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THE FEEDING OF COLORING MATTERS TO *PIERIS RAPAE* LARVAE

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INTRODUCTION

Since this paper is concerned with the feeding of dyes to the larvae of *Pieris rapae,* mention will be made of some of the work that has been done along similar lines with other insects.

The silkworm has been a favorite subject. Lombardi (1920) fed dyed leaves to the worms to time the retention of the leaf in the intestine. That aniline dyes were absorbed by the silkworm gut was mentioned by Goris and Muehlemann (1936). Jucci and Ponseveroni (1930) found that Berlin blue, safranin, gentian violet, methylene blue, Nile blue, neutral red, indigo carmine, alizarin, fuchsin, methyl orange, and other dyes did not show up in the silk. Only orcein migrated into the cocoon, while neutral red, Sudan III, methylene blue, and Nile blue migrated into the eggs. Campbell (1932) fed aqueous solutions of dyes to fourth-instar silkworms. Alternatively, fifth-instar worms were injected with measured volumes of the solutions. This interesting work will be referred to in the Discussion. An article by Edwards (1921) reviews attempts to produce colored silk by feeding dyes to silkworms and also will be referred to in the Discussion.

The selectivity of absorption of dyes by the midgut of *Machilis* (Thysanura) is shown by the fact that only fifteen of sixty-five dyes were taken up by the midgut caeca, and the uptake of several dyes by the midgut of various insects has been studied (Roeder, 1953). It was found by feeding dye-coated seeds to wireworms (Zacharuk, 1963) that the dyes most suitable for marking the living worms internally were Nile blue, rhodamine B, Sudan black B, brilliant cresyl blue, acridine orange N, coriphosphine O, and brilliant fat scarlet. External manifestation in the adult stage was prevented by the brownish-black integument

of the beetles. Also, various dyes (some mentioned in Discussion) have been fed to silverfish on ground wheat (Lindsay, 1940).

The literature as cited above suggested that certain dyes probably would be absorbed by the intestine of the *Pieris rapae* larva, but there is no real indication that a dye might appear on the resulting adult, especially superimposed on the conveniently white or off-white ground color of the wings. Therefore, the following experiments were made with the particular object of changing the color of the wings and the secondary objects of externally coloring the pupae, noting toxic effects, and making incidental observations on other effects of ingested dyes.

EXPERIMENTAL

1. REARING PROCEDURE

Thirteen females (collected at Flemington, N. J., on May 1, 1965) were confined in a glass jar (approximately a cylinder 3.5 inches in diameter and 5 inches in height) containing fresh cabbage leaves held in place by a wire screen over the top, and the jar was placed in an open window in direct sunlight. (Ordinary electric light from a 100 watt bulb close to the jar also was effective in stimulating oviposition.) The first day yielded about 280 eggs and the second day 105 eggs; only four butterflies remained alive at the end of the second day.

The larvae were kept in cardboard boxes (4.5 inches high by 9 inches square) with gauze windows (5.5 inches square) in the lids and were fed leaves peeled from refrigerated cabbage heads. As the larvae grew they were distributed among the boxes so that usually there were no more than fifteen per box by pupation. However, as many as 43 could be reared to pupation (to give 37 pupae all of which yielded adults) in a single box.

The control adults of the first brood were placed in a screen-covered cage (16 inch cube) in which they were exposed to fresh air and sunlight for a day to allow mating; then cabbage leaves (fresh each day) were hung up on the screen on the sunny side of the cage for three to four days to collect eggs. With 25 or more butterflies, hundreds of eggs were obtained in this manner (though production per female might be only on the order of 30 eggs since the butterflies were not fed). In this fashion four consecutive broods were reared during the experiments described below. Eclosion of the last brood was completed on Sept. 22, 1965.

In one case, new pupae were refrigerated at 1-3° C. for six days to delay the time of eclosion with no ill effects, and the 1 to 3-day-old larvae of the last brood similarly were refrigerated for two days because they could not be attended during this time.

Although really unnecessary, since photoperiod seems to have no effect above 20° C., the boxes were illuminated by electric light until 2 A.M. each morning to make certain that the pupae would not diapause. The room temperature varied from a low of 65° F. at night to a high of 90° F., and the relative humidity was always in the range 31-65%.

2. METHOD OF FEEDING DYES

At first, dyes in salt shakers were simply sprinkled onto the leaves at, very approximately, 2 mg./in.^{2} leaf surface; in such cases the dye is listed as "100% of blend" in Table 1. However, the desirability of lowering the ingestion levels of the more toxic dyes as well as the extent of consumption of expensive biological stains suggested that the dyes be extended with an inert ingredient. Organic compounds were extended in experiments in which a mixture of 10% compound and 90% kaolin was fed to silkworms (Ginsburg and Cavallito, 1936), and pesticides customarily are extended with clays. It has been suggested that malachite green compounded with clay might be used in insecticides (Campbell, 1932).

Five possible extenders were considered: Celite (Johns-Manville Products), P-12 Davenite mica (325 mesh; Hayden Mica Co.), Hydrite UF kaolin (Georgia Kaolin Co.), "lanolized" talcum powder, and ASP 400 P aluminum silicate pigment (Minerals and Chemicals Philipp Corp.). The talcum powder was rejected because it was not easily wetted by water, and the kaolin was eliminated because it tended to cake up and was not delivered readily from a salt shaker. The other three candidates were fed to larvae (at about 2 mg./in.^{2} leaf surface, applied to both sides of the leaf) as shown in Table 1. The aluminum silicate and the Celite seemed to have a "dehydrating effect" on the larvae in terms of appearance, for many of them shriveled up and died. Mica gave the best result, and its inertness to the larvae was demonstrated by the many cases (see Table 1) in which dye-mica blends allowed 90-100% yields of both pupae and adults.

The dye-mica mixtures were prepared by grinding thoroughly the dye and a portion of the mica with a mortar and pestle and then blending with the remainder of the mica by tumbling. The resulting blend was sprinkled, from a salt shaker, onto both sides

Table 1. Feeding of Coloring Matters to Pieris rapae Larvae.

Biolog. Stain, or Dye*	CI No.	% of Blend	Days Fed	Color Larvae	Yield Pupae	Color Pupae	Yield Adults
			NITRO				
Naphthol Yellow S*	10316	5	3-5	N	14/15	N	13/15
			MONOAZO				
Janus Green B	11050	5	to 9	deeper green	0/15	-	0/15
Oil Scarlet 6G	12140	5	3-8	N	12/15	v. sl. pink	12/15
Citrus Red #2*	12156	5	5-9	sl. pink?	14/15	N	13/15
FD&C Red #4*	14700	100	4-7	sl. pink	5/7	N	5/7
Amaranth	16185	5	2-6	N	14/15	N	14/15
FD&C Yellow #5*	19140	100	5-9	N	7/7	N	7/7
			DIAZO				
Bismarck Brown Y	21000	5	3-6	N	13/15	N	13/15
Bismarck Brown R	21010	5	3-5	sl. brown	15/15	sl. pink	15/15
Congo Red	22120	5	3-4	N	15/15	N	15/15
Brilliant Vital Red	23570	5	3-7	N	13/15	N	13/15
Trypan Blue	23850	5	3-5	sl. blue	15/15	N	15/15
Evans Blue	23860	5	4-5	sl deeper grn.	13/15	sl. green?	12/15
Sudan III	26100	5	3-6	sl. pink	14/15	pink	14/15
Sudan IV	26105	5	3-4	v. sl. pink	15/15	pink	15/15
Sudan Black B	26150	5	2-4	bluish	15/15	greenish-blk.	15/15
"	"	5	6(Note 1)	gray-grn.	9/20	deep blue	6/20
			DIPHENYLMETHANE				
Auramine O*	41000	5	4-9	sl. yellow	9/15	yellow-green	6/15
			TRIARYLMETHANE				
Victoria Green WB*	42000	5	to 4	N	0/15	-	0/15
Brilliant Green	42040	5	to 5	deeper green	0/15	-	0/15
Fast Green FCF	42053	5	3-6	deeper green	15/15	N	15/15
FD&C Blue #1*	42090	100	4-7	N	6/7	N	6/7
FD&C Green #2*	42095	100	4-9	N	7/7	N	7/7
Fuchsine Y*	42510	5	to 6	pink	0/15	-	0/15
Crystal Violet	42555	5	to 9	sl. bluish?	0/15	-	0/15
Aniline Blue	42755	5	3-6	sl. deeper grn.	15/15	N	15/15
			XANTHENE				
Acridine Red	45000	5	5-13	deep pink	11/15	pink	11/15
Rhodamine B*	45170	100	5	pink	0/7	-	0/7
"	"	1	3-6	pink	9/10	sl. pink	9/10
Fluorescein*	45350	100	7	N	1/7	N	1/7
Uranine*	45350	1	2-4	N	15/15	N	15/15
Rose Bengal	45440	5	2-3	N	15/15	N	15/15

Table 1. (Continued) (part 2)

Biolog. Stain, or Dye*	CI No.	% of Blend	Days Fed	Color Larvae	Yield Pupae	Color Pupae	Yield Adults
			ACRIDINE				
Acridine Orange	46005	5	3-6	N	2/15	orange-yellow	2/15
			METHINE				
Nabor Brill. Red 6B*	48020	5	to 5	sl. pink?	0/15	-	0/15
			AZINE				
Neutral Red	50040	5	4-7	pink	10/10	red	7/10
"	"	5	32 hrs.	(Note 2) pink	13/15	N to pink	12/15
Azocarmine G	50085	5	6-11	N	10/15	N	8/15
Azocarmine B	50090	5	5-8	N	11/15	N	9/15
Phenosafranin	50200	5	to 7	pink	0/15	-	0/15
Safranin, Bluish	50205	5	1-6	N	9/15	N	8/15
Safranin O	50240	5	to 7	pink	0/15	-	0/15
Naphthalene Red	50375	5	6-11	deeper green	13/15	N to violet	10/15
Indulin(Alc. soluble)	50400	5	5-10	N	13/15	N	12/15
Nigrosin(Water sol.)	50420	5	7-10	deeper green	7/15	N	7/15
			OXAZINE				
Brill. Cresyl Blue	51010	5	3-9	bluish	15/15	violet	15/15
Gallocyanine	51030	5	6-10	sl. deeper grn.	11/15	N	8/15
Celestine Blue B	51050	5	6-11	sl. deeper grn.	13/15	N	10/15
Nile Blue A	51180	5	3-4	bluish	4/15	blue-green	4/15
"	"	1	4-9	bluish	13/15	blue	10/15(Note 3)
"	"	5	32 hrs.	(Note 4) bluish	13/15	blue	8/15
			THIAZINE				
Methylene Blue*	52015	100	to 5	deeper green	0/7	-	0/7
"	"	1	3-7	deeper green	15/15	pale grn.-blue	15/15
Methylene Green*	52020	100	to 5	deeper green	0/7	-	0/7
"	"	1	3-5	sl. deeper grn.	14/15	sl. green	14/15
			ANTHRAQUINONE				
Alizarin	58000	5	3-4	N	14/15	N	14/15
			INDIGOID				
Indigo Carmine	73015	5	2-4	N	14/15	N	14/15

Table 1. (Continued) (part 3)

Biolog. Stain, or Other	CI No.	% of Blend	Days Fed	Color Larvae	Yield Pupae	Color Pupae	Yield Adults
NATURAL ORGANIC COLORING MATTERS							
Carmine (Alum Lake)	75470	5	3-5	N	15/15	N	12/15
Orcein	-	5	3-5	N	15/15	N	15/15
Paprika, imported	-	10	3-5	N	15/15	N	15/15
INORGANIC COLORING MATTERS							
Berlin Blue	77510	5	4-6	N	14/15	N	14/15
NEUTRAL RED - NILE BLUE A MIXTURE							
Neutral Red	50040	1					
Nile Blue A	51180	1	2 (Note 5)	blue	13/15	greenish-blue	12/15
INDICATORS							
Phenol Red	-	5	3-5	N	12/13	N	12/13
Brom Cresol Purple	-	5	2-6	N	14/15	N	14/15
Brom Thymol Blue	-	5	2-6	N	14/15	N	14/15
EXTENDERS ALONE							
P-12 Davenite Mica	-	100	4-6	N	5/7	N	4/7
Celite	-	100	6	N	2/7	N	0/7
ASP 400 P	-	100	4-8	N	3/7 (Note 6)	N	1/7
CONTROLS							
None	-	-	3-8	N	52/60	N	51/60
None	-	-	3-6	N	37/43	N	37/43
None	-	-	4-7	N	17/20	N	16/20

Notes

1. Larvae pupated 1-4 days after suspension of dye feeding.
2. Larvae pupated 3-6 days after suspension of dye feeding.
3. All but one adult failed to expand wings. There were only incidental failures to expand (up to 3 out of 15 specimens) in all other cases.
4. Larvae pupated 5-8 days after suspension of dye feeding.
5. Larvae pupated 4-6 days after suspension of dye feeding.
6. These pupae were dwarfed (12, 12, and 14 mm. in length vs. 17 mm. for normal pupae).

of each newly-added leaf at about 2 mg. blend/in.^{2} leaf surface.

Feeding was begun when the larvae had attained a minimum length of 5 mm. in the case of pure dyes (100% of blend) or 17 mm. in all other cases; the final length is about 25 mm. In all but four cases (see footnotes in Table 1) feeding was continued to pupation. That the dyes were being ingested was shown in nearly all cases by the color of the excrement.

3. EXPLANATION OF TABLE 1

All the coloring matters except the paprika (a kitchen item) were obtained from the National Aniline Division of the Allied Chemical Corp. Dyes (commercial colorants) are identified by an asterisk, as shown at the head of the column in the table. Except for the three indicators and the paprika, all the others are biological stains and are listed and described in the "National Biological Stains and Indicators Price List", 1964. In the present case, dyes are distinguished from biological stains on the basis of how they were obtained; for example, methylene blue happened to be procured as a dye sample, but it is also sold as a biological stain. For convenience, the coloring matters in general are apt to be called "dyes" elsewhere in this paper. The chemical structures of all the materials with a CI (Colour Index) number are given in the well-known Colour Index.

In the column headings, "% of blend" means the weight-% of dye in the dye-mica blend, and "days fed" indicates the time from the beginning of dye feeding to pupation (in all but four cases) and is, of course, a range due to differences among individual larvae. In the yield columns, 14/15, for example, means that 14 pupae or adults were obtained from the original 15 larvae. Under "Color", N means normal, as in the control group.

Following the Colour Index system, the dyes are grouped into chemical classes in the table.

RESULTS AND DISCUSSION

1. VITAL STAINING RESULTS

a. LARVAE

It should be noted that the judgment of external color was difficult because of the presence of mica-dye blend clinging to the integument.

Of the 56 coloring matters fed, 24 (four at 100% of blend, the rest at 5%) gave no color in larvae or pupae (or adults), and most of these allowed high yields of uncolored adults. Eight dyes (one at 100% of blend, the rest at 5%) were more or less doubtfully visible in the larvae and were not seen in the pupae. Of

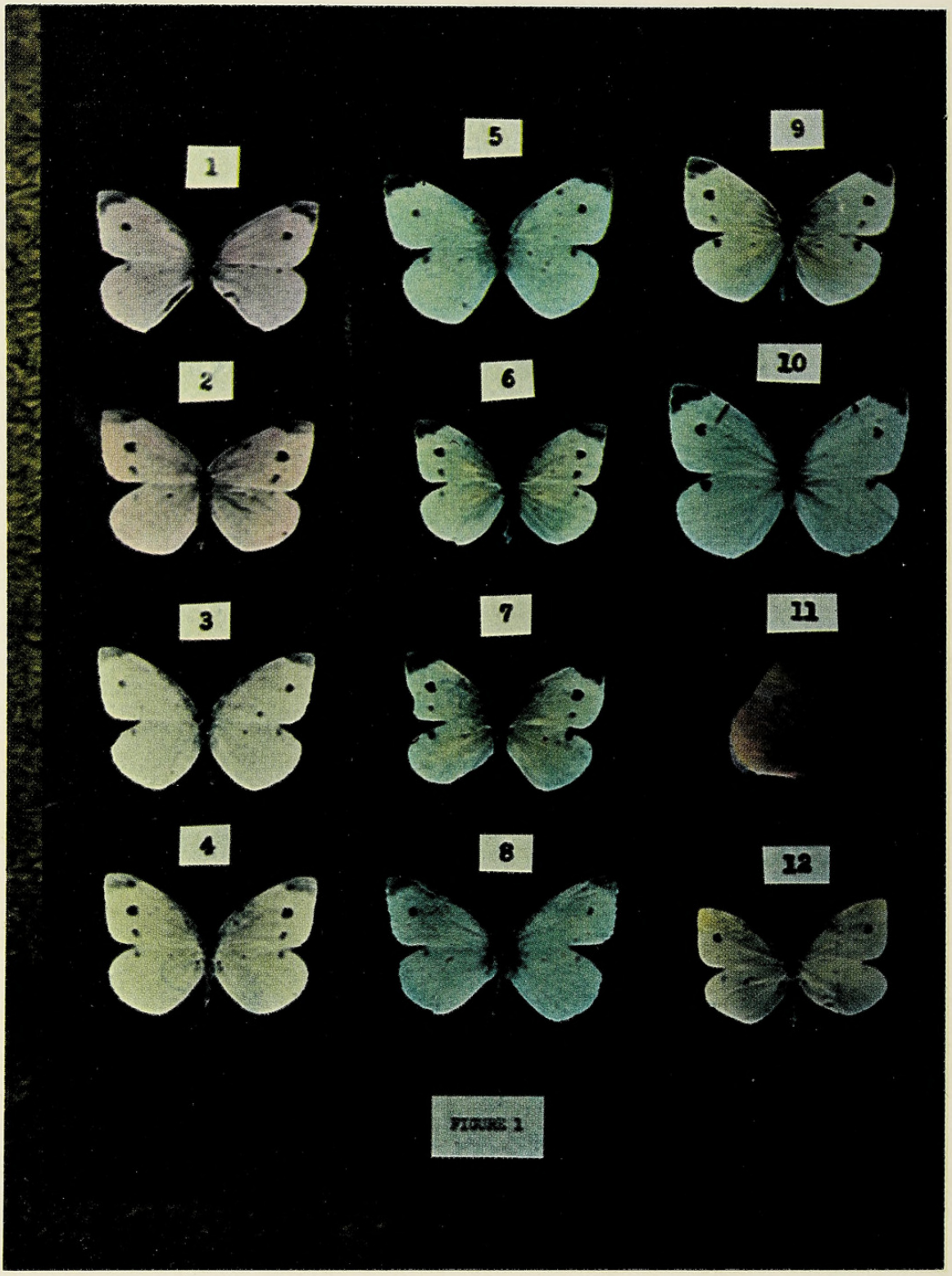

FIGURE 1

the eight dyes (at 5% of blend) so toxic or repellent that no pupae were produced only one did not give a real or suspected color in the larvae. In only two cases did color (orange-yellow, which is difficult to see in the green larvae, or very pale pink) appear in the pupae without being detected in the larvae. Only 14 dyes (three at 1% of blend, the rest at 5%) appeared in both larvae and pupae.

These results indicate that, in general, toxic dyes impart external color to larvae, which seems reasonable since toxicity should require absorption, and color will seldom appear in the pupa if it is not seen in the larva. Of the fifty-six dyes tried, thirty-one (including the two that gave colored pupae with no color having been noted in the larvae) gave real or suspected external colors in the larvae.

b. PUPAE

Results were more definite than with larvae because the powder-covered larval skin was shed to give an uncontaminated pupa.

Thirteen dyes at 5% of blend and three dyes at 1% gave external pupal colors ranging from faintly tinted to strongly colored. The following nine dyes gave conspicuous colors: Sudan black B (greenish-black), Sudan III (pink, especially on abdomen), Sudan IV (pink, especially on abdomen), auramine O (yellow-green), acridine red (pink), acridine orange (orange-yellow), neutral red (red), Nile blue A (blue), and brilliant cresyl blue (violet).

c. ADULTS AND THEIR EGGS

The effect of the dyes on external color (body, eyes, and wings) may be simply stated — Only Nile blue A and neutral red, discussed separately below, appeared externally in the adults.

EXPLANATION OF FIGURE 1 (cf. Table 1)

1. Male. Neutral red (5% of blend) fed 4-7 days.
2. Female. Neutral red (5% of blend) fed 4-7 days.
3. Male. Neutral red (5% of blend) fed 32 hours.
4. Female. Neutral red (5% of blend) fed 32 hours.
5. Male. Nile blue A (5% of blend) fed 3-4 days.
6. Female. Nile blue A (5% of blend) fed 3-4 days.
7. Female. Nile blue A (1% of blend) fed 4-9 days.
8. Male. Nile blue A (5% of blend) fed 32 hours.
9. Female. Nile blue A (5% of blend) fed 32 hours.
10. Male. Neutral red (1% of blend) and Nile blue A (1% of blend) fed 2 days.
11. Male, underside. Neutral red (5% of blend) fed 4-7 days.
12. Female. Celestine blue B (5% of blend) fed 6-11 days.

(Size Reference: Length forewing of specimen no. 10 is 21 mm.)

There is reason to suspect (see below) that Sudan black B may be transmitted to the eggs. This dye did give strong internal color, and some more or less intense internal coloration may have occurred with the other six dyes that gave strongly-colored pupae. Though larval feces were fluorescent, fluorescein and uranine failed to give external fluorescence in larvae, pupae, or adults.

The dyes had no obvious effect on the black markings of the wings, which are highly variable. There was a tendency in the last brood for the lower female forewing spot to be almost or quite missing whether or not dyes were fed. An example is shown in Figure 1 for celestine blue B. Curiously, the apical markings also are missing in this case.

d. CHEMICAL STRUCTURE AND ABSORPTION

In considering the dye-feeding results with wireworms, Zacharuk (1963) was unable to generalize vital staining properties in terms of dye structure. Monoazo, diazo, diphenylmethane, acridine, azine, thiazine, and thiazole types were all absorbed. The best dye for vital marking of wireworms was Nile blue (an oxazine, and the next best was rhodamine B (a xanthene).

In the present work (Table 1) dyes among the diazo, diphenylmethane, xanthene, acridine, azine, oxazine, and thiazine types were absorbed to give more or less strongly colored larvae and pupae, while toxic dyes (yielding no pupae) were found in the monoazo, triarylmethane, xanthene, methine, azine, and thiazine classes. These results are suggestive or qualitative only, of course, because dosage was not controlled in these experiments, which constitute essentially a screening program.

Because of the success of Nile blue A, three more oxazines were tried; none colored the adults, but brilliant cresyl blue gave strongly violet pupae. Similarly, following the success of neutral red, eight more azines were tested; some were quite toxic, while among the others only naphthalene red gave a hint of color in the pupa. Thus, among the azines and oxazines screened neutral red and Nile blue A were uniquely effective.

Methylene blue and Sudan III were absorbed by silverfish (Lindsay, 1940), and, in further agreement with the present work, carmine was not absorbed. Congo red, which was not absorbed by the *Pieris rapae* larvae, has been found not to be absorbed by the midgut of adult *Deilephila* (Roeder, 1953).

e. INDICATORS

Insects (Thysanura) have been fed indicators to show the pH of different regions of the gut (for example, Modder, 1962). In the case of wireworms (Zacharuk, 1962) phenol red stained the gut contents deep red, suggesting a pH above 8.4. In the present work, the excrement of larvae fed indicators was yellow-orange (pH 6.8-8.4) with phenol red, yellow-orange (pH less than 6) with brom thymol blue, and deep wine-red (pH above 6.8) with brom cresol purple. These results plainly are contradictory in defining the pH of the excrement. As seen in Table 1, the indicators had no efficacy as vital stains.

2. TOXICITY

The fact is that the larvae which were fed "toxic" dyes died before pupating, but it is difficult to distinguish between poisoning and starvation because, as has been pointed out (Ginsburg and Granett, 1935), some organic compounds may be repellent to larvae while others may be truly toxic. Injection might be necessary to make the distinction. In any event, rhodamine B, methylene blue, and methylene green were 100% fatal at 100% of the blend but harmless at 1%. Methylene blue, incidentally, in the nutrient medium is toxic to *Drosophila* larvae (David, 1963). Fluorescein was very toxic at 100%, but its sodium salt (uranine) was harmless at 1%.

The following dyes (at 5% of blend) were 100% fatal: Janus green B, crystal violet, brilliant green, Victoria green WB (malachite green), fuchsine Y, Nabor brilliant red 6B, phenosafranin, and safranin O. The following dyes (also at 5%) showed limited toxicity: Sudan black B, auramine O, acridine orange, safranin bluish, nigrosin, azocarmine B, azocarmine G, naphthalene red, Nile blue A, celestine blue B, and gallocyanine.

The theory that basic dyes are especially toxic to the animal organism is consistent with the results of Campbell (1932) with silkworms, e.g. malachite green, safranin bluish, brilliant green, and crystal violet were particularly toxic. Also, it was noted in other experiments with the silkworm (Ginsburg and Cavallito, 1936) that water-insoluble organic compounds are apt to show higher toxicity if they bear primary amino groups.

The eight dyes listed above as 100% fatal at 5% of blend all bear amino groups with substituents no larger than 2-chloroethyl. Of the eleven dyes that showed limited toxicity at 5% of blend, eight have amino, dimethylamino, or diethylamino groups, and the other three have disubstituted amino linkages. These facts agree with the generalization that amino and substituted amino groups

conribute to toxicity. However, many of the dyes harmless at 5% of blend, e.g. Bismarck brown Y and brilliant cresyl blue, are well endowed with amino and dialkylamino groups. Also, amino groups were not necessary for the absorption of dyes; of the nine dyes strongly manifested in the pupae two (Sudan III and Sudan IV) have only azo and hydroxyl groups.

Triarylmethane dyes are noted for toxicity, but two (see Table 1) had no effect even at 100% of blend (perhaps because they bear no amino, dimethylamino, or diethylamino groups).

In conclusion, no generalization is apparent except that the toxic dyes had amino or substituted amino groups but dyes with similar amino groups were not necessarily toxic.

Incidentally, some interesting deformities were noted in adults fed the partly-toxic (under the conditions) safranin bluish. These were (1) a dwarfed female (15 mm. forewing), (2) a female with deformed forewing, and (3) a male with eyes undeveloped and antennae, proposics, and adult palpi missing.

3. DYES OF PRINCIPAL INTEREST

a. NEUTRAL RED

Neutral red was the only dye of several tested that changed the external color of the silkworm (Campbell, 1932), and it was said not to be toxic to silkworms (Edwards, 1921) although it gave 80% mortality among wireworms vs. 20% for a control group (Zacharuk, 1963). The red color persisted for more than 40 days in wireworms when dye feeding was suspended but was eliminated within 18 days when silkworms were fed undyed leaves (Edwards, 1921).

In the present work, loss of dye from the larvae when dye feeding was suspended was indicated. The most effective way, therefore, to produce colored adults with a minimum of possible toxic effect may be to feed the dye only during the last two days or so of larval life. In the case of silkworms it was only necessary to feed the dye during the fifth instar to produce red cocoons (Edwards, 1921).

With *Pieris rapae*, the silken pupal girth was slightly pink. In the adult, the wing membrane of heavily-colored specimens was colorless, while the scales on wings and abdomen were pink (or, more accurately, violet-pink). The contents of the eye were dark-red instead of the normal dark-brown. As seen in Figure 1, increasing neutral red content in the butterfly is seen first in the eyes and abdomen (specimens no. 3 and 4) and later more and more intensely in the wings (nos. 1, 2, and 11).

The neutral red used in this work was the chloride (see Colour Index for formula) and was specified by National Aniline to have 70% minimum strength and 1.0% maximum water-insoluble content.

b. NILE BLUE A

This was shown by Zacharuk (1963) to be the most suitable dye for marking living wireworms internally and imparted more color (to fat) and was retained longer than neutral red (which stained tissues in general).

In the competitive experiment in which 1% each of neutral red and Nile blue A were present in the blend (see Table 1 and Figure 1, specimen no. 10), the adults showed only the influence of Nile blue A, i.e., they ranged from uncolored to pale-blue with blue-green eyes.

Nile blue A adults were colored internally as well as externally, the contents of the abdomen being blue and gray-blue.

The Nile blue A used in this work was the sulfate (see the Colour Index for the formula of the corresponding chloride) and was specified by National Aniline to have 70% minimum strength.

c. OTHER DYES

Brilliant cresyl blue seems promising for marking pupae (violet) because it gave strong coloration and was not at all toxic at 5% of blend.

Sudan black B was assimilated into the fat body of wireworms, coloring it greenish-blue to black (Zacharuk, 1963), and is a fat stain used for lipids (Roeder, 1953). In the present work it gave butterflies with no external color but whose abdomens were seen to be gray-blue internally on teasing open under the microscope. Some of the eggs laid by a group of females including some fed Sudan black B as larvae were blue-gray in color and produced pale blue-gray larvae (as opposed to very pale greenish in the normal case), but this suspected carry-over of the dye into the eggs needs confirmation.

CONCLUSION

It has been suggested (Campbell, 1932) that the staining of silkworm epidermis by neutral red might be applied to the indelible marking of insects for experimental purposes. The present work with *Pieris rapae* discloses dyes useful in marking larvae and pupae as well as adults (in the case of neutral red and Nile blue A). Neutral red seems particularly suitable for staining the wings of butterflies by means of feeding the dye, conveniently blended with mica or perhaps another extender, to the caterpillars.

Aside from the use of the dyes as a convenient marker, it might be of interest to note the effect of the colors on the behavior of adults. For example, *Pieris napi* males respond differently to the white form vs. the yellow form females (Petersen, Tornblum, and Bodin, 1932), and in line with this it might be interesting to observe the relative attraction of normal males to pink or blue females. Also, the concentration of neutral red, for example, in the eye might alter the response of the butterfly to various colors; red-eyed females might show different color preferences for oviposition than normal females (see Hovanitz and Chang, 1964). Many other experiments suggest themselves.

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