than that of hepatopancreas. In both the tissues the maximal activity was recorded at 20·00 h and the minimal activity was recorded at 08·00 h. But the pattern of rise and fall in total phosphorylase activity is different in the two tissues studied. In the muscle the rise is less pronounced. Only the peak period value at 20·00 h is significantly higher than the value at 08·00 h ($P < 0·002$). The decline also is gradual, the midnight value being not significantly different from 20·00 h value ($P > 0·8$) which the 04·00 h is significantly lower than 20·00 h value ($P < 0·05$). In the hepatopancreas the rise and fall in enzyme activity are more steeper, the value at each period being significantly different from the value at the previous interval.

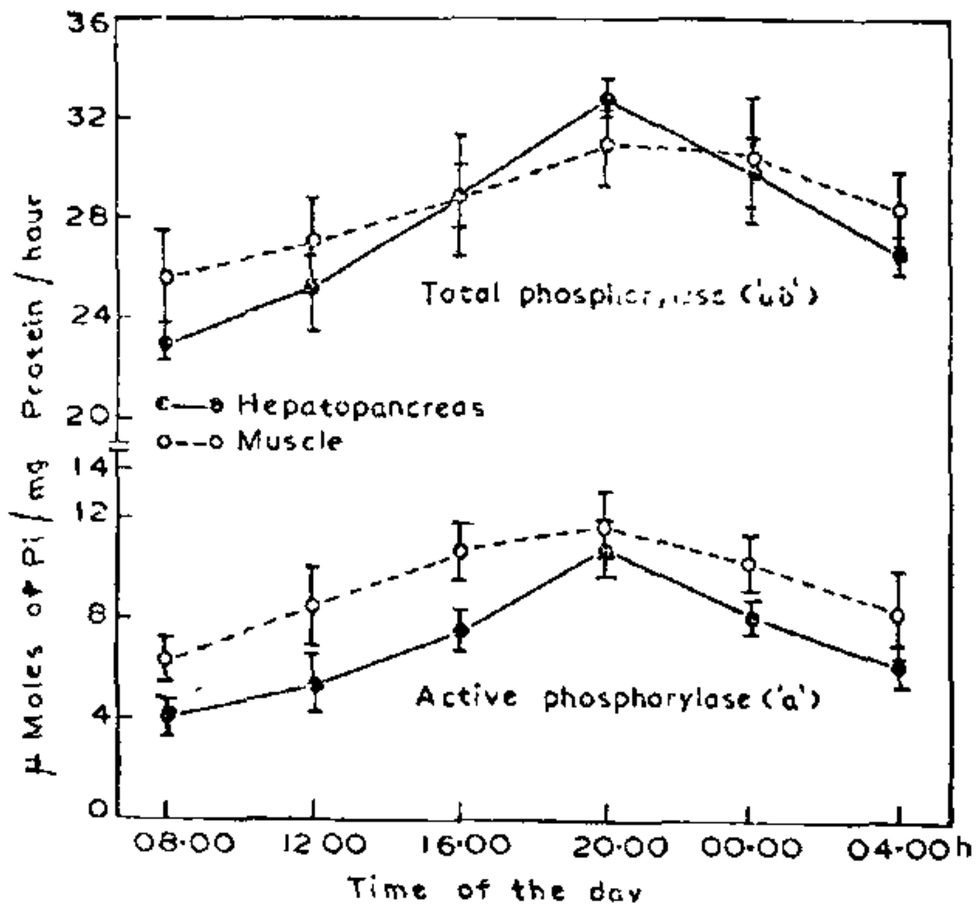

FIG. 1. Phosphorylase activity in the scorpion *Heterometrus fulvipes* as a function of time of the day. Each point represents the mean of six estimations ± S.D.

The general pattern of activity of phosphorylase *'a'* is very similar to that of total phosphorylase, the activity being maximal at 20·00 h and minimal at 08·00 h in both the tissues. It ranges from 6·06 to 11·53 μ moles Pi/mg protein/h in the muscle and from 4·05 to 10·67 μ moles Pi/mg protein/h in the hepatopancreas. Muscle active phosphorylase is consistently higher than that of hepatopancreas. In the muscle even though the peak enzyme activity is found at 20·00 h, the enzyme activity keeps continuously high between 16·00 h and 0·00 h. In hepatopancreas the rise and fall are more sharper.

The higher phosphorylase *'a'* activity in the muscle perhaps reflects glucose utilization for muscle contraction. Hepatopancreas is known to be the main organ of storage of nutrients. Synthesis and breakdown of glycogen in hepatopancreas are related to general metabolic needs of the animal. Both active and total phosphorylase activities were maximal at 20·00 h. It was reported earlier that blood glucose value was maximal at 20·00 h while the hepatopancreas glycogen content was minimal indicating mobilization of carbohydrate from hepatopancreas to blood[5]. The maximal phosphorylase activity reported in the present investigation adequately explains the earlier results. The minimal phosphorylase activity at 08·00 h is correlated to the maximal glycogen value in hepatopancreas and minimal blood sugar level. The pattern of variation in phosphorylase activity is of interest in the context of rhythmic patterns seen in other activities and the behaviour of the scorpion. The scorpion is a nocturnal creature having high locomotor activity during the night times[1]. The metabolic rate also is high between 16·00 h and midnight (0·00 h) with a peak around 20·00 h[1]. We have observed that the scorpions remain apparently inactive lurking under the stones during the most of the day time. They exhibit active feeding behaviour during the night time. Rate of heart beat and cholinesterase activity of the heart muscle are also higher during the dark hours with a peak activity at about 20·00 h[3]. The spontaneous electrical activity in the ventral nerve cord is reported to be maximum at 16·00 h while the electrical activity in the segmental nerves is maximal at 20·00 h[4]. The high phosphorylase activity in the scorpion during the night period at and around 20·00 h should be providing the necessary glucose from glycogenolysis, required for energetic needs of various physiological activities. This is particularly true of hepatopancreas phosphorylase, since the glycogen in this tissue is a more labile store of energy. Muscle phosphorylase, on the other hand, augments glycogenolysis yielding glucose

which would be mostly utilized as energy source for contraction process. After 20·00 h phosphorylase activity decreases as there would be enough glucose in the blood due to post-prandial absorption. The excess glucose is perhaps transported to hepatopancreas for glycogen synthesis. This is evident from the observation that the glycogen level in hepatopancreas gradually increases after 20·00 h reaching a maximal value at 08·00 h in the morning[5].

It is known in mammals that epinoephrine induces greater production of cyclic AMP in muscle resulting in increase of active form of phosphorylase which in turn augments glycogenolysis. It is of interest that a hyperglycemic principle has been identified in the extracts of scorpion cephalothrocic nerve mass[11]. Circadian rhythms in the neurosecretory activity in the scorpion has also been reported[2]. It is probable that the active principle from scorpion neurosecretory system with epinephrine-like action is responsible for activating the phosphorylase system.

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RETRACTOCEPHALUS—A NEW GENUS OF CEPHALINE GREGARINES (PROTOZOA: SPOROZOA) FROM INSECTS

KUDO[1], recognised 12 genera of cephaline gregarines (Protozoa : Sporozoa) under the family Gregarinidae Labbé, 1899. In our studies on this group of parasites from insects of this locality, we have found that the cephaline gregarines inhabiting the various parts of the mid gut of the beetles, belonging to the family Chrysomelidae possess sporonts in syzygy, simple globular retractile epimerite, cysts without ducts and barrel-shaped spores extruded in chains—characters absent in any known genus of the family Gregarinidae. A new genus, *Retractocephalus* is, therefore, proposed here to accommodate these gregarines. The genus is characterised by the following diagnostic features :

(1) initial development of the parasite is intracellular ;
(2) sporonts are in syzygy and the association is caudo-frontal in nature ;
(3) the epimerite is a simple symmetrical globular structure and retractile into the protomerite ;
(4) dehiscence of the cyst is by simple rupture ;
(5) the spores are barrel-shaped and extruded in chains.

The gregarine, *Retractocephalus raphidopalpii* obtained from the mid gut of the beetle, *Raphidopalpa* (= *Aulacophora*) *foveicollis* (Lucus) is designated as the type species of the genus. A brief description of the organism is given here. Details of its morphology and life history will be dealt with separately.

The parasite undergoes its initial development within the epithelial cells of mid gut of the host. With the development of the epimerite, it leaves the infected cell and remains attached with it by the epimerite for some time. Later, it frees itself from there, begins to live inside the gut lumen. A fully grown trophozoite (Fig. 1) has an elongated body. Its epimerite is a simple hyaline globular structure and occasionally retracts completely into the protomerite. The protomerite is elongated, cylindrical and has a depression to receive the epimerite. The deutomerite is the largest segment of the body and is separated from the protomerite by a thick straight septum. The nucleus is spherical or slightly oval enclosing one or two karyosomes.

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