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Gibberellins and Nodulation

RECENTLY, Fletcher, Alcorn and Raymond[1] presented evidence that gibberellins had no effect on the nodulation of white clover (*Trifolium repens*) grown either in aseptic agar or in soil. In concentrations from 1 to 1,000 p.p.m., gibberellic acid was also without effect on the growth of the nodule-forming organism *Rhizobium trifolii*. These combined results seemed to contradict a previous report of Thurber, Douglas and Galston[2], who had found a marked depressive effect of potassium gibberellate on nodulation in dwarf beans (*Phaseolus vulgaris*).

Although I have done no further work on this phenomenon since the original report, I have been in receipt of information from other workers in the field, who have corroborated and extended our findings. For example, Dr. P. W. Brian, of Imperial Chemical Industries, Ltd., has informed me that 10 and 100 p.p.m. sprays of gibberellic acid, applied daily or weekly, greatly depressed the number of nodules and the mean diameter of nodules of 14-day old plants of *P. vulgaris* var. Masterpiece grown in garden soil.

Similarly, Dr. N. P. Kefford, of the Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, Canberra, has found a depressive effect of gibberellic acid at 0·3 p.p.m. on nodule formation of lucerne (*Medicago sativa*, Hunter River) grown for 42 days on mineral agar, the acid in these experiments being added directly to the agar. In this case, the fewer nodules were each of larger volume than the controls, so that the nodule volume per plant was unaltered by treatment with gibberellic acid. Both Dr. Brian and Dr. Kefford plan to publish their results in detail.

In view of these results, involving independent demonstration of the same kind of phenomenon in three widely separated laboratories, it would seem that the original conclusion that gibberellins can inhibit nodulation is valid, and that some care is therefore required in the use of this product on legumes. It is also clear from the work of Fletcher *et al.* that not all legumes grown under all conditions will show this inhibitory effect of gibberellic acid. This difference in nodulation response is probably related to the already known fact that different genotypes of plants, presumably of varying endogenous gibberellin-levels, react quite differently to identical gibberellin applications.

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Quantitative Measurement of the Effect of Lysergic Acid Diethylamide on Mice and its Interactions with other Drugs

ALTHOUGH several species of animals (including spiders, fighting fish, pigeons and rats) have been used for the quantitative evaluation of the effect of hallucinogenic and psychotomimetic agents it is worth while to add to this list owing to the biochemical differences between species. The present favourite seems to be the rat[1]. This communication reports a test for the quantitative evaluation of the effect of psychotomimetic agents using mice chosen for their small size (useful if limited quantities of agent—for example, extracts from schizophrenic serum—are available) and ease of handling. A Latin square design was used employing four cages, each of 5 mice, and four modes of injection (saline, lysergic acid diethylamide, drug to be tested, and lysergic acid diethylamide and this drug together). The mice were injected subcutaneously and placed individually on the centre of a vertical pole at 1 min. intervals. The time (up to a maximum of 40 sec.) taken by the mice to reach lines drawn 1 in. from the top or bottom of the pole was then measured. Variability can be reduced by eliminating slow or erratic mice in preliminary tests. (The mice can also be trained to run down the pole under hunger drive. This procedure, however, introduces two new parameters—learning and hunger—which may be differentially affected by drugs.) Doses of lysergic acid diethylamide of 1 and 2 mgm./kgm. were used. The solutions were always freshly prepared and the effect tested of their interaction with adrenochrome, adrenolutin and the brom derivative of lysergic acid diethylamide. Fig. 1 shows the difference between the drug value and its matching saline value, with the latter corrected to a straight line at the level of the first saline value (maximum possible score = 200). All statistical work was done, however, on uncorrected results. As the results obtained were not normally distributed, all statistical evaluations were carried out using non-parametric methods (Wilcoxon's method for paired data).

It was found that lysergic acid diethylamide increases the time which the mice take to reach the end of the pole. This is maximum at 10–15 min. after injection and is still appreciable after 30 min. Bufotenin in doses up to 20 mgm./kgm. did not show any of this effect (nor any qualitative change). Adrenochrome in doses of 10 and 20 mgm./kgm. has no effect on mice demonstrable by this test or on the effect of lysergic acid diethylamide on mice. Adrenolutin inhibits the effect of lysergic acid diethylamide on mice but exerts no influence by itself in a dose of 20 mgm./kgm. (Fig. 1). This effect was confirmed in a further experiment using 40 trained hungry mice and 30 mgm./kgm. adrenolutin (P for the interaction of lysergic acid diethylamide and adrenolutin is between 0·02 and 0·05). The brom derivative of lysergic acid diethylamide in a dose of 1 mgm./kgm. produced a marked sedation of untrained mice and a delayed time for descending the pole. The same dose did not produce this effect in trained or hungry mice where, presumably, the sedative effect could be overcome by motivation or training. The morpholide derivative of lysergic acid diethylamide is less active but causes a late acceleration of the mice (at 30–60 min.: $P = 0{\cdot}02$).

The purpose of this test is to evaluate the effect of lysergic acid diethylamide and other psychoto-

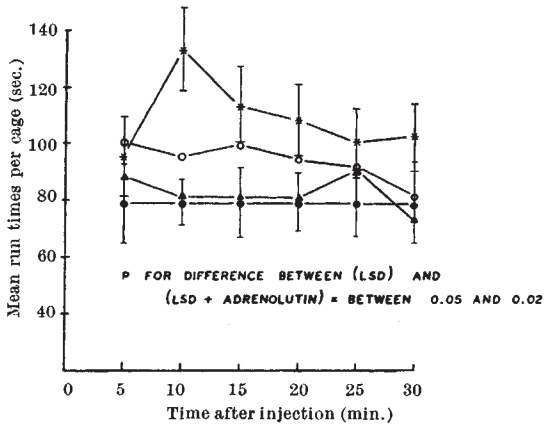

Fig. 1. Showing the effect of lysergic acid diethylamide (LSD), adrenolutin and their interaction. Cages *A–D*, each point representing 40 mice: ●, saline; ★, LSD (1 mgm. per kgm.); ▲, adrenolutin (20 mgm. per kgm.); ○, LSD + adrenolutin

mimetic agents quantitatively and to measure their interaction in mice. Inferences from these results to humans can only, of course, be made with caution. These experiments indicate that adrenochrome and adrenolutin (in the doses used) do not act on mice as lysergic acid diethylamide does and that they do not potentiate this action of lysergic acid diethylamide or vice versa (it has been claimed[2] that lysergic acid diethylamide in man potentiates the effect of adrenolutin). However, bufotenin, a known human hallucinogen, has no demonstrable effect either in mice even in large doses.

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Antibody Formation by Isolated Spleen Cells transferred to Recipients in Absence of Homotransplantation Reaction

It is generally agreed that the internal environment in new-born animals is not favourable for antibody formation to occur, as has been postulated by Dixon[1,2]. It has, however, been demonstrated in our laboratory[3–5] that new-born animals are more suitable recipients for transferred cells producing antibodies—during the period when they are not capable of their own active antibody synthesis[6]—than X-irradiated adult animals. We suppose that the homotransplantation reaction is the main factor limiting antibody formation by cells transferred to young animals[7]. Simonsen[8] has shown that spleen cells of adult hens are tolerated if transferred to chicken embryos three days before hatching. We assumed, therefore, that by using the chicken embryo as recipient it will be possible to obtain fully developed formation of antibodies to bacterial antigens and also a positive primary response to a protein antigen. According to Dixon's assumption, this should, on the other hand, lead to a further decrease in the intensity of antibody formation in embryos.

Normal White Leghorns weighing 1·5–2 kgm. were used as donors of spleen cells, which were isolated as described in a previous report[4]. Spleen cells mixed with antigen *in vitro* were transferred either by intraperitoneal route to chickens 2 days after hatching or into the chorioallantoic vein of 18-day-old chicken embryos. 6×10^7 cells were transferred to chickens and 5×10^7 cells to embryos. The number of cells was determined by counting in a Bürker's chamber. A heat-inactivated suspension of *Brucella suis* (in a ratio of two bacterial cells to one spleen cell) and bovine serum albumin (1 mgm. per embryo or chicken) were used. Cells were transferred simultaneously with antigen; to controls, animal cells only or antigens only were given. Blood for determination of antibody titres was taken at different time-intervals according to the antigen used. This was usually 10, 14, 20 and 28 days after transfer in the case of bovine serum albumin and 5, 7, 9, 12, 16 and 24 days after transfer when using *Brucella suis*. Antibodies were determined by agglutination of the suspension of *Brucella suis* or tanned sheep erythrocytes sensitized by bovine serum albumin. 73 per cent of chickens hatched from the embryos to which spleen cells had previously been transferred died within 15 days after transfer with typical symptoms of the runt disease. The decrease of blood hæmoglobin in chickens before death was even lower than 10 per cent. Symptoms of the runt disease served at the same time as indicator of a successful cell transfer.

The experiments were performed as follows. In the first group, cells with the *Brucella* antigen were transferred to two-day-old chickens and 18-day-old embryos. The antibody formation occurred in all the 20 transfers to chickens, with a maximum titre 1 : 128 (M = 1 : 32). Out of 17 transfers to embryos there was a positive antibody response in all experimental chickens, with a maximum titre 1 : 130,000 (M = 1 : 4,096). The transfer of cells only (13 embryos, 7 chickens) or antigens only (12 embryos, 9 chickens) was always negative during the critical period, that is, up to the fifteenth day of life. This group of experiments is illustrated in Fig. 1. In the second group of experiments bovine serum albumin was used as antigen under the same experimental conditions. In none of the transfers to

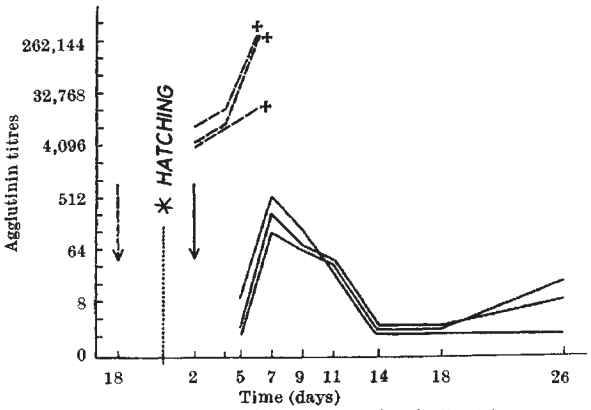

Fig. 1. Antibody formation following transfer of cells with *Brucella* antigen to embryos and chickens. Full line, transfer to 2-day-old chickens; interrupted line, transfer to 18-day-old embryos. A cross indicates that animals died with symptoms of the runt disease