

Fig. 1. Relationship between count rate and effective thickness, with the same source-detector characteristics and geometry as for experimental measurements.

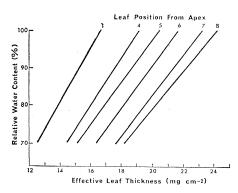


Fig. 2. β -Gauge calibration curves, for a series of cotton leaves numbered from the apex, obtained with Eqs. 2 and 4.

known value of R. Since, for the particular source-detector geometry and characteristics used in a β gauge, Dcan be uniquely related to count rate, calibration can therefore be simply achieved by determining only: (i) the count rate-effective thickness relationship for the system being used; (ii) the weight (W_s) of a disc sample of known area at R = 100, and when oven dried (W_a) .

The calibration procedure we adopted was to use the β gauge as set up for experimental measurements. The relationship between count rate and effective thickness, which is independent of absorber characteristics (5), was first established with the use of leaf discs of varying water content or aluminium foil of varying thickness. For this purpose, discs punched out of foil were mounted in small plexiglass holders with Mylar windows, each holder being arranged, in turn, in the β gauge so that the foil discs were in the same position as a leaf used for experiments. The relationship is shown in Fig. 1. A series of measurements could then be made with a leaf in position, no further count rate-effective thickness calibrations being required as long as source-detector geometry and characteristics remained unchanged.

At the end of each experimental run, a disc sample of known area was punched out of the leaf being monitored and was then rehydrated to the stage R = 100 by use of the procedure outlined by Barrs and Weatherley (3). Each disc was then placed in one of the standard plexiglass holders and a count rate was obtained. The sample was then weighed to obtain W_s and oven-dried to obtain W_d . The slope dR/dD was then obtained from Eq. 4 and the intercept from Eq. 2, W_s being substituted for W_t . A range of typical calibration curves for a series of leaves of different age on a single cotton plant is shown in Fig. 2.

The accuracy of the calibration curves was checked by measuring the count rate of samples of leaf discs having a range of relative water contents. The results obtained for two leaves of another cotton plant are given in Fig. 3. The points plotted as open circles relate relative water content to count rate and reflect the curvilinear relationship characteristic of most calibrations at present in the literature. The points plotted as filled circles relate relative water content to effective thickness, as in the present calibration procedure.

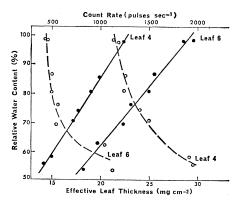


Fig. 3. The relationship between relative content, effective leaf thickness (solid lines, filled circles), and count rate (dashed lines, open circles) for a young, thin cotton leaf at position 4 from the apex and an older, thicker leaf at position 6. Each curve is based on separate estimates from two samples of ten discs, 1 cm in diameter, which were allowed to lose water slowly over several hours.

The standard error of the estimate of R for leaf 4 and leaf 6 did not exceed 1.19 percent and 1.25 percent relative water content, respectively.

> P. G. JARVIS R. O. SLATYER

Division of Land Research, CSIRO, Canberra, A.C.T., Australia

References

- W. R. Gardner and R. H. Nieman, Science
 143, 1460 (1964); H. J. Mederski, Soil Sci. 92,
 143 (1961); F. S. Nakayama and W. L. Ehrler, Plant Physiol. 39, 95 (1964); P. C. Whiteman L. Wilson, Australian J. Biol. Sci. 16, 140 (1963).
- Slatyer and S. A. Taylor, Nature 187,
- H. D. Barrs and P. E. Weatherley, Australian J. Biol. Sci. 15, 413 (1962).
 R. O. Slatyer, Bull. Res. Council Israel Sect. D 8, 159 (1960).
- R. A. Faires and B. H. Parkes, *Radioisotope Laboratory Techniques* (George Newnes, London, 1958), pp. 16-24.
- 9 May 1966

Influence of the Lethal Yellow (Ay) Gene on Estrous Synchrony in Mice

Abstract. Introduction of an adult male induces partially synchronous estrus in female laboratory mice that have been caged in groups. In the inbred YS/ChWf strain, this effect was observed only when the male was nonyellow (aa), while males heterozygous for the lethal yellow allele (Aya) failed to induce synchrony.

Estrous cycles are prolonged and irregular in female laboratory mice housed in groups. Introduction of a male shortens the cycle and elicits heat. Because of partial synchrony of this process more females mate on the third night after being placed with the male than on any other night (1). Yellow $(A^y a)$ and nonyellow (aa)males of the inbred YS/ChWf strain were compared for effectiveness in this respect. The Whitten effect (estrous synchrony) (2) was observed only when the male was nonyellow.

All mice used were from the YS/ ChWf strain maintained by sib matings with forced heterozygosis for the lethal yellow (A^y) and nonagouti (a) alleles at the agouti locus; they were housed and fed in the manner described (3). Females were weaned when 3 to 4 weeks old and housed three to ten per cage. Several weeks later, matings of two females (mean age, 8.6 weeks) and one young adult male were set up in clean cages. During the next 6 to

Table 1. Pattern of plug formation by aa and $A^{y}a$ males.

| Genotype | | Plugs on days after placement with the male (No.) | | | | | |
|----------|---------------------|---|-------|------------------------|-------|--------------------|--|
| Male | Female (and number) | Day 1 | Day 2 | Day 3 (and percentage) | Day 4 | and later Day 5 | |
| аа | aa(51) | 11 | 13 | 19(37%) | 4 | 4 | |
| аа | $A^{y}a(134)$ | 28 | 27 | 50(37%) | 22 | 7 | |
| A^ya | aa(125) | 26 | 25 | 28(22%) | 18 | 28 | |
| $A^{y}a$ | $A^{y}a(52)$ | 12 | 15 | 11(21%) | 7 | 7 | |

Table 2. Statistical analysis of number of females remaining unfertilized at the end of

| Item | Day 2 | End of day 3 | Day 4 |
|---|--------|-----------------|-------|
| Total χ^2 (df:3) | 3.20 | 10.86* | 9.18‡ |
| aa o o | 1.25 | 0.56 | 0.40 |
| $A^{\eta}a^{\dagger} \stackrel{+}{\circ} \circ$ | 1.18 | .52 | .37 |
| Between QQ (df:2) | 2.43 | 1.08 | .77 |
| Within Q Q (df:1) | 0.77 | 9.78† | 8.41* |
| aa 2 2 + + | .87 | 4.47 | 4.38 |
| A "a & & | .91 | 4.68 | 4.59 |
| Between A A (df:2) | 1.78 | 9.15* | 8.97* |
| Within & & (df:1) | 1.42 | 1.71 | 0.21 |
| | D 4 04 | d. D | 03.4 |

P = .01 to .02. $\dagger P < .01$.

10 days the females were checked daily for the presence of vaginal plugs. Conditions of the experiment were kept constant from one series to the next, and data from several consecutive runs were pooled for analysis.

The data (Table 1) were analyzed by means of a χ^2 test on the number of females remaining unfertilized in each category each day; the expected numbers were calculated from the initial group and from the number remaining unfertilized at the end of each day; presence of the Whitten effect would cause significant departure from randomness of the number of females fertilized on the 3rd day. Partitioning of χ^2 (Table 2) showed deviations from randomness at the .01 level "within females" and "between males" on days 3 and 4; there were no significant deviations "between females" or "within males." In matings of aa males with both genotypes of females, a higher proportion were fertilized on day 3 and a smaller proportion on day 4 than in matings of A^ya males with either aa or A^ya females. Thus estrous synchrony was induced by aa males but not by $A^{y}a$ males and was not influenced by the genotype of the females.

Among females that mated during the experiment, 94 percent of those placed with aa males mated during the first 4 days, while only 80 percent of those placed with A^ya males mated during this period ($\chi^2 = 15.6$, P <.001).

The lethal yellow (A^y) gene in heterozygous state affects hair pigmentation, fat and cholesterol metabolism, development of spontaneous and induced tumors, and normal growth (4). In the YS/ChWf strain, reproductive function is also affected by this gene (3). Because of higher embryonic mortality, litter size is smaller in A^ya females mated with aa males than in the reciprocal matings. Yellow females mated to yellow males produce, on the average, only half as many litters as (1.3:2.5) and cease production at an earlier age (18.0:23.7 weeks of age at birth of last litter) than when mated to nonyellow males.

These data indicate that, in the YS/ChWf strain, yellow (A^ya) males do not elicit synchronous estrus in females previously kept in groups, while nonyellow (aa) males do so regularly. The genotype of the female (aa or $A^{y}a$) does not significantly affect this phenomenon. The Whitten effect is believed to be caused by the olfactory stimuli provided by a mature male (1). Possibly Aya males differ significantly from aa males in the production of the odoriferous substance concerned and, consequently, do not provide sufficient olfactory stimulation to influence the estrous cycle to a measurable extent.

> Andrzej Bartke* GEORGE L. WOLFF

The Institute for Cancer Research, Philadelphia, Pennsylvania 19111

References and Notes

- 1. H. M. Marsden and F. H. Bronson, Science
- H. M. Marsden and F. H. Bronson, Science 144, 1469 (1964).
 W. K. Whitten, J. Endocrinol. 18, 102 (1959).
 G. L. Wolff and A. Bartke, J. Heredity 57, 14 (1966).
- G. L. Wolff, *Amer. J. Physiol.* **209**, 632 (1965). Supported by PHS grants GM-10112 and CA-06927 and American Cancer Society grant IN-49F. We thank G. T. Rudkin for aid with the statistical analysis.
- Present address: Department of Animal Genetics, Jagiellonian University, Krakow, Poland,
- 8 April 1966

Mechanism of a Reaction in Vitro Associated with **Delayed-Type Hypersensitivity**

Abstract. The cell type responsible for inhibition by antigen of migration in vitro of peritoneal exudate cells obtained from tuberculin-hypersensitive guinea pigs was studied. Exudate populations were separated into component cell types, the lymphocyte and the macrophage. Peritoneal lymphocytes from sensitive donors were the immunologically active cells in this system, the macrophages being merely indicator cells which migrate. Sensitized peritoneal lymphocyte populations, upon interaction with specific antigen in vitro, elaborated into the medium a soluble material capable of inhibiting migration of normal exudate cells.

The nature of delayed-type hypersensitivity remains a problem for two reasons. The cell type or types which effect the delayed-type reactions are not established with certainty (1), and the biochemical basis of the response is almost completely unknown. In order to approach these problems under defined conditions, a great deal of work has been directed towards developing methods for studying delayed-type hypersensitivity in vitro (2). The discovery (3) that migration of cells from splenic explants from hypersensitive guinea pigs was specifically inhibited by antigen has served as the basis for a quantitative assay (4) which is antigen-specific, reproducible, and apparently independent of the antibody response. In this method, peritoneal exudate cells are allowed to migrate from capillary tubes onto cover slips in small culture chambers, and the area of cell migration is measured. The migration of exudate cells obtained from hypersensitive guinea pigs is markedly inhibited by the presence in the medium of specific antigen. This inhibition of migration seems characteristic of cells from animals with delayed-type hypersensitivity, since exudate cells obtained from nonhypersensitive animals immunized to produce only circulating antibody are not inhibited by antigen (4, 5). In addition, the results obtained from this assay in vitro correlate well in