Table 1. Biological activity of fractions A and B after paper chromatography. The number of plus signs is a rough measure of the intensity of the response.

Final concn. (mg/ml)		Absorbance at 265 mμ		Biological activity
A	B	A	B	
1.3	0	.560		–
1.3	0.7	.560	.280	++++
1.0	1.0	.420	.420	++
0.7	1.3	.280	.560	–
0	1.3		.560	–

Table 2. Inactivation of acrasin by neutral extract (NE). The acrasin solution contained 3 mg/ml of crude powder and was mixed with neutral extract in the ratio of 10:1 (vol/vol).

Mixture	Biological activity	
	Zero time	4 hr
Acrasin + neutral extract	+++	–
Acrasin + boiled neutral extract		++
Acrasin + H_2O		++
H_2O + neutral extract		–

1 summarizes the data from one separation. Note that the relative proportions of the two components were critical for demonstration of biological activity. The absolute amounts are also important since excessive concentration or dilution of the mixture yielded negative results. Too great a supply of the agents would tend to diminish the relative concentration gradients in the system. Previous results (*2*) do indicate that the myxamebas depend on a gradient in order to know in which direction to move.

The A and B fractions have also been detected by spot chromatography, using a water-butanol-ethnol (50 : 30 : 17) solvent. The R_f values were 0.48 and 0.42, respectively.

The reason for the instability of acrasin in the crude state has been investigated. At the outset of this study, the most likely possibility was held to be the existence of an extracellular enzyme that catalyzes the destruction of acrasin. It was upon this preconception that we chose the extraction procedure with the expectation that *p*H 3.5 might be either in the isoelectric range of the enzyme or else far removed from its *p*H optimum. Subsequent experiments, while admittedly not crucial by rigorous biochemical criteria, strongly support this view.

To obtain the hypothetical enzyme, cells from eight growth plates were extracted with 50 ml of ice water (hereafter called neutral extract). A solution of crude powder from the acrasin concentrate (3 mg/ml) was mixed 10 : 1 with the neutral extract and incubated at 25°C. The mixture and appropriate controls were assayed for biological activity by the Shaffer procedure and were run in bar chromatograms with 80-percent ethanol. Table 2 shows the data. Although it was initially active, the mixture lost all activity after 4 hours of incubation. In contrast, boiled neutral extract failed to inactivate the crude powder. The chromatograms revealed that the inactivated acrasin had lost its B fraction while the A fraction had increased in quantity.

The neutral extract was subjected to ammonium sulfate precipitation. The precipitate that was brought down by adding between 50 and 55 percent ammonium sulfate was washed and redissolved in water. This preparation could inactivate the crude powder precisely as described in the previous paragraph, and it also caused the disappearance of the B fraction. Further, the crude powder that had been inactivated by these means was mixed with additional B fraction which had been eluted from paper chromatograms. The Shaffer test revealed that inactivated powder plus B fraction was active, while inactivated powder plus A fraction was not active. Thus the putative enzyme destroyed the B fraction of acrasin but left the A fraction intact.

Preliminary experiments have been performed on the partially purified A and B fractions themselves. The neutral extract was shown to have no effect on fraction A, but it converted fraction B into something that was identical with fraction A, both biologically and chromatographically.

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References and Notes

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4. This work was aided by funds supplied through the Office of Naval Research and the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.
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Properties of Acrasin

Although it had been suspected for nearly half a century that aggregating amebas of the cellular slime mold *Dictyostelium* oriented to a chemical gradient, Bonner (*1*) was the first to demonstrate this convincingly; he named the chemical *acrasin* but was unable to isolate it *in vitro*. Amebas could be made to orient to the gradient set up by an artificial source, which consisted of the cell-free liquid obtained from the immediate surroundings of a natural source, once a method had been devised for renewing it at frequent intervals with this liquid, if it was freshly collected (*2*). At room temperature, this acrasin solution lost its activity within a few minutes, an instability that was mainly responsible for the chemical's elusiveness.

In an experiment to confirm Runyon's finding (*3*) that the stimulus to aggregation would pass through cellophane dialysis membrane, natural sources were grouped on a raft of this material and liquid was periodically added to, and collected from, the underside of it; I found that it oriented the amebas; moreover, this cell-free acrasin solution, unlike that obtained earlier by filtering through a Millipore membrane, was stable. I concluded that acrasin activity was associated with molecules small enough to pass rapidly through cellophane and that loss of activity was due to reaction with larger molecules, possibly those of an enzyme, also present extracellularly.

During my tenure of a Dill fellowship at Princeton University in 1954, I was able to develop a procedure for extracting acrasin chemically. Cold methanol was poured over culture plates in which aggregation was general; this was dried in a vacuum at – 10°C, and the residue was extracted with a small volume of methanol, which was then filtered and again dried. An aqueous solution of the product had a very high acrasin activity, which was retained after boiling for more than 1 hour and after exposure to 0.1*N* hydrochloric acid or 0.01*N* sodium hydroxide. The solubility of dry acrasin decreased rapidly in passing up the alcohol series.

A cell-free aqueous extract of an aggregating culture had the power to inactivate acrasin but lost it after boiling: the fraction that could be precipitated with ammonium sulfate was shown to be responsible. These findings made it probable that the original instability was due to an extracellular enzyme (*4*).

The action of acrasin was not confined to the orienting of sensitive amebas: it also caused them to secrete acrasin. The functional significance of this is clear: it is the basis of a chain reaction that permits chemotactic attraction to operate over distances much greater than those over which diffusion processes are normally effective in biological systems.

Sussman and Noel (*5*) thought it likely that the capacity to initiate a center of aggregation was not identical with the ability to produce acrasin during aggregation; it now appears that the essential thing for initiation is for some cell or cell group to be able to start the production of acrasin and so to excite the system of acrasin relays. The rhythmic movements during aggregation described

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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1. J. T. Bonner, *J. Exptl. Zool.* 106, 1 (1947).
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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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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.