epidermal surface. It can also be used to obtain information regarding the after-effects of pesticides on plant surfaces.

H. F. LINSKENS
H. KRNER
Department of Botany, University, Nijmegen,
The Netherlands.

A Second Gibberellin-like Substance in Immature Barley Seed

IN an earlier paper it was shown that gibberellic acid occurs in the immature seed of barley, and it was mentioned that a second gibberellin-like substance was found in the extracts[1]. This second substance was eluted from a charcoal : celite column with 90 per cent aqueous acetone. When chromatogrammed on paper using *n*-butanol–1·5 N ammonia (3 : 1) or benzene–glacial acetic acid–water (4 : 2 : 1) it had the same R_F as gibberellins A_4 and A_7.

These preliminary experiments were carried out with samples gathered one week and four weeks after anthesis. There appeared to be more activity in the younger sample, so the following year a large batch was gathered about ten days after anthesis. Extraction of the bulk of this yielded only gibberellic acid, but a small part of the sample was extracted separately by a slightly different procedure, and both substances were found on this occasion. Bioassay of the second substance by dwarf pea seedlings[2] indicated an activity of 0·5 μg gibberellic acid equivalent per kg fresh weight of seed, whereas the activity shown by the cucumber hypocotyl assay[3] was 24 μg gibberellin A_9 equivalent per kg. Part of the sample was further purified by elution from a silica : celite column (1 : 2 w/w) with varying proportions of ethyl acetate and chloroform. Most of the activity was found in those fractions in which gibberellins A_4 and A_7 would be expected. Part of this repurified sample was tested by the lettuce seedling assay[4], in which only a trace of activity was found, and part was tested on dwarf maize seedling[5] (cultivar d_1), no significant activity being found.

The high activity in the cucumber assay, the low activity in the pea seedling test, and the R_F evidence all suggested that the substance could be gibberellin A_4 or A_7, but the lettuce assay result appeared to eliminate gibberellin A_7, which is highly active in this assay, while the activity seemed too low even for gibberellin A_4. Both gibberellins A_4 and A_7 are as active as gibberellic acid in the d_1 maize assay, so this assay also failed to confirm the identity of the unknown substance.

Thin-layer chromatography of the sample was carried out by the method previously described, but no fluorescent spot could be detected after spraying with sulphuric acid. It is possible that some fluorescence may have been masked by impurities, but the extracts appeared to be fairly highly purified at this stage. Most and Vlitos[6] have reported the extraction from sugar cane of a group of substances with similar properties, that is, they are biologically active especially in the cucumber assay but do not fluoresce on thin-layer chromatograms. This lack of ability to fluoresce after reacting with sulphuric acid may imply that these substances do not have a gibbane skeleton, but it is not yet known whether all gibbanes fluoresce, and these substances may be gibberellins in structure as well as activity.

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M. E. RADLEY
Imperial Chemical Industries,
Pharmaceuticals Division,
Alderley Park, Macclesfield.

[1] Jones, D. F., MacMillan, J., and Radley, M., *Phytochem.*, **2**, 307 (1963).
[2] Brian, P. W., and Hemming, H. G., *Physiol. Plant*, **8**, 669 (1955).
[3] Brian, P. W., and Hemming, H. G., *Nature*, **189**, 74 (1961).
[4] Frankland, B., and Wareing, P. F., *Nature*, **185**, 255 (1960).
[5] Phinney, B. O., *Plant Growth Regulation*, 489 (1961).
[6] Most, B. H., and Vlitos, A. J., *Actes Coll. Intern. C.N.R.S.* (123), 287 (1963).

RADIOBIOLOGY

Previous Reproductive History and the Susceptibility to X-ray-induced Congenital Anomalies

THE suspicion that primiparous mice are more apt to produce anomalous offspring, following foetal X-irradiation, than are multiparous mice, has led to the necessity of determining the normal incidence of such anomalies among large numbers of control mice of both categories. This investigation deals with the normal primiparous and multiparous *CF*1 mice of exactly the same age (7–9 months) and the analysis of the condition of their offspring. In direct contrast with this is presented a parallel study of pregnant primiparous and multiparous *CF*1 mice X-irradiated at a specific time and to a specific dose to determine whether a single variable (primiparity or multiparity) might alter the incidence of anomalies induced by X-rays. This paper reports data from 225 litters (2,700 implantations).

The strain of mouse used was the *CF*1 which we have found to be satisfactory for many years. All mice for this experiment were received at one time from the source, and were kept under identical conditions of temperature, humidity, food and water for the duration of the experiment. All females used were from 7 to 9 months of age (the range being due to the fact that pregnancies for the multiparous mice could not always be immediately achieved). The multiparous females were kept pregnant from 2 months of age, and every one had been pregnant four times followed by a 2-week period of rest before the experimental pregnancy.

Pregnancies were achieved by exposing the females to males of the same strain for a period of 2 h (8–10 a.m.) and females found to have vaginal plugs were segregated and labelled as to the time of conception. At 18 days gestation each pregnant mouse (controls or irradiated) was dissected and the contents of the uterus exposed and analysed, and the sex of each foetus determined.

For X-irradiation, paired tubes were used in a cross-fire of rays, with the pregnant mice placed equidistant between the tubes so as to be exposed uniformly to the rays. The dose was calculated as the dose delivered to the foetuses within the gravid uterus. (The physical factors of irradiation were 184 kVp. and 30 m.amp; half value layer, 0·6 mm copper, filtration 0·28 copper and 0·50 aluminium, distance from the tubes to the uterus 72 cm; and dose rate 50 r./min). A single exposure-level of 150 r. was chosen and delivered at exactly 8 days gestation because previous experience had shown that this stage and dose would definitely provide a wide gamut of congenital anomalies[1–3].

The anomalies observed besides resorption and death were: exencephaly, cephalic blisters, microcephaly, brain haemorrhage, eye and jaw defects, persistent exteriorized intestines, and stunting. There was no attempt to determine inner histological alterations.

The experimental data involve 125 control and 100 experimental pregnancies, giving 1,484 control implantations and 1,216 irradiated implantations. There appeared to be little difference in the condition of the foetuses among the controls except that there was a higher (3·3 per cent) incidence of anomalies among the primiparous than among the multiparous females (1·5 per cent). There were a few more dead foetuses among the multiparous controls (1·8 per cent) than among the primiparous controls (0·6 per cent), but the percentage of apparently normal foetuses was quite similar.

The mouse embryo at 8 days gestation is just beginning massive differentiation of almost all its tissues so that it is quite susceptible to the insult of X-rays[4]. An exposure of 150 r. at this time will invariably produce anomalies, so that similar treatment of the primiparous and the multiparous females should indicate whether previous