Michigan, USA. Foetal bovine (FS) and human serum (HS) were heat-inactivated at 56 °C for 30 min. Lipid-free bovine serum albumin (BSA) was obtained from the Sigma Chemical Co., USA.

In 10% HS or FS, PGE_1 and PGE_2 produced a concentration-dependent type 2a response and were of similar potency (fig. 1). In some cases, significant differences from controls could be detected at 10^{-9} M. $PGF_{2\alpha}$ was comparatively ineffective and produced a type 2a response only at concentrations of 10^{-5} M and above. When the prostaglandins were removed the morphology reverted to normal within 60 min. In the presence of serum, type 2b responses were limited to a small proportion of the total. Examples of PG-induced type 2a and 2b cells are illustrated in figure 2. In many experiments of the type shown by figure 1 the type 2a response was comparatively small (< 10%) and persisted for only 4–5 h, suggesting inhibition by some sera. In BSA (4 mg/ml) without serum a pronounced type 2b response occured in the untreated controls, which was characterized by tightly contracted astrocyte-like forms. This effect was markedly increased by PGE_1 and PGE_2. The type 2a response was comparatively slight but could be enhanced, with simultaneous reduction of the type 2b proportion, by allowing 48 h or more between passaging and addition of the prostaglandins. The effects in BSA were persistent for at least 72 h but reversed upon removal of the agents.

In unpublished experiments in this laboratory PGE_1 and PGE_2 were found to be potent activators of adenylate cyclase in synovial cells, whereas $PGF_{2\alpha}$ was less effective. This is in agreement with the effect of these agents on cAMP in explant-derived synovial cells[10]. Since cAMP is known to control the organisation of microfilaments and microtubules of the cytoskeleton[11], changes in cAMP levels can account for the morphological effects of prostaglandins, The type 2b effect in synovial cells is probably an intensification of the 2a phenomenon, since apparently intermediate forms can usually be observed. A fenestrated appearance produced in iris epithelial cells by agents which stimulate intracellular cAMP[12], is nonetheless distinctive from the type 2a response in synovial cells and studies so far suggest that this phenomenon might be specific for synovial cells.

1 This work was supported by the National Health and Medical Research Council of Australia.
2 N. Williamson, K. James, N.R. Ling and L.P. Holt, Ann. rheum. Dis. *25*, 534 (1966).
3 P. Barland, A.B. Novikoff and D. Hamerman, J. Cell Biol. *14*, 207 (1962).
4 J.R.E. Fraser, B.J. Clarris and E. Baxter, Ann. rheum. Dis. *38*, 287 (1979).
5 J. LeMarshall, J.R.E. Fraser and K.D. Muirden, Ann. rheum. Dis. *36*, 130 (1977).
6 F.A. Kuehl, Prostaglandins *5*, 325 (1974).
7 J.R.E. Fraser and K.J. Catt, Lancet *2*, 1437 (1961).
8 J.R.E. Fraser and J.F. McCall, Ann. rheum. Dis. *24*, 351 (1965).
9 B.J. Clarris and J.R.E. Fraser, Aust. J. exp. Biol. med. Sci. *45*, 549 (1967).
10 C.W. Castor, J. Lab. clin. Med. *85*, 392 (1975).
11 J.R. Dedman, B.R. Brinkley and A.R. Means, Adv. cyclic nucl. Res. *11*, 131 (1979).
12 J.R. Ortiz, T. Yamada and A.W. Hsie, Proc. natl Acad. Sci. USA *70*, 2286 (1973).

Role of kinetin in the dormancy of *Cercis siliquastrum* seeds[1]

Paola Gastaldo, Paola Profumo and Liliana Caffaro Corti

Institute of Botany, University of Genova, Corso Dogali 1-C, I-16136 Genova (Italy), 16 July 1981

Summary. Studies conducted on *Cercis siliquastrum* seeds treated with kinetin confirm that this hormone does not interrupt dormancy in either whole seeds or those decoated at the radical pole. Seeds totally decoated or decoated at the cotyledon pole only demonstrated atypical germinations linked to cotyledon growth, permitting the embryo to escape the inhibitory action present in the endosperm; this does not occur when the cotyledon surface is experimentally reduced.

Within the realm of the problem regarding factors inhibiting germination of *Cercis siliquastrum* seeds[2], kinetin treatment has proven to have made an important contribution[3]. Kinetin does not interrupt dormancy in the seed but, at high concentrations, it causes the development of characteristic cuts in a circular pattern; this normally indicates germination, but here is not followed by radicle protrusion. Results are described of further experiments with the same substance, associated with mechanical action on both the integument and endosperm as a means of deepening knowledge of the effect of kinetin on factors responsible for dormancy.

Materials and methods. During the entire course of the experiment, which lasted for a period of 2 years, seeds collected from the preceding year were always used.

The technique was the same as that used in the above-cited study[3]. Aqueous extracts of endosperm homogenates were obtained by applying a technique reported in a preceding study[2]. Experiments were repeated on average 6 times each. Kinetin was supplied by the Sigma Chemical Co., USA.

Results. Kinetin treatment of totally decoated seeds, free of integument, determined different percentages of atypical germinations (with cotyledon protrusion), varying according to concentrations; optimum effect at 10^{-5} M (fig.). This was however not always followed by the normal seedling development that is invariably obtained with low temperature pre-treatment (30–40 days at 6 °C) or by the action of gibberellic acid (GA_3) in whole seeds. On the 20th day when some samples treated with kinetin show elongated seedlings free of endosperm, other seeds in which the cotyledons have partially protruded maintain a certain integrity, and the cotyledons even though belonging to healthy seedling are unable to go out of the seed completely.

When a partial removal (about $^1/_3$) of the integument is made, liberating the radical portion of the endosperm, kinetin does not induce germination even when two small incisions are made in proximity of the radical pole of the endosperm, although the endosperm is extremely thin in the area of the radical apex.

If instead the cotyledon portion is completely freed from the integument (approximately $^1/_3$), kinetin (with an optimal effect at 10^{-4} M) induces atypical germination analogous to

that seen in cases of total decortication. In fact a good percentage of germination was demonstrated, often lacking, however, a complete seedling development.

When embryos are isolated and allowed to grow in vitro under the action of kinetin (10^{-6} M or 10^{-5} M) this substance determines cotyledon growth stimulation, presumably by distension, while the embryonic axes resulting are shorter than those of control seedlings.

Embryos isolated in vitro and treated with aqueous endosperm extracts, in a 1 ml/4 endosperm ratio, with or without kinetin, fail to grow, and die within a few days.

Another type of treatment consisted of the removal of about $\frac{1}{3}$ of the cotyledons in decoated seeds. Such an operation required extraction of the embryo from the endosperm followed by partial removal of the cotyledons which were reinserted in the endosperm, split twice at the radical pole. In this case lots of seeds were placed in a 10^{-5} M kinetin solution while control groups were placed in water. Following 10 days of incubation (± 20 °C) no signs of germination were detected in either group.

Discussion and conclusions. The above-reported results confirm that kinetin at various concentrations is unable to interrupt dormancy in whole seeds with scarified integuments. As referred to elsewhere[3] recent researches indicate that the action of the substance varies according to the seed treated. In the case of *Cercis siliquastrum* the action of kinetin differed under low temperature[4] and GA_3[5] treatment, macroscopically producing in both cases only one sign associated with germination: a circular break of the integument at the radical pole.

If, on the other hand, the integument is removed, kinetin does show some action, inducing a certain percentage of atypical germinations[5,6] similar to those in seeds treated by us with fusicoccin (FC). Evidently kinetin, like FC, in some way removes mechanical obstacles to germination by increasing the growth force of the embryo. This is illustrated in the kinetin treatment of isolated embryos which determined cotyledon growth, a typical effect of kinetin noted by several authors[7–9]. While embryos of the same year's harvest treated by us with FC showed increased growth of the embryonic axis, kinetin was only able (and to a lesser degree than FC) to cause an increase in cotyledon distension (table 1) contrary to results found by Pienfield and Stobart[10] in *Acer pseudoplatanus* seeds.

This may explain the different behaviour of seeds decoated in various ways containing whole endosperm treated with both substances.

Results of experiments conducted with kinetin on seeds decoated in various ways stimulated comparisons between low temperature and various types of treatment already conducted on the *Cercis* seed and illustrated in table 2.

It may be concluded that the action of chilling and GA_3 treatment is not comparable with the effects caused by FC and kinetin. In particular, since kinetin was demonstrated to be unable to induce germination it is unlikely that the dormancy break is due only to an increase, obtained by low temperature pre-treatment, of endogenous kinetin[10–12].

The possibility of seedling development, in our case deriving from atypical germination, seems to be linked to the speed of growth; rapid growth causes a more rapid seedling release from the endosperm, escaping from the action of inhibitors. This is further confirmed by experiments conducted on decoated seeds with cut cotyledons: the smaller surface of the mutilated cotyledons constrains the embryo to a longer permanence in the endosperm, exposing it to the action of inhibitory substances liberated by the endosperm itself.

In *Cercis siliquastrum* seeds kinetin does not seem to be able to reverse inhibitory chemical action, but rather acts by increasing cotyledon growth acting upon the mechanical resistance offered by the endosperm.

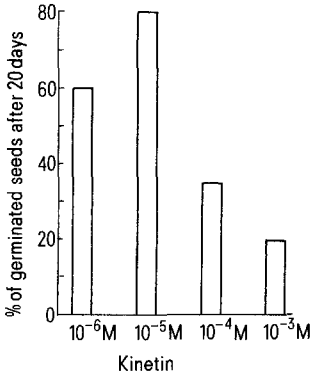
This underlines the fact that dormancy is linked to mechanical and chemical factors, as suggested in our previous work[13]; comparison of the role of kinetin and other types of seed treatment conclude that the above-cited factors are correlated among themselves with a time function also.

Table 1. Average length of the embryonic axis and cotyledons 8 days after in vitro seeding

Treatment	Embryonic axis length (mm)	Cotyledons length (mm)
H_2O	27.21	6.65
K 10^{-5}M	25.5	8.8
FC 10^{-5}M	28.08	12.45

Table 2. Germination modality of seeds decoated in various ways (with whole endosperm)

Treatment	Totally decoated seeds	Seeds decoated at radical pole	Seeds decoated at cotyledon pole
6 °C	Typical	Typical	Typical
GA_3 10^{-5} M	Typical	Typical	Typical
FC 10^{-5} m	Typical and atypical	Typical	Atypical and typical
K 10^{-5} M	Atypical		Atypical



Kinetin effect at various concentrations on decoated seeds (seeds, with whole endosperm, atypically germinated at 24 °C).

1 This investigation was supported by the Italian National Research Council (C.N.R.) Project 'Biology of Reproduction'.

2 P. Profumo, P. Gastaldo and R. Martinucci, Experientia *35*, 1452 (1979).
3 P. Gastaldo, P. Profumo and L. Caffaro Corti, Boll. Soc. ital. Biol. sper. *56*, 507 (1980).
4 P. Gastaldo and P. Profumo, G. Bot. ital. *109*, 39 (1975).
5 P. Profumo and P. Gastaldo, G. Bot. ital. *111*, 211 (1977).
6 P. Profumo, P. Gastaldo and V. Parisi, Accad. naz. Lincei *63*, 135 (1977).
7 E. Marrè, R. Colombo, P. Lado and F. Rasi Caldogno, Pl. Sci. Lett. *2*, 139 (1974).
8 R.F. Norris, Pl. Sci. Lett. *6*, 63 (1976).
9 J.R. Dunlap and P.W. Morgan, Pl. Physiol. *60*, 222 (1977).
10 N.J. Pinfield and A.K. Stobart, Planta *104*, 134 (1972).
11 J. Van Staden, D.P. Webb and P.F. Wareing, Planta *104*, 110 (1972).
12 N.A.C. Brown and J. Van Staden, Physiol. Plant. *28*, 388 (1973).
13 P. Profumo and P. Gastaldo, G. Bot. ital. *112*, 474 (1978).