group, for rhodopsin is much more stable than opsin (*13*). If, as seems likely, acetylation removes numbers of amino groups that formerly had been engaged in hydrogen bonding, this would loosen the structure of the protein, making it more susceptible to denaturation. Presumably, the structure in the neighborhood of the prosthetic group is still intact, as evidenced by the unchanged optical properties. The bleaching process, by dissociating the prosthetic group, removes a further stabilizing influence, and the liberated acetyl opsin readily denatures.

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References and Notes

1. R. Hubbard, *J. Gen. Physiol.* 37, 381 (1953–54).
2. ——— and G. Wald, *ibid.* 36, 269 (1952–53); G. Wald and P. K. Brown, *Nature* 177, 174 (1956).
3. This research was supported by a grant to George Wald from the Rockefeller Foundation.
4. R. C. C. St. George, *J. Gen. Physiol.* 35, 495 (1952).
5. G. Wald and P. K. Brown, *ibid.* 35, 797 (1951).
6. G. Albrecht, in preparation.
7. G. Wald, *Science* 113, 287 (1951).
8. ——— and P. K. Brown, *J. Gen. Physiol.* 37, 189 (1953).
9. H. Fraenkel-Conrat, K. S. Bean, H. Lineweaver, *J. Biol. Chem.* 177, 385 (1949).
10. H. S. Olcott and H. Fraenkel-Conrat, *Chem. Revs.* 41, 151 (1947).
11. V. J. Harding and R. M. MacLean, *J. Biol. Chem.* 20, 217 (1915).
12. J. S. Ram *et al., Arch. Biochem. and Biophys.* 52, 464 (1954).
13. C. M. Radding and G. Wald, *J. Gen. Physiol.* 39, 909, 923 (1955–56).

30 October 1956

Route of Elimination of Anthracene and 9-Methylanthracene Involving Protein Binding

The emphasis of current research on the mechanism of carcinogenesis has been on the binding of the carcinogen or its metabolites with tissue protein. The relationship between binding and carcinogenic potentiality has been studied by changing the chemical agent (for example, carcinogenic, weakly carcinogenic, and noncarcinogenic agents, azo dyes, polynuclear hydrocarbons, an aromatic amine, and an acridine), and the tissue (for example, liver of rat, mouse, guinea pig, rabbit, cotton rat, and chicken; and skin of mouse, hamster, rat, rabbit, and guinea pig).

From their correlations with azo dyes, Miller and Miller proposed a protein- or enzyme-deletion theory of carcinogenesis (*1, 2*). These authors considered not only the maximum value of protein binding but also the rates of increase and decrease of the maximum (*2*). Following the demonstration that the carcinogens 3,4-benzpyrene (*3*) and 1,2,5,6-dibenzanthracene (*4*) were bound to mouse-skin protein, three groups reported on the relationship between skin protein binding of hydrocarbons and the carcinogenic potential of the hydrocarbon-tissue combination. Moodie, Reid, and Wallick concluded that noncarcinogens are either not bound to protein at all or are much more weakly held than are carcinogens (*5*). Woodhouse (*6*) found no relationship between protein binding and carcinogenic potentiality. Heidelberger and Moldenhauer (*7*) found a positive relationship between the maximum value of protein binding and carcinogenic potential in seven out of eight instances.

It was established that the binding of 9,10-dimethyl-1,2-benzanthracene-9,10-C^{14} with mouse-skin protein was dependent on the dose of carcinogen per unit area of skin and related to the weight response of the treated skin (*8*). This study has now been extended to several hydrocarbons, each of which was applied in a series of graded doses (*9*). A significant difference was observed in the rates of elimination from mouse-skin protein between two trinuclear hydrocarbons and two tetranuclear hydrocarbons. Twenty-four hours after application to mouse skin, the protein binding of anthracene-9,10-C^{14} (a noncarcinogen) and of 9-methylanthracene-9,10-C^{14} (a noncarcinogen) (Tables 1 and 2) was shown to be much lower than the protein binding of 9,10-dimethyl-1,2-benzanthracene-9,10-C^{14} (*8*) (a potent carcinogen) and of 1,2-benzanthracene-9,10-C^{14} (*10*) (a weak carcinogen, *11*).

The relatively rapid elimination of anthracene-9,10-C^{14} and of 9-methylanthracene-9,10-C^{14} might have taken place through a transient protein-bound phase or might not have involved any appreciable protein binding. The previous observations (*8, 10*) with the series of graded doses showed binding to be dose-dependent and hence suggested that measurements made within 24 hours after the application of the highest dose would be necessary to detect a transient protein-bound phase.

The experimental procedure has been described (*8*). The data (Tables 1 and 2) demonstrate that the relatively rapid elimination of anthracene-9,10-C^{14} and 9-methylanthracene-9,10-C^{14} from mouse skin involve a transient protein-bound phase of at least the same order of magnitude as that observed with 9,10-dimethyl-1,2-benzanthracene-9,10-C^{14}. Thus, the maximum value of protein binding is independent of the carcinogenic activity and structure of the hydrocarbon. Data were obtained by feeding rats the five azo dyes 3′-methyl-4-dimethylaminoazobenzene, 4-dimethylaminoazobenzene, 4′methyl-4-dimethylaminoazobenzene, 3-methyl-4-monomethylaminoazobenzene, and 2-methyl-4-dimethylaminoazobenzene. The last three dyes were of low carcinogenic potentiality, and two of these three experiments did not support a positive relationship between carcinogenicity and extent of binding with liver protein (*2*). The results with the polynuclear hydrocarbons differ from the azo dye data (*2*); no positive relationship was established between the rate at which the maximum of protein binding was attained and carcinogenic potential. Also in contrast with the azo dyes—specifically the very weakly carcinogenic dyes 2-methyl-4-dimethylaminoazobenzene and 3-methyl-4-monomethylaminoazobenzene (*2*)—the protein binding of anthracene and of 9-methylanthracene receded faster from their observed maxima than the protein binding of 9,10-dimethyl-1,2-benzanthracene and 1,2-benzanthracene.

Thus there is no experimental demon-

Table 1. Incorporation of radioactivity by mouse skin following the application of anthracene-9,10-C^{14} (0.208-percent series). Each determination was made on the pooled tissues of four mice. Each dose, 104 μg (1860×10^3 count/min) was applied over 3.46 cm^2 of skin. The analyzed dose was 76.3 μg (1365×10^3 count/min) of hydrocarbon over 2.54 cm^2 of skin. Each result is the average of two determinations.

Time after application (hr)	Total activity of whole homogenate (count/min)	Specific activity (count/min mg)	
		Particulate protein	Supernatant protein
0.5	414,000	543	1150
17	62,800	205	667
24	42,100	76	157

Table 2. Incorporation of radioactivity by mouse skin following the application of 9-methylanthracene-9,10-C^{14} (0.225-percent series). Each determination was made on the pooled tissues of four mice. Each dose, 113 μg (1860×10^3 count/min) was applied over 3.46 cm^2 of skin. The analyzed dose was 83.7 μg (1365×10^3 count/min) of hydrocarbon over 2.54 cm^2 of skin. Each result is the average of two determinations.

Time after application (hr)	Total activity of whole homogenate (count/min)	Specific activity (count/min mg)	
		Particulate protein	Supernatant protein
0.5	1,490,000	1410	1600
17	31,400	35	50
24	18,400	30	*

* One determination yielded 43 count/min mg; the other was too low to measure.

stration of a relationship between gross protein binding of hydrocarbons and a metabolic route that is specific to the carcinogenic process (*12*). In this study of contact carcinogenesis, there has been no identification of a specific metabolic route distinguishable from detoxication. The degradation of a hydrocarbon applied to the skin should involve enzymes, in view of which the results are reasonable. It is of interest that two examples of high metabolic activity in the skin have been found.

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References and Notes

1. E. C. Miller and J. A. Miller, *Cancer Research* 7, 468 (1947); 12, 547 (1952).
2. E. C. Miller *et al., ibid.* 9, 336 (1949); E. C. Miller and J. A. Miller, *J. Natl. Cancer Inst.* 15, 1571 (1955).
3. E. C. Miller, *Cancer Research* 11, 100 (1951); C. Heidelberger and S. M. Weiss, *Cancer Research* 11, 885 (1951).
4. W. G. Wiest and C. Heidelberger, *ibid.* 13, 250 (1953).
5. M. M. Moodie, C. Reid, C. A. Wallick, *ibid.* 14, 367 (1954).
6. D. L. Woodhouse, *Brit. J. Cancer* 8, 346 (1954); 9, 418 (1955).
7. C. Heidelberger and M. J. Moldenhauer, *Proc. Am. Assoc. Cancer Research* 2, 24 (1955); *Cancer Research* 16, 442 (1956).
8. V. Darchun and H. I. Hadler, *Cancer Research* 16, 316 (1956).
9. This research was supported by grant C-2399 and cancer control grant CS-9212 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.
10. H. I. Hadler *et al., Proc. Am. Assoc. Cancer Research* 2, 113 (1956).
11. P. E. Steiner and J. H. Edgcomb, *Cancer Research* 12, 657 (1952).
12. The experiments indicating the nonbinding of anthracene (*5*) and phenanthrene (*7*), which are both noncarcinogenic hydrocarbons with three rings, were not designed to detect a transient protein-bound phase.

9 July 1956

Sex Difference in Eosinophil Counts in Tail Blood of Mature B_1 Mice

Eosinophil counts in tail blood of mice vary with the phase of the daily adrenal cycle (*1–4*) in males (*1, 4, 5*) and in females (*6*) of several inbred strains and their hybrids. Strain differences in characteristic eosinophil levels—obtained at the daily time of high counts—have also been described (*7*). The study reported here (*8*) led to the additional detection of sex differences in the eosinophil count of mature mice of an inbred strain.

The mice studied were of a subline of the C_{57}-black stock (B_1) which had been maintained in the Division of Cancer Biology at the University of Minnesota by brother-to-sister mating for more than 20 generations. The animals were 8¼ months ± 1 week of age at the time of study. For 7 days prior to the study, they were singly housed in a room maintained at 25.6 ± 0.6°C and illuminated from 6 A.M. to 6 P.M. and darkened from 6 P.M. to 6 A.M. Purina Fox Chow and tap water were available to the mice from the time of weaning and throughout the study. Except for the activities associated with cleaning the cages, feeding the mice, and filling the water bottles, the animals were not intentionally disturbed from birth until the time of study. The study was begun at 8:30 A.M. and ended at 8:30 A.M. the following day. It involved eosinophil counts on tail blood obtained from separate groups of mice at 4-hour intervals. Each mouse was thus used for venesection only once. The assembly-line procedures employed for eosinophil counts have been described (*7*).

Figure 1 shows the mean count ± 1 standard error for the two sexes. The 24-hour eosinophil rhythm stands out clearly for each sex, in agreement with the results of earlier work on this subject. But the level around which the mean count cycles in the two sexes, which was previously not compared, is not the same in the two sexes as far as the stock and age-group studied are concerned (Fig. 1). The females exhibit lower counts than the males, without any overlap of mean counts throughout the 24-hour period. Subsequent work on the same mice revealed that the mean paired adrenal weights of the females were higher than those of the males and that the ascorbic acid concentrations in glands from females were higher than in glands from males.

The observation of a sex difference in adrenal weight of B_1 mice extends to this stock observations on the sexual dimorphism of the adrenals, which was reported earlier for several animal forms (*9*). The sex difference in eosinophil count deserves more study, for it may be related to sex differences in adrenal secretory behavior, as anticipated but not yet reliably established (*9*).

The possible relation of the sex difference in eosinophil level to a sex difference in adrenal secretory activity comes to mind in view of the known eosinopenic effect of both cortical and medullary adrenal hormones (*2*). The role played by interaction of the longer estrus cycle (*10*) with the daily adrenal cycle (*2, 4, 6*) which maintains the eosinophil rhythm must also be considered. But whatever may underlie the differences noted, it seems fair to conclude that, in B_1 mice of the age group studied, eosinophil counts can be shown to be a function not only of strain and of phase of daily cycle but also of sex.

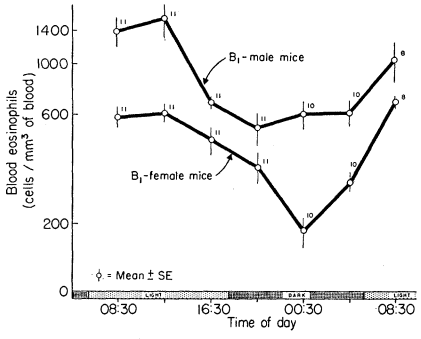

Fig. 1. Sex difference in mean blood eosinophil counts of B_1 mice approximately 8 months old. The number of mice used given above each point; serially independent sampling.

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References and Notes

1. F. Halberg and M. B. Visscher, *Proc. Soc. Exptl. Biol. Med.* 75, 846 (1950).
2. F. Halberg, *J.-Lancet* 73, 20 (1953).
3. ——— and M. B. Visscher, *Endocrinology* 51, 329 (1952); F. Halberg, *Acta Med. Scand. Suppl.* 302, 117 (1955).
4. H. E. Brown and T. F. Dougherty, *Endocrinology* 58, 365 (1956).
5. H. Panzenhagen and R. Speirs, *Blood* 8, 536 (1953); C. Louch, R. K. Meyer, J. T. Emlen, *Proc. Soc. Exptl. Biol. Med.* 82, 668 (1953).
6. F. Halberg, M. B. Visscher, J. J. Bittner, *Am. J. Physiol.* 174, 109 (1953).
7. F. Halberg, J. J. Bittner, M. B. Visscher, *Blood* 6, 832 (1951).
8. This investigation was aided by the Elsa U. Pardee Foundation and by research grants from the U.S. Public Health Service (G-4158 and B-980), the American Cancer Society, and the Graduate School of the University of Minnesota.
9. F. A. Hartman and K. A. Brownell, *The Adrenal Gland* (Lea and Febiger, Philadelphia, 1949), p. 325; R. Bachmann, in *Handbuch der mikroskopischen Anatomie des Menschen* (Springer, Berlin, 1954), vol. 6, p. 952.
10. G. Bourne and S. Zuckermann, *J. Endocrinol.* 2, 268 (1940).

29 October 1956

Tentative Correlation of Alaskan Glacial Sequences, 1956

A tentative correlation of the Cook Inlet and other Alaskan glacial sequences was published in 1953 (*1*). Since then, additional field and radiocarbon data have accumulated that necessitate some revisions in the 1953 correlation chart. These revisions (Fig. 1) include additions to earlier published glacial sequences and changes in correlation with the type Pleistocene chronology.

Correlations with the standard North American Pleistocene chronology have been revised in accordance with new radiocarbon data relating to both the Mid-continent and Alaskan sequences. Reruns of radiocarbon samples W-76, W-77 (*2*), and also W-174 (*3*) by the more accurate gas-counting method (these samples were originally analyzed by the solid-carbon counting method) indicate that the lower boundary of the