least a few times, but that eggs did not reach the blastoderm stage.

In experiments with *Drosophila melanogaster* unfertilized eggs laid by virgin *y w sn*^{3} *lz*^{50e30} females were taken as hosts (*y*, yellow body colour, yellow larval mouth hooks; *w*, white eye colour, unpigmented Malpighian tubes; *sn*^{3}, singed bristles; *lz*^{50e30}, lozenge shaped eyes). After they had been collected these females were kept in culture bottles where they began to deposit unfertilized eggs after 3 days. As an additional precaution culture bottles from which host eggs were taken were observed for several days after the time at which larvae would have normally hatched: no larvae developed.

Fertilized donor eggs came from a Berlin-wild stock. Eggs 5 min old or younger were collected, dechorionated, rinsed in sterile *Drosophila* Ringer solution[4] and kept on a slide under paraffin oil. Here development was allowed to proceed until the time of transplantation when unfertilized host eggs which had been similarly treated were also placed in oil on the same slide. The ages of host eggs ranged from 20 min to 1 h; the ages of donor eggs from 1·75 h to 3 h. For each single successful transfer the times are given in Table 1. At the time of transplantation donor eggs contained between about 200 and 2,000 nuclei, depending on their age. Table 1 indicates that an attempt was made to specify the region of the donor eggs from which nuclei were taken. The number of nuclei which were transferred into a single egg could not be counted exactly. There were usually about ten, never less than five and never more than fifteen. All transplantations were carried out with a modified Leitz-Micromanipulator set.

Table 1. DATA FOR ELEVEN SUCCESSFUL NUCLEAR TRANSFERS

Transpl. No.	Donor Age* (h)	Donor Origin of nuclei†	Host age* (h)	Development proceeded to stage‡ 1	2	3
14	2¼	p.c.+pb.n.	½	+		
22	2½	p.c.+pb.n.	¾		+	
25	2	Ventral pb.n.	½	+		
29	2¼	p.c.+pb.n.	¾	+		
89	2¼	p.c.+pb.n.	½	+		
91	1¾	p.c.	¾	+		
93	1¾	p.c.	½	+		
94	2	p.c.+pb.n.	½			+
95	2	p.c.+pb.n.	½	+		
110	1¾	p.c.+pb.n.	½		+	
114	2	p.c.+pb.n.	½	+		

* Interval between egg deposition and time of transfer.
† p.c. = pole cells; pb.n. = preblastoderm nuclei.
‡ For characterization of stages see text.

After transplantation, host eggs remained under paraffin oil and were raised at 25° C. The only larva that hatched was removed from the oil, rinsed in Ringer solution and transferred to standard *Drosophila* medium.

Pricking 139 unfertilized eggs did not result in any parthenogenetic development. In eleven out of 118 transplantations, development of the host eggs was observed. These eleven cases can be placed in three groups according to the degree of their development (Table 1). Stage 1: eggs died 18–26 h after deposition. Muscular contractions, the beginning of segmentation and the presence of tracheae could be observed; but development had not proceeded to a stage which is characteristic at this age. Stage 2: embryo No. 110 died 28 h and embryo No. 22 died 36 h after egg deposition. Segmentation was more advanced; the two main tracheal trunks were formed and so were the Malpighian tubes, but the head regions were not differentiated. In embryo No. 110 the tubes had not yet developed to a stage where pigmentation normally sets in. In embryo No. 22 these tubes were normally pigmented, indicating that they had been developed by injected nuclei. Host nuclei are white (*w*) and show no pigment formation in the tubes. Stage 3: one larva (No. 94; Fig. 1) hatched 25 h after egg deposition and died after having reached the second larval instar. Its phenotype was wild-type, that is, the Malpighian tubes were pigmented and the mouth hooks were black. Two characters thus indicated that the transplanted nuclei were responsible for the development.

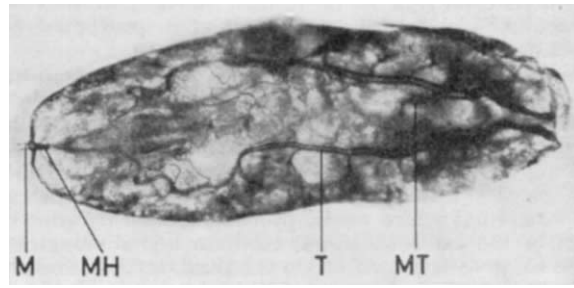

Fig. 1. Embryo No. 94, 24 h after egg deposition, in dorsal view. M, Micropyle; MH, mouth hooks; T, tracheal trunks; MT, Malpighian tubes. This embryo hatched 1 h after the picture was taken and developed into the second larval instar. (×*c*.140.)

The reasons for the difference in head development between the two embryos of stage 2 and the single embryo of stage 3 are still conjectural. Different amounts of cytoplasm lost during transfer and/or slightly different regional origin of nuclei might be responsible. The reasons for lack of development in the remaining 107 transplants are not known. Technical faults are almost certainly among them. This is indicated by the fact that later sets of experiments were more successful than earlier ones (Table 1).

Experiments to test the capacities of transplanted nuclei from different developmental stages and of nuclei from different regions within the embryo are being undertaken.

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[1] Gurdon, J. B., *Adv. Morphogen.*, **4**, 1 (1964).
[2] DuPraw, E. J., *Glean. Bee Cult.*, **88**, 104 (1960).
[3] Schnetter, W., *Zool. Anz. Suppl.*, **30**, 494 (1967).
[4] Ephrussi, B., and Beadle, G. W., *Amer. Nat.*, **70**, 218 (1936).

Germination of Achira Seed (*Canna* sp.) Approximately 550 Years Old

DURING the excavation of a tomb at the archaeological site of Santa Rosa de Tastil, Argentina (24° 25′ S., 65° 50′ W.), we found a necklace made out of nuts of *Juglans australis*. Inside each nut was a seed of *Canna* sp., making a rattle. Samples from bones of cameloids in the upper strata of garbage from the site were taken for carbon-14 analysis by Groningen Laboratories (Holland), and they were dated at about 530 yr (AD 1420). The bones probably belong to the last period of occupation of the city by the Indians. No Incaic elements were found, and the carbon-14 dating implies that the city was developed before AD 1420.

We were interested in germinating the achira seeds found inside the necklace, because embryos of this species are protected by resistant nutritious reserve substances and have impermeable teguments, which we thought might have helped to preserve their viability.

Our material was limited to three seeds. We began by placing one of them on a wet filter paper in a precipitation glass covered with polyethylene. The glass was kept in darkness at 27° C. After a few days, a rhizome appeared, reached a length of 1–2 mm and then ceased to grow. To activate its growth, a solution of gibberellic acid—and later one of indole-3-acetic acid—were added, but with no effect. This result showed that the seeds were still viable and we assumed that growth regulators

had been destroyed in the course of time, or that this particular seed had a deficiency that prevented the continuation of germination.

Another seed was aseptically sown in sterile conditions in a test tube in Fox and Miller's mineral medium[1], together with indoleacetic acid (0·1 mg/l.), 6-furfurylaminopurine (0·01 mg/l.), gibberellin (GA_3) (0·01 mg/l.), thiamine (0·4 mg/l.) and extract of incipient lactic fermentation of ripe maize (7·0 ml./l.) (ref. 2) as regulators.

Two normal achira seeds, probably *Canna indica*, were sown in the same nutritious medium in individual test tubes to serve as controls. On the third day, the archaeological seed and the control germinated normally at 27° C in darkness. First the rhizomes sprouted and on their extremities the roots grew. Later the first leaves appeared. They were gradually exposed to the light to allow chlorophyll synthesis and were then transferred to a chamber at 21° C.

On the tenth day, the archaeological plantule had developed a leaf of 3·3 cm which was still tubular, and a good root system, though this showed disturbances in its geotropism. Lateral roots had also started to grow. The control plantule which germinated on the same day had a 1·5 cm leaf, a root system showing normal geotropism and no lateral roots. On the fourteenth day, the plantules were taken out of the test tubes and planted in quartz gravel with a nutritious solution and kept in a greenhouse. They continue to grow and develop.

The differences between the leaves of the plants may be varietal or specific characteristics, because the identity of the archaeological plant cannot yet be determined. The geotropic irregularity in its root system may be attributable to hormone deficiency. Root geotropism is common among higher plants. The fast growth of lateral roots could corroborate this fact.

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[1] Fox, E., and Miller, C., *Plant Physiol.*, **34**, 577 (1959).
[2] Nakayama, F., *Rev. Fac. Agric.*, **42**, 1 (1966).

Photochemically Active Chromoprotein isolated from the Blue-green Alga *Anabaena cylindrica*

While carrying out a biochemical study of the photosynthetic system of *Anabaena cylindrica*, we have found that a chromoprotein isolated from the algal cells is active in the photo-oxidation of ascorbate. We report here the methods for isolation of the active chromoprotein, and some of its characteristics.

Cells of *Anabaena cylindrica* were grown as described previously[1]. Algal cells were sonicated for 15 min (200 W, 10 kc) in 0·1 M *tris*-HCl buffer (*p*H 7·5) containing 0·35 M NaCl and 0·001 M ethylenediamine tetraacetic acid. Proteins in the supernatants after centrifugation at 27,000*g* were fractionated by acetone precipitation (80 per cent, −20° C) and ammonium sulphate precipitation between 30 and 60 per cent saturation. Further purification was achieved by gel-filtration on 'Sephadex *G*-100' in 10^{-3} M phosphate buffer, *p*H 7·5. The active chromoprotein ran through the column slightly faster than phycocyanin. The fractions rich in the active chromoprotein were combined, and the proteins were concentrated by ammonium sulphate precipitation. The sample in 0·1 M *tris*-HCl buffer was placed on a DEAE-cellulose column equilibrated with the same buffer. The column was developed with 0·15 M *tris*-HCl buffer. The active chromoprotein moved faster than phycocyanin and was clearly separated from the latter. Repeated chromatography on a column of DEAE-cellulose yielded the chromoprotein preparation, which formed a single band in 'Sephadex' gel-filtration (Fig. 1). The active chromoprotein has a larger molecular weight, estimated by 'Sephadex' gel-filtration, than *c*-phycocyanin (molecular weight, 134,000) (ref. 2) and *c*-phycoerythrin (213,000) (ref. 2) in the same experimental conditions. On a protein basis the specific activity of the preparation was 150 to 270 times greater than for the crude extracts (Table 1).

Table 1. PURIFICATION OF THE ACTIVE CHROMOPROTEIN

Treatments	Specific activity (μmoles/mg protein/h)	(μmoles/ OD_{620} ml./h)	Total activity (μmoles/h)	Recovery (%)
Acetone-treatment	3·2	1·5	30,600	100
Ammonium sulphate precipitation	5·8	2·1	24,300	80
'Sephadex *G*-100' chromatography	33·5	6·7	24,000	79
DEAE-cellulose chromatography I	—	169·0	12,300	40
DEAE-cellulose chromatography II	574·0	234·0	10,890	37

The activity was estimated manometrically by O_2 uptake induced by light. The reaction mixture contained, in 2·4 ml., 48 μmoles of sodium ascorbate, 120 μmoles of *tris*-HCl buffer, *p*H 7·5, an appropriate amount of test sample; KOH (0·1 ml., 20 per cent), in the centre well. Light intensity was 15,000 lux, and the temperature was 26° C.

The absorption spectrum of the active chromoprotein is similar to that of phycocyanin, except for the characteristic minor peaks at 430, 450, 480 and 695 mμ (Fig. 2).

When excited by light of wavelength 590 mμ, the chromoprotein gave an emission spectrum with peaks at 646 and 700 mμ (Fig. 3*A*). In the same experimental conditions, phycocyanin gave a single emission peak at 646 mμ. When the active chromoprotein was excited

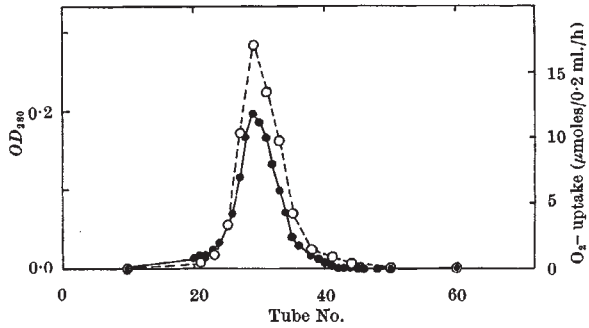

Fig. 1. Patterns of the active chromoprotein shown by column chromatography of 'Sephadex *G*-200'. The column of 'Sephadex' (4 × 45 cm) was equilibrated with 0·1 M *tris*-HCl buffer (*p*H 7·5) containing 0·2 M NaCl. The column was developed with the same buffer. Each fraction was of 8 ml. (●), *OD* at 280 mμ; (○) activity for ascorbate photo-oxidation.

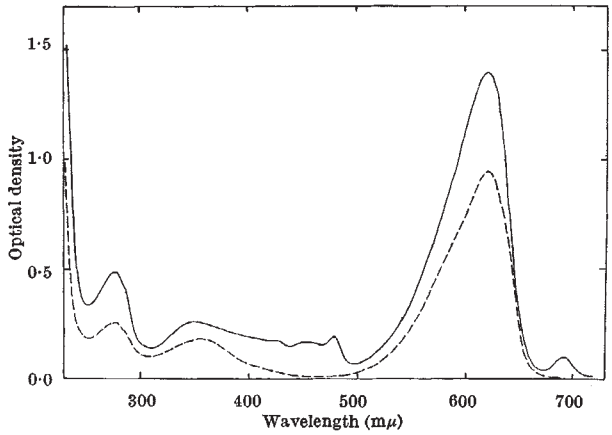

Fig. 2. Absorption spectra of the active chromoprotein and phycocyanin in 0·1 M *tris*-HCl buffer, *p*H 7·5. ———, Active chromoprotein; - - -, phycocyanin purified by combined use of calcium phosphate gel adsorption and DEAE-cellulose column chromatography.