This work was supported by grants from the National Science Foundation (*GB*-2411) and from the National Institutes of Health (*HD*-00215).

ROGER P. ATWOOD
LAWRENCE RAZAVI

Channing Laboratory, Boston City Hospital,
Harvard University Medical School.

[1] Moorehead, P. S., Nowell, P. C., Tallman, W. J., Batipps, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).
[2] Chu, E. H. Y., and Swomley, B. A., *Science*, **133**, 1925 (1961).
[3] Chu, E. H. Y., and Bender, W. A., *Science*, **133**, 1399 (1961).
[4] Chu, E. H. Y., and Bender, W. A., *Ann. N.Y. Acad. Sci.*, **102**, 253 (1962).
[5] Hamerton, J. L., Klinger, H. P., Mutton, D. E., and Lang, E. M., *Cytogenetics*, **2**, 240 (1963).
[6] Bender, W. A., and Chu, E. H. Y., in *Evolutionary and Genetic Biology of the Primates*, edit. by Buettner-Janusch, J. (New York, 1963).
[7] Makino, J., *Chromosoma*, **3**, 220 (1948).
[8] Robertson, W. R. B., *J. Morphol.*, **27**, 179 (1916).
[9] Corfman, P. A., and Richart, R. M., *Nature*, **204**, 502 (1964).

Brilliant Cresyl Blue as a Stain for Plant Chromosomes

THE usefulness of cresyl violet as a stain for rapid preparations of root tip squashes has been recently reported[1].

For the past two years I have used the related stain brilliant cresyl blue in aqueous solution, in preference to conventional staining procedures which have generally been more time-consuming. More than forty species of flowering plants have been tested with aqueous brilliant cresyl blue without any difficulties being encountered.

In Amirkhanian's method[1], cresyl violet was dissolved in 50 per cent acetic acid. This solvent proved unsuitable with brilliant cresyl blue which was finally prepared as a 10^{-3} M solution in distilled water.

Consequently the same solution of brilliant cresyl blue may be used with vital staining of dividing nuclei as well as with normal fixation procedures. It is thus possible to compare fixed and living chromosomes (or other cellular components) which may be an important consideration in some cytological studies.

Brilliant cresyl blue is classified as a quinone-imine dye belonging to the sub-group of oxazins. According to Conn[2] "they are not stains having very general application". The dye marketed under the name of cresyl violet is of uncertain chemical structure, but is probably closely related to cresyl violet acetate, the formula of which has been characterized from the chemical synthesis of the material. Since the chemical formula of brilliant cresyl blue is definitely known, it would be preferable to use this dye in place of cresyl violet in cytological studies where a knowledge of the underlying histochemical reactions is desirable.

In squash preparations involving refractory material (for example, shoot and root meristems of grasses) brilliant cresyl blue gives good results after pectinase treatment[3]. It is also compatible with colchicine and other chemicals used in pre-treatment[4].

The procedure in squashing is as follows: After pretreatment (if any) root tips or other meristematic tissues are fixed in aceto-alcohol (1 part glacial acetic acid : 3 parts ethanol). Other appropriate fixatives may be used[4].

After fixation, the specimen is hydrolysed in 1 N HCl for 5 min or longer at 60° C and transferred to a drop of brilliant cresyl blue on a slide (a 10^{-3} M solution in distilled water, or stronger solutions up to 10^{-2} M). The specimen is teased out, tapped and squashed under the coverslip in the usual way[4]. By warming the slide gently over a spirit lamp the cytoplasm may be cleared to the degree desired. Permanent preparations may be made by inverting the slide in 80 per cent ethanol in a smearing dish, dehydrating rapidly in 100 per cent ethanol, and mounting in 'Euparal' or Gurr's neutral mounting medium. Other methods of making the preparation permanent may equally well be applied[4].

Brilliant cresyl blue in dilute aqueous solution provides the means for rapid squash preparations and is applicable to a wide range of different species of flowering plants and, if required, the concomitant metachromatic staining of living cells[2].

An aspect of added interest is the report of retardation of the growth of tumours by brilliant cresyl blue[2]; further work with this dye might throw valuable light on the course of cell division, not only in cancerous growths but also in normal tissues.

B. C. ARNOLD

Department of Botany,
University of Canterbury, Christchurch.

[1] Amirkhanian, J. D., *Nature*, **201**, 319 (1964).
[2] Conn, H. J., *Biological Stains* (Williams and Wilkins, Baltimore, 1961).
[3] de Latour, G., *N.Z. J. Sci.*, **3**, 295 (1960).
[4] Darlington, C. D., and La Cour, L. F., *The Handling of Chromosomes* (Allen and Unwin, London, 1960).

VETERINARY SCIENCE

Restricted Protein Intake and Avian Atherosclerosis

ALTHOUGH low-protein diets in the presence or absence of supplementary dietary cholesterol give rise to a marked hypercholesterolaemia in Leghorn cocks, nevertheless, we observed[1,2] a reduction in the severity of abdominal atherosclerosis after 10 or 20 weeks on the experimental low-protein diets. We have examined the effects of prolonged restrictions (20 months) of protein intake in relation to plasma cholesterol, body composition and abdominal aortic and coronary atherosclerosis.

Day-old Leghorn cocks from the same random-bred strain used in previous investigations of avian atherosclerosis[3,4] were divided into two groups of sixty, one being given an adequate and the other an inadequate protein-level. The basic diet was composed of soy-bean meal, glucose, maize oil, vitamins and minerals. Its protein content was continuously diminished with increasing age and the average daily intake per bird throughout the experiment is listed in Table 1.

Mortality was 31 and 25 birds from the adequate and inadequate protein-intake groups, respectively. During the course of the examination another 14 and 20 cocks from each group were removed for nitrogen metabolism studies. Blood was taken from the 15 survivors in each group for plasma-cholesterol analysis prior to the termination of the experiment. The birds were killed with chloroform, and the liver and aorta removed for further analysis. Liver cholesterol was determined[5] on a portion of the lipid extracted with chloroform–methanol (2 : 1). The aorta was macroscopically graded[4] on a scale of 1–3 and then examined and graded histologically[4], as were also the

Table 1. COMPARISON BETWEEN SEVERITY OF AORTIC AND CORONARY ATHEROSCLEROTIC LESIONS IN LEGHORN COCKS ON NORMAL OR RESTRICTED LEVELS OF DIETARY PROTEIN OVER A PERIOD OF 20 MONTHS

Measurements	Protein intake	
	Normal	Restricted
Number of birds: at start	60	60
removed*	14	20
died	31	25
Protein intake (g/bird/day):		
1 day–1 month	4·1	2·5
1 – 2 months	7·7	4·6
2 – 4·5 months	11·2	7·1
4·5– 7 months	13·1	7·8
7 –14 months	11·2	6·2
14 –20 months	7·4	4·3
Number of survivors	15	15
Body-weight, final (g)	2,504 ± 38†	2,234 ± 49
Carcass N (g/100 g dry weight)	9·6 ± 0·3	9·7 ± 0·3
Carcass extractable lipid (g/100 g dry weight)	20·9 ± 2·2	19·8 ± 1·5
Plasma cholesterol (mg/100 ml.)	110 ± 5	135 ± 7
Liver cholesterol (g/100 g wet weight)	0·52 ± 0·02	0·51 ± 0·03
Abdominal aorta:		
Macroscopic score	2·07 ± 0·15	2·07 ± 0·15
Weight-area ratio	68·1 ± 2·3	65·6 ± 2·4
Histological score	1·53	1·52
Coronary arteries:		
Histological score	1·46 ± 0·12	0·78 ± 0·10
Number of birds with severe scores	8 (53%)	1 (7%)

* For nitrogen metabolism investigations.
† Mean value with its standard error.