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Original Article

Localization of regenectin in regenerates of American cockroach *(Periplaneta americana)* legs

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ABSTRACT The localization of regenectin, a sucrose-binding C-type lectin, in the regeneration of the cockroach leg was investigated by immunoblotting and immunofluorescence studies. Regenectin was found to appear transiently around developing muscle cells in regenerating legs in the late stage of regeneration. With maturation of the muscles, it disappeared and was not detectable in completely regenerated legs. These findings suggest that regenectin is a cementing substance connecting developing muscle cells. Regenectin was not detected in embryos or nymphal legs at various developmental stages, suggesting that it might not be involved in normal development of embryos and legs.

KEY WORDS: *regenectin, regeneration, muscle formation, cockroach*

Introduction

Many humoral lectins have been found in various invertebrates (Marchalonis and Edelman, 1968; Hammarström and Kabat, 1969; Baldo *et al.*, 1978; Yeaton, 1981; Umetsu *et al.*, 1984; Giga *et al.*, 1985; Muramoto *et al.*, 1986; Ravindranath *et al.*, 1985; Qu *et al.*, 1987; Suzuki *et al.*, 1990). Some of these lectins are known to be involved in the defense systems of invertebrates (Komano *et al.*, 1980; Komano and Natori, 1985; Lackie, 1980), but the biological roles of most of these lectins are unknown. The hemolymph of the American cockroach *Periplaneta americana* has been found to show appreciable hemagglutinating activity (Scott, 1971, 1972; Lackie, 1981). To investigate the biological roles of the hemagglutinins in the adult *Periplaneta*, we are trying to purify the proteins with hemagglutinating activity. So far, we have purified two lectins (Kubo and Natori, 1987; Kubo *et al.*, 1990) and one lipopolysaccharide-binding protein with affinity for *Escherichia coli* lipopolysaccharide (Jomori *et al.*, 1990). We purified *Periplaneta* lectin first (Kubo and Natori, 1987), and then isolated regenectin as a protein that cross-reacted with *Periplaneta* lectin antibody (Kubo *et al.*, 1990). These lectins have the following common features: they exist as large aggregates of a single subunit and they require Ca^{2+} for hemagglutinating activity. But the primary structures of their subunits and their hapten sugars are different.

Cockroach legs are known to regenerate when amputated in the nymphal stage (Cowden and Bodenstein, 1961; Penzlin, 1963; Kunkel, 1981; Turby, 1983; Bullière, 1985). Both these lectins are normally present in the hemolymph of adult cockroaches, but we found that only regenectin was detectable in regenerates of metathoracic legs and that it appeared transiently between day 17 and 26 after amputation (Kubo *et al.*, 1990). As no regenectin was detectable in the regenerates before or after this stage, its appearance at this stage seems to be important for the morphogenesis of regenerating legs.

To gain more direct insight into the biological role of regenectin in leg regeneration, we performed an immunofluorescence study with antibody raised against regenectin. Regenectin was found to be localized around newly emerged muscle cells, suggesting that it plays a role in muscle formation in regenerating legs.

Results

Regenectin in cockroach leg regeneration

Previously we found that regenectin, a lectin with sucrose-binding activity, appeared transiently in the regenerating nymphal legs of *P. americana* in the late stage of regeneration (Kubo *et al.*, 1990). This finding suggested that regenectin might be a mediator of cellular interaction in all morphogenic processes in the cockroach. To examine this possibility, we performed immunoblotting experiments using various tissue samples obtained during development. For this purpose, we first raised antibody against regenectin. This antibody cross-reacted with *Periplaneta* lectin, just as *Periplaneta* lectin antibody cross-reacted with regenectin. However, it was possible to trace regenectin with this antibody, because the molecular masses of the subunits of regenectin and *Periplaneta* lectin are different.

Abbreviations used in this paper: SDS, sodium dodecyl sulfate; FITC, fluoresceinisothiocianate.

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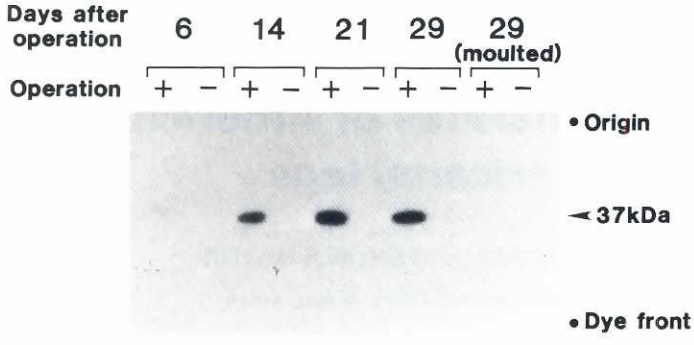


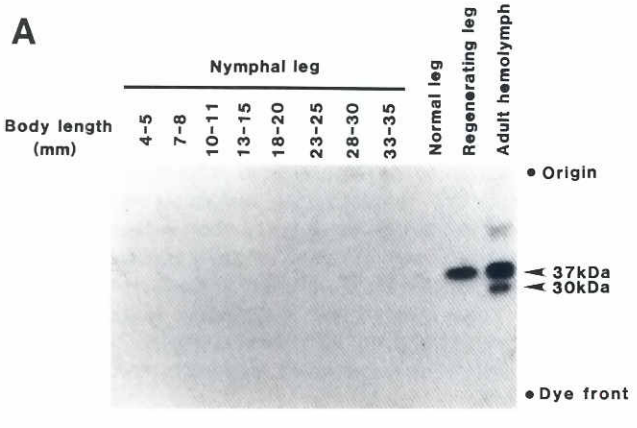
Fig. 1. Immunoblotting analysis with regenectin antibody of homogenates of regenerating legs at various times after amputation. *Regenerating tissue of metathoracic legs collected on the indicated days after amputation (operation +) and normal legs collected from the same nymphs (operation -) were homogenized and subjected to immunoblotting analysis with affinity-purified regenectin antibody. Each lane contained 50 μg of protein. Lane 29 (moulted) means moulted insects collected on day 29.*

As shown in Fig. 1, the 37 kDa subunit of regenectin was detected in homogenates of regenerating legs harvested between day 14 and 29 after amputation, but not in those of normal legs obtained at the same times, confirming the results obtained with *Periplaneta* lectin antibody (Kubo *et al.*, 1990). To examine the participation of regenectin in leg development, we analyzed the homogenates of normal nymphal legs harvested at various developmental stages. Nymphs were classified into eight developmental stages on the basis of their body length. Legs harvested from nymphs at various developmental stages were homogenized, and the resulting homogenates were subjected to electrophoresis and immunoblotting. As shown in Fig. 2A, no significant regenectin was detected in normal nymphal legs harvested at any of the eight developmental stages. As positive controls, we analyzed adult hemolymph and a homogenate of regenerating legs. The 30 kDa protein in the hemolymph that cross-reacted with regenectin antibody was a subunit of *Periplaneta* lectin. These results suggest that regenectin may not be involved in normal development of legs. Therefore, we next tested regenerating legs from nymphs at two different developmental stages (body length 10-15 mm and 25-30 mm). Regenerating legs were collected on days 17 and 26, respectively, after amputation. These regenerating legs contained regenectin, as shown in Fig. 2B. Thus, regenectin probably plays a role in the regeneration of lost legs, irrespective of the developmental stage of nymphs, but not in the development of normal legs.

Cockroaches can regenerate legs amputated in nymphal stages, but not in the adult stage (Bodenstein, 1955). Therefore, we tested whether regenectin appeared in coxa when the femur of adult legs was amputated. After wound healing, muscle cells in the coxa are destroyed and are replaced with mononuclear cells. Samples were prepared at various times after amputation and subjected to immunoblotting. As shown in Fig. 3, faint spots of regenectin became detectable 14 to 26 days after amputation, but the amount of regenectin was much lower than that detected in regenerating nymphal legs. Thus, regenectin probably plays a role in regeneration of lost parts and not in wound healing of the remaining part.

Regenectin in the development of Periplaneta

We next tested whether regenectin participates in embryogenesis in the cockroach. For this, we collected embryos at various developmental stages. Eggs hatch 60 days after oviposition under our



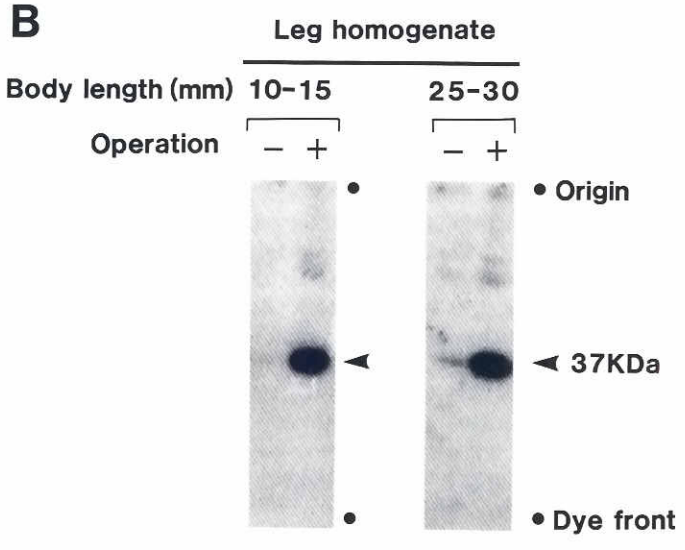


Fig. 2. Immunoblotting analysis of nymphal legs at various developmental stages. (A) *Nymphs were classified into eight developmental stages on the basis of their body length and regenectin in normally developing legs was examined by immunoblotting. Each lane contained 50 μg of protein. As controls, homogenates of regenerating legs and normal legs, and adult hemolymph were examined. Arrowheads at 37 kDa and 30 kDa indicate the positions of subunits of regenectin and* Periplaneta *lectin, respectively.* **(B)** *Metathoracic legs of nymphs at two developmental stages (body lengths are 10-15 mm and 25-30 mm) were amputated. Regenerating legs were isolated 17 and 26 days later, respectively, and analyzed by immunoblotting. Normal legs of the same animals were analyzed as control. Each lane contained 50 μg of protein.*

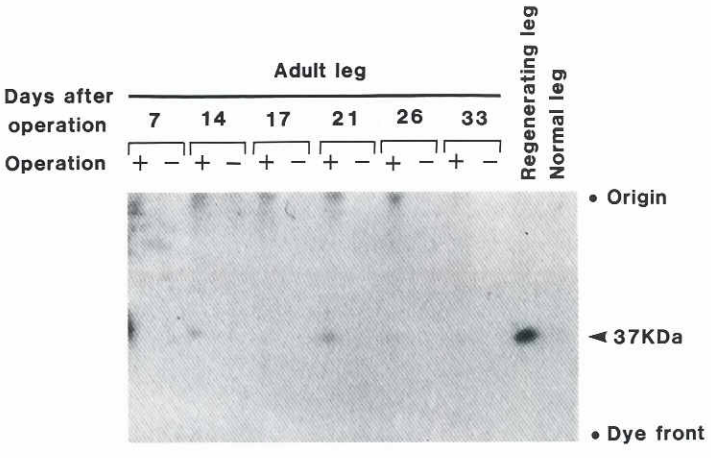


Fig. 3. Immunoblotting of amputated adult legs. *A metathoracic leg of adult* Periplaneta *was amputated between the coxa and femur. The coxa were isolated from the operated leg (+) and a normal leg (-), respectively, on the indicated days after amputation and analyzed by immunoblotting. Regenerating nymphal legs and normal legs were analyzed as controls. Each lane contained 50 μg of protein. The arrowhead at 37 kDa indicates the position of the subunit of regenectin.*

rearing conditions, so we collected embryos of different developmental stages during this period. As is evident from Fig. 4A, regenectin was not detected in any embryos tested. The signal detected in the day 1 sample is not regenectin, judging from its mobility. These results suggest that regenectin may not be required during normal embryonic development.

Regenectin was originally found in the hemolymph of adult *Periplaneta*. So we tested whether nymphal hemolymph contains regenectin. Hemolymph was collected from nymphs at various developmental stages and analyzed by immunoblotting. Developmental stages were classified according to the body length as described above. As shown in Fig. 4B, the content of regenectin in the hemolymph was very low in young nymphs, but increased significantly in the final nymphal stage when the body length became more than 28 mm. Thus, regenectin in the hemolymph clearly increases during nymphal development.

Localization of regenectin in regenerates

We examined the localization of regenectin in regenerates by the indirect immunofluorescence method. Sections of regenerating legs were treated with affinity-purified regenectin antibody, and the antibody was located with fluorescence-labeled second antibody. Regenectin was first detected inside the regenerate surrounded by epidermal cells. Fig. 5 shows a section of a regenerate harvested on day 26 after amputation. The regenerates are filled with mononuclear myoblast-like cells which are stained with Toluidine blue (panel C), and the fluorescence in this region (panel A) was much greater than the nonspecific fluorescence (panel B). In a more advanced stage of regeneration, just before the next moult (day 33 after amputation), myoblasts had differentiated into muscle cells (Fig. 6, panel C), and significant fluorescence was detected around these muscle cells (panel A). These findings suggest that regenectin participates in the construction of muscles in the regenerate as a cementing substance of muscle cells. However, unidentified structures indicated by arrows 1 and 2 in panel A were also stained significantly.

Regenectin in muscle construction

As the above findings suggested that regenectin plays a role in the construction of muscles in regenerating legs, we investigated this process in detail. Muscle formation occurs after day 20 following amputation. First, mononuclear cells, thought to be myoblasts, appear in the regenerate (Cowden and Bodenstein,

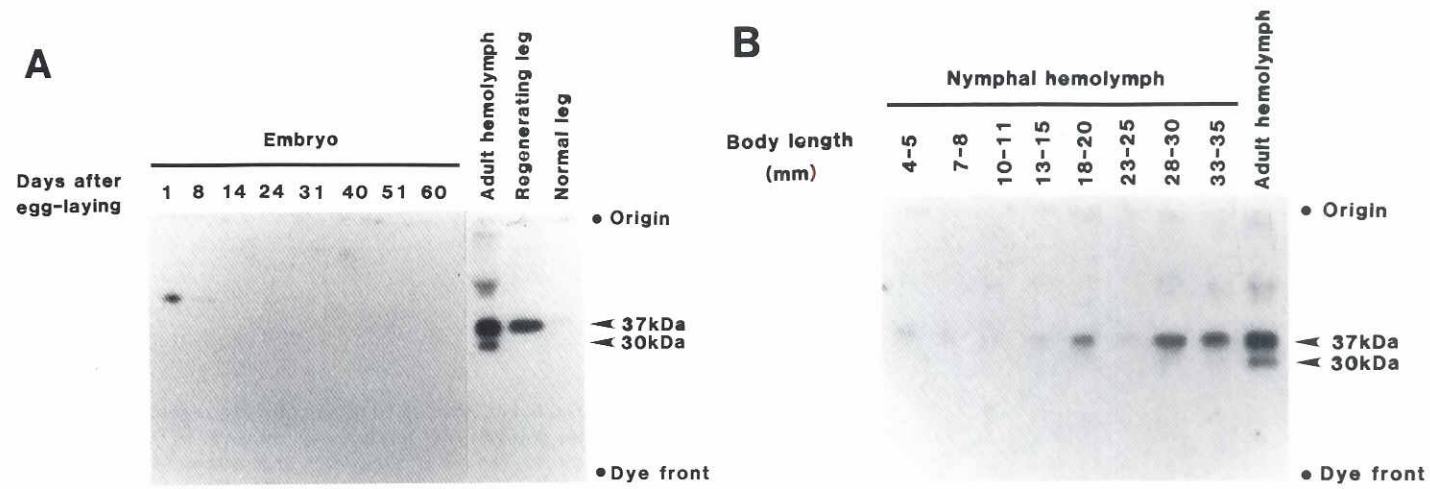


Fig. 4. Immunoblotting analyses of embryos and nymphal hemolymph. (A) *Embryos were collected at the indicated times after oviposition and analyzed by immunoblotting. Usually, nymphs hatch on day 60 under our experimental conditions. Each lane contained 50 μg of protein.* **(B)** *Hemolymph was collected from nymphs at various developmental stages and analyzed by immunoblotting. As controls, regenerating nymphal legs, normal legs and adult hemolymph were analyzed. Each lane contained 50 μg of protein. Arrowheads at 37 kDa and 30 kDa indicate the positions of subunits of regenectin and* Periplaneta *lectin, respectively.*

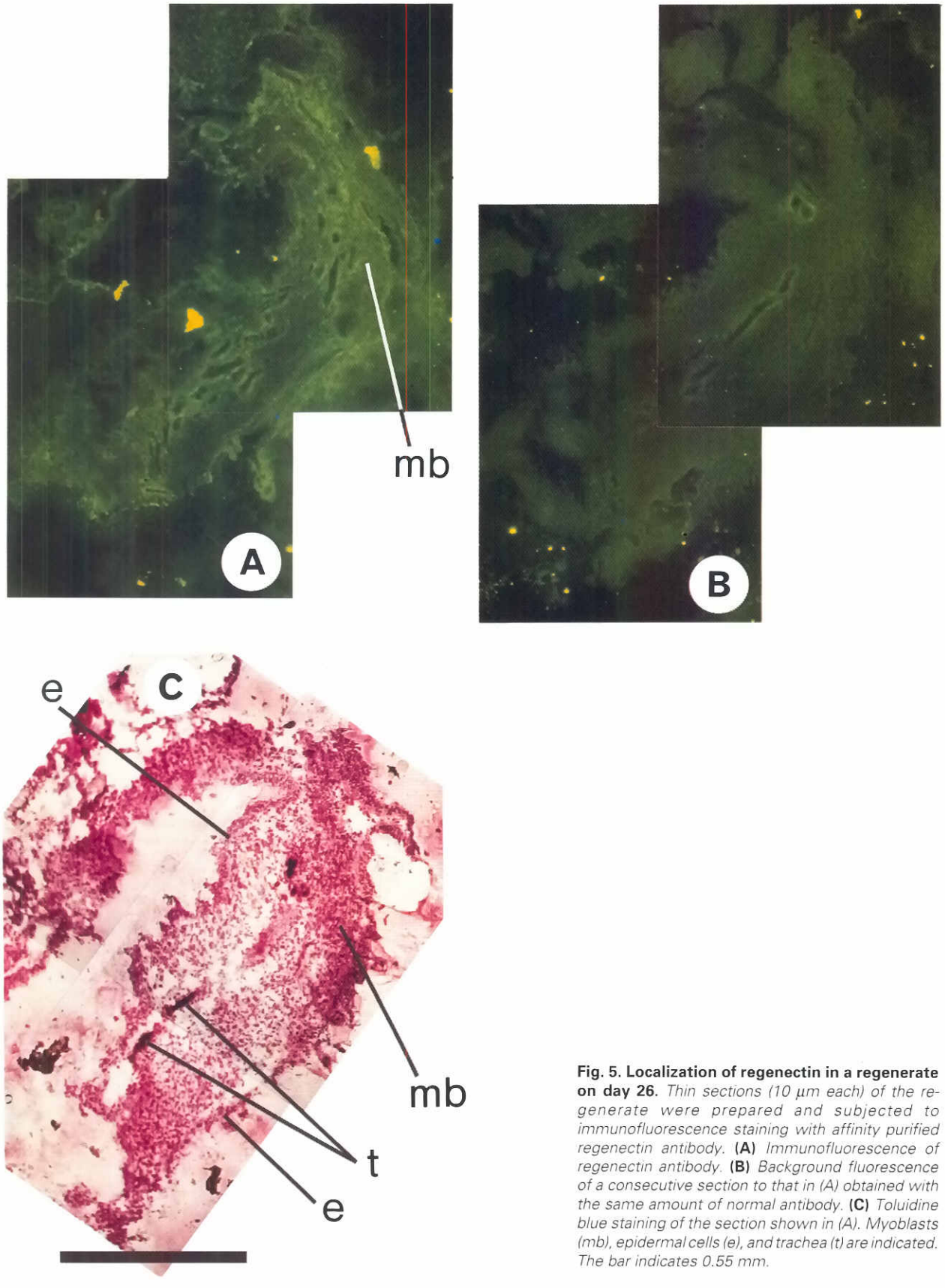


Fig. 5. Localization of regenectin in a regenerate on day 26. *Thin sections (10 μm each) of the regenerate were prepared and subjected to immunofluorescence staining with affinity purified regenectin antibody.* **(A)** *Immunofluorescence of regenectin antibody.* **(B)** *Background fluorescence of a consecutive section to that in (A) obtained with the same amount of normal antibody.* **(C)** *Toluidine blue staining of the section shown in (A). Myoblasts (mb), epidermal cells (e), and trachea (t) are indicated. The bar indicates 0.55 mm.*

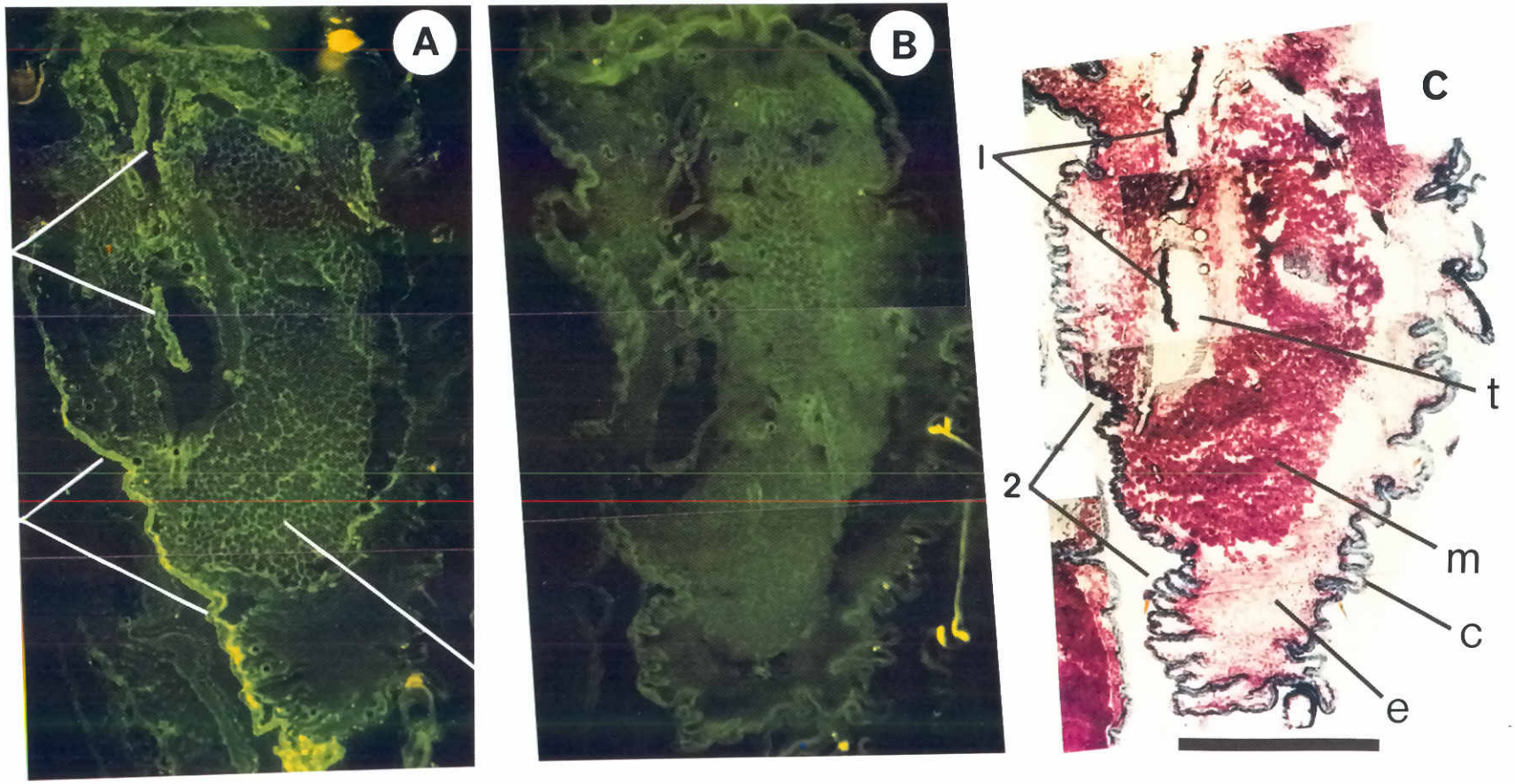


Fig. 6. Localization of regenectin in a regenerate on day 33. (A) *Immunofluorescence of regenectin antibody.* **(B)** *Background fluorescence of a consecutive section to that in (A) obtained with normal antibody.* **(C)** *Toluidine blue staining of the section shown in (A). Muscle cells (m), epidermal cells (e), trachea (t) and cuticle (c) are indicated. The bar indicates 0.55 nm. 1 and 2 are unidentified structures stained with regenectin antibody.*

1961; Penzlin, 1963; Kunkel, 1981; Turby, 1983; Bullière, 1985). These cells differentiate into muscle cells and finally form muscle fibers. The regenerate containing myoblasts was stained with regenectin antibody, but the boundaries of the cells were not clear (Fig. 7A). With progress of regeneration, myoblasts differentiated into muscle cells. Concomitantly, the boundaries of the cells seen by immunofluorescence staining became clearer (Fig. 7D and G). On the the other hand, fluorescence was not detected in mature muscle of normal leg (Fig. 7J). Therefore, regenectin seems to appear specifically in regenerating muscle, and to interact with developing muscle cells. Once muscle fibers are established, it rapidly disappears from the regenerate.

Discussion

In an immunofluorescence study, we found that regenectin is localized around the developing muscle cells in regenerating legs of *P. americana*. Regenectin seems to be a cementing substance filling gaps between muscle cells when muscle cells are premature. Regenectin was easily detected by immunoblotting, and was shown to appear in regenerates in a late stage of regeneration coinciding with the time of muscle formation. After amputation of a nymphal leg, wound healing takes place rapidly. Then epidermal cells increase inside the scab. Next, mononuclear myoblasts appear, and epidermal cells enclose these myoblasts forming a premature regenerate. Subsequently, myoblasts differentiate into muscle cells, and muscles are formed from these cells in the late stage of regeneration just before the moult.

We could not detect regenectin in legs harvested from intact nymphs at various developmental stages. Thus, regenectin may not play a role in normal leg development, but only in regeneration of legs. This is an important finding, because it might indicate that the molecular mechanisms of normal leg formation and regenerating leg formation are different, although finally the same structures are formed. Regenectin was also not detected in normal embryos throughout their development. Therefore, regenectin may not be important for formation of first instar nymphs.

In this study, we used antibody against regenectin. This antibody cross-reacted with *Periplaneta* lectin, although its reactivity with regenectin was more than that with *Periplaneta* lectin. The fluorescence observed in the immunofluorescence study was probably due to regenectin and not the 30 kDa subunit of *Periplaneta* lectin because the latter was not detected by immunoblotting of homogenates of these regenerates.

We found that the content of regenectin in the hemolymph increased in later stages of nymphal development. The source of regenectin in the regenerate is unknown, but at least two possibilities are conceivable. One is that it may be translocated from the hemolymph to the regenerate, and the other is that it may be synthesized *de novo* in the regenerate. In any case, there must be a mechanism to accumulate regenectin in the regenerate at a specific regeneration stage. Regenectin is present in the adult hemolymph, but as adults have no ability to regenerate legs, it probably has another role in adult hemolymph.

An immunofluorescence study revealed that regenectin is first distributed all over regenerating tissue, but later becomes localized around muscle cells. Possibly binding sites for regenectin appear on the surface of muscle cells at this stage. As regenectin was not detected in fully regenerated legs, it might be degraded selectively when regeneration of legs is completed, or its conformation might change in such a way that it does not react with its antibody. Biochemical studies are required on the binding sites for regenectin and the proteinase that degrades regenectin selectively.

Various insect lectins are known, but their biological roles are not clear. This is the first demonstration that a lectin participates in regeneration. As regeneration ability is restricted to certain species of insects, regenectin may be a common lectin in insects with regeneration ability. Amputated urodele limbs are also known to regenerate, so possibly a similar lectin participates in muscle formation during their regeneration.

Materials and Methods

Animals and preparation of regenerating legs

A colony of American cockroaches *(P. americana)* was maintained in a plastic container at 27°C with dog biscuits and water. Nymphs of 1.5-2.5 cm body length were anesthetized on ice 0-1 day after a moult, and their left metathoracic legs were amputated at the junction between the coxa and femur with fine scissors. Wound healing took place and a regenerated portion appeared at the tip of the amputated coxa by the time of the next moult (about 30 days after the operation). The amputated leg was removed at the bottom of the coxa at various times after the amputation for use in subsequent experiments. Right metathoracic legs of the same animals were used as intact control legs.

Collection of embryos

Eggs laid on the bottom of the container were collected every day and incubated at 27°C. Eggs were hatched 60 days later under these conditions. Embryos at various developmental stages were collected after 1 to 60 days.

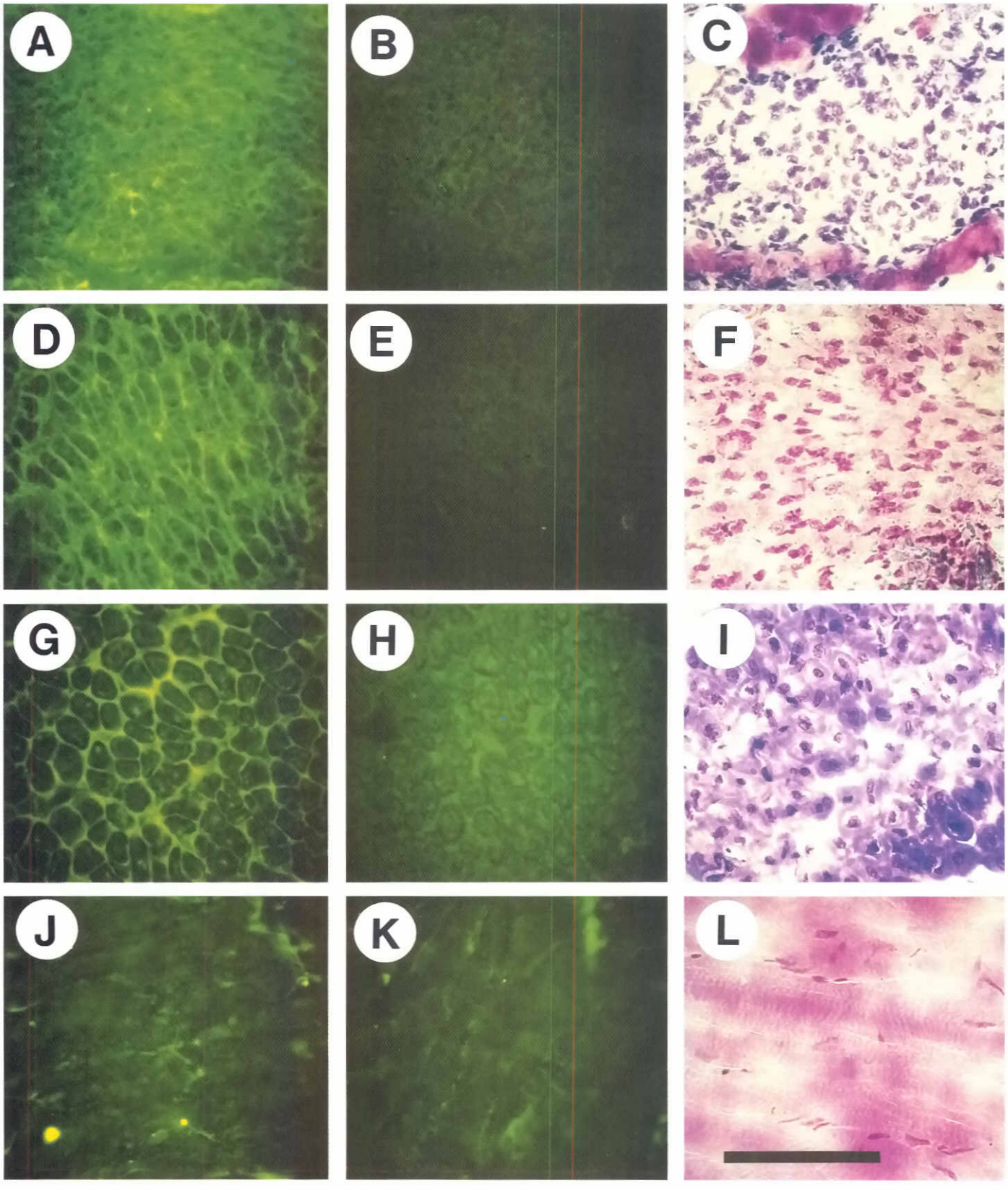
Preparation of tissue homogenates

Homogenates of various tissues were prepared by homogenization in 5 ml of buffered insect saline (10 mM Tris-HCl buffer, pH 7.9, containing 130 mM NaCl, 5 mM KCl and 1 mM $CaCl_2$) in a glass homogenizer on ice. The homogenates were centrifuged at 700 x g for 10 min and the resulting supernatants were used for further analyses. Protein was determined by the method of Lowry *et al.* (1951).

Regenectin and affinity purification of its antibody

Regenectin was purified to homogeneity as described before (Kubo *et al.*, 1990) and antibody against regenectin was raised in a male albino rabbit by injection of 44 μg of the purified regenectin in complete Freund adjuvant, followed 14 days later by a booster injection of the same amount of protein. Affinity purification of the resulting antibody was performed with purified regenectin. For this, regenectin (50 μg) was first separated by electrophoresis on 12.5% polyacrylamide gel containing 0.2% SDS[1], and then the protein was blotted onto a nitrocellulose filter. The small region of the filter on which regenectin was concentrated was excised and treated with skim milk

Fig. 7. Detailed analysis of immunofluorescence. (A and D) *Immunofluorescence of myoblasts in a regenerate on day 26.* **(B and E)** *Background fluorescence of the same regenerate observed in consecutive sections to (A) and (D).* **(C and F)** *Toluidine blue staining of the sections shown in (A) and (D), respectively.* **(G)** *Immunofluorescence of muscle cells of a regenerate on day 33.* **(H)** *Background fluorescence of a regenerate on day 33.* **(I)** *Toluidine blue staining of (G).* **(J)** *Immunofluorescence of a normal mature leg.* **(K)** *Background fluorescence of a mature leg.* **(L)** *Toluidine blue staining of (J). The bar indicates 0.11 mm.*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solution (20 mM Tris-HCl buffer, pH 7.9, containing 5% skim milk). Then the strip of filter paper was incubated in 10 ml of 10-fold diluted antiserum in rinse solution (10 mM Tris-HCl buffer pH 7.9, containing 150 mM NaCl, 1 mM EDTA, 0.1% Triton-X 100, 0.01% sodium azide, and 0.25% skim milk) at 4°C for 15 h with gentle shaking. The strip was rinsed well with rinse solution and cut into pieces, and the antibody specifically bound to regenectin was extracted with 1 ml of 0.2 M glycine-HCl buffer, pH 2.8. The resulting extract was neutralized with 1 M KOH, and a final concentration of 1% bovine serum albumin was added.

Electrophoresis and immunoblotting

Electrophoresis on SDS-polyacrylamide slab gel was carried out by the method of Laemmli (1970). Samples were denatured by heating them for 2 min at 100°C in 1% (w/v) SDS and 2% (v/v) 2-mercaptoethanol. After electrophoresis, the gels were stained by the method of Fairbanks *et al.* (1971).

Proteins separated by electrophoresis were transferred electrophoretically from the gel onto a nitrocellulose filter (BA-85, Schleicher & Schuell), and the filters were washed for 15 min at room temperature with rinse solution to remove SDS. Then the filters were immersed in skim milk solution for at least 1 hr, transferred to 5 ml of the rinse solution containing affinity purified antiserum against regenectin (diluted 300-fold) and kept at 4°C for 6 hr. They were then washed well with rinse solution, transferred to 5 ml of rinse solution containing radioiodinated anti-rabbit IgG (2×10^6 cpm) and kept for 4 hr at 4°C. Finally they were washed well with rinse solution, dried and subjected to autoradiography with Kodak XAR film.

Immunofluorescence staining of the regenerates with antibody against regenectin

Regenerates of metathoracic legs were removed 26 and 33 days after amputation, and examined by immunofluorescence staining to locate regenectin. For this, the regenerates were frozen in Tissue Tek and 10 μm sections of frozen regenerates were prepared on a gelatin-coated, fluorescence-free glass slide. Samples were fixed in 50 mM phosphate buffer, pH 6.0, containing 1.5% formaldehyde for 15 min, and washed in buffered insect saline. Then they were incubated with 30 μl of affinity-purified antibody solution for 1 hr at room temperature. As negative controls, serial sections were treated with normal IgG. The preparations were then washed well with buffered insect saline and incubated with 30 μl of FITC-conjugated goat anti-rabbit IgG for 20 min. Finally they were immersed in 90% fluorescence-free glycerol and mounted with coverslips for examination.

The same samples used for the immunofluorescence study were stained with Toluidine blue. For this, after removal of cover slips, the samples were treated in 1% (w/v) boric acid solution containing 0.5% (w/v) Toluidine blue for 2-3 min, and then washed well.

Acknowledgments

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References

BALDO, B.A., SAMYER, W.H., STICK, R.V. and UHLENBRUCK, G. (1978). Purification and characterization of a galactan-reactive agglutinin from the clam *Tridacna maxima* (Roding) and a study of its combining site. *Biochem. J. 175:* 467-477.

BODENSTEIN, D. (1955). Contributions to the problem of regeneration in insects. *J. Exp. Zool. 129:* 209-224.

BULLIÈRE, F. (1985). *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* vol. 2 (Eds. G.A. Kerkut and L.I. Gilbert). Pergamon Press, Oxford, pp. 371-424.

COWDEN, R. and BODENSTEIN, D. (1961). A cytochemical investigation of striated muscle differentiation in regenerating limbs of the roach, *Periplaneta americana. Embryologia 6:* 36-50.

FAIRBANKS, G., STECK, T.L. and WALLACH, D.F.H. (1971). Electrophoretic analysis of the major polypeptides of the erythrocyte membrane. *Biochemistry 10:* 2606-2617.

GIGA, Y., SUTOH, K. and IKAI, A. (1985). A new multimeric hemagglutinin from the coelomic fluid of the sea urchin *Anthocidaris crassispina. Biochemistry 24:* 4461-4467.

HAMMARSTRÖM, S. and KABAT, E.A. (1969). Purification and characterization of a blood-group A reactive hemagglutinin from the snail *Helix pomatia* and a study of its binding site. *Biochemistry 8:* 2629-2705.

JOMORI, T., KUBO, T. and NATORI, S. (1990). Purification and characterization of lipopolysaccharide-binding protein from the hemolymph of American cockroach *(Periplaneta americana). Eur. J. Biochem. 190:* 201-206.

KOMANO, H., MIZUNO, D. and NATORI, S. (1980). Purification of a lectin induced in the hemolymph of *Sarcophaga peregrina* on injury. *J. Biol. Chem. 255:* 2919-2924.

KOMANO, H. and NATORI, S. (1985). Participation of *Sarcophaga peregrina* humoral lectin in the lysis of sheep red blood cells injected into the abdominal cavity of larvae. *Dev. Comp. Immunol. 9:* 31-40.

KUBO, T., KAWASAKI, K. and NATORI, S. (1990). Sucrose-binding lectin in regenerating cockroach *(Periplaneta americana)* legs; its purification from adult hemolymph. *Insect Biochem. 20:* 585-591.

KUBO, T. and NATORI, S. (1987). Purification and some properties of a lectin from the hemolymph of *Periplaneta americana* (American cockroach). *Eur. J. Biochem. 168:* 75-82.

KUNKEL, J.G. (1981). *The American Cockroach* (Eds. W.J. Bell and K.G. Adiyodi). Chapman and Hall, London, pp. 425-443.

LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:* 680-685.

LACKIE, A.M. (1980). Invertebrate immunity. Parasitology 80: 393-412.

LACKIE, A.M. (1981). The specificity of the serum agglutinins of *Periplaneta americana* and *Schistocerca gregaria* and its relationship to the insect's immune response. *J. Insect Physiol. 27:* 139-143.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193:* 265-275.

MARCHALONIS, J.J. and EDELMAN, G.M. (1968). Isolation and characterization of a hemagglutinin from *Limulus polyphemus. J. Mol. Biol. 32:* 453-465.

MURAMOTO, K. and KAMIYA, H. (1986). The amino-acid sequence of a lectin of the acorn barnacle *Megabalanus rosa. Biochim. Biophys. Acta 874:* 285-295 .

PENZLIN, H. (1963). Über die Regeneration bei Schaben (blattera). I. Das Regenerationsvermogen und die Genese des Regenerates. *W. Roux Arch. Entwmech. Org. 154:* 434-465.

QU, X.-M., ZUANG, C-F., KOMANO, H. and NATORI, S. (1987). Purification of a lectin from the hemolymph of Chinese oak silkmoth *(Antheraea pernyi)* pupae. *J. Biochem. 95:* 239-245.

RAVINDRANATH, M.H., HIGA, H.H., COOPER, E.L. and PAULSON, J.C. (1985). Purification and characterization of an O-acethylsialic acid-specific lectin from a marine crab *Cancer antennarius. J. Biol. Chem. 260:* 8850-8856.

SCOTT, M.T. (1971). A naturally occurring hemagglutinin in the hemolymph of the American cockroach. *Arch. Zool. Exp. Gen. 112:* 73-80.

SCOTT, M.T. (1972). Partial characterization of the hemagglutinating activity in the hemolymph of the American cockroach. *J. Invertebr. Pathol. 19:* 66-71.

SUZUKI, T., TAKAGI, T., FURUKOHRI, T., KAWAMURA, K. and NAKAUCHI, M. (1990). A calcium-dependent galactose-binding lectin from the tunicate *Polyandrocarpa misakiensis. J. Biol. Chem. 265:* 1274-1281 .

TURBY, P.R. (1983). Blastema formation and cell division during cockroach limb regeneration. *J. Embryol. Exp. Morphol. 75:* 151-164.

UMETSU, K., KOSAKA, S. and SUZUKI, T. (1984). Purification and characterization of a lectin from the beetle, *Allomirina dichotoma. J. Biochem. 95:* 239-245.

YEATON, R.W. (1981). Invertebrate lectins: I. Occurrence. *Dev. Comp. Immunol. 5:* 391-402.

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