and photographed by Arndt (*6*) may well be due to periodic reexcitation of this system, after its recovery, either by the same cells as initially or by others with similar properties (*7, 8*).

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7. These matters are considered further in papers on (i) the properties of aggregating amebas, (ii) patterns of aggregation, and (iii) integration, disintegration, and initiation.
8. I greatly appreciated M. Sussman's interest in my procedure for stabilizing acrasin when I was at Princeton, and it is encouraging to learn that he and his coworkers were able to extend my findings in such a brilliant way, while other duties temporarily prevented me from continuing with this work.

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16 May 1956

Inasmuch as the editors were informed of B. M. Shaffer's unpublished work on acrasin, they asked him to prepare the foregoing paper to be published simultaneously with that by M. Sussman, F. Lee, and N. S. Kerr.

Resistance to Organic Phosphorus Insecticides of the Housefly

Organic phosphorus insecticides have been used for fly control on Danish farms since 1951–52, when the houseflies (*Musca domestica* L.) had developed resistance to the chlorinated hydrocarbons on practically all farms (*1, 2*).

Parathion (0,0-diethyl 0-*p*-nitrophenyl thionophosphate) was used from 1951, illegally, on a great many farms as a residual spray or in baits. In 1952 parathion-impregnated gauze strips were officially approved for fly control in animal houses, and in the following years this method was used on a large scale (*2*). Thus, it was estimated that fly control with parathion-strips was carried out on 10 to 15 percent of the farms in 1952, on about 50 percent in 1953, and on 75 percent or more in 1954 and 1955.

Diazinon (0,0-diethyl 0-2-isopropyl-4-methylpyrimidyl-6 thionophosphate) has been employed as a residual spray for fly control on farms since 1953 but only on a limited scale compared with the parathion-strips.

The third organic thionophosphate used was Bayer 21/199 (3-chloro-4-methyl-umbelliferone-0,0-diethyl thiophosphate). In extensive laboratory tests with DDT-resistant flies, it had shown good residual effect on building materials, and a few field trials in 1954 had been very promising. From May 1955 it was used as a residual spray in certain areas.

Even by July 1955, however, we got reports of failing effect on many farms, and tests showed that the flies were highly resistant to deposits of Bayer 21/199. The investigated cases that most clearly indicated this were from the area around Skelskør in the island of Zealand. Here a pest-control company had used phosphorus insecticides for fly control in cowsheds and pigsties on about 1000 farms as follows.

"1953: parathion-strips with good results.

"1954: 2–3 residual sprayings with Diazinon wettable powder with varying results.

"1955: At the end of May one treatment with Bayer 21/199 wettable powder (Resitox) (0.2–0.4 g active material per m^2). This gave generally a good control for about two months. However, a second treatment at the end of July failed to control the flies, and later treatments with higher concentrations were reported to be without residual effect."

Biological tests of surfaces in pigsties on two farms, Nos. 128 and 129, that were sprayed 12 days previously with Bayer 21/199 showed the expected effect on a DDT-resistant laboratory strain 17b. Confined on the surface in shallow cellophane cages, these flies were paralyzed within 3 to 4 hours on woodwork and within 6 hours on the walls. However, when local flies, caught in the pigsty, were exposed to the sprayed surfaces in the same way, more than half of them survived 20 hours' exposure (52 percent on farm No. 128 and 61 percent on farm No. 129).

Similar results were obtained when flies from four other farms were exposed in the laboratory to wood sprayed with a wettable powder to give 0.2 g Bayer 21/199 per square meter. The resistance was further determined by topical application of 1-mm^3 acetone solutions on the mesonotum of female flies, the mortality being observed after 24 hours. LD_{50} was determined graphically on log-probability paper. Some results are given in Table 1.

The strains Nos. 98, 127, and 129, which were from the Skelskør district, showed an LD_{50} that was 8 to 45 times that of the laboratory strains. By testing flies collected in other districts, similar and higher resistance to Bayer 21/199 was found in strains (examples, Nos. 74, 149, and 150) that had never been exposed to this compound but had been exposed to parathion and Diazinon. A still higher resistance (LD_{50} up to 500 times normal) was found in flies from a farm (No. 79) where the stables had been sprayed with parathion in 1953 and 1954, and where Bayer 21/199 had not been used until 2 weeks before collection of the flies.

After the demonstration of resistance to Bayer 21/199, investigations of resistance to Diazinon and parathion were carried out. Even by 1954 we had had some reports of a failing residual effect of Diazinon used in a dosage of about 0.2 g/m^2. However, this failure had been ascribed to other causes than resistance. From July 1955 we received many new reports of unsatisfactory effects of Diazinon from farms where the insecticide had been used in 1954 and 1955. Flies from several of these places were tested for resistance in the laboratory (examples, strains Nos. 149, 150, and 151 in Table 1).

By topical application, these strains showed a tolerance 3 to 15 times that of laboratory strains. Although this does not seem to be a serious degree of resistance, it was significant from a practical point of view, as was shown by tests in which flies were exposed continuously on wood or blotting paper sprayed with the normal field dosage of a wettable powder (0.25 g Diazinon per square meter). Under these conditions, all flies of the DDT-resistant laboratory strain 17b

Table 1. Resistance to phosphorus compounds in houseflies on Danish farms. (Locations: J, North Jutland; Z, South Zealand; F, Funen. Compounds: P, parathion; D, Diazinon wettable powder; B, Bayer 21/199 wettable powder.)

Strain (district and No.)	Collected	Exposure to phosphorus insecticides in field				LD_{50} by topical application, Oct.–Dec. 1955 (μg per female fly)		
		1952	1953	1954	1955	Bayer 21/199	Diazinon	P
Laboratory								
9, 17	1949–50	None	None	None	None	0.02–0.06	0.03–0.04	0.015–0.023
Field								
J 74	July 1955	?	P*	P*	P*	1.7	0.11	0.06
J 79	8 July 1955	?	P*	P*	P† B‡	5–11	0.13	0.09
Z 98	3 Aug. 1955	None	None	D	B‡	0.9		0.03
Z 127	15 Sept. 1955	None	P†	D	B‡	0.9	0.17	0.05
Z 129	15 Sept. 1955	None	P†	D	B‡	0.5	0.09	0.05
Z 149	11 Oct. 1955	P†	P†	D	D‡	0.6	0.3	0.06
Z 150	11 Oct. 1955	None	P†	D	D‡	1.3	0.5	
F 151	23 Nov. 1955	None	None	D	D‡	0.06	0.13	0.04

* Sprayed as an emulsion. † Impregnated on gauze strips. ‡ Report of failing effect.

were definitely paralyzed in less than 4 hours, while in strains 151, 127, and 150, 2 to 23 percent of the females survived for more than 24 hours. These results seem to explain the lack of residual effect in the stables.

Tests of resistance to parathion in several field strains (see Table 1) by topical application of the compound generally showed an LD_{50} of about 2 to 3 times normal in each strain. In strain 79 from a farm where parathion has been used as a residual spray for at least 2 years, the parathion resistance was about 5 times normal.

The practical significance of this resistance is not yet quite clear. On most farms the parathion-strips still seem to work well when used according to our directions. However, preliminary tests indicated that some resistant flies (strains Nos. 74 and 79) required a contact period on the parathion strips about 4 times longer than did the laboratory strains.

In conclusion, our results with Bayer 21/199 and Diazinon have shown that development of resistance may impede the control of houseflies even with organic phosphorus compounds. So far, the future use of this group of insecticides for fly control has been regarded with optimism in view of the apparently modest resistance (LD_{50} 10 to 20 times normal) obtained by long-term selection in the laboratory (*3*). However, as is shown in this report, an increase in tolerance of only 10 times may be of significance for the control of flies with residual sprays (*4*).

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4. Further investigations are in progress, and a more detailed report is in preparation.

9 February 1956

Inhibition of Enzymatic Browning of Chlorogenic Acid Solutions with Cysteine and Glutathione

Weurman and Swain (*1*) have demonstrated that chlorogenic acid is one of the substrates involved in the enzymatic browning of both apple and pear homogenates and that three new fluorescent compounds were formed during the reaction. Work in this laboratory (*2*) with apples confirms the disappearance of chlorogenic acid in aerated homogenates as well as during the development of storage scald, but no new fluorescent spots were observed on paper chromatograms of the browned solutions. However, further studies have resulted in evidence for the formation of fluorescent *o*-quinone-sulfhydryl addition complexes.

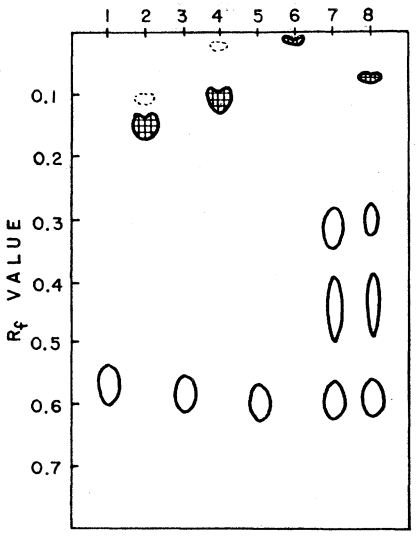

Fig. 1. Chromatographic patterns of fluorescent compounds found after 1 hour of aeration in various reaction mixtures: (i) apple enzyme + chlorogenic acid + cysteine, boiled, *1*, and unboiled, *2*; (ii) apple enzyme + chlorogenic acid + glutathione, boiled, *3*, and unboiled, *4*; (iii) apple enzyme + chlorogenic acid + coenzyme A, boiled, *5*, and unboiled, *6*; (iv) cabbage extract, boiled, *7*, and unboiled, *8*.

In this study, dilute aqueous extracts of Grimes Golden apple tissue, pulp and peel, were used as a source of polyphenoloxidase. Enzyme solutions were prepared by macerating 25 g of tissue with 200 ml of distilled water for 2 minutes in a Waring Blendor, allowing the solids to settle, and decanting the liquid portion. Inactive enzyme solutions were prepared by macerating the tissue in boiling water. When 1 drop of a concentrated chlorogenic acid solution was added to 1-ml aliquots of both the active and inactive enzyme solutions, rapid browning was observed in the aliquots containing the active enzyme and no browning in the aliquots containing the inactive enzyme. After 1 hour, approximately 100-lambda aliquots were spotted on Whatman No. 1 filter paper and separated by descending chromatography with the organic phase of butanol - acetic acid - water (4 : 1 : 5). Examination of the dried chromatograms under ultraviolet light revealed an intense blue-white fluorescent spot (R_f = 0.57) corresponding to chlorogenic acid in the nonbrowned, inactive enzyme solution. Neither chlorogenic acid nor any new fluorescent products of the browning reaction were detected on chromatograms of the browned, active enzyme solutions. The addition of ascorbic acid to mixtures of chlorogenic acid and active apple enzyme inhibited browning as well as the disappearance of the chlorogenic acid spot on the chromatograms. Ascorbic acid is known to reduce quinones back to phenols (*3*) and also to have an inhibitory effect on the enzyme (*4*).

The inhibition of browning reactions of chlorogenic acid with cysteine or glutathione appears to proceed by a different mechanism. The addition of cysteine or glutathione to a mixture of chlorogenic acid and active apple enzymes resulted in inhibition of browning; as may be seen in Fig. 1, there is a complete disappearance of chlorogenic acid and the appearance of a new, yellow fluorescent spot (R_f = 0.16 for cysteine-chlorogenic acid; R_f = 0.11 for glutathione-chlorogenic acid). The coenzyme-A solution tested did not prevent browning, but a new, faint yellow fluorescent spot (R_f = 0.02) appeared on the chromatogram. Since the sulhydryl content of this preparation was not known, the data only support the suggestion that coenzyme A participates in these reactions. The chromatographic spots of the cysteine- and glutathione-chlorogenic acid complexes gave an immediate reddish-brown color on spraying with ammoniacal silver nitrate, a blue color with ninhydrin, and a white spot against a pink background with the KI-H_2PtCl_6 reagent for sulfur compounds (*5*). Cysteine (R_f = 0.04) and glutathione (R_f = 0.20) were also detected on the chromatograms by the latter two spray reagents. These data indicate that these sulfhydryl compounds inhibit *in vitro* browning by removal of the quinone produced by polyphenoloxidase from further participation in the browning reaction. The nonsulfhydryl amino acids—glycine, alanine, and glutamic acid—did not inhibit browning. Glutamic acid did not appear to increase

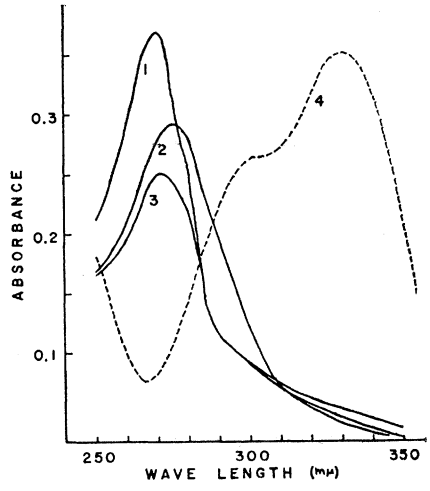

Fig. 2. Ultraviolet absorption spectra of the addition products of various sulfhydryl compounds with the quinone of chlorogenic acid: *1*, aerated cabbage compound; *2*, cysteine addition product; *3*, glutathione addition product; *4*, chlorogenic acid alone.