

affect the mean number of eggs deposited. The presence or absence of the seed coat affected the preferential oviposition by the gravid insect to a maximum extent in French bean and hence the seed coats of this legume were used in the subsequent experiments.

Ten g of finely ground French bean seed-coat was extracted with 250 ml of distilled water for 3 h. The slurry was filtered. Filter paper, which normally does not induce egg laying, and the chickpea seeds on which no significant difference was recorded for the preferential oviposition (Table I), were chosen in the subsequent experiment. The filter paper strips of 3 cm by 2 cm were made into the cylinders of about 8 mm diameter. One lot

of 50 filter paper cylinders was immersed in a beaker containing the extract for 30 min and another lot of cylinders in distilled water for the same duration. These 2 lots were dried overnight at 28°C. 5 cylinders from each lot were mixed and placed together in a petridish covered with muslin and 25 pairs of newly emerged adults were released for oviposition. The number of eggs laid on cylinders of each lot were counted after 10 days (Table II).

A lot of 50 chickpea seeds was soaked in French bean seed coat extract for 3 h, and another lot in distilled water for the same duration. The seeds from the 2 lots were then dried overnight at 28°C. 5 seeds from each of these lots were mixed together and placed in a petridish covered with muslin and 3 pairs of newly emerged adults were released for oviposition. The number of eggs deposited were counted at various time intervals upto 120 h (Figure).

The ovipositional attractant activity in French bean seed coat for *C. maculatus* is here reported for the first time<sup>8</sup>. Since this insect lays very few eggs on inert surface, and significant differences exist in the rate of oviposition on seeds with and without seed coats of other legume hosts as well, it appears that the occurrence of chemical ovipositional attractants is fairly common if not universal<sup>9</sup>. The isolation and chemical characterization of these attractants from the host seeds, followed by breeding to eliminate or reduce these substances in the seed varieties, may provide an effective method for the control of this insect.

*Zusammenfassung.* Nachweis, dass die Samenhüllen gewisser Leguminosenarten die Eiablage des Bohnenkäfers *Callosobruchus maculatus* stimulieren und die Wasserextrakte attraktiver Samenhüllen die Oviposition auch auf inertem Filterpapier anregen.

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Table I. Seed coat as a factor for the ovipositional preference of *Callosobruchus maculatus* (Fabricius)

Seeds	Mean number of eggs oviposited	
	Seeds with seed coat *	Seeds without seed coat *
French bean	66.40 (59-90)	5.00 (1-12)
Red gram	49.40 (31-86)	20.60 (8-31)
Cowpea	88.60 (61-115)	14.40 (1-29)
Pea	39.80 (30-62)	15.20 (10-17)
Green gram	35.60 (23-50)	24.60 (12-40)
Chickpea	26.60 (10-36)	19.00 (9-41)
Lentil	15.80 (7-33)	4.60 (1-14)

\* Values in parentheses indicate the range of maximum and minimum number of eggs laid in the replicates. S.E. (pulses)  $\pm$  5.646; S.E. (Treatment)  $\pm$  3.018 C.D. (5%) 15.969; C.D. (5%) 8.536

Table II. Ovipositional attractant activity of French bean seed coat extract for *C. maculatus*

Treatment	Mean No. of eggs oviposited on 5 cylinders
French bean seed coat extract	115.6
Distilled water (control)	11.3
t-value 17.200.	

<sup>8</sup> Acknowledgment. We thank Mr. P. N. MATHUR for his useful suggestions and Mr. S. SUNDER for his help.

<sup>9</sup> V. G. GOKHALE, Doctoral thesis, University of Udaipur, Udaipur (1971).

## Regeneration of *Drosophila melanogaster* Male Leg Disc Fragments in Sugar Fed Female Hosts

Proliferation is a prerequisite for epimorphic regeneration. In order to test if regeneration in leg discs<sup>1</sup> is also dependent on proliferation, we cultured leg imaginal discs with excised end knobs (called the remainder pieces) into adult females which were kept on a protein-free sugar diet 4 days prior to receiving the implant. This environment prevents the imaginal cells from multiplying<sup>2</sup>. The implant was cultured for 2, 4, or 8 days in sugar fed animals and then tested for differentiation by transplanting into a larval host for metamorphosis.

The Table shows that regeneration fails when remainder fragments were cultured in starved adult hosts. However regeneration does occur frequently in well fed hosts<sup>1</sup>. But, the ability to regenerate is not lost irreversibly during culture under stressed conditions. If implants from starved females are injected into 72-h-old larvae the time before metamorphosis is sufficient to allow regenera-

tion of claws in  $\frac{1}{3}$  of the cases. As culture time in sugar-fed flies increases the size of the implanted fragments decreases. This is also reflected by a decrease of the bristle numbers in the proximal segments (Coxa, Trochanter and Femur). The number of these bristles increases dramatically when the cultured fragments are injected into 72-h-old larval hosts again giving them some time to grow.

Moreover, after remainder pieces were cultured for one day in flies which were fed only sugar, we brought the hosts for 2 days onto normal food and then injected the implants into old (105 h) larval hosts. In 42% ( $n = 12$ )

<sup>1</sup> G. Schubiger, *Devl Biol.* 26, 277 (1971).

<sup>2</sup> A. GARCIA-BELLIDO, *Wilhelm Roux Arch. EntwMech. Org.* 158, 212 (1967).

Differentiation and regeneration (claw formation) of remainder pieces after culture in starved adult hosts

Culture time in starved adults (days)	Age of larval hosts (h)	n	Coxa	Trochanter	Femur	Claw formation (%)
0	105	18	14.9 ± 2.3	10.1 ± 1.9	101.7 ± 11.5	0
2	105	9	10.9 ± 4.2	6.0 ± 2.8	53.0 ± 23.2	0
4	105	9	10.7 ± 4.8	6.4 ± 3.1	62.5 ± 20.9	0
8	105	9	4.4 ± 3.9	2.3 ± 2.7	17.7 ± 13.6	0
8	72	9	10.7 ± 6.3	7.8 ± 5.1	78.4 ± 35.4	33

Average number of bristles in Coxa, Trochanter and Femur.

of the cases the tissue had regenerated including the claw and the bristle number in the proximal segments was the same as in controls.

We can conclude that regeneration is dependent on proliferation which is inhibited in sugar-fed flies. In such

hosts the implants become smaller but the damage is repairable<sup>3</sup>.

*Zusammenfassung.* Fragmente von Beinimaginalscheiben regenerieren nicht, wenn sie in restriktiv ernährten Wirtsn kultiviert werden, wo Proliferation unterdrückt wird. Die Regenerationshemmung ist reversibel, da solche Fragmente in gut ernährten Wirtslarven normal regenerieren.

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Histones from the Red Alga, *Rhodymenia palmata*?

While it is clear that histones of the type most fully characterized in calf thymus are found only in eucaryotes, it is becoming apparent that not all encaryotes possess such histones<sup>1-5</sup>. This raises a number of questions. At which stage in the development of living organisms did histones appear? Prior to this stage, did other proteins fulfil their role in relation to the structure and properties of chromatin? If so, what is the nature of these proteins?

It is thought that development of a typical nuclear envelope occurred in the transition from the blue-green algae to the red algae<sup>5</sup>. Therefore, it seemed advisable to examine a member of the red algae in an initial attempt to answer these questions. Because of its ready availability, *Rhodymenia palmata* (L.) Grev.<sup>6</sup> was chosen.

*Materials and methods.* Calf thymus histones, standard proteins and 2-deoxy-adenosine were obtained from the Sigma London Chemical Co. Ltd. All other reagents used were of Analar grade where-ever possible.

Fresh samples of the alga were collected from the coast of East Lothian and homogenized in distilled water by two passages through a 25 ml capacity X-press (Biotec

Ltd.). Following this, proteins were extracted from the homogenate by 3 techniques normally used to obtain histones, viz. serial pH titration<sup>7</sup>, direct acid extraction with 0.25 M sulphuric acid following removal of ribosomal proteins at pH 2.8, and differential salt extraction<sup>8</sup>.

DNA was estimated by the diphenylamine reaction<sup>9</sup> following extraction by the method of SCHMIDT and THANNHAUSER<sup>10</sup>. Protein was measured in extracts by the method of WARBURG and CHRISTIAN<sup>11</sup>. Protein bound phosphate was determined by the method of AMES<sup>12</sup>. Amino-acid analysis was carried out on a Technicon Auto-Analyser following hydrolysis of the proteins in 5.65 M hydrochloric acid for 24 h at 105 °C.

Polyacrylamide gel electrophoresis was either as described by JOHNS<sup>13</sup> for histones, or as described by WEBER

Table I. Relative amounts of protein obtained by pH titration

pH	Protein obtained (mg)
2.8	93.4
2.1	9.3
1.8	16.0
1.3	10.5
1.0	6.9

<sup>1</sup> G. J. M. TONINO and Th. H. ROZIJN, *Biochim. biophys. Acta* **124**, 427 (1966).

<sup>2</sup> J. H. DUFFUS, *Biochim. biophys. Acta* **228**, 627 (1971).

<sup>3</sup> J. H. DUFFUS and C. S. PENMAN, *J. gen. Microbiol.* submitted.

<sup>4</sup> J. D. DODGE, *Arch. Mikrobiol.* **48**, 66 (1964).

<sup>5</sup> E. J. DUPRAW, in *DNA and Chromosomes* (Holt, Rinehart and Winston Inc., New York 1970), p. 86.

<sup>6</sup> M. PARKE and P. S. DIXON, *J. mar. Biol. Ass. U. K.* **48**, 783 (1968).

<sup>7</sup> K. MURRAY, G. VIDALI and J. M. NEELIN, *Biochem. J.* **107**, 207 (1968).

<sup>8</sup> H. BUSCH, in *Methods in Enzymology* (Eds. L. GROSSMAN and K. MOLDAVE; Academic Press Inc., New York 1967), vol. 12, part B, p. 421.

<sup>9</sup> K. BURTON, *Biochem. J.* **62**, 315 (1956).

<sup>10</sup> G. SCHMIDT and S. J. THANNHAUSER, *J. biol. Chem.* **167**, 83 (1945).

<sup>11</sup> O. WARBURG and W. CHRISTIAN, *Biochem. Z.* **310**, 341 (1941).

<sup>12</sup> B. N. AMES, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press Inc., New York 1966), vol. 8, p. 116.

<sup>13</sup> E. W. JOHNS, *Biochem. J.* **104**, 78 (1967).