

DIFFERENTIAL STAINS OF INSECT TISSUES¹

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In entomological work there is a great need for stains which will pick out specific organs and tissues. This is especially true for the work of gross dissection and the recognition of the boundaries and shape and form of the internal organs. In the dissection of mammals the lungs are pink, the muscles a darker pink, the liver a brownish red, the kidneys a grayish red, the testes yellow, etc. Few anatomists realize the tremendous positive aid such differences of color give the research student. In the insects, nearly all internal organs and tissues are white so that color values in the recognition and differentiation of parts are absent.

This lack of color help has tended to limit studies of the internal anatomy of insects to the larger organs, such as the sexual, digestive, muscular and nervous systems. A review of the literature shows that the heart has seldom been observed even in some elaborate published studies of internal organs. The heart wall is one cell thick! This organ actually disappears in many dissections especially of preserved material.

This review of stains for specific tissues and organs is a first attempt to locate stains which when injected in dilute solution into the live insect color only specific organs against the general white background. This procedure would be classed as intra-vitam staining. It prepares the internal structure for gross dissection.

It was found necessary to develop a technique to include the handling of the subjects as well as the application of the material. One method has been worked out and will be explained along with the results produced by a number of stains in its application. In using a number of different stains it was found possible to determine to some extent whether those so-called "vital stains" from other fields of investigation could be so classified in entomology. In certain cases some were found usable that are not usually considered for viable tissue.

The use of the American cockroach *Periplaneta americana* for the experimental subject was due to its generalized structure and ready availability. For the purposes of a control other forms were used as will be explained.

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MATERIALS AND METHODS

EXPERIMENTAL ANIMAL.

The basic experimental subject was the American cockroach *Periplaneta americana*. Data were more easily obtained by using the adult form as the strength of solutions used was high and forms younger than six months of age did not survive except in a very small percentage. The sex of the insect did not prove to be a differentiating factor as was shown in a number of cases where the reaction to a stain was found to be identical in both the male and female forms. However, more males were used in the work because of the available supply and the sparing of some females for colony perpetuation.

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EQUIPMENT.

Small size battery jars served as retaining chambers for insects undergoing the tests. Each housed only those subjects containing one of the stains. The jars were marked as to the stain being used as well as the date of injection. From each were taken the subjects that had passed the time interval required, for each observation stage. These were examined, and then the data recorded.

Three materials were used for lessening the activity of the experimental insects during the injection of the staining materials and at the time of the observational dissection. One of these was chloroform, the first used and least satisfactory. Its use was discontinued mainly due to the high mortality rate in the roach population. If the insects were left in the anesthetizing chamber a minute more than the time required to cause the cessation of rapid leg movement, the possibility of recovery was only 50 percent. More successfully used was ether. Complete cessation of movement (external) could be awaited with an expectation of 95 percent recovery. Complete relaxation of the insect being used permitted the injection of the stain and the transfer to the housing jar with an additional few minutes before visible activity was again evident.

When a number of roaches were required at one time or when excessive excitement in the culture prevented the easy removal of desired specimens, carbon dioxide gas was employed. The entire culture was exposed for one minute. After that only some head and leg movements were visible and the desired specimens could be removed and the gas driven off the remainder to permit recovery. The effect lasted one to five minutes and then normal activity was again observed.

For injection, pipettes scaled to deliver 2-3 cc. were first used, but these were abandoned, because the amount of staining material proved to be excessive and the internal organs were often injured as well as the cuticular body covering. A hypodermic needle (1 cc.) using a small needle was the replacement. With this 0.1 cc. injections were made.

Injection of the stain by means of a hypodermic needle proved to be not only the most successful means of application but also the easiest. The point of injection used throughout was at the base of the third abdominal segment on the ventral side, a little off the center of the body. In the attempts to use immature forms under six months the piercing of internal organs proved a serious cause of death.

A binocular microscope was employed to study the internal structures and the observations recorded. The insect was still alive while the dissection and subsequent study were made and it was possible to observe the reactions of the visceral structure (as parts of the digestive system and the heart).

EXPERIMENTAL TECHNIQUE.

The first step after the insect was removed from the stock culture was to apply an anesthetizing agent, preferably ether, until all violent external movement ceased. This was accomplished by placing the animal in a closed jar containing a small piece of cheesecloth saturated with ether. Then after being placed on a small dissecting pan and held steady by the investigator's hand, the hypodermic needle was inserted at the base of the third abdominal segment, and the stain being used was injected.

After the injection was made, the insect was placed in a housing jar properly labeled as to type of stain used and the date. As will be observed, a definite time interval schedule was set up. These were one-half hour, one day, two days, and three days. These periods were used not only as an equalizing measure but also as a test of speed and viability. After retention over the time interval desired, the dissection was made and the gross results obtained.

Before observation began ether was again used; then upon removal to a dissecting pan the legs and wings (if present, on female specimen) were removed. The body was opened by a slit through the center of the ventral side from the

base of the head to the tip of the abdomen. The body was then spread open and pinned so the internal area was entirely exposed.

All the stains used were made up at the percentage of one gram of dry material used to 100 cc. of normal salt solution. This was a fairly high concentration for staining material and may play a definite role in those types upon which it was impossible to obtain data except over a very short time interval. The problem arises in this connection as to what dilution the stains might be carried in order to obtain like results, but this was not determined.

EXPLANATIONS

TERMS.

V-shaped—The heart wing muscles: color extending along tracheae from the heart region in shape of a V with the large end toward the heart.

Fractions—Refer to percentages of total amount or number of particular organ or group of same type of organs affected.

All stains tested are classified under four headings:

1. Outstanding Results.
2. Results Fair.
3. Lack Good Results.
4. Incompletes.

Each stain is taken in order and has under it all organs and tissues that showed results. Those organs and tissues not mentioned were found to be of the normal color and condition. Opposite this listing or following it is the description of the appearance after a definite time interval that was allowed. All the stains are classified in the same intervals of time.

The findings classified under *Incompletes* have been the results of death appearing at the time intervals described. Under repeated testing the mortality rate continued to appear so high, it was found impossible to gain results at the concentration of staining material used.

OUTSTANDING

BLUE DE LYON O.

Two days testing proved best for number of structures shown and variation of color. The gizzard and crop were blue tinged; the heart light blue outlined; malpighian tubules pale blue to lavender; salivary glands very pale blue; and small intestine pale lavender. The thorax muscles were blue near the dorsal side while the fat bodies were blue about the injection point, and the dorsal wall material was blue (pale) for a short distance on either side of the heart. In half an hour the organs shown were the gizzard, caecae, heart outline, tracheae (partially), malpighian tubules, and intestines; in one day were shown the gizzard, heart outline, crop, edges of caecae, salivary glands, thorax muscles and digestive tract; for three days only the caecae and heart outline showed.

ANILINE BLUE.

Here half an hour gave the best results. Scattered portions of the fat bodies were light blue; the thorax muscles grey; the dorsal wall material a dead white. The caecae and tracheae (in the thorax) had faint blue outlines. The gizzard was blue tinged, the heart faintly so; while the salivary glands had a faint grey-blue tinge. After one day less distinction but some color was in a few fat bodies, malpighian tubules, heart outline, while the dorsal wall material and femurs were colorless; two days presented caecae, heart and crop; and the third, the heart and tracheae.

AZUR II.

For structures half hour results are taken. Light lavender fat bodies, lavender outlined heart, and tracheae (color from heart) showed easily. The thorax muscles

were blue; salivary glands dark blue; caecae (swollen) blue; blue tinge on crop; and greenish blue on malpighian tubules. For color variance one day proved good. Here was easily seen the lower halves of the crop and gizzard, dorsal wall material, fat bodies, salivary glands, heart, and caecae; two days presented caecae, gizzard, intestines, fat bodies, salivary glands, malpighian tubules, and dorsal wall material; in three days was still shown the fat bodies, dorsal wall material, salivary glands, gizzard, and large intestine.

BRILLIANT CRESYL BLUE.

Here two days testing proved best for structures and color. The gizzard, caecae, salivary glands, large intestines were lavender while the malpighian tubules were a mixture of this and yellow. The dorsal wall material was dark blue; the fat bodies bright blue with lavender marks; the heart blue-tinged (not all through thorax); the femurs green; and thorax muscles bluish-green; except for parts mentioned the digestive tract was deep blue. With half an hour the large intestine and most of the other internal structures were colored; with one day the gizzard, caecae, intestine, half the malpighian tubules, crop, fat bodies, dorsal wall material, heart, and salivary glands were designated; likewise on the third day.

TOLUIDIN BLUE.

For structures and color one day timing proved most productive. Lavender was the color of the thorax muscles, salivary glands, while it tinged the crop and the gizzard. The tracheae were blue-tinged near the heart while the heart itself had blue outline varying from pale to dark. The ovaries were light blue and the fat bodies deep blue; the malpighian tubules and caecae green. In half an hour reactions were seen on fat bodies, caecae, salivary glands, gizzard, and heart; in two days thorax muscles, fat bodies about intestines, salivary glands, and dorsal wall material were clear; blue predominated with three days on fat bodies, salivary glands, and heart outline.

TRYPAN BLUE.

With this stain half hour timing showed the most structures and color variance. The salivary glands and ovaries were pale lavender; the caecae blue tinged; the malpighian tubules lavender tinged. After one day the stomach, tracheae, and heart were shown; two days only the heart appeared touched; this was the same on the third day as the second.

ROSE BENGAL.

Three days timing proved most effective for showing the greatest number of structures. The crop and gizzard were deep red; the caecae orange-red; the thorax muscles red streaked. The salivary glands were deep pink while the ovaries were pink. The dorsal wall material was light scarlet, and half of the malpighian tubules and fat bodies were bright scarlet. The heart was deep wine. Two days was best for color variation. Here appeared the crop, gizzard, caecae, heart, dorsal wall material, salivary glands, ovaries, malpighian tubules, part of the fat bodies, and femurs; with half an hour all the insides were colored; one day showed dorsal wall material, malpighian tubules (most), tracheae, and ovaries with some other scattered coloring.

RESULTS FAIR

BIEBRICH SCARLET.

The most results were gained from one day tests. The heart, fat bodies and dorsal wall material were scarlet; the small intestine red; the malpighian tubules bright orange; and the salivary glands bright pink. Half hour testing produces the most color. There showed up the malpighian tubules, digestive tract, salivary glands, thorax muscles, and heart; two days showed malpighian tubules, heart, and

light outlining of the tracheae; three days produced results on dorsal wall material, heart, malpighian tubules, and salivary glands.

GENTIAN VIOLET IMP.

After one day the greatest color variance and results were obtained. Violet predominated, showing in the crop, gizzard, fat bodies, heart, dorsal wall material, small intestine, and caecae. The salivary glands were a deep lavender and half the malpighian tubules were lavender. After half an hour there showed the crop, gizzard, heart outline, salivary glands, malpighian tubules and fat bodies; with two days were traces on crop and some fat bodies; like traces were seen on the third day on salivary glands, fat bodies, gizzard, malpighian tubules, and small intestine.

CONGO RED.

Two days work was the best for structure and color results with this stain. Dark brown flakes were scattered in the body cavity. The fat bodies and dorsal wall material were orange-brown colored; crop and gizzard dark red-brown; the caecae dark red with a dull red coating over the heart; and the salivary glands dull lavender. Half an hour produced results on salivary glands, digestive tract, fat bodies, dorsal wall material, and heart; one day on salivary glands and gizzard, fat bodies, crop, and heart; three days on heart, caecae, malpighian tubules, and salivary glands.

BORDEAUX RED.

With this, one day brought more structures to attention. Pink predominated, being on the salivary glands, crop, gizzard, small intestine, and outer two-thirds of the tracheae. The malpighian tubules were dull red (granular in appearance) as was the heart (near the tracheae). The thorax muscles had a few red streaks. Half hour testing produced more color variance. Here showed up clearly the heart, salivary glands, malpighian tubules, caecae, dorsal wall material, and thorax muscles; two days showed caecae, small intestine, and heart; while three days showed salivary glands, fat bodies, crop, and heart.

SAFRANINE O.

With this stain more structures showed up in a day. The pink of the upper half of the crop, dorsal wall material, heart outline, fat bodies, and gizzard showed as deep pink on the salivary glands and thorax muscles. The small intestine and caecae were bright scarlet; the malpighian tubules bright orange. The color variance was best in two days. The salivary glands, caecae, malpighian tubules, thorax muscles, small intestine, and heart outline were seen; with half an hour was seen the malpighian tubules, crop, thorax muscles, and caecae, salivary glands, dorsal wall material and heart outline; in three days was seen these: small intestine, fat bodies, thorax muscles, salivary glands, and heart outline.

DAHLIA B.

Half hour results showed more structures and color. Two-thirds of the fat bodies, malpighian tubules, and sides of the heart were lavender; gizzard, salivary glands, and lower end of crop violet. In one day could be seen the salivary glands, half the fat bodies, dorsal wall material, and thorax muscles; in two days the anterior half of the fat bodies, heart, caecae, and a few malpighian tubules; in three days salivary glands, fat bodies, caecae and small intestine.

METHYL VIOLET B.

One day proved the optimum timing. The fat bodies, malpighian tubules, and dorsal wall material were colored rose lavender; gizzard and small intestine light violet. The salivary glands were lavender with violet colored "veins," and the heart outline was violet. Half hour results were for salivary glands, crop, gizzard, fat bodies, heart outline, and malpighian tubules; two days for heart outline (in

the thorax), caecae, salivary glands, digestive tract, and fat bodies; three days for large intestine, malpighian tubules, and fat bodies.

LIGHT GREEN S F YELLOWISH.

Half hour results were best on structure and color counts. The ovaries were spotted with pale green; the malpighian tubules brilliant green colored; the gizzard dark green as well as the heart outline; the salivary glands pale blue. The crop, dorsal wall material, and thorax muscles (at base of legs 1 and 2) were green tinged. In one day we find malpighian tubules, heart outline, salivary glands, femurs, gizzard, large and small intestines, and crop; two days find salivary glands, malpighian tubules, heart, crop, thorax muscles and small intestine, tracheae and femurs; three days find heart outline, dorsal wall of heart, half malpighian tubules, large intestine, and thorax muscles.

FAST GREEN F C F

Half hour work produced the greatest number of affected structures. Bright green predominated as shown in the fat bodies tinge, salivary glands, gizzard, intestines, and ovaries. There were traces of green on the crop and malpighian tubules as well as the tinge on the thorax muscles. Half the caecae were green; the heart covered with dark green; and the dorsal wall material had a blue-green tinge. Two days produced more color variation, on salivary glands, femurs, dorsal wall material, crop, gizzard, heart, caecae, tracheal outlines, malpighian tubules, fat bodies, and intestines; one day showed on fat bodies, malpighian tubules, femurs, dorsal wall material, crop, gizzard, intestines, heart, caecae, and stomach; three days on fat bodies, salivary glands, thorax muscles, heart, femurs, base of antennae, caecae, malpighian tubules, crop.

CHLOROZOL BLACK E.

Three days was best for structures. The crop, heart, tracheae (V-shaped outline), and femurs were black; the malpighian tubules, caecae and gizzard were grey with the fat bodies a dark grey as also the salivary glands. The dorsal wall material was almost black while the intestines had a dark lavender tinge. One day work had more color variation. Namely, on crop, heart, caecae, dorsal wall material, fat bodies, malpighian tubules, and salivary glands; half an hour on crop, fat bodies, dorsal wall material, salivary glands, malpighian tubules; two days on fat bodies, crop, malpighian tubules, salivary glands, and heart outline.

AZUR I.

One day proved best for structures and color here. The salivary glands were a true blue, while the thorax muscles were light blue; the malpighian tubules ranged from light blue to deep violet; and both the heart outline and the tracheae were blue tinged. Both the fat bodies and dorsal wall material were pale greenish blue and the caecae and gizzard had faint lavender tinges. After half an hour was seen crop, salivary glands, dorsal wall material, fat bodies, caecae, and ventral nerve cord; after two days was seen fat bodies and dorsal wall material only; but three days had fat bodies, dorsal wall material, salivary glands, and large intestine (posterior end).

COTTON BLUE.

In this stain one day was the best for all results. Loose fluid stain was present in the body cavity. The gizzard was pale blue as was also the salivary glands and the crop (here dark blue streaks present). The fat bodies were blue (at the injection point), as also the caecae and small intestine. The heart was deep blue and the dorsal wall material blue tinged. In half an hour the heart, gizzard, dorsal wall material, and thorax muscles were seen; in two the gizzard, salivary glands, crop, caecae, few malpighian tubules, heart, fat bodies, and intestines were seen; while on three days testing only the heart, salivary glands, caecae and fat bodies.

FUCHSIN BASIC.

The results were brief and best shown after half an hour. The salivary glands, crop, and gizzard (lower half) were light pink; the caecae (half of them) were pink; the malpighian tubules dark pink. In one day was seen half the malpighian tubules, salivary glands, and stomach; in two days only the heart and its outline; then on the third day trials the malpighian tubules, fat bodies (near the digestive tract) and stomach tracheae.

CRYSTAL VIOLET.

Best results for structures and color were gained after one day. Light pink showed in the "veins" of the salivary glands, the stomach, and intestines. The fat bodies near the crop were light lavender, and the heart had a brown outline. Half hour results were on salivary glands, caecae, gizzard, and fat bodies; two days on stomach and dorsal aorta; three days on salivary glands, half the malpighian tubules, caecae, and intestines.

PYRONIN.

More structures were shown after one day. There was a pink tinge on the crop and half the fat bodies. The caecae were pale pink; the dorsal wall material deep pink; a few malpighian tubules deep peach; and the heart deeply outlined in pink (out V-shaped along tracheae). Two days showed color variance, in the dorsal wall material, salivary glands, half the malpighian tubules, stomach, fat bodies, and crop; three days produced fat bodies, dorsal wall material, ovaries, one-third of the malpighian tubules, heart and its outline; in half an hour all the insides (scarlet).

ALDEHYDE GREEN.

Three days was best for structures and variability of color. The female reproductive organs and second and third leg femurs were light green; dark green showed on the fat bodies, dorsal wall material, and heart. Blue-green, light for the thorax muscles and dark for the malpighian tubules showed well. The anterior end of the crop was black. The fat bodies were deteriorated; this condition showed also in one and two-day results. Half hour results were caecae, malpighian tubules, three-fourths of the fat bodies, digestive tract, heart, dorsal wall material, and salivary glands; one day were malpighian tubules, caecae, digestive tract, dorsal wall material, femurs, heart and thorax muscles; two days were digestive tract, malpighian tubules, dorsal wall material, femurs, thorax muscles, and heart.

BORAX CARMINE.

Half hour results indicated the most structures. Scarlet accounted for the dorsal wall material (rosy shade), gizzard, intestines, caecae; fat bodies, salivary glands, and heart went from rose to pink to dull red. Color variance was obtained with three days on heart, posterior end of crop, dorsal wall material and thorax muscles, caecae, and gizzard; one day showed leg muscles, thorax muscles, gizzard to end of the digestive tract, heart; two days showed digestive tract (gizzard on), heart, thorax muscles, and tracheae.

ACID FUCHSIN.

For structures one day was best for timing. The posterior end of the crop and the caecae were wine colored, and the heart and its outline was very deep wine. The dorsal wall material was pink; half the malpighian tubules dull pink. Tinges, pink on thorax muscles and scarlet on the intestines, were also present. Color changes predominated in three days work on thorax muscles, fat bodies, dorsal wall material, heart, intestines, salivary glands; half an hour resulted in fat bodies, heart, salivary glands, dorsal wall material, digestive tract, crop, and two-thirds of the malpighian tubules; two days resulted in fat bodies, digestive tract, dorsal wall material, gizzard and salivary glands.

LACK GOOD RESULTS

ORANGE I.

One day testing proved best for number of structures shown and color variation. The caecae and stomach were reddish; the salivary glands slightly brown; and the thorax muscles red-brown. After half an hour run these showed up, i.e., malpighian tubules, digestive tract, and heart outline; two days, only the heart (tinged); three days, the caecae and traces on dorsal wall material.

AURENTIA.

The best structure and color results were found after one day. Here the caecae and small intestines were reddish; the dorsal wall material and fat bodies had an orange-brown tint; the salivary glands were yellow as was the upper half of the crop (lower half, brown). Half an hour produced fat bodies, dorsal wall material, salivary glands, and caecae; two days produced fat bodies, dorsal wall material, salivary glands, thorax muscles, and caecae; three days produced caecae, small intestine, fat bodies, salivary glands, and heart outline.

THONIN.

One day results showed more structures and color. The large intestine was purple speckled; the fat bodies were dark blue at the injection point; the tracheae a faint lavender (near the heart). Half an hour had only thorax muscles; two days no results; three days had digestive tract, caecae.

ALIZARINE GREEN G.

Half hour timing was best for both number of structures and variation of color. Light green appeared in the salivary glands, dorsal wall material (traces), and fat bodies (near injection point); the crop was dull green. One day had only malpighian tubules and heart outline; two days only malpighian tubules; three days showed salivary glands, malpighian tubules, and heart outline.

ORANGE G.

More structures and color variance appeared after half an hour. The caecae and stomach were orange; the thorax muscles with an orange-brown tint, and the dorsal wall material brown-tinge. One day lacked color only in fat bodies and malpighian tubules; two days had fat bodies, dorsal wall material, heart, stomach and hind gut; three days had heart, intestines, caecae, and dorsal wall material.

PHLOXINE.

One day's work proved best for coloration and structure showing. Variations of pink dominated; from bright pink of the malpighian tubules and fat bodies (half of them); the pink of the salivary glands and caecae; to the pink outline of the heart. All the viscera were a rosy pink. Three days produced results on malpighian tubules, digestive tract, caecae, fat bodies, and heart.

QUINOLINE YELLOW.

In this stain the half hour group was best for number of structures shown. The fat bodies and dorsal wall material were yellow, while the digestive tract showed an even clearer, bright yellow. The thorax muscles and salivary glands were light orange. For color variations two days was best as on fat bodies, dorsal wall material, caecae, and heart; one day had fat bodies, dorsal wall material, heart, caecae, and digestive tract; three days had fat bodies, dorsal wall material, heart, and digestive tract.

BISMARCK BROWN.

With this stain three days proved the optimum with color and structures. The caecae and small intestine were red-brown and the heart had a red-brown tinge. Along the tracheae the dorsal wall material was brown tinged (in the

abdomen). The fat bodies were brownish pink at the point of injection. Half an hour had tinged fat bodies; two days, fat bodies, dorsal wall material, and tracheae; one day, fat bodies, caecae.

METHYLENE BLUE.

Here one day results were best for number of structures shown. Pale blue showed on the fat bodies and salivary glands while the ovaries had a pale blue tinge as the nerve cord (between the ganglia). The thorax muscles were deep blue as was the heart outline and the caecae tips. The crop showed blue (lower half); the gizzard a purple tinge. Two days showed the most color changes as on intestines, malpighian tubules (few), heart outline, tracheae, salivary glands, caecae, gizzard, and fat bodies; half an hour showed thorax muscles, crop, nerve cord, heart, half the malpighian tubules, ovaries, salivary glands, fat bodies, and gizzard; three days showed crop, gizzard, and fat bodies.

EOSIN Y.

Half hour testing was best for structure number and color. The color went from red of caecae (fluid filled), bright pink malpighian tubules, orange pink salivary glands, salmon pink fat bodies, to orange outlined heart and pink tinged digestive tract and testes. For one and two days all the insides were colored; for three days, only the caecae, dorsal wall material, half the fat bodies, some malpighian tubules.

MAGENTA ROTH.

Extent of coloration and variance showed up in half an hour. The malpighian tubules were scarlet, the fat bodies rosy scarlet, dorsal wall material bright pink, thorax muscles pink; and the caecae were deep wine in color. One day results produced salivary glands, dorsal wall material, and fat bodies; two days, intestines and caecae; the same occurred for three days.

AURAMINE O.

After two days more structures appeared stained. Bright yellow accounted for the crop, malpighian tubules, and female reproductive organs. In the thorax region the heart was deep yellow, while the fat bodies were a creamy yellow and the dorsal wall material slightly yellow tinged. After half an hour all the insides were bright yellow; one day followed suit; three days varied some on caecae, fat bodies, salivary glands, and heart.

RHODAMINE 6G.

Here two days proved best for structure staining and color change. The dorsal wall material and fat bodies were pink and the digestive tract had a slight pink tinge. Salmon accounted for the thorax muscles and ovaries with the malpighian tubules and caecae being lavender. Half hour testing showed fat bodies, malpighian tubules, caecae, dorsal wall material, salivary glands, and digestive tract; one day colored all except tracheae; three days, the intestines, fat bodies, dorsal wall material.

ORANGE II.

After half an hour more structures were shown. The intestines, caecae, malpighian tubules were bright orange; the dorsal wall material, and thorax muscles light orange. The fat bodies and salivary glands had a light orange tinge while the heart had a dark orange outline. The greatest color variation was shown after three days, this on intestines, gizzard, crop, ovaries, and heart; one day showed on malpighian tubules, thorax muscles, femurs, digestive tract, dorsal wall material, and heart; two days showed on heart, malpighian tubules, crop, caecae, and thorax muscles.

WATER BLUE.

Three days presented the best color picture on the greatest number of structures. The caecae and gizzard were blue; the dorsal wall material and femurs a light blue; and thorax muscles were deep blue. The heart had a blue outline (out V-shaped along tracheae). The malpighian tubules were greenish blue. Half an hour showed dorsal wall material, a few malpighian tubules, crop, and caecae; one day showed at least tinges of color on all viscera; two days resulted in heart outline, caecae, gizzard, and ovaries.

INDIGO CARMINE.

More structures were shown after half an hour. Most of the malpighian tubules and the crop were deep blue. The intestines and dorsal wall material were light blue as was the covering of the heart. A few fat bodies were blue tinged. After two days the range of color covered the greatest area, this on half the malpighian tubules, caecae, salivary glands, heart outline and femurs; one day showed one-third of the malpighian tubules, caecae, fat material; heart and malpighian tubules succeeded on three days.

ORCEIN.

In this case one day results proved best for all counts though evidence was meager. The caecae were a very faint pink; the small intestine lavender; and the edges of the heart were yellow tinged. Half an hour showed only the gizzard; two days, the intestines and caecae; three days showed heart outline and few malpighian tubules.

INCOMPLETES**METHYLENE GREEN.**

Half hour testing proved best from the standpoint of number of structures shown and color variation. The salivary glands were pale green; the heart was outlined through the thorax region with green. One day showed the malpighian tubules (half), and ovaries (bleached).

MALACHITE GREEN

Only half hour testing gave results. The salivary glands, caecae and thorax muscles were dark blue; the malpighian tubules and heart were dark green while half the fat bodies were brilliant green. Femurs showed green and tracheae had a narrow blue outline.

PHLOXIN RED.

This also had only half hour data. The dorsal wall material and fat bodies were rosy scarlet; the tracheae had a scarlet tinge; the malpighian tubules were bright red.

NILE BLUE SULPHATE.

Here one day testing proved best for both numbers of structures shown and color variance. The salivary glands, caecae, gizzard, and small intestine were light blue; the dorsal wall material and crop were deep blue; the femurs and malpighian tubules were green. The fat bodies went from deep purple to yellow-green while the heart was violet. Half an hour showed femurs, thorax muscles, digestive tract, fat bodies, dorsal wall material, salivary glands, heart, caecae, and malpighian tubules.

JANUS GREEN B.

Half hour results were best on structure count and color. Deep scarlet showed on the crop and dorsal wall material and combined with blue shades in the fat dies. The malpighian tubules were deep blue as also the caecae; the heart was

more a navy blue; the thorax muscles were blue tinged. Gizzard and salivary glands were lavender. One day results were femurs, thorax muscles, heart, malpighian tubules, dorsal wall material, salivary glands, crop, and fat bodies.

ERYTHROSIN BLUISH.

The half hour work was best in structure and coloration counts. The gizzard was true pink and the ovaries deep pink tinged and a slight pink tinge on the digestive tract. The fat bodies varied from deep to light rose; the salivary glands a deep rose. The caecae were scarlet and the malpighian tubules orange-red. One day presented caecae, heart, thorax muscles, dorsal wall material, malpighian tubules, salivary glands, and fat bodies.

VITAL RED HR.

One day information proved best for structure and color showings. Brilliant scarlet was on fat bodies, gizzard, caecae, malpighian tubules; a duller scarlet for thorax muscles, dorsal wall material, and femurs. The tracheae and salivary glands were pink, while the heart was dark pink with flakes of scarlet lying over it. The intestines had a reddish tinge. Half an hour produced fat bodies, heart, and most of the other viscera.

PO. COCHINEAL.

Half an hour was best with pink tinged small intestine (near the injection point) and dorsal wall material. One day had scattered dull green spots on salivary glands and fat bodies.

RECOMMENDED STAINS FOR PARTICULAR TISSUES

- 1) Heart—Toluidin Blue ($\frac{1}{2}$ hour); Trypan Blue (1, 3 days).
- 2) Tracheae—Fuchsin Basic (3 days); Borax Carmine (2 days).
- 3) Ventral Nerve Cord—Azur I ($\frac{1}{2}$ hour).
- 4) Gizzard—Acid Fuchsin (2 days); Azur II (2 days).
- 5) Caecae—Aldehyde Green (1 day); Acid Fuchsin (1 day).
- 6) Salivary Glands—Brilliant Cresyl Blue ($\frac{1}{2}$ hour, 2 days); Trypan Blue ($\frac{1}{2}$ hour).
- 7) Crop—Bordeaux Red (3 days); Blue de Lyon O (1 day).
- 8) Fat Bodies—Methyl Violet B (3 days); Azur II ($\frac{1}{2}$ hour, 2 days).
- 9) Thorax Muscles—Safranin O (2 days); Toluidin Blue (1, 2 days).
- 10) Malpighian Tubules—Azur II (2 days); Light Green SF Yellowish ($\frac{1}{2}$ hour, 1 day).
- 11) Dorsal Wall Material—Toluidin Blue (2 days); Aniline Blue ($\frac{1}{2}$ hour, 1 day).
- 12) Digestive Tract—Congo Red ($\frac{1}{2}$ hour); Aldehyde Green ($\frac{1}{2}$ hour).
- 13) Hind Gut—Azur II (3 days); Brilliant Cresyl Blue ($\frac{1}{2}$ hour).
- 14) Ovaries—Trypan Blue ($\frac{1}{2}$ hour); Aldehyde Green (3 days).

STAIN MANUFACTURERS

Coleman & Bell Co.	Norwood, Ohio
Dr. G. Grubler & Co.	Leipzig
National Aniline & Chemical Co., Inc.	New York, N. Y.

LITERATURE

- Conn, H. J. 1940. *Biological Stains*, Fourth edition, Biotech Publications, Geneva, New York.
- Emig, W. H. 1941. *Stain Technique*, Science Press Printing Co., Lancaster, Pennsylvania.
- Guyer, M. F. 1936. *Animal Micrology*, Fourth edition, University of Chicago Press.
- Lee, Bolles. 1937. *The Microtometist's Vade-Mecum*, Tenth edition, edited by Gatenby and Painter, P. Blakiston's Son & Co., Inc., Philadelphia, Pennsylvania.
- McClung, C. E. 1937. *Handbook of Microscopical Technique*, Second edition, Medical Book Dept., Harper & Bros., New York.