MARKING BIRDS AND MAMMALS WITH DYES FOR FIELD IDENTIFICATION PURPOSES

Albert J. Anderson

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By

Albert J. Anderson

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TABLE OF CONTENTS

INTRODUCTION

Recent demands are becoming increasingly urgent for a better method of marking live birds and mammals for field identification. In response to these demands an investigation of the possible application of chemical dyes has been initiated.

Several methods of marking have been tried with varying degrees of success and it is evident no one method is entirely satisfactory. Marking methods tried have been banding, tagging and various kinds of mutilation. In this study, a method of chemical dyeing has been developed and appears to offer a better solution to the problem.

This study, still unfinished, is in the nature of a progress report.

PROCEDURE

A thorough search of the literature and correspondence with various dyestuff manufacturers showed clearly that relatively little information pertinent to the use of dyes in marking live animals was available. Several of the dyestuff manufacturers cooperated by recommending and furnishing dyes, which, in their opinion, offered the greatest possibilities for experimentation.

During the preliminary experiments, the applications of these dye materials were limited to pieces of white fur and white feathers. In this way unnecessary handling of live animals was avoided.

The number of live birds and mammals used in the experiments were necessarily limited, because time for live-trapping wild animals was lacking. Therefore, the species used were those most easily obtained and capable of enduring captivity. Cottontail rabbits trapped in the field, ringneck pheasants obtained from the State Game Farm at Mason, white mice and white rats secured through the courtesy of several experimental laboratories at the University were all subjected to marking experiments with chemical dyes.

Necessary records were kept for the kinds of dyes used, how applied, and the effects of each upon the materials. Additional notes were taken upon the resistence and duration of the color and upon distance that it was visible under field conditions.

BRIEF HISTORY OF PREVIOUS WORK

Workers interested in marking live animals in the past have used a variety of methods by which they hoped to identify the individual at some future date. Early writers mentioned the use dyeing, painting, imping of colored wing and tail-feathers and many other methods including mutilation of body parts.

Later work supplemented the first crude attempts at marking by a more intensive application of each method. One of the outstanding pieces of research was carried out by Wilber K. Butts (1927) who used dyes as an aid to studying bird ranges. Butts (1930) concluded from experiments in his "Study of the Chickadee and Nuthatches by Dyes and Paint", that dyes are useful for a period of 10-14 days and a maximum of three weeks. The following table after Butts summarizes the work.

Table I

Species	Stain Used	Period of Time In days	Condition of Color
Tree Sparrow	Artist's oil color	9	Bright
" "	Fuchsin	15	Faint
" "	"	16	Bright
" "	"	22	Very faint
" "	"	23	Faint
Chickadee	"	18	Faint
"	Artist's oil color	52	Faint
Junco	" " "	14	Faint
1 Downy Woodpecker	Fuchsin	11	Bright
1 Downy Woodpecker	"	34	Very faint
1 Downy Woodpecker	"	54	Practicall gone
2 Nuthatches	"	10	Bright
2 "	"	26	Bright
2 "	"	42	Very faint
3 "	Artist's oil color	32	Bright
3 "	" " "	61	Very faint
3 "	" " "	90	Gone

Note: The terms used above to illustrate the condition of the dyes at the last observation are interpreted to mean:
Bright - easily recognized, enough to attract ones attention, and color nearly the same as when applied.
Faint - color faded considerably, identified fairly easily.
Very faint - color faded so that it is recognized only under ideal conditions of light and distance.

From the preceding table it is noticeable that only one dye offered possibilities for marking. A list of the dyes and other materials that gave no appreciable results is as follows: silver nitrate, iodine, picric acid, indigo, orange G, pontamine yellow, pontamine red, acid fuchsin, basic fuchsin, Congo red, methyl blue, methyl green, Hoffman's green, anthaflavone GC, thio indigo red B, identhrene golden orange G, and artist's color. In all the experiments Butts used only one method of application. Dyes were dissolved in alcohol if it were possible, and if not they were dissolved in water. Artist's colors were mixed with carbontetrachloride.

In general, experimentation with dyes has been upon birds, however, some efforts to use dyes on mammals have also been tried.

A hair dye was tried by Emmett T. Hooper of the University of Michigan Museums. His work was limited to one dye called jet black. This dye is one which produces its color by oxidation of a coal tar product. Hooper's method of application was provided for in directions obtained with the product.

A deep black color was produced on the fur of three different chipmunks. It was found that some of the dyed hair still remained on the animals in the form of small patches of black six months after application. Skins of the chipmunks used in the experiments are preserved at the museum for reference.

Other work done upon mammals may also be found in a method developed by R. E. Trippensee (1940). His technique involves the use of colored discs made of a plastic called Pyralin. These discs are attached to the ears of mammals by means of monometal clips. Birds are also marked by a similar method of fastening the markers upon the feather vane.

THEORIES OF DYEING

An understanding of the different theories explaining the phenomenon of dyeing is essential if one is to appreciate the difficulty involved in dyeing. Of the five accepted theories, two are generally credited with explaining most dyeing processes. However, with the progress being made in new fields of dyeing, each theory has some value in helping one understand why a material is dyed.

A strictly mechanical explanation of all the dyeing processes is far from being satisfactory because it fails primarily to recognize the complex nature of chemical reactions. Thus, it becomes necessary to understand the chemistry of dyeing.

The chemical theory was advanced from the belief that animal fibres possessed more acid and basic properties, than vegetable fibres. Therefore animal fibres have a greater affinity for dyestuffs than plant fibres. And likewise, dyestuffs had the same properties and were able to absorb acids, alkalies and certain salts from their solutions. In this manner dyestuffs produced color by combining with the fibre. In a like manner, the dyeing of wool is believed to be due to a salt formation between the fibre and dyestuff. Recent research along this line tends to substantiate this belief. An analysis of the measurement of heat evolved by the reaction of alkalies and acids in the dyebath, lead chemists to believe that the dyeing of wool is a process associated with salt formation.

Another theory that has received much attention is the electrical theory. This theory is explained by the fact that positive and negative charges are found on colloidal particles and their union by the attraction of opposite charges result in the permanent condition of the color upon the fibre. It has been conclusively demonstrated that negative charge and the ability of a fibre to absorb dyestuff are definitely related to temperature (Horsfall and Lawrie, 1927).

The maximum negative charge on cotton, silk and wool is at 40 degrees C. correspondingly the maximum absorption of dye by fibre was reached at the same temperature 40 degrees C. From this we might conclude that temperature controls the efficiency of the dyeing process.

The colloidal theory is closely related to the above theory in that electronic charges are the primary reason for both theories. However, due to the union of dyestuffs with fibres carrying an opposite ionic charge and producing color of a permanent nature. Reactions of this sort also take place in the presence of electrolytes, that is, solutions carrying a charge.

Special consideration was given to the composition of fibres in the Solid-Solution theory. It was explained that fibre acted as a solvent for the dyestuff, and was considered better than the medium water. It was further pointed that keratine and fibrine are good solvents and cellulose is poor. This explains the relative affinities of plant and animal fibres for dyestuffs and is unique in that it explained the reason for artificial silk having such a poor affinity for acid and basic dyes.

A more complete discussion of the different theories is found in "The Dyeing of Textile Fibres" by Horsfall and Lawrie (1927).

DYESTUFFS

Dyestuffs are classified into three general kinds, mainly, Natural, Mineral, and Artificial Organic. Natural dyes are those dyes found in nature in plants. Some of the better known dyes of this class are, Brazil wood, Indigo, Cuba wood or Fustic, Cutch or Gambier, Logwood, Madder and Berberine, this latter being the only natural basic dye.

The Mineral dyestuffs are Iron-Buff, Khaki, Chrome Yellow, and Prussian Blue. For a complete discussion of the Natural and Mineral dyes refer to the encyclopaedia Americana.

The Artificial Organic dyes are rather recent in development. Perkins in 1856, working under Hofmann in Germany, discovered that the oxidation of aniline produced a beautiful violet of great tinctorial powers. Thus, the discovery of Mauve began a new era of dyeing. Up until this time, only the natural dyes and the mineral dyes were used. The new field offered many new shades, greater fastness to light, washing, etc. Ease of application was the most notable factor. It is significant that these same dyestuffs offer the best application to the problem of dyeing live birds and mammals. As all dyes used in the experiments were samples from companies that manufactured these products, a list of these dyes will be found useful and appear in table II on the following page.

A classification of the Organic dyes most commonly used by the dyers is given below:

1. Basic dyestuffs
2. Resourcine dyestuffs
3. Acid dyestuffs
4. Mordant dyestuffs and true alizarine

Table II

Dyestuffs Used in Experiments

Dyestuff	Class	Company
Rhodamine 6 GDN extra	Basic color	Du Pont
Rhodamine B	Basic	"
Sulfogene Brilliant Green 2 G	Sulfur color	"
Orange II Conc.	Acid color	"
Anthraquinone Blue S. E.	Acid color	"
"Polyform" Scarlet RF	Direct color	"
"Pontamine" Diazo Green 3G	Developed color	"
Auramine Conc.	Basic color	"
"Pontacyl" Fast Violet 10B	Acid color	"
"Pontamine" Fast Pink BL	Direct color	"
"Sulfogene" Carbon 2BCF	Sulfur color	"
"Ponsol" Blue GD Double Powder	Vat color	"
Fuchsin Powder Special	Basic color	Nat. Aniline
Rhodamine B	Basic color	" "
Brilliant Green B crystals	Basic color	" "
Methyl Violet 2 B Powder	Basic color	" "
Calcozine Yellow Ox (Auramine)	Basic color	Calco Chem. Co.
" Red Y Ex. Conc. (Safranine)	Basic color	" " "
" Red BX (Rhodamine)	Basic color	" " "
" Blue GXX (Methylene Blue)	Basic color	" " "
Napthol As-Sw With R Salts	Azoic	Amer. Aniline
Fast Scarlet R Salt		" "
Fast Red GL Salt		" "
Bordeaux GP Salt		" "
Fast Blue B Salt		" "
Fast Black B Salt		" "
Aerosol OT Aqueous 10%	Wetting agent	Amer. Cyanamid

The above dyestuffs can be obtained from the addresses listed below:

E. I. DuPont de Nemours & Co.
Organic Chemicals Department
Wilmington, Delaware

American Aniline Products, Inc.,
50 Union Square
New York, New York

Calco Chemical Division
American Cyanamid Co.,
Bound Brook, New Jersey

Amer. Cyanamid & Chem. Co.,
30 Rockerfeller Plaza
New York, New York

National Aniline & Chem. Co.,
40 Rector Street
New York, New York

5. Direct cotton dyestuffs
6. Sulphur dyestuffs
7. Azoic dyestuffs
8. Vat dyestuffs

The above classification is the basis for discussion that it to follow.

METHOD OF PROCEDURE

Since the problem involves different materials than those used in the commercial dyeing processes, the dyeing of live birds and mammals necessitates changes in methods of application. To prevent pain to the animals we must guard against a number of obvious features. The hot or boiling dyebath is automatically eliminated, and the use of strong acids, caustic alkalies and poisonous compounds must be avoided or these products handled with great care. Prolonged treatment makes the application impractical; therefore, speed is essential to application. Thus we find that the use of information obtained from the commercial practices for different dyestuffs serve only as hints and specific proceedings had to be developed.

Killing Agents

This term is misleading in some respects as it is not synonymous with the definition used in histology, but is used in the sense of preparing the fibre rather than fixing it. It may be defined as any chemical used in dyeing, to clean, degrease, and prepare the fibre for the application of the dyestuff. A killing agent produces mechanical changes as well as chemical changes in its application to the fur and feathers. Its main function is to soften the tough horny nature of the materials that are naturally resistent to water solvents. We find that acids have a swelling effect and cause the cuticular scales to extend outwards from their normal position, thus aiding in the penetration of the dyestuff. Sodium hydroxide tends to dissolve fur, and should be used cautiously for this reason. In my work I have considered the use of killing agents the most important method of producing results. As the killing agent is first applied to the material it should be given first consideration.

The problem of using a killing agent centered itself around using a very strong agent under control, to try to produce similar results obtained by commercial methods. For this reason, sodium hydroxide was used intensively.

Bleaching Agents

The use of bleaching agents was first tried because their action is classed as a killing process. Although warned by manufacturers that no degree of success could be expected, some consideration was given to their use. It was hoped that they could be combined with the dyestuff in the dyebath and be applied in this manner, but time required for bleaching eliminated their practical use.

It was found that their effects were negligible in destroying the pigment in fur and feathers. On feathers some of the stronger solutions resulted in a complete disintegration of barbs and barbules. Bleaching agents tried were as follows: hydrogen peroxide, calcium hypochlorite, potassium permanganate, commercial

Chlorox, and oxalic acid, which is a reducing agent rather than a bleaching agent.

Wetting Agents

A wetting agent as defined by Sluhan is any substance added to water or solution of various kinds, that enables the fibre, powder or substances, to be wetted more easily than would otherwise be possible. C. A. Sluhan (1940) defines wetting as "the phenomenon of a liquid and a solid coming into contact so as to form a liquid-solid interface". It is considered complete when the contact angle between the two surfaces is zero. From this we can say that in general, the lower the interfacial tension, the more rapid is the wetting process. Sluhan, 1940, defines interfacial tension as "the surface tension at the interface between two immiscible liquids or a liquid and a solid". One of the most valuable effects created by the use of wetting agents in dyeing, is the swelling of the fibre which in turn is due to the speed in which the fibre is wet. This rapid swelling of the fibre increases the penetration of the dyestuff, which is in direct favor with the purely mechanical theory of dyeing.

Another desired effect of the wetting agent in the current problem is reduction of surface tension aiding in the speed of dyeing caused by the limitations of a water solution. In this manner it is possible to obtain better effects with a ten percent solution of the wetting agent than could be obtained if a 75 percent alcoholic solvent for the dyestuff were used. This means that dyestuffs not easily soluble can be made so, and that dyes not soluble in alcohols or similar solvents can be used with a wetting agent applied to the solution. Thus we find the use of a good wetting agent is advantageous in obtaining speed in dyeing.

In selecting a wetting agent it must be noted the kind of wetting agent needed. If we are going to use basic dyes it means that the wetting agent must be of the cation type, and likewise, if we are using acid dyes, the anion type must be used. This selection is necessary from the fact that acid dyes are similar to the anion surface active compounds, and that the color ion carries the negative charge. And likewise the basic dye color-ion carries the positive charge. It follows that if the wrong type is used with a selected dyestuff, a precipitate will be formed upon the mixture of the two substances. With the proper wetting agent for the dyestuff, the liquid remains clear. The selection of a wetting agent must be correlated then to the kind of dyestuff that we intend to use.

The oldest known agent is soap, which is very effective, but it has the disadvantage that it cannot be used with acids, because in combination with them it produces a gummy, sticky substance.

Commercial products are now on the market that have remarkable powers of wetting a material. In this respect, I limited myself to one product, namely Aerosol OT. This product is produced by the American Cyanamid & Chemical Corporation.

Effects Upon Fibre:

Although we are primarily interested in the effects of dyestuff and chemicals upon hair and feathers, some of our information is obtained from fibres dyed commercially. The effect has been determined primarily by the use of the microscope. In this respect it must be remembered that there are certain limits that can be reached in the examination of the material.

Wool presents the most interesting data, as it is most nearly related with the general method of dyeing fur. Wool is composed of the elements, hydrogen, oxygen, nitrogen, and sulphur. The composition of wool is likened to that of horn. The complex protein keratine, composes the greater percent of the wool fibre. Keratine is an insoluble compound having definite chemical characteristics that are taken advantage of in dyeing. In wool there is found languginic acid which plays an important part in the dyeing of wool as the acid has the power to precipitate coloring matters and mordants.

Wool differs primarily in structure from silk in that it has two and sometimes three kinds of cell structure. Silk has none, as it is purely a secretion. Chemically it is the same, but possesses no acid. Wool has the typical central medulla of large round cells forming a central core. Just outside of the medulla is found the cortex that contains the cell pigments. This portion of the fibre is made up of spindle-shaped cells. The outermost layer consists of horny scales overlapping each other and projecting upwards. The edges of the overlapping scales have roughly serrated margins.

All hair has this same general structure, but may vary upon the same mammal and between species. Two types of hair compose most fur, guard hair and fur hair. Most noticeable in the variations is the arrangement and size of the cuticular scales and the difference in the size of the medullary cells. Some hair has no medulla. The character of hair having the scaley structure is one of the most important facts in the dyeing process. Control over these scales by chemicals and killing agents governs the success of the dyeing operation to some degree. Thus in wool and other animal hair there is a penetration of the dyestuff, while silk has the dyestuff deposited as a film upon the surface.

It has been pointed out that killing agents and wetting agents have the characteristic of effecting the cuticular scales and therefore should be used in almost all dyeing operations. In addition to this fact we must consider the possibility of dyes penetrating into the cells of the cortex of hair. Deeper penetration of the dyestuff is desired, but is rarely attained.

Feather structure varies considerably from that of hair. The mechanical structure of feathers is complex and is responsible for some color sensations, feathers having color of two kinds, those due to pigment, and those due to structural properties. Pigment colors depend upon the chemical nature of the material and are due to the absorption of certain wave lengths by the molecules. Structural colors depend upon or are modified by the physical arrangement of matter. Color is produced by prismatic structures made by the arrangement of the various parts of the feather. Chief among these parts is the barbule.

Bancroft - 1923, classified feathers into two classes, those having their color changed by the addition of a liquid of the proper refractive index, and those that are unchanged by the addition of a liquid. There are two reasons for the whiteness in feathers: first, the effect may be due to surface refraction of light being scattered by a difference in indices of refraction. Second, the effect may be due to a structural phenomena such as cells filled with juices or the presence of air spaces within, thus scattering the light very completely, giving the sensation of white. In both cases the white sensation is produced by a multitude of minute surfaces of optical inhomogenity. The former may have their color destroyed by the addition of balsam or cresol; the structure becoming practically invisible. Feathers of the second type are unchanged due to the structure. This type is found on the shaft of the feathers of white turkeys and the domestic hen. The shaft has a keratinous sheath that is fibrous, consisting of many elongated cells packed closely together. Since the whiteness is due to two solids, the addition of a liquid will not alter the effect.

Blue feathers may also be classified as two types, non-metallic and metallic. The non-metallic are structural colors, and are due to minute air-bubbles in the horny mass of the feather. The blue color is, in structural feathers, due to the transmission of light. An example of this blue is called Tyndall blue and is commonly seen in cigarette smoke. The smoke, when viewed from the top is white, when seen from the side, it is bluish. The sky is another example. Common birds having Tyndall blue feathers are the Bluejay, Purple Gallinule, and the Indigo Bunting. Metallic blue feathers are due to the presence of pigment. The

The color green was found by Krukenberg (1881) as being due to structural blue and yellow pigment. Thus, if we add yellow dye to a blue feather of this structural type, green should be produced. The green color is located in the barbs of the feather and is similar to blue feathers in this respect. The green feathers are the same as blue except that they have yellow pigment in the outer sheath of keratine, while blue has the colorless keratine sheath. Other colors in feathers are due primarily to the presence of color pigments.

ANALYSIS OF DYEING EXPERIMENTS

A list of the dyes used in the experiments has already been given in Table II, and only those that have shown results will be discussed at this time.

Basic Dyestuffs

With one exception, Orange II, the basic colors have been found to offer the best success. The method of application offering the best results is the use of an acid dyebath. Basic dyes have been described as being fugitive, that is, their color fades rapidly. However, they appear to be the only practical dyes because of their high affinity for animal fibres. The desired affinity has made the application of these dyes comparatively simple.

Acid Dyestuffs

The acid dyestuffs tried in the experiments are limited to three. Orange II conc. gave the best results from the records on mice in Table V. Orange II is also applied from the acid dyebath although it is possible to do so from the neutral bath. The other two dyestuffs gave poor results, only the Fast Violet 10 B dyed the chicken feathers satisfactorily. Upon live birds neither were good.

Mordant Dyestuffs

Few experiments were carried out for the possible use of this class of dyestuff, as none of the companies manufacturing dyestuffs would recommend it. The only experiments carried out was an effort to determine, what was first thought to be the effects of a mordant upon dyed feathers. It was found that bright streaks of the color were present upon one or two of the barbs, and as the feathers were dried in contact with a metal screen over a radiator. It was thought that it might be due to a mordanting effect. It was concluded that this was not the case, because the same results were obtained on feathers not dried in contact with metal.

Other reasons for the avoidance of this kind of dyestuff was the prolonged soaking necessary with a mordant. Prolonged heat is another disagreeable factor considered. It is possible to mix the mordant with the dyebath and apply it in one operation, but this method does not apply well to animal fibre.

Sulphur Colors

Sulphur colors were not used as they are primarily a dye developed for plant fiber. However, some trials were made with a sample of the dyestuff with different killing agents. Each proved unsatisfactory.

Direct Colors

This too is another dyestuff used upon plant fibres, and has a poor affinity for animal fiber. Trials made, produced negative results.

Vat Color

The use of dyes applied by the vat system upon textile fibres requires prolonged boiling, which is inimical to the problem under investigation. Some trials were given the dye, but all gave negative results.

Developed Color

This type of dyestuff requires the use of a special developer, which is entirely impractical to apply to this problem. A sample of the dyestuff was tried, but without the aid of the developer, it was of no value.

Azoic Dyestuffs

This distinguished group of dyestuffs are not applied as dyes but are actually produced within the fibre itself. They are also known as "ice colors" as ice is used in their manufacture, the ice is used in preparing the second component of the dye called the R salts or the diazo component. The base (first component) of the samples used is mixed according to directions, added to the fur or feather that has been previously cleaned, after a period of soaking, the R salt is added. The color is purely a chemical reaction between these two components.

Trials with this method of producing color were not encouraging. The full development of the color was not obtained upon the white hair of rats and mice. Intense colors, however, were produced on dead feathers.

DYESTUFFS PRODUCED BY OXIDATION (ALSO AZOIC)

Dyestuffs of this class can be applied on fur as shown by Hooper in his experiment upon chipmunks (see page 4) but have been eliminated from the study as being impractical. The colors produced by this method are attained by using poisonous chemicals to both man and the animal. Another reason for avoidance is that "flash" colors are not obtained.

For a complete discussion of these dyes refer to T.R.V. Parkin (1934), E. Beeley (1933) and W. E. Austin (1922). These references are complete reviews of commercial application of colors to fur and feathers.

Drawbacks in this method are the exactness to which the directions must be carried out and the trouble involved in mixing. Solutions mixed must be used fresh as it was noticed that they were relatively unstable upon standing.

The method recommended by the manufacture for the product tried produces poor results upon hair, the chief being its susceptability to rubbing. On sample feathers excellent results may be obtained, providing the reaction is let go to completion. On the live bird, however, it does not seem to do this as well, however, the effect of air upon the treated hair before adding the R salt may have accounted for it.

Wallwork,(1929) reports that satisfactory results for the use of Azoic dyes on fur have been found. This method involves the use of a soap-soda solution instead of the sodium hydroxide solution. The method also involves too much time to be practical for its application upon the live specimen. It has one possible advantage and that is the increased affinity that it has for the fibre when the soap-soda solution is used. He reports that shades are as bright as those from acid colors.

METHOD OF APPLICATION

Solvent

I have found that for this purpose, distilled water offers excellent results. Accordingly I have used distilled water in all of the experiments for the actual mixing of the dyebath. For washing and rinsing the tap water was sufficiently pure. If only hard water is available, the addition of a small amount of acetic acid (2%) is helpful.

Temperature of the Dyebath

The dyebath was heated until it boiled. After cooling to about 180 degrees Fahrenheit, the dye was added to the specimen that had been previously cleaned and the killing agent added. No noticeable difference was found whether the temperature at the time of dyeing was 150 degrees or 180 degrees. It must be noted that if the dyebath were added at temperature over 180°F, trouble might occur through injury to the skin. The actual temperature on the hair or feathers is considerably less as the colder liquids left from the killing process is responsible for the reduction.

The advantage of keeping the dyebath as warm as possible is to maintain the maximum penetration and the swelling effect caused by the use of hot liquids. In no case was there any serious symptoms of shock recorded by the action of the animal during the dyeing process.

Application of Killing Agent

It was found that by eliminating the washing process with soap and water, that the use of sodium hydroxide cleaned and killed at the same time. In this manner one step in the dyeing operation has been saved. The solution is best applied by using a small piece of natural sponge, one inch square, held by a pincher-type clothespin. This method eliminates the harsh effects of the alkali upon the fingers. Sodium hydroxide has a decided softening effect upon the skin and burns severely in cuts or abrasions upon the hands.

Brushing on the solution is best done in a scrubbing motion against the grain of the fur. This is done so that a more rapid wetting is obtained. This insures more perfect penetration of the dyebath upon the hair. If this operation is slighted the matting effect of the hair may prevent a good application of the dye. A continued stroking motion, toward the tip of the feathers is advisable. Care must be taken that all of the feathers are thoroughly wet.

Measurement of time for the period that the killing agent must remain on the fiber is taken from the time after the entire area, being dyed, is properly wet. If a period of over five minutes is needed for proper preparation of the hair, added applications should be made.

<u>Application of the Dyestuff</u>

The dyebath should be cooled after it has just come to the boiling temperature for two or three minutes. If the killing process is timed properly, the whole operation can be continuous. The dyebath can best be applied in the same manner as with the killing agent. Clothespins of the pincher-type, holding a one inch piece of sponge can be used. This method has been found to color better than using a brush. Separate clothespins should be reserved for each dyestuff used.

<u>Rinsing</u>

It is well to rinse the dyed spot after it has cooled for a few minutes, however, it is not absolutely necessary. A warm rinse with a little acetic acid or ammonia is recommended. The dye spot can be soaped to remove all of the excess dye, but this too is not necessary. The excess dye can be blotted off with no trouble at all by using a soft paper or a piece of cheesecloth. No harmful effects were encountered by any of the animals by their habit of licking or preening off the dyebath.

The use of acetic acid is advocated because it has the effect of exhausting the dyebath. Oxalic acid has the same effect. If either of these acids have been used in the dyebath, their presence in the rinse is not too essential.

Drying

In some cases it is best to let the animal dry first before releasing. This is especially so in the case of the birds. If the dye has been applied to the wing, by all means make certain that it is dry, as the wetting of the primary feathers may seriously effect the flight.

An example of this was experienced with a female pheasant. The bird was released about one hour after the application of the dye. Upon being liberated from the burlap sack, she attained a maximum height of only four feet before falling to the ground. Releasing a bird in this condition subjects it to unnecessary danger.

DIRECTIONS FOR APPLICATION

Directions for the successful application of dyestuffs used in the experiments will follow as an aid to those wishing to use the same dyes in further experiments.

Basic Dyes

To prepare a dyebath of 100 cc., proceed as follows: Add 1 to 2 grams of dyestuff to 100 cc. distilled water. Warm until dissolved and add 3 grams of Glauber's salt (sodium sulphate) and 5 cc. of 30% strength oxalic acid. Since 100 cc. will be sufficient for 50 mice, a smaller amount can be prepared in the same proportion if so desired. Raise to a boil and let cool to proper temperature (150-180°F).

The fur or feather is first thoroughly wet with a solution of sodium hydroxide (2 gms./100 cc. H_2O). This is left on from 3 to 5 minutes for moderately resistant fur and 5 to 7 minutes for resistant fur. On feathers it may be left on from 5 to 10 minutes in most cases.

The hot dyebath is then applied to the fur or feather by means of a one-inch square of natural sponge held in a pincher-type clothespin. They dye is left on for 3 to 4 minutes and dye is added during this time to maintain a warm application.

At this time, the dyed spot is rinsed with a 2 to 3% glacial acetic acid solution.

Variation

Acetic acid can be used in the dyebath and also in the rinse. Oxalic is recommended for the dyebath as it has a reducing property. If a wetting agent such as Aerosol OT is on hand the fur or feathers can first be wet with a 2% solution. If trouble is encountered in getting the dyestuff to dissolve in warm or hot water, add some of this the same strength wetting agent to the dyebath.

Acid Dyes

The same directions apply for the acid dyes listed in Table II. If the addition of the wetting agent, Aerosol OT precipitates the dyestuff in the bath it cannot be used.

Azoic Dyes

These dyes consist of two solutions used successively. The following directions are given by the manufacturer of the product:

Table III

Stability of Dyestuffs

Dyestuff	Color Produced	Stability of Dye to Date (days)		
		Mice	Pheasants	Rabbits
Calcozine Yellow OX	Yellow	74	27	13
Picric Acid	Yellow	--	--	38
Orange II	Orange	74	10	--
Orange II (redyed)	Dull orange	--	3	--
Rhodamine 6 GDN extra	Pink (red)	19	10	57
Rhodamine B	Pink	75	--	--
Brilliant Green Crystals	Green	--	1	--
Calcozine Blue GXX	Dark blue	31	10	--
Calcozine Red BX	Red	--	1	--
Naphtol AS-SW and Fast Red salt.	Red	--	1	--
Rhodamine B and Yellow OX (redyed)	Red	--	3	--
Rhodamine B and Yellow OX	Scarlet	--	3	--

Note: The date for the last observation for the above is June 10, 1941. The number of days represent only the total number of days and is not meant to mean that the color existed for just that time.

Table IV

Dyeing Data on Cottontail Rabbits

Sex	Dyestuff	Color	Date of Dyeing	Date Observed	Time Elapsed	Condition of Dye
M[1]	Picric acid	Yellow	3-27-41	5-4-41	38 days	Easily visible
F[2]	Rhodamine 6 GDN ex.	Red	3-29-41	5-25-41	57 days	Easily visible
F[3]	Calcozine Yellow OX	Yellow	4-6-41	4-19-41	13 days	Color unchanged

1-- Observation reported by student of School of Forestry and Conservation.

2-- Tail dyed red; released with a broken front leg.

3-- Dye applied to tail and underparts.

Table V

RECORD OF DYED WHITE MICE

Date Dyed	Color	Dyestuff Used	Record of last Observation: Date	Day Elapsed	Condition of Color
5-22-41	Scarlet	Rhodamine 6 GDN extra	6-10-41	19	Perfectly brilliant.
5-22-41	Scarlet Yellow	Rhodamine 6 GDN Yellow OX	"	19	Perfectly brilliant.
5-22-41	Yellow	Yellow OX	"	19	Brilliant color.
5-17-41	Scarlet	Rhodamine B and Yellow OX	"	24	Spotty and faded. Color bright.
5-17-41	Orange	Orange II	5-29-41	12	Fairly bright.
5-15-41	Scarlet	Rhodamine 6 GDN extra	6-10-41	26	Spotted and faded only upon back.
5-10-41	Blue	Calcozine Blue GXX	6-10-41	31	Very faint trace of color left behind head.
5-10-41	Blue	Calcozine Blue GXX	5-18-41	8	Good color for 4 days; fur started to fall out.
5-10-41	Blue	Calcozine Blue GXX	6-10-41	31	Blue color just as good as when first applied.
5-10-41	Blue	Calcozine Blue GXX	6-10-41	31	Color changed very little, slightly faded.

Table 5 (continued)

4-28-41	Red & Blue	Naphtol AS-SW & Red GL & Blue B Salts.	6-10-41	43	Red faded to a reddish-brown and blue to purplish. (Old Stock Used).
4-11-41	Yellow & Blue	Calco Yellow OX & Blue GXX	6-10-41	60	Green first prod. now a yellow and blue is green. Colors excellent.
4-1-41	Yellow	Calco Yellow OX	6-10-41	70	Color in very good condition Faded to a lemon color.
3-28-41	Orange	Orange II	6-10-41	74	Color still good, faded but easily recognized.
3-28-41	Blue	Calco Blue GXX	6-10-41	74	Hair lost due to fights. Some spots still very good, mostly spotted.
3-27-41	Pink	Rhodamine B	6-10-41	75	Color still good, faded to a light pink.

*Mouse a brown color.

Table VI

Record of Dyed Chinese Ringneck Pheasants

Band No.	Sex	Color	Date Dyed	Date Released	Dyestuff Used
39814	M.	Dark blue	5-10-41	5-20-41	Calco. Blue GXX
39806	M.	Yellow	"	"	Calco. Yellow OX
39822	M.	Orange	"	"	Orange II
39807	M.	Pink	"	"	Rhodamine 6 GDN extra
39819	M.	Orange(dull)	5-17-41	"	Orange II (redyed)
39829	M.	Red	"	"	Rhod. B.& Yellow OX
39801	M.	Yellow	4-23-41	"	Calco. Yellow OX
39820*	M.	Scarlet	5-17-41	"	Rhod. B.& Yellow OX
39818	M.	Pink	5-15-41	"	Rhodamine 6 GDN extra
39825	M.	Red & Yellow	5-22-41	"	Rhod.6 GDN & Yellow OX
39830	F.	Red (rump) Green(L.wing)	1-11-41	"	Calco. red BX & Bril.Gr. Crystals
39832	F.	Red (breast) Green (rump)	"	"	Naphtol AS-SW + Fast red And Brilliant Green

* Color may be observed as yellow, after rhodamine fades out.

** This bird was unable to fly due to wetting the primary feathers.

The first solution, Amanil Naphtol AS-SW is prepared in the following manner - add 3 cc. Ethyl alcohol to 1 gram of Naphtol AS-SW. Add to this paste, 0.5 gram of caustic soda flakes (sodium hydroxide) that has been dissolved in 10 cc. of distilled water. (The Naphtol AS-SW will go into solution immediately). To this add another 0.5 grams caustic soda flakes dissolved in 90 cc. of distilled water. Immediately afterwards add 1 cc. of formaldehyde (30%).

Apply this solution to the feathers or fur that have been treated with a sodium hydroxide solution (02 gms/100 cc. H_2O) for 5 minutes. Sponge or squeeze off before adding to next batch.

Add the second solution, the R salt, 3 grams per 100 cc. of cold water, to the feathers already treated with the first component. Leave this on for 3 to 5 minutes and rinse with an acetic acid solution (3 grams glacial in 100 cc. distilled H_2O).

A solution of ammonium acetate can be used for the rinse.

EFFECTIVENESS OF DYES USED

For a record of dyed animals refer to tables 3, 4, and 5.

Calcozine Yellow OX

Although this is a basic dye and all references indicated that it would be badly faded after a month, data found upon dyeing pheasants and white mice prove otherwise.

A pheasant dyed with this color on April 23 was kept in captivity for exactly 28 days. At the end of this period, the bird was released. There was no decernible difference between that color and the same color dyed on a male bird one day previously.

Calcozine Yellow OX applied to a white mouse on April 1, 1941 examined 66 days afterwards had retained a good yellow color, although it faded from a deep yellow to lemon color. The color is recognized at a glance and should be easily identified for quite some time yet. On other white mice there is no perceptible change in the color after 20 days.

Calcozine yellow dyed upon a white mouse and covered by Blue GXX produced a green. The green faded and a rich yellow color is left in its place. Areas colored blue by the dye at the time of dyeing has changed to a bright green. From this experiment it is believed that yellow can be protected by covering with other dyes of the more fugitive nature. In this manner the life of the dye can be increased considerably. A green produced in this manner would start fading at the end of four weeks after this time the yellow would become prominent.

Rhodamine B

This dyestuff applied upon a white mouse on March 27, 1941 produces a deep pink color, and is still easily recognized after 75 days. It has faded from a bright pink to a light pink color. First signs of fading were noticed at the 3 week period. See Tables III and V.

Rhodamine 6 GDN

This dye produces a scarlet color that is extremely bright. A satisfactory record for this dye was obtained by dyeing the tail of a cottontail rabbit. This rabbit was live-trapped, dyed, and released on March 30, 1941; exactly eight weeks later I saw this cottontail and identified it very easily by the dyed tail. The color was still a bright red. See tables III and IV.

Rhodamine 6GDN

This color dyed upon the white ring of a male ringneck pheasant produced a bright pink that remained unchanged for a period of ten days. At this time it was released to obtain observations in the field. This bird has band #39807 on its leg. Another pheasant with band #39818 showed similar results. See tables III and VI.

Orange II

This dye produced a good orange color dyed on white mice and on ringneck pheasants. Dyed applied on a white mouse on March 28, 1941, is still a prominent color but has faded badly. A total of 74 days has elapsed since the dyeing. See table V.

No change had taken place in the color dyed on a ringneck pheasant #39822 in the ten days before release. See tables III and VI.

Calcozine Blue GXX

This color has had a varying degree of success upon dyed mice and ringneck pheasants.

A brown colored mouse was dyed with this dyestuff on May 10, 1941; it produced a deep blue color. The dyed hair is still in very good condition, and no fading is noted after 31 days. See tables III and V.

The ringneck of a male pheasant, #39814, was dyed on May 10, 1941 and no change had taken place in the color in the ten days of captivity before releasing on May 20, 1941 for field observations. See table III.

Brilliant Green B Crystals

The use of brilliant green was limited to dyeing two female pheasants. The application here was entirely satisfactory. No records have been secured for the durability of this color. Upon dyed feathers of the domestic fowl, this color is unfaded, but has been put to no weathering test.

Other Dyestuffs (Refer to Table II)

Anthraquinone Blue SE., Auramine Conc., Pontacyl Fast Violet 10 B, Fuchsin Powder-Special, Methyl Violet 2B Powder and Red Y are satisfactory, but no records upon live birds or mammals substantiate their use. They have proved possibly successful by their application upon hen feathers and bits of white rabbit fur.

It is believed that these dyes will produce approximately the same results as the ones experimented with upon live specimens.

EXTENT OF APPLICATION

Upon what birds and mammals can these dyes be successfully applied is the question that must be next answered. The limitations can be easily placed in two classes. First, those birds and mammals having natural white on their bodies, and second, those having natural colors that can be altered by the addition of the darker dyes.

Class number one, natural white, offers the greatest range for application, because even the light colors such as pink and yellow can be discerned with ease. Examples of these are: herring gulls, swan, various species of ducks and geese, the cottontail rabbit, white-tail deer, opposum, skunk, snowshoe hare, weasel, bluejay, bobolink, meadowlark, field sparrow, night herron, American egret, and many others.

Dyes applied upon the white of the above mentioned species would give the better results than those in class two, birds and mammals having natural colors that can be altered by dyes.

Class two is not expected to produce results comparable to those in class one, because the natural pigment is not covered up as in the case of painting. The experiment upon two female pheasants showed that dyes applied in large blotches of color are recognized fairly easy. Reds, greens, and blues offer the best success in this respect.

Examples of birds and mammals in this class are: mourning doves, sparrows, female duck species, female cowbirds, many species of sparrows, robin vireos, thrushes, shrikes and chickadees.

APPLICATION TECHNIQUE

Equipment

Necessary equipment for applying dyes is listed below:

Sponge - 12 pieces, 1 inch square. 6 pieces, 2 inches square.

Chothespins (pincher type) - one dozen.

Rubber gloves - not tight fitting type, heavy rubber is best.

Rubber apron - obtain type recommended by Chemical Supply Co.

Beakers - 2 or 3 50 cc. and 1 200 cc. pyrex glass.

Graduate cylinder - 1 100 cc.

Thermometer Fahrenheit scale or centigrade.

Glass dishes - 6 holding at least 150 cc. good glass.

Stirring rods - 3

Test tubes - 6 pyrex

Bottles of 150 cc. capacity and 300 cc.

Cheese cloth   a good supply.

Cloth hoods - 12, of varying sizes and of various thicknesses. Each equipped with "pucker strings".

Cloth sacks - 6, of different sizes. Tie string and cord.

Burlap or grain sack - only one is needed; tie strings sewed on.

Stretcher boards - one for real small animals - one for larger.

Bird funnel - for pheasant etc., big enough to hold the bird with a two inch opening for head.

Record Cards (according to personal taste).

<u>Chemicals</u>

Sodium Hydroxide in flakes or pellets (N_aOH)

Acetic Acid, commercial 30% (CH_3COOH)

Oxalic Acid, powder form ($C_2H_2O_4 + 2H_2O$)

Glauber's Salt, powder form (Sodium sulphate, ($Na_2SO_4\ 10H_2O$)

Soda Ash, powder form. (sodium carbonate) (Na_2CO_3)

Ammonia carbonate - lump form.

Wetting agent - Commercial product, Aerosol OT aqueous 10%

Liquid soap.

Natural sponge has been found to work very well in handling both killing agents and hot dyes. The smaller pieces are used with the clothespins for dipping into the dyebath. One should be reserved for each dye color. The larger pieces of sponge are used in the bleaching and rinsing solutions. Precautions should be taken to see that everything is as clean as possible.

Rubber gloves are not especially necessary if the pins are used regularly. If one finds that they are needed, it is advisable to have the larger and heavier kind, because they must be taken off quite often during work.

The use of cloth hoods over the bird or mammals heads are very helpful in keeping the animal quiet. The heavier material type are recommended for those mammals capable of biting seriously. Small mammals like mice can be handled very well without this precaution, but care must be taken not to get the liquids in the eyes. A few mice have died of fright, some have fainted, and others have struggled so that excessive gripping and squeezing has caused their death. Pucker-strings on the hoods drawn too tight have caused their death. Care must be taken to keep the pucker-strings dry, because once wet they are hard to loosen in time to prevent death from choking.

The "stretcher board" is an idea designed to handle mammals when a man is working alone. The board must be larger than the mammal and be provided with four pegs or holes to which the legs are tied. In combination with this device a cloth sack over the head should help keep the animal quiet. Cloth tie-strings should be used instead of cord to prevent unnecessary chafing. Recommended that chloroform or ether be used as an aid to handling. Use with caution.

A cottontail rabbit can easily be handled without the use of a stretcher board, but smaller and more active animals such as chipmunks and squirrels are best handled by the use of a "stretcher-board". Cottontails are safely handled by using a cloth hood over the head or enclosing front feet and head. As the tail and hind-quarters are the logical place for marking, this method is recommended.

Field Kit

A small wooden or metal box 14" x 10" x 8" will hold all the necessary equipment needed in the field. It can be equipped with compartments and racks to prevent breaking glassware. The cloth hoods, sacks and sponge can be used to an advantage in this respect also. Chemicals and dyestuffs should be tightly sealed and kept in a separate compartment.

The heating unit of a field kit offers the most concern, because it is so necessary. Practically, it should be enclosed within the kit, this however, may not prove possible. A separate unit, specifically for the heating method can be used, an example of this is a gasoline stove (oneburner).

Examples of what may be used can be summed up as follows: open fire with grate, alcohol burner with rack, gasoline stove, small blow-torch with holder for dishes, and canned heat. The matter of choice is personal, but some easily handled method should be adopted.

EFFECTS OF DYESTUFF UPON THE LIVE SPECIMEN

Effects Upon Fur

No harmful effects were noted from the application of dyes when the right strength killing agent was used. The effect of strong NaOH is shown to slight degree by the hair taking a wavy appearance. Harmful effects are shown after a period of two to four days in which the hair and the outer layer of epidermis falls off. Precautions should be taken against this danger. If one is to use strong sodium hydroxide, it must not remain on the fur 3 minutes before applying dye. The operation of dyeing must be timed to meet this requirement.

Effects Upon Feathers

It was found that stronger solutions of sodium hydroxide could be used upon feathers then on fur and precaution was taken so that very little reached the skin. Feathers when properly killed with Na OH lose all natural arrangement of the barbs and barbules, become very limp in appearance, and are likely to mat so that the application of dyes is hindered by their arrangement. However, it cannot be expected to maintain the feathers in their normal positions. The precaution taken, must be mainly that of seeing that the dye reaches all parts of the treated feathers.

A very remarkable thing was noticed about the feathers of the neckring of the male ringneck pheasant that had been dyed. After a few hours the feathers regained their normal positions, luster, and arrangement of all barbs. So remarkable was this rearrangement that some observers ignorant of the fact, thought that a new variety of pheasant was being kept in the cages.

This fact was especially encouraging as all previous observations pointed to a badly bedraggled appearance. The return of the natural luster and oils should serve as a protective element to the dye. The rejuvenation of the feathers after a harsh treatment, took place with in a twelve hour period.

It follows then that if a bird were to be dyed on the primary feathers of the wing, it would be certain of flight in that time. In view of this fact, the female ringneck pheasant #39830 dyed on January 11, 1941 (see table 3) was able to fly the following morning.

Upon no occasion were any of the dyed feathers observed to have fallen out of the birds.

Effects Upon the Normal Behavior

It was observed that when white mice were dyed, they became noticeably irritable. They preferred to be alone and not bothered by the curious mates in the same cage. In the case of younger mice they sought the mother out who immediately started to help the youngster dry its fur. If the mouse was kept in a separate cage until dry, only the natural curiosity was noted, but no objection was made to its presence.

The mouse remained irritable for only a short time.

Invariably the dyed animals sought to dry themselves at the first opportunity. The drying process started in the head region, then the front feet, sides and last, the belly. Some of the mice acted dejected in behavior. This was probably due to the effect of hot dyestuff upon the skin and the chemical effect of NaOH that has a drying effect when once dry.

Effects Upon Relationship with Other Animals.

Observations upon penned birds indicated that no attention was paid to the change in color. Birds released in the pens, sought cover and usually hovered in one corner of the pen. No preening of feathers was noticed as the birds were dyed and returned to the pens and left undisturbed.

An observation by Watson (1908) in using Artist's oil paints upon the behavior of the terns when released. In the breeding colony, the marked bird was promptly driven away from the nest at each approach. Only through persistence did the marked bird finally win out. After this reconciliation, no further indications were observed that the unmarked bird notices the color.

A personal observation upon a Herring Gull on Isle Royal Michigan in 1932 indicated that their was no harmful effect endured. Evidently a humorous fisherman that had caught a gull in some manner or other, had painted the entire breast of the bird with a red paint. The gull was observed in the company of many other birds and no indication of dislike by the others could be detected. The bird was diligently removing the paint with its bill.

Cottontail rabbits released in the same locality where live-trapped were observed there later, which indicates that they were not driven out or were they killed due to the increased disclosure by a bright color.

No observation has been made in this respect for the ring-neck pheasants released. It is expected, however, that local males that had set up crowing areas, will drive out the intruders whether colored or not.

No observations have been made upon the effect of colors on breeding activities, because concentration of time was upon the application of dyestuffs. Records of this interrelationship are more appropriately correlated with strictly life history studies.

PRACTICAL APPLICATION OF METHOD

Field Application

It is expected that some difficulty will be encountered in applying dyes under field conditions. However, this should not be considered a limiting factor. The worker who is really interested in the problem, will certainly overlook inconveniences. The point to consider is how to save time. In the laboratory the time required for dyeing a single specimen is approximately 10 minutes. This time does not include the preliminary time needed to set up the equipment. There should be very little difference then between field time and laboratory time .

Limitations of Color

It must be realized that the dyeing for this purpose is limited to a few definite colors. Those best identified are: red or scarlet, blue, pink, orange, yellow, and green. This makes a total of six colors that can be used.

Combinations of these six colors could be placed upon different parts of the body, thus giving 36 different combinations by only one color at each position. The use of two colors in a group and all six on the animal would increase the number of ways considerably, but this and the former idea is certainly impractical. This is true because under field conditions, it would be next to impossible to identify an animal so marked. A practical application would be two or three positions using 2 colors in a group.

The idea of position colors is the most practical method, but is limited as these should be confined to parts of the body most readily seen in the field. An example of this is the tail of the cottontail rabbit.

The use of two colors adjacent to each other upon a prominent part of the body is ideal as they offer a striking contrast that magnifies the effectiveness upon the eye. With this method 30 different combinations could be obtained with the six colors. Using only five colors with the adjacent arrangement would give 20 different arrangement would give 20 different arrangements, thus making it possible to identify twenty animals. An example of this method could be easily illustrated by marking the tail of the cottontail rabbit using the five colors instead of six as the combination of pink and red could be easily confused. The color red could be applied on the tip end of the tail and the color blue on the base end, altering these positions would give you two possibilities and thus altering the colors the total of twenty could be attained, reducing this to the four colors, blue, red, orange, and yellow, twelve different combinations could be dyed. This reduces the marking possibility to a practical number for good field identification.

The possibility of using different color arrangements for identification would further increase the range of individuals that could be easily identified in the field. A tail dyed yellow could have a dot of blue applied thus making a green spot on a yellow background. This might possibly work with the addition of red on yellow.

It must be noted that the control of dyeing is not this simple on the live animal. The above combinations would work only after the base color had dried. One exception to this would be the green spot on yellow. However, this is hard to control as when the hair is wet the dyestuff will run, making a mass of color rather than something definite and easily recorded. This difficulty is increased by the moving of the animal during the application of the dye. Thus we find that dyeing in mass color with the combination of two contrasting colors adjacent to each other is about the only practical means of marking. The only other alternative is the method of dyeing on different positions of the specimens body. This, it must be realized, is limited due to the fact that only certain parts of the body are readily observed in the field. Birds offer the best exception to this rule.

There are three limitations that must be recognized in dyeing and these are as follows: First, only light natural colors can be covered over with the darker flash colors. In most cases this is blue. Red may serve the purpose in some instances, and the use of violet might prove successful. Second, upon the the addition of a dark base over the natural browns or buff, no light flash-color like yellow or pink can be added to show contrast. Some method to remove the original color completely in a spot may some day be worked out, but at present it is non-existent. Third, there is no method by which all of the natural pigment can be removed completely so that the dye used would be most effective, and thus we find it most practical to use the natural white areas for marking purposes.

One promising method by which a color could be protected for an additional period thus increasing the total time that a dyed animal could be recognized in the field. This was found by the addition of blue dyestuff over a previously dyed yellow. The produced green served as a buffer color for the yellow. This should increase the life of the dye by at least four weeks. Other combinations may offer the same prospect. In general, the method would be the application of a more fugitive color over a more stable color.

To Problems

The use of color can be used along with live-trapping as an given season. The extent of utilization of types within this range could be determined best by this method. This would give a higher number of observations and a greater number of records for different animals using the same type with in the range. It would show clearly the extent of overlapping of range.

This method is a key to studying the variation in the activity of sexes that are impossible to identify under normal observation. This would apply to the favorite example, the cottontail. It also would apply to a host of others too numerous to mention. A study of the sex activity may help solve some of our management problems. An example of this application to sex would be the dyeing of the white "flags" of the deer, so that identification of sex would be positive during otherwise doubtful periods. This would be done by dyeing the bucks one color and the females another.

Period of Time

It has been proven that the period of time that one could expect to identify an individual specimen that has been dyed, is eight weeks. There is a possibility that this may be longer, but no data are available to substantiate this.

Brilliant effects can be easily obtained without any degree of difficulty for a period of 3 weeks. After this period, some of the more fugitive dyes will start to fade. A yellow basic dye will not begin to fade until after a four week period.

SUMMARY

Throughout this research, certain facts have been disclosed that place the use of chemical dyes for identification purposes on live birds and mammals, on a practical basis with certain reservations.

It now appears that the use of dyes is more practical than earlier research has indicated. The work has been extended to include mammals as well as birds. And although some progress has been made, more extensive field investigations to test visibility and permanence should be carried out.

A list of summarized data follows:

1. As compared to others, basic and acid dyes have the greatest affinity for hair and feathers of live birds and mammals.

2. No harmful effects were evident by the application of acid and basic dye upon the skin of the live specimen.

3. Heat is essential in applying the dyestuff except in the case of Azoic dyes where it is unnecessary.

4. White and light-colored parts of the animal can be successfully dyed.

5. Yellow dyes apparently have the greatest affinity for fur and feathers.

6. Combinations of more than two colors were not practical in the majority of cases.

7. The use of mass color in a conspicuous location presents the most practical method for using dyes.

8. The period that a dye may serve as a means of identifying an individual in the field was not fully determined but was in excess of eight weeks.

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