

not more than one-third of the damage can be ascribed to the 'direct' effect.

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¹ Halssinsky, M., and Lefort, M., *C.R. Acad. Sci., Paris*, **230**, 1156 (1950).

² Read, J., Annual Report Brit. Emp. Canc. Camp., 1951, p. 247.

³ Gray, L. H., and Scholes, M. E., *Brit. J. Radiol.*, **24**, 348 (1951).

⁴ Gray, L. H., *Brit. J. Radiol.*, **24**, 26 (1951).

⁵ Gray, L. H., *Acta Radiol.*, **41**, 63 (1954).

⁶ Read, J., *Brit. J. Radiol.*, **27**, 154 (1953).

Deoxyribonucleic Acid Content of Marsupial Nuclei

THE deoxyribonucleic acid content of nuclei of different species of animals has been listed¹; there appears to be a fairly regular increase from the sponges to the mammals. In the sponges the amount is about 0.1×10^{-12} gm. per diploid nucleus, in mammals it is about 6.0×10^{-12} gm. In birds the amount is only a little more than a third of that found in mammals, being about 2.3×10^{-12} gm. As pointed out by White², there is a distinct break in the distribution of chromosome numbers of the mammals, the Eutherian mammals with few exceptions having a haploid number of the order of 20-30, the marsupials of the order of 6-12. The birds and monotremes both have considerably larger numbers, and resemble the reptiles in this respect. There are, then, at least two major characters by which the higher vertebrates differ: first, the deoxyribonucleic acid content of the nucleus, and secondly, the number of chromosomes. The two do not necessarily parallel one another; but there seems to be a tendency for the groups with many chromosomes to have less deoxyribonucleic acid. The deoxyribonucleic acid content of marsupial nuclei has not heretofore been measured, and investigations of this group are reported here.

Three species of marsupial were investigated. Fresh testes of *Macropus rufus* (kangaroo) were kindly provided by Mr. Edgell, of Sydney, who removed them from three specimens, sliced them into two or three pieces and shook them in saline. Formaldehyde to a final concentration of 4 per cent was then added for preservation. Sperm were separated from this material by low-speed centrifugation and washed. The final preparations contained

Table 1

Species	Tissue	Deoxyribonucleic acid per nucleus (gm. $\times 10^{-12}$)	Diploid chromosome number
<i>Macropus rufus</i>	Sperm	2.9	18 + XY
		3.1	
		3.4	
<i>Trichosurus vulpecula</i>	Kidney Spleen Intestine Liver	6.6	18 + XY
		6.0	
		6.4	
		5.0	
<i>Perameles nasuta</i>	Kidney Spleen Intestine Liver	8.9	12 + XX
		9.9	
		8.8	
		9.4	

less than 10 per cent of other nuclei as judged by Feulgen-stained smears. The number of extra nuclei was estimated from the smears and included in the final count. Liver, gut mucosa, kidney and spleen nuclei were isolated from young *Trichosurus vulpecula* (possum) and *Perameles nasuta* (bandicoot) by the method of Marshak³ and counted. Deoxyribonucleic acid was estimated by the method of Davidson, Frazer and Hutchison⁴. The results are shown in Table 1, with chromosome numbers (Sharman, G. B., personal communication).

The deoxyribonucleic acid content of marsupial nuclei is very similar to that of Eutherian mammals and different from that of birds. We cannot account for the rather high amount found in the nuclei of *Perameles*; it is certainly not less than in Eutherian mammals. We may conclude that the big increase in nucleic acid content of the nucleus which differentiates birds and mammals and appears to accompany a difference in chromosome number took place before mammals were differentiated into Eutherian and Marsupial forms.

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¹ Mann, T., "The Biochemistry of Semen" (Methuen and Co., London, 1954).

² White, M. J. D., "Animal Cytology and Evolution" (Camb. Univ. Press, 1954).

³ Marshak, A., *J. Biol. Chem.*, **139**, 607 (1950).

⁴ Davidson, J. N., Frazer, S. C., and Hutchison, W. C., *Biochem. J.*, **49**, 311 (1951).

Crystalline Cytochrome c from the King Penguin

CYTOCHROME c was prepared from skeletal (mainly pectoral) muscle of the king penguin (*Aptenodytes patagonica*) by the method of Keilin and Hartree^{1,2} and further purified with the aid of the ion-exchange resin 'Amberlite XE-64', by the method of Margoliash³.

Attempts were made to crystallize the protein by saturating 1-2 per cent aqueous solutions with ammonium sulphate at various pH's. From solutions of ferri-cytochrome only spherulitic globules were obtained, which did, however, exhibit birefringence between crossed nicols. But if a neutral solution of cytochrome, almost saturated with ammonium sulphate, was reduced by adding a trace of sodium dithionite, the reduced cytochrome was precipitated slowly in the form of true crystals possessing flat faces and exhibiting straight extinction between crossed nicols. The crystals have the form of thin plates, as shown in Fig. 1. Crystalline preparations of cytochrome c have not hitherto been reported in the literature.

The precipitate, which was almost completely crystalline, was centrifuged off and its enzymatic activity compared with that of purified horse cytochrome c. Two methods were used: (a) the cytochrome oxidase system with ascorbic acid as reducing