

eighth (not one-fourth) of a turn with the marking dot down.

4. Raise the whole manometer, keeping it slightly tilted, and turn the stopcock another eighth of a turn thus allowing air to enter the end of the stopcock and the excess mercury to flow back towards the reservoir. Detach the tubing from the manometer after replacing the clamp.

5. Allow the mercury to flow into a tared vessel and divide the net weight by 13.54 to obtain V_M . Disregard the small amount of mercury in the channel of the stopcock as this is below the level of significance. A permanent record of the V_M values should be made for future reference.

The above maneuver requires perhaps 15 min of practice before repeatable results are obtained, but once mastered permits rapid calibrations in duplicate.

References

1. BURRIS, R. H. in *Manometric techniques* of W. W. Umbreit, R. H. Burris, and J. F. Stauffer. Minneapolis: Burgess, 1947, 50.
2. DIXON, M. *Manometric methods*, New York: Macmillan, 1943.
3. DIXON, M. *Biochem. J.*, 1945, **39**, 427.
4. DIXON, M. *Nomograms for manometric constants*, New York: Macmillan. Pamphlet.
5. KREBS, H. A. in *Die methodik der fermente* of C. Oppenheimer, Leipzig, 1929.
6. MUNZER, E., and NEUMAN, W. *Biochem. Z.*, 1917, **81**, 319.
7. WARBURG, O. *Biochem. Z.*, 1923, **142**, 317.

Effect of Radioactive Phosphorus (P^{32}) in the Culture Medium on Growth and Viability of *Phomopsis citri* and *Diplodia natalensis*

Erston V. Miller and Jerome J. Wolken

Department of Biological Sciences,
University of Pittsburgh

Some interesting results were obtained while studying the effects of radioactive phosphorus on the physiology of *Phomopsis citri* and *Diplodia natalensis*, the fungi commonly causing stem-end decay in oranges.

Potato dextrose agar was prepared in two lots that were identical in every respect, except that one lot contained 20 ml per l of a solution of radioactive phosphorus. The radioactivity of the solution introduced into the agar ranged from 0.156 to 0.245 $\mu\text{c}/\text{ml}$, with a half-life of 14.3 days. The control lot of agar was treated with an equal amount of 0.1% solution of NaH_2PO_4 . Mycelial tufts of the fungi were transferred from pure cultures to the center of the agar in Petri plates, and the daily rate of growth of the cultures was determined by measuring increase in diameter. During four replications of the experiment the P^{32} showed a tendency to retard the growth of *Diplodia natalensis*, whereas the results with *Phomopsis citri* were not very consistent.

When transfers of the organisms were made from the Petri plates to orange fruits, the fungi that had been grown on radioactive media were slower in producing decay than were the control lots. California oranges were selected for inoculation to avoid the possibility of natural infection in the fruits, since the two fungi under consideration are rarely found in oranges produced under irrigation. A total of 400 fruits were employed, 200 of each variety. There were four lots of 50 fruits in each

TABLE 1
STEM-END DECAY IN CALIFORNIA ORANGES INOCULATED WITH FUNGI GROWN ON RADIOACTIVE AND CONTROL MEDIA

Variety	Days following inoculation	Percent showing decay					
		<i>Phomopsis citri</i>			<i>Diplodia natalensis</i>		
		Grown on radioactive media	Grown on control media	Chi square	Grown on radioactive media	Grown on control media	Chi square
Valencia	4	48	80	11.0	70	88	5.1
Washington navel	6	18	52	12.7	26	52	7.1

experiment. Inoculations were made by removing the stem-buttons, cutting a slit in the exposed stem with a sterile scalpel, and then forcing some of the agar and mycelia into the slit. Navel oranges were inoculated with mycelia from cultures in which the P^{32} had shown definite inhibitory effects on the rate of growth of the two fungi. In the experiments in which Valencia oranges were used, the inoculum was taken from cultures in which no significant retardation of either fungus had been produced by the P^{32} . Tests were made with navel oranges in April and with Valencia oranges in November, so that each variety was inoculated near the end of its commercial season, when one would not expect much resistance on the part of the fruits to pathogens.

It will be noted in Table 1 that in all instances there was more decay in the oranges when inoculated with fungi from control media than when the inoculating organisms had been grown on radioactive media. The effect was not so pronounced with Valencia oranges as with the Washington navel variety. With the navels, the control fungi caused at least twice as much decay as did the fungi that had been cultured on radioactive media. However, the results in all instances were highly significant.

The ability of the fungi to cause decay was reduced by exposure to P^{32} , irrespective of whether or not growth of the organisms on culture media had been previously retarded. Furthermore, the radioactivity of the solutions employed was not great. The original solutions, with an activity of 0.156 to 0.245 $\mu\text{c}/\text{ml}$, were diluted 50 times in making up the culture media. At the time that the Petri plates were poured and inoculated, 1 ml of the agar emitted 10,000 to 11,000 counts per min. Inoculations were made into oranges a week or ten days later, and the amount of inoculum employed weighed approxi-

mately 20 mg. No radioactivity could be detected in the inoculum at the time that the oranges were inoculated. The delayed action of the fungi in infecting the fruits therefore appeared to be the result of previous exposure to P^{32} rather than to any actual radioactivity conveyed to the fruits in the inoculum. The very low activity of the original solutions suggests the possibility that greater dosages might be employed without too great an increase in health hazards.

It would be interesting to observe the effects of radioactive phosphorus on the subsequent decay of oranges, if the phosphorus were applied in the grove either as a spray or as a fertilizer. Although fungicidal sprays are employed in the groves, in commercial practice the most concerted efforts to control decay are made in the packing houses. However, control of stem-end decay of citrus fruits is made much more difficult because the two causal organisms become established in the stem tissues, and few fungicides will penetrate these tissues without injuring the fruits.

Progesterone in Blood Plasma of Cocks and Nonovulating Hens¹

Richard M. Fráps

Bureau of Animal Industry, USDA, Beltsville, Maryland

Charles W. Hooker² and Thomas R. Forbes

Department of Anatomy, Yale University

Tests utilizing the method of Hooker and Forbes (4) have indicated the occurrence of progesterone in blood plasma of the regularly ovulating hens at least during certain phases of the ovulatory cycle (3). In extending these original observations, we thought it desirable to test raw plasma samples from males, and also from reproductively quiescent hens, as controls against possibly untoward reactions of the mouse endometrium to presumably progesterone-free plasma of avian origin. Preliminary tests unexpectedly yielded positive, and altogether typical, progesterone reactions. Additional assays confirmed these findings—the first direct evidence, so far as we are aware, of progesterone in the blood stream of normal males of any species.

The sex, breed, and age of the seven fowl furnishing plasma samples are recorded in Table 1, together with assay findings. Undiluted plasma (0.33 $\mu\text{g}/\text{ml}$) from the New Hampshire male did not give a positive reaction, possibly because of uterine distension or loss of material from the ligated segment. The questionable result from one of the females at the 1.0 $\mu\text{g}/\text{ml}$ level may be accounted for similarly. All other findings are consistent among themselves; plasma from the two capons was com-

pletely negative at all levels tested.

The maximal levels of progesterone recorded in Table 1 are relatively high, ca. 3 $\mu\text{g}/\text{ml}$ plasma, or about half the concentration most frequently encountered by Forbes and Hooker (2) and Hooker and Forbes (5) in mammals

TABLE 1
RESULTS OF ASSAYS FOR PROGESTERONE IN PLASMA FROM MALE, NONLAYING FEMALE, AND CASTRATED FOWL

Source of plasma			Progesterone, $\mu\text{g}/\text{ml}$ *			
Sex	Breed†	Age	0.33	1.0	3.0	5.0
Male	W.L.	15 months	+	+	n.t.	n.t.
"	W.L.	16 "	+	+	+	—
"	N.H.	18 "	—	+	+	—
Female	W.L.	16 "	+	+	n.t.	n.t.
"	W.L.	16 "	+	±	+	±
Capon‡	N.H.	7 "	—	—	—	—
"	N.H.	ca. 4 years	—	—	—	—

* Plus and minus signs indicate presence and absence respectively of progesterone at indicated assay levels; n.t. no test at this level.

† Breeds: W.L., White Leghorn; N.H., New Hampshire.

‡ Castrated at 6 weeks of age. We are indebted to the Poultry Department, University of Maryland, for placing these capons at our disposal.

with active corpora lutea. The surmise that progesterone at these concentrations exercises some specific, if presently unknown, function seems reasonable.

We cannot exclude the possibility that the substance in avian blood eliciting in the mammal the same reaction as progesterone may in fact not be progesterone. However, two of us (6) have recently completed tests of some 25 substances believed most likely to duplicate the action of progesterone; none of them did so in our tests. It is of especial relevance that testosterone and desoxycorticosterone acetate did not reproduce the effects of progesterone by the mouse assay (4).

Progesterone has been isolated from the adrenal glands of oxen (1), and pregnanediol concentrations in the urine of bulls are reported to exceed those normally found in the urine of pregnant cows, mares, or women (7). These and numerous other observations of an indirect nature have suggested that progesterone is not necessarily limited in occurrence to the female. The actual finding of progesterone in the blood stream of male fowl substantiates these views.

More extended investigations of avian and mammalian plasma from males, nonpregnant females, and castrates are currently in progress.

References

1. BEALL, D., and REICHSTEIN, T. *Nature, Lond.*, 1938, **142**, 479.
2. FORBES, T. R., and HOOKER, C. W. *Science*, 1948, **107**, 151.
3. FRAPS, R. M., HOOKER, C. W., and FORBES, T. R. *Science*, 1948, **108**, 86-87.
4. HOOKER, C. W., and FORBES, T. R. *Endocrinology*, 1947, **41**, 158-169.
5. HOOKER, C. W., and FORBES, T. R. In press.
6. ———. In manuscript.
7. MARKER, R. E., WITTE, E. L., and LAWSON, E. J. *J. Amer. chem. Soc.*, 1938, **60**, 2931-2933.

¹ 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.

² Present address: Department of Anatomy, Emory University School of Medicine, Emory University, Georgia.