

- F. S. Hammett, *Growth* 3, 211 (1939); H. S. Burr, *Yale J. Biol. and Med.* 14, 580 (1942).
4. E. J. Lund, *Bioelectric Fields and Growth* (Univ. of Texas Press, Austin, 1947).
  5. Applied Physics Laboratory, Johns Hopkins University, "Tumor regression by means of an external electrical source: announcement of preliminary findings," C. E. Humphrey, E. H. Seal, M. C. Garbow, 18 Dec. 1958.
  6. This research was performed in part under the auspices of the Applied Physics Laboratory and in part under Public Health Service grant CY 3739. Appreciation is expressed to Dr. Ander-vont and others of the National Cancer Institute for their valuable help, to Drs. Darden, Armbricht, and Ruback of Georgetown University Medical School for their help and histological analyses, to Dr. Lester Harris of Washington Missionary College for temporary laboratory space, and Dr. S. N. Stein of the Naval Medical Research Institute for laboratory space and helpful suggestions.

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## X-ray Dosimetry and Contact Microradiography with Color Film

**Abstract.** The effect of x-rays at various voltages and intensities, with monochromatic and polychromatic beams, on Ektachrome daylight and artificial-light film was investigated. The colors were rated according to the Munsell color system and ranged over all the spectrum except red. The color in terms of hue, value, saturation, and chroma was a function of wavelength as well as intensity, and thus the method may be useful in dosimetry as well as in radiography. Microradiographs of metals and wood were remarkable in showing detail not obtainable with conventional black-and-white photographic emulsions.

For some time prior to recent reports of the use of color film to distinguish between radioactive isotopes (1) and for registration of electron micrographs (2) we have been interested in investigating the possibility of increasing the information provided by contact microradiographs registered on color film beyond that derived with the conventional fine-grained, black-and-white photographic emulsions. Also, in the course of our investigation there appeared a paper by Blum (3), describing two types of sensitive coatings which permit registration in arbitrary colors of the action of x- or  $\gamma$ -rays of different wavelengths or of ionizing particles of different energies.

It is the purpose of this report to summarize briefly our experience with Kodak Ektachrome daylight and artificial-light film which we could process.

The action of x-ray beams of varied spectral quality or wavelength distribution (in some instances essentially monochromatic) generated at 40 kv by cobalt, chromium, copper, and molybdenum target tubes on the film alone was studied. Copper and molybdenum radiation at 30, 20, 10, 7, and 5 kv, and at 35 and 30 kv, respectively, was also investigated.

The colors produced were classified visually according to the Munsell color

system. Colors ranging from blue, blue-green, green, yellow, yellow-red, and purple-blue to gray were obtained. Red, however, was not obtainable.

The reproducibility of the colors under similar exposure conditions was shown—that is, the reciprocity law holds for color film. However, the method of film storage before exposure, as well as change in x-ray tubes having the same target, seems to have affected the colors somewhat. The artificial-light film produced more saturated colors than the daylight film. For the same value of  $E$ , the same voltage yielded the same color, while a different voltage yielded a different color. The hue varied more from one target to another at the same voltage and  $E$  value in daylight film. The hue remained the same after exposure to radiation from any given target for one kind of film. It was easier to overexpose daylight film than artificial. The color property of value decreased with decreasing  $E$ . There were greater shifts in value and chroma with decreasing  $E$ . Sometimes even a change of hue was evidenced. Herein lies the key to the use of color film in the evaluation of x-ray dosage.

With a newly designed camera, x-ray beams from copper and molybdenum targets were transmitted through various metal and wood specimens in contact with color film, and the resulting shadow images were examined for color and resolution of the fine structure of the specimen.

Artificial-light film brought out the phase structure of metals at several of the higher voltages, especially with the copper radiation, with remarkable clarity and with differentiation of composition far beyond that in black-white images. Softer radiation was needed to bring out the structure in the wood specimens. Specimens containing elements of large atomic number seemed to render the film more sensitive in resolving structure when higher voltages were used.

Green and blue-green images were obtained for metal specimens on daylight film, while blue was obtained for metal and wood specimens on artificial-light film.

The processing time is the same as that required in the conventional method of microradiography on black-and-white film, although more manipulation is necessary. Because of the rather large grain size, extensive enlargement of the color microradiographs is not feasible. However, the use of these transparencies as slides is very convenient.

Because of the complex nature of the film and the fact that the formulation is highly confidential and not disclosed by the manufacturer, such problems as the inability to obtain red coloration

must be answered with conjecture. Thus, it is possible that the radiation may have destroyed the dye or its carrier. Further, it is also possible that variations from one emulsion lot to another may have been responsible for certain variations in color which were unexplainable. The balancing necessary because of the greater saturation of the yellow dye which is used may also be a contributing factor.

Besides producing encouraging results, indicating interesting and valuable applications in radiography and dosimetry, and even for Laue diffraction patterns made with polychromatic x-radiation, this investigation has opened new areas of research in the field of radiation chemistry and may perhaps contribute to the development of new color-film formulations more specifically adapted to radiation in the x- and  $\gamma$ -ray ranges of the electromagnetic spectrum.

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### References and Notes

1. G. W. Buckaloo and D. V. Cohn, *Science* 123, 333 (1956).
2. T. F. Anderson, *Bull. microscop. appl.* 6, 152 (1956).
3. J. M. Blum, *Sci. et inds. phot.* 29, 211 (1958).

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## Chromosomal Translocation in Domestic Fowl Induced by X-rays

**Abstract.** The cytological appearance and behavior of an x-ray-induced reciprocal translocation between the first and second chromosome of the domestic fowl is described, and its relevance to the further definition of linkage studies in the fowl is observed.

Although there are possibly better organisms among the vertebrates for cytological and genetical investigations than the domestic fowl, economic considerations have led to its frequent utilization, especially for genetic studies, and a large amount of breeding data has been accumulated. Five autosomal groups and one sex-linkage group, with a substantial number of genes in each group are known (1). The cytology of fowl, however, has been said to be less well known than that of other vertebrates (2), and the chromosome complement has been variously estimated to be between 40 and 80, with the mode near the latter. If these estimates were correct, the prospects for any cytological correlation of genetic data would be indeed dim. But recent studies utilizing a smear tech-

nique, pretreatments of tissues prior to fixation, and a new killing fluid (3, 4) have shown that the fowl carries only 12 chromosomes in the male and 11 in the female. The other elements, designated as microchromosomes by previous investigators, are shown by their origin, their heterochromaticity, their structure, their variability in numbers (due to fusion or fragmentation or both), and their disappearance at the end of the meiotic cycle to be not chromosomes but elements of an adjuvant nature and function. I have called them chromosomoids (4, 5). Thus, the fowl apparently carries a haploid number of six chromosomes which correspond to the six known linkage groups, and it now seems possible and feasible to attempt the association of the linkage groups with specific chromosomes.

This study (6), while it has not succeeded in associating a known linkage group with its respective chromosome, indicates the feasibility of such an association and presents a cytological demonstration of a reciprocal chromosomal translocation in the fowl involving the first and second chromosomes.

The fowl sent to us for this study was an S. C. White Leghorn male XP781, 22 months of age when killed, and derived from a project on x-ray induction of genetic variability carried on by I. Michael Lerner, Everett R. Dempster, and Nobuo Inouye at the University of California, Berkeley. The bird was produced from unpedigreed random matings in which the semen was exposed to 1000 r-units per generation in each of four generations. It was suspected of being a carrier of a translocation on the basis of hatching performance of the offspring when it was mated to presumably normal mates. Its offspring, tested in turn in a similar way, were found to segregate for normal and suspected translocation behavior.

The bird was killed by pithing through the palatal aperture and the gonads were removed, sliced, and diced into cubes approximately 5 mm on a side. Tissues were pretreated in distilled water for 10 minutes (7) before fixation (3). Tubules were then dissected and removed to a slide for smearing with propionic carmine. Tissues which may seem too firm for smearing after prolonged fixation will soften to their original consistency if allowed to remain in a drop of 45-percent propionic carmine on the slide for the length of time it takes one to clean and dry a cover slip for the smear.

The normal meiotic complement of six bivalent chromosomes in the male is shown in Fig. 1A. Evidences for the non-chromosomal nature of the other chromatic bodies, though irrelevant to this study, have been indicated (4, 5). The

position of the centromeres in chromosomes 1 and 2 which are involved in the translocation is indicated by the bend in the J-shaped chromosomes (Fig. 1A). Figures 1B and 1C show chromosomes

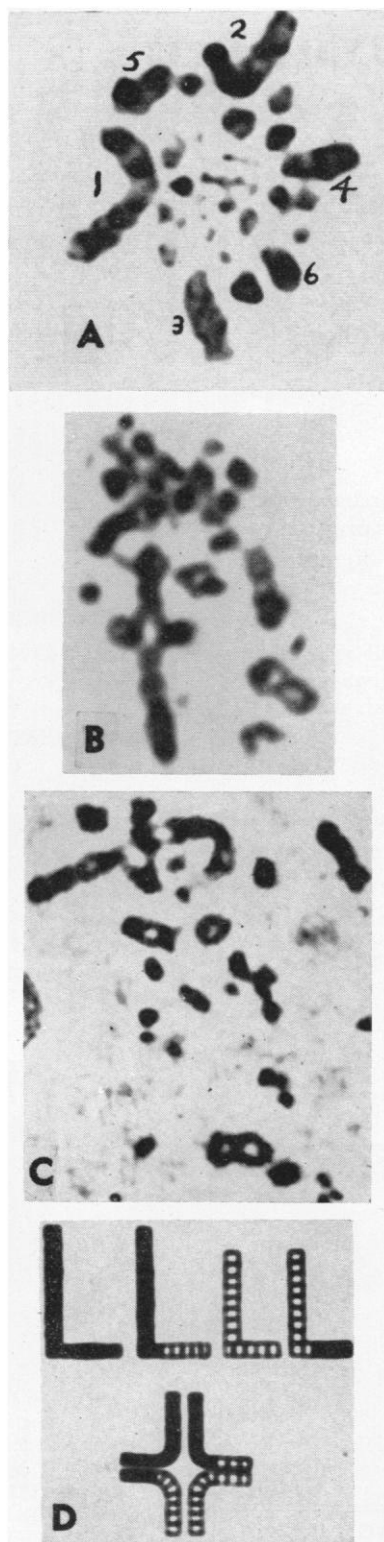


Fig. 1. A, Normal meiotic complement of six bivalent chromosomes in the male. B, C, Chromosomes 1 and 2 associated as a quadrivalent. D, Diagram of translocations.

1 and 2 associated as a quadrivalent forming a cross with a hole between the short arms subtending their respective centromeres. The short arms of chromosomes 1 and 2 were broken just distal to the centromere, and the fragments were translocated as shown diagrammatically in Fig. 1D. The bird is heterozygous for the interchange; both bivalent chromosomes 1 and 2 carry one normal chromosome and one with a translocated segment. During synapsis the translocated segments are of sufficient length to permit pairing, producing the characteristic configuration and quadrivalent association shown in Figs. 1B and 1C and indicated in the diagram of Fig. 1D. The net result of this association is one quadrivalent and four bivalent chromosomes, observed from diplotene to metaphase.

At the stage of diakinesis, the chiasmata between the interchanges appear to terminalize normally, and regular segregation might be anticipated. However, the chiasmata often either do not resolve or do so belatedly at metaphase, producing an end-to-end association or a cross with short arms. These configurations experience considerable difficulty in anaphase separation, producing bridges and unequal disjunction resulting in heteromorphic daughter nuclei.

Since the chromosomes of the fowl at the second meiotic division are too condensed for detailed morphological study, the results of unequal segregation cannot be adequately followed, but the presence of large masses of abnormal spermatids indicates that the differentiation of spermatids into spermatozoa is partially blocked. Thus it appears that, in addition to the expected semisterility caused by the normal segregation of deficient gametes due to the interchange and crossovers between them, there is an added generational sterility resulting from a partial failure of the chromosomal mechanism. There is also the possibility that, in addition to the segmental interchanges produced by x-rays, other undetectable changes, such as small deletions or inversions, might have occurred, but no cytological evidences for these were found.

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#### References and Notes

1. F. B. Hutt, *Genetics of the Fowl* (McGraw-Hill, New York, 1949).
2. R. Matthey, *Les Chromosomes des Vertébrés* (Library of the University of Lausanne, 1949).
3. E. H. Newcomer, *Science* 118, 161 (1953).
4. —, *J. Heredity* 48, 227 (1957).
5. —, *Cytologia (Tokyo)*, in press.
6. This investigation was supported by research grant C-3479 from the U.S. Public Health Service.
7. J. Makino and I. Nishimura, *Stain Technol.* 27, 1 (1952).

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