

a substance which causes follicle stimulation in the white rat is not present in the royal jelly of the honeybee. However, more detailed work may demonstrate the presence of a gonadotropic substance which may or may not be associated with caste production in the honeybee.

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CRYSTALLINE DROSOPHILA EYE-COLOR HORMONE

THE development of eye color in *Drosophila* is known to be controlled by specific diffusible substances designated as v^+ and cn^+ hormones. Khouvine, Ephrussi and Chevais¹ and Tatum and Beadle² have shown that these substances are amino acid-like in nature. The former authors in testing various amino acids for v^+ hormone activity obtained results which indicated that tryptophane, when added to the larval food, was concerned with hormone production. This "tryptophane effect" was found to be due to the production by certain bacteria of a substance with v^+ hormone activity.³

This bacterially produced v^+ hormone has now been obtained in a pure crystalline state. The bacteria were grown on an agar medium containing dead yeast, sugar and l-tryptophane. The agar and yeast were precipitated in 80 per cent. alcohol. The hormone was then taken up in a mixture of butyl alcohol, ethyl alcohol and water, and was finally precipitated from absolute butyl alcohol. It was then crystallized from 90 per cent. ethyl alcohol. The crystals are very light yellow, elongated plates, usually forming in rosettes. The elementary analysis (made under the direction of Dr. A. J. Haagen Smit, of the California Institute of Technology) supports the empirical formula $C_{21}H_{34}N_2O_{14}$.

The crystalline hormone has an activity of approxi-

TABLE I
BIOLOGICAL ACTIVITY OF CRYSTALLINE v^+ HORMONE¹ (0.28 γ INJECTED PER LARVA)

Times hormone recrystallized	Maximum eye-color value	v^+ units per fly	v^+ units per gram hormone
1	3.1	6	21,400,000
2	3.1	6	21,400,000
3	3.3	7	25,000,000
4	2.9	5	17,900,000

¹ Y. Khouvine, B. Ephrussi and S. Chevais, *Biol. Bull.*, 75: 425, 1938.

² E. L. Tatum and G. W. Beadle, *Jour. Gen. Physiol.*, 22: 239, 1938.

³ E. L. Tatum, *Proc. Nat. Acad. Sciences*, 25: 486, 1939.

mately 20,000,000 v^+ units per gram⁴ when a solution is injected into vermilion brown test larvae. Table I shows that within the limits of accuracy of the biological test, repeated recrystallization does not change the activity.

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EFFECT OF EMANATIONS FROM SEVERAL SPECIES OF FUNGI ON RESPIRATION AND COLOR DEVELOPMENT OF CITRUS FRUITS

THE vapors given off by single moldy lemons inoculated from pure cultures were passed at a constant rate through cotton tubes into jars filled with 50 to 60 sound fruits. Respiration was determined by absorbing the CO_2 evolved in a solution of barium hydroxide. During this determination, which consisted of three one-hour tests, the container with the moldy fruit was disconnected from the jar. Hence the respiration values are for the sound fruit only and do not include the carbon dioxide given off by the moldy fruit.

The most pronounced effects were produced by the emanations from fruits inoculated with *Penicillium digitatum* (green mold), as demonstrated by a typical case in Fig. 1. The response was similar in 12 other

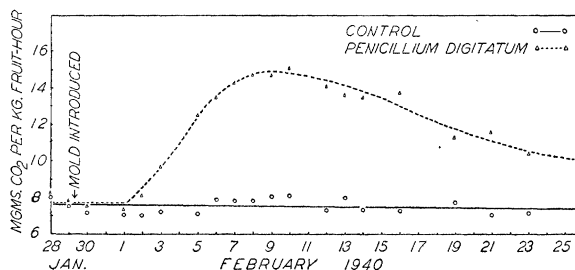


FIG. 1. Respiration of lemons as affected by the vapors of *Penicillium digitatum*.

experiments on lemons and in one test on oranges. The increase in CO_2 evolution by fruit subjected to green mold vapors over the control varied from 50 to 100 per cent. The activity of this fungus is definitely inhibited by low temperatures ($2^\circ C.$), while higher temperatures ($25^\circ C.$) do not appear to cause any accelerated effect as compared with $14.5^\circ C.$ used normally for these experiments. It seems that there are no specific substances present in the fruit which are essential for the production of the active vapor, because *Penicillium digitatum* grown on an agar medium (to which dextrose and potato broth was added) brought about the same results.

Another striking feature of these tests is the very

⁴ See reference cited in footnote 2 for significance of eye-color values and units.

TABLE I
RELATIVE RESPIRATION VALUES OF LEMONS UNDER THE INFLUENCE OF THE VAPORS OF SEVERAL FUNGI

Treatment	January														
	5	6	7	8	9	10	11	12	13	15	17	19	20	23	27
Control	100	99	99	101	100	97	99	99	88	91	89	90	85	78	82
<i>Penicillium digitatum</i> ..	100*	113	129	151	233	196	176	176	157	172	169	187	176	143	178
<i>Penicillium italicum</i> ...	100*	102	102	103			106			107		113		98	101
<i>Sclerotinia sclerotium</i> .	100*	93	97	105			104			102		110		89	83
<i>Aspergillus niger</i>	100*	94	94	102			99			95		103		84	83

* Mold placed in series with jars.

much reduced activity or complete lack of response by the fruit to the emanations of the other fungi as evidenced by Table I.

P. italicum (blue mold) appears to produce a slight effect, much less than that of green mold, though these two species are closely related physiologically. The other species, as well as *Oospora* and *Alternaria* which were tested in other experiments, did not seem to bring about any acceleration of the CO₂ evolution.

Rapid yellowing of green lemons was observed in all cases in which emanations of green mold were passed over the fruit. A slight increase in the rate of

color development took place under the influence of blue mold, with no effect produced by the vapors of the other fungi. Shedding of stem ends was caused by green mold only. Apparently the responses of the fruit to the gaseous products of *P. digitatum* are similar to the effects of ethylene. Definite proof as to the production of this substance by the fungus is hoped to be obtained by direct chemical analyses. Detailed discussion of these experiments will be forthcoming in the *American Journal of Botany*.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CONVENIENT APPARATUS FOR THE MANIPULATION OF EGGS IN THE STUDY OF THE CHORIO-ALLANTOIC MEMBRANE

IN the study of the lesions produced by viruses of vaccinia and ectromelia on the chorio-allantoic membranes of chick and duck embryos using annular oblique incident illumination, it was found by Himmelweit¹ that it was necessary to raise the membrane until it was in close contact with a coverslip which was sealed over the opening in the shell. He found that the natural air sac could be inflated by exerting gentle pressure, by means of a rubber tube, to the opening which had previously been made in the wall of the natural air sac for the purpose of lowering the membrane. During our study by high power microscopy of the virus of infectious myxomatosis on the chorio-allantoic membranes of eggs the piece of apparatus here described was developed.

After culturing the virus of infectious myxomatosis on the chorio-allantoic membrane according to the technique of Goodpasture and associates,² the shell was broken down to allow an opening of from 2 to 3 cm in diameter, and a layer of vaseline and paraffin mixed in equal parts was built up around this opening to a height of approximately 2 mm above the cut edge of the egg-shell. A few drops of saline were then placed

on the membrane and a coverslip was sealed to the paraffin wall according to the method described by Himmelweit. The egg was sealed onto a spoon holder as described by one of us.³ A piece of 7 mm glass tubing, the end of which had been previously flared to increase its diameter, was sealed over the hole in the wall of the natural air sac by means of paraffin, not by Ash's Model Cement as used by Himmelweit. This glass tube was held firmly in position by means of an ordinary wooden clothespin fastened to a No. 5 cork stopper, as shown in Fig. 1. A rubber tube provided

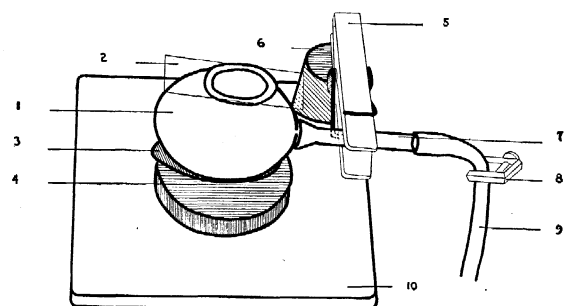


FIG. 1. 1. egg; 2. coverslip; 3. spoon; 4. metal lid; 5. clothespin; 6. cork; 7. glass tube; 8. screw clamp; 9. rubber tubing; 10. wooden base.

with a screw clamp was fitted to the other end of the tube. The entire apparatus was securely fastened to a small wooden base which could be held in the hand.

³ R. E. Hoffstadt, Stephen Pilcher and Elizabeth Osterman, *SCIENCE*, 86: 356, 1937.

¹ F. Himmelweit, *Brit. Jour. Path.*, 19: 2, 108-123, 1938.

² A. Woodruff and E. Goodpasture, *Amer. Jour. Path.*, 8: 209-222, 1931.