patibility with the plant cuticle. Some will act in the dust form, where solubility in cuticle is not complicated by the presence of water.

The cuticle does not form a perfect covering, especially on young leaves. Only xerophytes like cactus have nonpervious cuticle; young leaves of mesophytic plants lose considerable water through the cuticle. This is why arsenic and chlorate from 1 or 2% solutions penetrate young leaves and also why the sodium salt of 2,4-D is so effective on young plants.

Roots are specialized for absorption of ions. Recent tests in Puerto Rico prove that sodium pentachlorophenate as a pre-emergence treatment through the soil is more effective than pentachlorophenol. The sodium salt is effective as a selective soil sterilant against weeds in robust crops such as corn or sugar cane. Pentachlorophenol in oil emulsion is more useful as a pre-emergence contact spray in onions and carrots. The sodium salt in the topsoil injured these fragile plants; pentachlorophenol was noninjurious (2).

Salts of 2,4-D are more readily absorbed from soil than the esters; the salts caused crippling of oats and corn to a much greater extent than esters. On the other hand, 2,4-D acid, its sodium salt, and its methyl ester caused equal toxicity to oats, sunflowers, and peas in California soils compared on an acid-equivalent basis (4). Apparently the acid is sufficiently soluble to cause normal toxicity to the highly sensitive roots; the ester hydrolizes to an equivalent concentration. No leaching occurred from the cultures.

There are two possible pre-emergence methods: (1) pre-emergence contact spray to kill weed seedlings that have grown since preparation of the seed bed; (2) selective sterilization of the soil to kill weeds with little or no harm to the crop. Pre-emergence contact sprays can be used safely provided a nonpolar or water-insoluble toxicant is employed. Sodium cresylate and pentachlorophenate have proved harmful to seedlings of lettuce, onions, and carrots when a light shower occurred soon after application. Corn, cotton, and soybeans may not be injured.

Successful selective soil sterilization depends on profound differences between the crop and weed in susceptibility, root location, or some other factor. Certain corn varieties allow treatment with enough 2,4-D to kill grass seedlings without injury to the crop (1). Sodium pentachlorophenate was used successfully in corn and sugar cane in Puerto Rico. Leonard (6) found ammonium dinitrobutylphenylate effective in cotton in Mississippi. Current use of 2.4-D in cane proves that the pure acid applied dry is more effective under humid conditions than the sodium salt applied in solution. Solubility as related to solution rate and loss by leaching is probably the critical factor. The salt was effective for two weeks, but with over 3" of rain it was leached out, whereas the acid remained effective for over a month. To use preemergence treatments successfully one should understand the nature of the selectivity upon which success depends.

A valuable generalization concerning absorption of herbicides is: for penetration of the cuticle and absorption by foliage, nonpolar compounds should be used; for absorption by roots, polar compounds. Although solubility and differential wetting (dinitro selectives) may limit the usefulness of toxicants, and cost, availability, and other economic considerations may justify exceptions, the above rule provides a sound theoretical basis for predicting the herbicidal uses of various toxic materials.

## References

- ANDERSON, J. C., and AHLGREN, G. Down to Earth, 1947, 3, 16; ANDERSON, J. C., and WOLF, D. E. J. Amer. Soc. Agron., 1947, 39, 341-342.
- CRAFTS, A. S., and RAYNOR, R. N. West. Grow. Ship., 1944, 25, 10-11, 26-28.
- CRAFTS, A. S., and REIBER, H. G. Hilgardia, 1945, 16, 487-500.
- 4. HARVEY, W. A. Hilgardia, in press.
- 5. HARVEY, W. A., and CRAFTS, A. S. Results of tests on 2,4-D weed killers in California, 1945. (Unpublished.)

6. LEONARD, O. A. Private conference.

## Progesterone in Blood Plasma of the Ovulating Hen<sup>1</sup>

RICHARD M. FRAPS

Bureau of Animal Industry, USDA, Beltsville, Maryland

CHARLES W. HOOKER and THOMAS R. FORBES

Department of Anatomy, Yale University

The occurrence of progesterone in the avian ovary, or the presence of luteal tissue implying secretion of this hormone by the bird, has long been a debatable subject (2, 5) of much interest not only in connection with avian reproduction, but also because of more general phylogenetic implications emphasized by the remarkably divergent histories of the ova of birds and mammals following ovulation. All previous attempts to identify luteal tissue or progesterone in the bird's ovary have led to either inconclusive or negative findings (see 2 and 5 for summaries). The results of tests reported here, however, demonstrate beyond reasonable doubt the presence of progesterone in the blood stream of the regularly ovulating hen, presumably still a proper representative of Aves.

The 10 plasma samples examined came from 3 White Leghorn hens in which the interval between successive ovulations was ca. 26.5 hrs. Timing of withdrawal of blood samples was referred to second ovulation of the clutch (Table 1), but the same samples may also be considered as having been taken between 2 consecutive ovulations, ca. 6.5 hrs elapsing between withdrawal of samples 10 and 1. The choice of relatively close intervals between times of withdrawal of samples 2-8 was predicated upon the supposition that changes in progesterone levels might most likely occur within this period.

Each plasma sample was prepared from 0.95 ml of blood plus ca. 0.05 ml of sodium citrate, centrifuged as

<sup>1</sup>This investigation was aided by grants from the Committee for Research in Problems of Sex, National Research Council, and from the James Hudson Brown Memorial Fund of the Yale University School of Medicine. soon after blood collection as possible, and kept at freezing temperature until tested. The samples were tested according to a recently described method capable of detecting 0.0002  $\mu$ g of progesterone in 0.0006 ml of plasma and apparently specific for progesterone (4). In the purely qualitative tests reported on here, a positive reaction indicates a progesterone concentration greater than 0.33  $\mu$ g/ml of plasma; a negative reaction signifies that, if progesterone is present, its concentration is less than 0.33  $\mu$ g/ml of plasma.

The samples yielded 8 positive and 2 negative reactions (Table 1). Some samples gave a positive reaction indicating progesterone concentrations well above  $0.33 \ \mu g/ml$  of plasma, but no attempt was made to quantitate this series. The essential point is that the positive reactions afford apparently unquestionable evidence for the occurrence of progesterone in the blood stream of the ovulating hen.

It is significant that the 2 negative reactions were given by samples taken at about the time of, or shortly follow-

TABLE 1 PROGESTERONE REACTION OF PLASMA SAMPLES TAKEN WITH REFERENCE TO ESTIMATED TIME OF SECOND OVULATION OF THE CLUTCH

Sample No.	Hen	Time of blood withdrawal referred to 2nd ovulation			Progesterone reaction*
1	A	11.5	hrs	before	+
2	Α	9.5	"	**	+
3	A	8.0	4	44	+
4	A	7.0	**	<b>66</b>	+
5	A	6.0	**	46	-
6	в	3.7	•• `	"	_
7	в	1.7	**	46	+
8	A	0.5	**	after	+
9	С	4.5	**	"	+
10	С	8.5	**	"	+

\*A plus sign indicates a progesterone concentration of more than 0.33  $\mu$ g/ml of plasma; a minus sign, a concentration of less than 0.33  $\mu$ g/ml.

ing, release of ovulation-inducing hormone in so far as this has been estimated by injection procedures  $(\mathcal{S})$  and hypophysectomy (6). The occurrence of low values at so critical a phase of the ovulatory cycle may be taken as substantial evidence for ovarian, and most likely follicular, origin of progesterone, an inference in good accord with recent views of the mammalian follicle (1).

The observations which encouraged undertaking the tests reported here will be accounted for elsewhere in some detail. Further tests are contemplated to determine the site or sites of origin of progesterone in the bird, as well as exact concentrations of progesterone throughout the ovulatory cycle.

## References

- ALLEN, E. Glandular physiology and therapy. Chicago: American Medical Association, 1942. Chap. 10.
- 2. FELL, H. B. Quart. J. micr. Sci., 1925, 69, 591-609.
- FRAPS, R. M., RILEY, G. M., and OLSEN, M. W. Proc. Soc. exp. Biol. Med., 1942, 50, 313-317.
- HOOKEE, C. W., and FORBES, T. R. Endocrinology, 1947, 41, 158-169.
- RIDDLE, O., and SCHOOLEY, J. P. J. Wash. Acad. Sci., 1944, 34, 341-346.
- 6. ROTHCHILD, I. Anat. Rec., 1946, 96 (No. 4), 46.

## Phosphorylase in Guard Cells

H. C. YIN and Y. T. TUNG

Botany Department, National Peking University, Peiping, China

In a previous report (5) a histochemical method was described for the detection of phosphorylase in plant cells and tissues. Briefly, the method consists of incubating free-hand sections of plant parts, previously freed





FIG. 1

of starch, in a buffered medium containing glucose-lphosphate and subsequent staining with iodine in potassium iodide. Using this method, a number of experiments have been made. The results will be published elsewhere. The present note gives a brief summary of observations on plant epidermis.

Leaves of tobacco and broad bean were starved in darkness to free them of stored starch. The epidermis was stripped off and treated with glucose-l-phosphate as described above.

Very intense starch formation is found in the guard cells after 4-5 hrs of incubation, indicating the presence of strong phosphorylase activity. Little or no reaction is shown by the epidermal cells (see Fig. 1). Incubation with glucose instead of glucose-l-phosphate with or without the addition of magnesium salts and adenosinetriphosphate fails to give rise to starch even after 24 hrs. The result indicates that the guard cells