

dove is apparently lower than that of the domestic fowl,⁸ a much larger bird.

(2) Not enough data have yet accumulated to justify a general statement regarding the effect of sex on the heart rate in birds. In some species, such as the domestic fowl,⁸ there appears to be a distinct difference between the sexes; in many other species little or no difference is evident from data so far accumulated.

(3) In wild birds, a seasonal difference in basal heart rate has been demonstrated for at least one species, the black-capped chickadee, the basal rate being 89 ± 25.1 per minute higher in summer than in winter.⁵

(4) The relation of age to basal heart rate is complicated; much depends on the air temperature being considered and the status of the temperature regulatory mechanism. In altricial species which are hatched completely cold-blooded, the heart rate at hatching varies directly with air temperature as in a frog, then gradually becomes inversely related to the temperature as temperature regulation becomes established. Interestingly enough, at a thermal neutral temperature the heart rate of nestlings of all ages, juveniles and adults of the house wren is about the same, 450/min. At 21° C. (70° F.), however, the heart rate rises from 150/min. at hatching to 600/min. at 9 days of age (when heat loss control is poor) and drops to about 490/min. in the adult⁴—reflecting in a dramatic way the ontogenetic recapitulation of poikilothermism to homeothermism.

(5) There are two types of inherent variations in the heart rate of birds as follows: (a) The rate usually decreases slightly at the peak of lung and air sac inflation and increases between breathing cycles. In mammals this relation of breathing to heart rate is apparently the reverse.⁹ (b) Slower, more or less rhythmic but not regular cycles of slow and fast rate which I have called "oscillatory variations" can usually be detected. These cycles occur about 2 to 15 times per minute and the degree of oscillation varies considerably with the individual sometimes amounting to 10 per cent. of average rate for a minute period. Similar variations, presumably related to vagal periodicity, are known in man.

(6) Any factor which abnormally lowers the heart rate below basal level tends to produce sinus arrhythmia, which also occurs in some individuals at basal level. When the heart speeds up in these individuals, arrhythmia disappears.

(7) Heart rate-air temperature curves are very similar to CO₂ production-air temperature curves,⁴ indicating that heart rate is a rough index to the rate of metabolism at least as far as temperature effects are concerned.

(8) At lower temperatures breathing rate is usually directly correlated with heart rate, but at high temperatures the relation may become inverse as a result of reflexive acceleration of breathing rate coming before a rise in body temperature accelerates the heart, since increased ventilation is a principal means of heat loss in birds.

(9) The ratio of breathing rate to heart rate appears to be significantly different in small birds and mammals, being greater than 1 to 6 in birds and less in mammals. In general, small birds breathe less rapidly but have a somewhat higher heart rate than small mammals of the same size, although comparable data are as yet few.

(10) By placing the pick-up crystal under the nest it has been possible to record the heart beat of an entirely wild bird during normal incubation in the field. During a 24-hour recording period the heart rate in the house wren ranged from 950 when the bird had just returned to the nest after a period of active flying and feeding, to 550 after the bird had remained quiet on the nest. A rate as low as the 450/min. basal recorded under controlled conditions was not recorded in the field. Also, strangely enough, the heart rate was actually higher at night than when the bird was resting quietly in daytime. This was apparently to be explained by the fact that the temperature was 20° lower at night. To get a rate as low as 450/min. under natural conditions apparently the night temperatures would have to approach thermal neutrality; during the day activity, feeding, etc., would keep heart rate above basal regardless of temperature.

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APPLYING COLCHICINE TO PLANTS BY THE AEROSOL METHOD

BOTH the published work¹ and experience of the authors indicate that a fairly sharp distinction exists between the response to colchicine application by mature meristems such as occur in trees, shrubs or herbaceous plants and the response of juvenile meristems such as the plumule. While the results with germinating seeds or very young seedlings on the whole have been satisfactory, results with more mature plants have been meager. For example, complete immersion of very young seedlings of certain species

see Young, *Jour. Parasit.*, 23: 424-484, 1937, and Nice, Nice and Kraft, *Wilson Bulletin*, 47: 120-124, 1935.

⁸ E. P. Boas and Walter Landauer, *Amer. Jour. Med. Sci.*, 185: 654-664, 1933.

⁹ G. V. Anrep, W. Pascual and R. Rössler, *Proc. Royal Soc. London*, 119(B): 191-230, 1936.

¹ H. Dermen, *Bot. Rev.*, 6: 599-639, No. 11, 1940.

in 0.1 per cent. aqueous solution of colchicine for 4 hours has usually yielded 50 per cent. or more of tetraploids, whereas treatment of the apical meristems of half-grown plants of the same species has usually yielded none or a very small percentage of tetraploids. Since many of the most important horticultural plants are clonal varieties which can not be reproduced from seed and present only the mature type of meristem for treatment, it is desirable that a method be devised that will yield better results with this type of meristem. The striking results recently reported by Hamner, Schomer and Goodhue² in applying growth-regu-

duced into the bell jar from below through a rubber stopper in the table top. When the valve was opened the colchicine "smoke" or aerosol was directed upward in the bell jar chamber and it settled on all exposed surfaces. The entire operation was carried out under a chemical hood to avoid the danger of breathing the colchicine-laden aerosol. Polyploid plants were detected principally by pollen examination but some were determined by inspection.

The range of dosage and the number and types of polyploids obtained are indicated by the data in Table 1. The total number of plants examined was

TABLE 1

FREQUENCY AND TYPES* OF POLYPOIDS IN TEN POPULATIONS OF STOCK, *Matthiola incana* R. Br., PLANTS, EACH TREATED AT THE SEEDLING STAGE WITH DIFFERENT DOSAGES AND EXPOSURES OF COLCHICINE AEROSOL

Grams of 0.5 per cent. colchicine solution used	Number of plants treated†	Number of plants killed	Number of affected plants	4n	4n internal	mixoploid‡	4n epidermal	"blind"§	Percentage affected plants among those surviving
4.7	93	0	1	0	0	1	0	0	1.1
4.8	96	0	5	1	1	3	0	0	5.2
5.8	96	0	23	7	1	9	3	8	29.2
6.7	91	0	16	1	0	14	1	0	17.6
7.0	90	0	24	5	5	10	3	1	26.7
7.6	96	20	23	4	3	16	4	1	36.3
8.6	92	0	15	4	1	8	2	0	16.3
9.2	100	81	8	2	0	2	4	0	42.1
12.7	100	89	5	2	0	2	1	0	45.4
15.9	100	79	6	1	0	2	3	0	23.6

* Based on examination of the inflorescence.

† Imperfect germination accounts for populations having less than one hundred plants.

‡ Includes all other types of sectorial chimeras.

§ Plants with arrested meristems. Presumably too strongly affected for subsequent growth.

lating substances to plants by means of the aerosol method and the efficacy of the aerosol method in dispersing certain insecticides as shown by Goodhue³ suggested that this technique might be useful in applying colchicine to plants. This report gives the results of applying colchicine in aerosol form to small seedling plants of stock (*Matthiola incana*, R. Br.) to induce polyploidy.

Approximately one hundred seedlings in the cotyledon stage in an 8-inch pan were placed under a bell jar made from a 5-gallon bottle which was sealed to a small table top by means of calking compound. In a small steel cylinder were placed one-half gram of colchicine dissolved in four and one-half grams of cyclohexanone to which was added under pressure 95 grams of methyl chloride (boiling point -23.7° C. at one atmosphere), thus making a "colchicine bomb." The "colchicine bomb" was provided with a nozzle consisting of a 10-inch length of capillary copper tubing (.014 inch inside diameter) which was intro-

duced into the bell jar from below through a rubber stopper in the table top. When the valve was opened the colchicine "smoke" or aerosol was directed upward in the bell jar chamber and it settled on all exposed surfaces. The entire operation was carried out under a chemical hood to avoid the danger of breathing the colchicine-laden aerosol. Polyploid plants were detected principally by pollen examination but some were determined by inspection.

The range of dosage and the number and types of polyploids obtained are indicated by the data in Table 1. The total number of plants examined was 685, and 27 or 3.9 per cent. of these were pure tetraploids. Plants that were polyploid in part or all of the inflorescence totaled 136, or 19.8 per cent. of the total that survived the treatments. The greatest proportion of polyploids was obtained among survivors of treatments in which more than three fourths of the plants were killed. For example, in treatment 9, Table 1, five affected plants were obtained out of 11 survivors, or 45 per cent., and 89 seedlings were killed.

That particle size may be important in effecting penetration of the drug is indicated by the fact that several treatments in which a 2 per cent. colchicine solution was used gave a much smaller proportion of polyploids than was obtained in the main experiment. Particle size in the aerosol produced by the 2 per cent. solution is approximately twice as large as that produced by the 0.5-per cent. solution used in the main experiment.

The results of applying colchicine by the aerosol method as used were not outstanding as compared with those formerly obtained by other methods. Likewise, the aerosol method characteristically produced a high proportion of mixoploids, many of which would

² C. L. Hamner, H. A. Schomer and L. D. Goodhue, *SCIENCE*, 99: 85, 2561, 1944.

³ L. D. Goodhue, *Ind. and Eng. Chem.*, 34: 1456-1459, 1942.

not lead to the establishment of a tetraploid race, whereas the immersion method produced only a minor proportion of such individuals. The chief feature of the aerosol method for applying colchicine to plants appears to be that the drug may be dissolved in toxic penetrating agents of various kinds and spread on plant surfaces in sublethal amounts in a highly dispersed form, a result that could be attained in no other way. It is possible that a penetrating agent may be found that will carry colchicine into tree buds and other complicated meristems of plants when applied in aerosol form, and further work is in progress with this objective.

It should be pointed out that this method of applying colchicine to plants should not be used except under carefully regulated conditions because of the danger of breathing the poisonous aerosol.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETECTION OF SPERM IN THE EGGS OF INSECTS

It is frequently desirable in genetic studies to know whether all eggs laid by impregnated females have been inseminated. The usual method of preparing sections for this purpose is entirely unsatisfactory, for besides the great amount of labor involved in making such preparations, many eggs are injured or entirely destroyed in the process. What is needed is a rapid method which permits the accumulation of accurate, quantitative data that will be statistically significant.

In connection with our studies in the genetics of *Drosophila*, we have developed such a method which should be equally useful in similar studies on other insects. The method is as follows: Three or four eggs, which have been removed from the culture by means of a needle with a spatula-like tip, are placed on a clean slide at a distance of about one third its width from the anterior margin and arranged, properly spaced, in a row with their micropile ends all directed away from the observer. A cover-slip is then rested on the eggs and one or two drops of a physiological salt solution placed at the edge of the cover glass. Capillary attraction pulls the cover-slip down and forces the contents of each egg out through a rupture at or near the micropile. This gives a uniformly thin smear, which is more or less circular in outline.

By means of a mechanical stage, such preparations can be very quickly searched under a high-dry objective, and if sperm are present, they are easily detected. Inseminated eggs of *Drosophila* usually contain two or more sperm, but even though only a single sperm is present, it can be observed readily. The detection of spermatozoa in the egg of *Drosophila* is facilitated by the fact that they become coiled into ring-like configurations, apparently soon after entering the egg. In general, it is best to examine freshly laid eggs, although it is possible to detect the sperm as late as eighteen hours after they have been laid.

We have found this technique useful as a means for clearing up various points in genetics. It has been especially useful as an analytical method in determining zygotic viability. In certain interspecific crosses it has been possible to show by this method that secretions of the female reproductive ducts inactivate or kill the foreign sperm within twenty-four hours after mating has occurred. It is also a simple method for determining whether mating has been successful, without having to destroy the female in order to see if her sperm receptacles contain spermatozoa.

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DESTRUCTION OF FOAM IN VOLUMETRIC FLASKS

In the preparation of samples for vitamin assay we have been much troubled by foaming. Under circumstances in which we did not want to add a surface active substance, this greatly delayed accurate dilution in volumetric flasks. We have found that the foam can be quickly broken by alternate suction and its quick release.

An appropriate size of single-hole stopper is attached to a short length of glass tubing. This is thrust into a rubber hose connected with an ordinary water pump. When the foam begins to rise in the neck of the volumetric, the stopper is quickly withdrawn. The inrush of air destroys the foam bubbles.

Care must be taken not to draw off some of the foam, but one soon learns to judge the correct amount of suction to apply. The solution should also come well up into the neck of the flask to minimize the danger of implosion.

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