two organisms for substrate or even production of toxic products by the eperythrozoa. This direct effect of the eperythrozoa is reinforced by an indirect one associated with the early anemia and subsequent reticulocytosis. There is evidence indicating that, if reticulocytosis is high at the time when malarial parasitemia is high, the malarial infection is less severe, and fewer mice succumb. The number of reticulocytes is high on day 7 and increases as the parasitemia slowly approaches the peak (Fig. 1). This is associated with low mortality. Although not shown, the time when the number of reticulocytes of group 4, in the experiment represented in Fig. 3, was high did not coincide with the time of high parasitemia. Reticulocytosis was greatest on day 10, but it had already decreased appreciably by day 12, the day of greatest parasitemia. The malarial parasitemia and the mortality rate of this group were greater than in any other instance of the dual infection. This

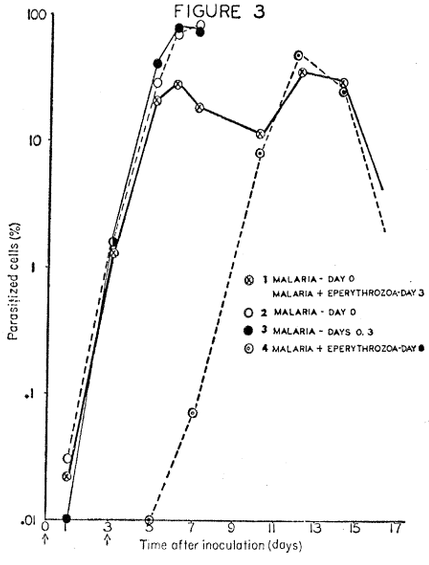

Fig. 3. Course of infection of *Plasmodium chabaudi* in four groups of eight mice: (1) Infected on day 0 with 1×10^6 cells parasitized with plasmodia from a donor mouse free of eperythrozoa and superinfected on day 3 with 1×10^5 parasitized cells plus large numbers of eperythrozoa from a contaminated donor mouse; (2) infected on day 0 with 1×10^6 parasitized cells from an eperythrozoan-free donor mouse; (3) infected on day 0 with 1×10^6 and superinfected on day 3 with 1×10^5 parasitized cells from eperythrozoan-free mice; and (4) infected only on day 3 with the inoculum of large numbers of eperythrozoa and small numbers (1×10^5) of malaria-parasitized cells identical with the superinfecting dose given to group 1. All inoculations were intravenous. The arrows indicate the days of infection and superinfection.

correlation between lack of severity of the malarial infection and reticulocytosis has been repeatedly observed. In a separate experiment, even when reticulocytosis was stimulated with phenylhydrazine, the phenomenon was seen. Eighty percent of a group of animals treated with phenylhydrazine survived the otherwise invariably fatal infection *(7)*.

The *Plasmodium chabaudi* infection in mice heretofore described as a low-level, chronic one, causing death only occasionally, was found to take this course because of the presence of eperythrozoa. When the contaminating organisms were eliminated, the malarial infection was found to resemble that of *Plasmodium vinckei*. The description of the infection of *P. vinckei (5)* could easily describe that for *P. chabaudi* in mice free of eperthyrozoa in that it causes fulminating infection invariably fatal within 5 to 7 days after intraperitoneal inoculation of a million parasitized red blood cells. Maximum parasitemias range between 51 and 96 percent. The fact that neither *P. vinckei* nor *P. chabaudi* preferentially invade reticulocytes also adds to their resemblance. In addition, the disc-electrophoretic patterns of a single sample of *P. chabaudi* extract reportedly closely resembles that of *P. vinckei (8)*. These observations might call for a reevaluation of methods for differentiating one species from another.

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3. Two strains of *P. chabaudi* are maintained in our laboratory; both are contaminated with eperythrozoa. Dr. Meir Yoeli of New York University School of Medicine provided the strain used in our work, and the other was obtained from Dr. Irene Landau through the courtesy of Dr. Elvio H. Sadun's laboratory at Walter Reed Army Institute of Research.
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9. This paper is contribution No. **149** from the Army Research Program on Malaria (grant DA 49-193-MD 2747). We thank Mrs. Nelda Davis and Frederick J. Levy for technical assistance.

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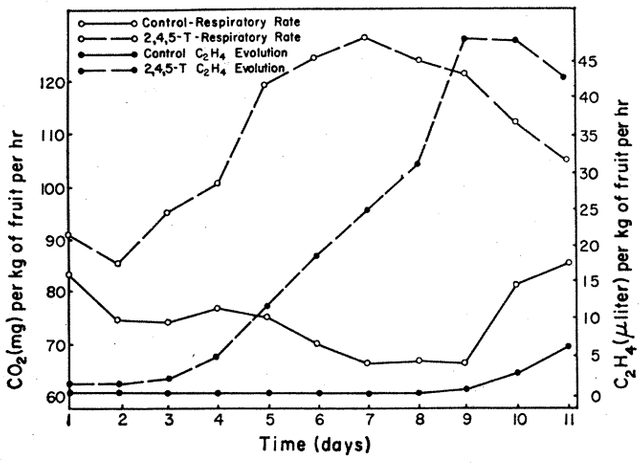
2,4,5-Trichlorophenoxyacetic Acid: Effect on Ethylene Production by Fruits and Leaves of Fig Tree

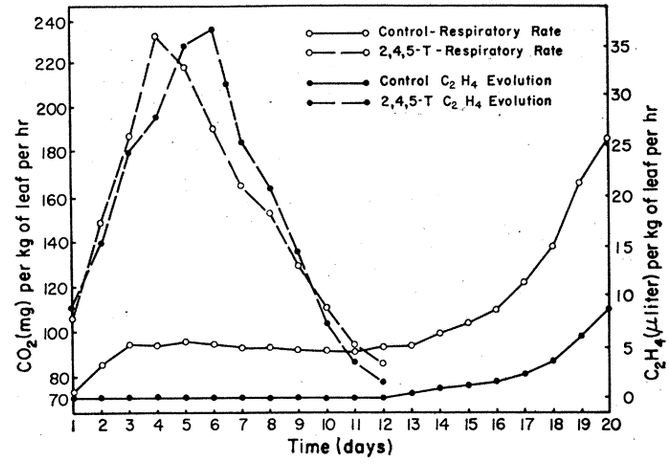
Abstract. *Production of ethylene by fruits and leaves of the fig tree was stimulated by the application of 2,4,5-trichlorophenoxyacetic acid (20 parts per million) to levels that apparently were responsible for hastened maturation of fruit, epinasty, and senescence of leaves.*

Several phenoxy growth regulators stimulate growth and hasten the maturation of fig fruits as much as 60 days *(1)*. Increase in growth is a characteristic response of plant tissues to auxins *(2)*, but the stimulation of maturation in fig and other fruits by these regulators has not been explained. Evidence presented here indicates that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) induces production of ethylene (C_2H_4) by fig fruits and thus stimulates their maturation. Exogenously applied C_2H_4, at concentrations of 0.1 part per million (ppm) or more, stimulates ripening in many fruits, and it has been suggested that this compound is a ripening hormone *(3)*. Auxins stimulate production of C_2H_4 in plant tissues *(4)*, and while maturation must not be equated with ripening, 2,4,5-T may stimulate maturation indirectly through induced production of C_2H_4.

Large branches on each of three Calimyrna fig trees were sprayed on 21 June 1966 with an aqueous solution of 20 ppm of 2,4,5-T. Controls were comparable branches on untreated trees. Samples of 20 fruits and 50 leaves were taken from each tree 24 hours after spraying and periodically thereafter. All fruits from either treated or untreated trees were mixed and then divided equally into three samples. Daily measurements of the production of CO_2 and C_2H_4 were made in triplicate. Leaf samples were handled in the same way. Respiratory rates at 20°C were measured on each continuously aerated lot by the method of Claypool and Keefer *(5)*, and the rates of C_2H_4 production simultaneously by the method of Maxie *et al. (6)*.

Figure 1 shows the effect of 2,4,5-T on the rates of production of CO_2 and C_2H_4 by fig fruits sampled 24 hours after treatment. Treated fruits had a higher initial respiratory rate and showed a climacteric-like rise and fall, reaching a peak on the 7th day. Untreated fruits showed a progressive decline in respiratory rate until the 10th





Figs. 1 and 2. Fig. 1 (left). Effect of 2,4,5-trichlorophenoxyacetic acid (20 ppm) on the production of CO_2 and C_2H_4 by fig fruits. Fig. 2 (right). Effect of 2,4,5-trichlorophenoxyacetic acid (20 ppm) on the production of CO_2 and C_2H_4 by fig leaves.

day, when a rise began; fruit rot on the 11th day terminated the experiment. Production of C_2H_4 by fruits from treated trees was detectable on the 1st day after treatment; the rate rose rapidly to a peak on the 9th and 10th days and declined thereafter. No measurable C_2H_4 was released by untreated fruits until the 9th day, when a slight amount was produced. Gas samples withdrawn immediately from the central cavity through the ostiole of treated fruits harvested 1, 3, 5, 9, and 14 days after treatment contained 1, 5, 6, 6, and 7 ppm of C_2H_4, respectively, whereas gas samples withdrawn from untreated fruits contained no measurable C_2H_4. These amounts of C_2H_4 have been shown to cause ripening in other mature fruits (*3*); the fig fruits in this study, however, were undergoing growth and assimilation associated with maturation. No studies have yet been made of the effect of C_2H_4 on these processes.

Sprayed fruits sampled 1 day after treatment showed what appeared to be a climacteric in CO_2 and C_2H_4 evolution but did not ripen normally. There was a loss of green and a development of yellow color, but the tissues were firm and lacked the characteristic flavor of ripe figs. This probably reflects lack of adequate sugar in the fruits to give them characteristic flavor, a condition associated with immaturity (*7*). The appearance of the fruits was like that of senescent leaf tissues rather than that of normally ripened fig fruits. Fruits harvested 9 days after treatment subsequently ripened normally even though they were not fully grown, which indicates rapid transition from an immature to a mature condition.

Fruit samples taken from treated branches between 28 June and 5 July showed progressively increasing rates of production of CO_2 and C_2H_4, which suggests that they were undergoing a climacteric on the tree. Fruits sampled on 5 July were fully developed and ripe, while untreated fruits did not reach a comparable stage until the 3rd week in August.

Within 24 hours after treatment with 2,4,5-T, the leaf petioles showed a slight epinasty that increased with time, while untreated leaves displayed none. The effect was similar to that reported for the action of C_2H_4 on tomatoes (*4*). Figure 2 shows the effect of 2,4,5-T on the rates of production of CO_2 and C_2H_4 by leaves harvested 24 hours after treatment. Untreated leaves exhibited a slight increase in respiratory rate for 2 days after sampling, a steady rate for 10 days, then a rise to the 20th day when rot forced termination of the experiment. Leaves treated with 2,4,5-T had a higher initial respiratory rate, which increased rapidly and reached a peak 4 days after treatment. Thereafter, the rate declined until the 12th day when rot appeared on the samples. The green color faded rapidly from the treated leaves, which were completely senescent when discarded. There was some loss of green color in the untreated leaves after 15 days, but they showed fewer symptoms of senescence even at 20 days than the treated leaves did at 7 days. Production of C_2H_4 by untreated leaves could not be detected until the 13th day; thereafter the rate of production evolved in a manner paralleling the respiratory rate. Treated leaves showed an initial high rate of production that increased markedly, reaching a peak on the 6th day, followed by a progressive decline thereafter.

We have not conducted experiments to ascertain the relative contributions of 2,4,5-T and C_2H_4 to the growth, maturation, and ripening of fig fruits. Fruits harvested immediately after treatment senesced in the same way the leaves did, but did not ripen, while those harvested when they had reached almost ultimate size ripened normally. The capability of C_2H_4 to stimulate ripening in fruits is well established, and the amounts of this gas produced by fig fruits treated with 2,4,5-T are adequate to cause ripening in other mature fruits (*3*). However, until exogenous C_2H_4 treatments are applied to fig fruits in the same stage of development as those we studied, we will not know the effect of C_2H_4 on growth and maturation per se, as compared to its effect on mature fruits.

Leaves treated with 2,4,5-T yellowed prematurely, showed epinasty, and easily abscissed. These responses are typical of the effects of C_2H_4 on leaves (*4, 9*), and amounts of the gas produced by fig leaves treated with 2,4,5-T were adequate to account for them. It is probable that the epinasty exhibited by apricot leaves and those of other fruit trees (*8*) in response to auxin treatments is caused by induced production of C_2H_4.

Burg and Burg (*9*) recently proposed that C_2H_4 produced as a result of auxin application may account for many of the responses exhibited by plant tissues originally thought to be due to auxin alone. Our data indicate that this is the case with fig fruits and leaves.

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10 January 1967

Phosphorylase b Kinase Inheritance in Mice

Abstract. *The gene for phosphorylase* b *kinase, a skeletal muscle enzyme, has been located on the X chromosome of mice. The inheritance of the enzyme through two generations from original matings between one inbred strain of mice, the I, which lacks the enzyme, and another strain, the* C_{57}, *follows the classical Mendelian pattern.*

The gene for phosphorylase *b* kinase, a skeletal muscle enzyme, has been located on the X chromosome of the mouse.

The inheritance of the enzyme was followed through two generations of offspring from matings between two inbred strains of mice, the I/FnLn and the C_{57}BL/FnLn. The I-strain mouse expresses no activity of the enzyme whatsoever in skeletal muscle (*1–3*), but is, otherwise, a normal, healthy laboratory animal with gross characteristics similar to those of the C_{57}-strain mouse.

Table 1. Distribution of phosphorylase *b* kinase in the F_1 and F_2 generations from matings of I- and C_{57}-strain mice.

Generation	Sex	No. of mice	Phos. *b* kinase*	Phos. *a* (%)†
		I-female × C_{57}*-male*		
F_1	♂	16	None	2.1±0.4‡
F_1	♀	10	476±72	24.7±2.6
F_2	♂	18	None	1.7±2.5
F_2	♂	26	883±47	38.2±3.3
F_2	♀	21	None	0.7±0.5
F_2	♀	19	500±43	26.0±1.8
		C_{57}*-female* × *I-male*		
F_1	♂	16	569±30	14.7±0.8
F_1	♀	17	394±41	23.4±2.1
F_2	♂	19	None	0.5±0.4
F_2	♂	21	784±49	37.7±3.4
F_2	♀	19	590±42	27.1±2.1

* Units (*4*) per milligram of protein (*12*). † (Units without 5′-AMP/units with 5′-AMP) × 100 (*5*). ‡ Standard error of the mean.

The presence or the absence of phosphorylase *b* kinase in the skeletal muscle of the offspring from these matings was determined by two separate analyses. First, phosphorylase *b* kinase was assayed directly in a muscle homogenate by following the conversion of a highly purified preparation of phosphorylase *b* to phosphorylase *a* (*2, 4*). The substrate and the product may be conveniently differentiated by a cofactor requirement; the *b* form has an absolute requirement for 5′-adenosine monophosphate (5′-AMP) whereas the activity in the absence of the nucleotide is arbitrarily taken as the amount of phosphorylase *a* (*5*). Second, phosphorylase, which catalyzes the breakdown of glycogen in the muscle cell (*6*), was assayed with and without 5′-AMP (*1, 5*). The relative amount of phosphorylase *a* in a muscle homogenate is considered to be a direct reflection of the activity of this kinase, since phosphorylase *a* increases in the muscle following electrical stimulation or the administration of epinephrine (*7, 8*).

Furthermore, this specific kinase reacts with such facility that the *b* to *a* conversion will take place during the process of homogenization of the frozen sample of muscle unless the homogenizing vessel is cooled to −20° to −30°C (*8*). This precaution was observed when the effect of epinephrine was studied in mice of the F_1 generation. For confirmation of the presence or the absence of the kinase in mice of the F_2 generation, the conversion of phosphorylase *b* to the *a* form was allowed to proceed in a homogenizing vessel under controlled conditions. Samples of frozen muscle were homogenized in a ground glass unit (*9*) at 0° to 2°C for 18 to 24 seconds.

Owing to the lack of phosphorylase *b* kinase, the parental I-strain mouse exhibits no increase in phosphorylase *a* under any stimulus tried so far (*1–3*). One group of mice in the F_1 generation, the male offspring from I-strain females and C_{57}-strain males, resemble the I strain in this respect. Phosphorylase *b* kinase was absent (Table 1), and an intravenous dose of epinephrine failed to produce an increase in phosphorylase *a* in these males (Fig. 1). In contrast, F_1 males from matings of C_{57}-strain females and I-strain males responded to epinephrine with a rapid and sustained rise in phosphorylase *a* (Fig. 1). No data are given but all of the females in the F_1 generation responded to epinephrine as would be expected from the presence of the kinase (Table 1). Throughout these studies the absence of phosphorylase *b* kinase was always associated with an absence of phosphorylase *a;* the presence of the kinase was always associated with increases in phosphorylase *a*.

The two groups of male mice in the F_1 generation were identical in appearance, size, and vigor. Except for a slight dilution in the markings, all of the mice in the F_1 generation resembled the jet black C_{57}-strain parents. The fur of the I-strain mouse is predominately white; two large patches of sandy brown fur nearly cover the dorsal surface, and these two patches are separated by a band of white fur in the lumbar region.

This discrimination between the

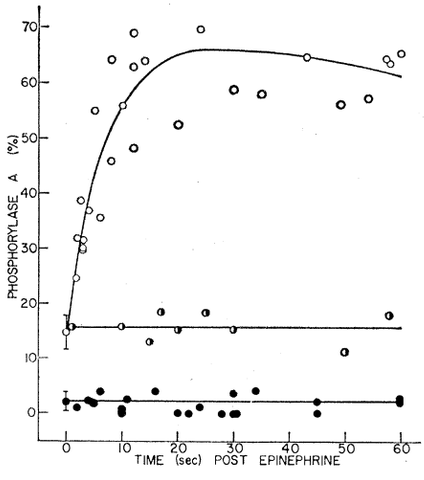

Fig. 1. The effect of epinephrine on phosphorylase in the gastrocnemius muscle of hybrid mice: F_1 males from C_{57}-females and I-males (open circles), and F_1 males from I-females and C_{57}-males (closed circles). One muscle was removed from the mouse under deep anesthesia (*2*), epinephrine (5 μl of a 5.46 μ*M* solution per gram of body weight) was injected into the tail vein, and the exposed contralateral muscle was removed within 1 minute. The muscles were frozen immediately in isopentane chilled in liquid nitrogen or between blocks of CO_2, and then stored in liquid nitrogen. Except at zero time each point represents one animal. The averages and the standard deviations (vertical bars) of the percent phosphorylase *a* (see legend of Table 1) in the muscles taken before epinephrine are plotted at zero time; each point represents 16 mice. The half-filled circles represent muscle taken from C_{57}-males after an injection of 0.85 percent NaCl (5 μl per gram of body weight) in the tail vein.