4. National Cancer Institute, *Monograph 21* (1966), p. 1.
5. Styrene divinylbenzene "uniform latex particles"; Dow Chemical Co., Midland, Mich. The manufacturer specifies that the particles range in diameter from 6 to 14 and from 12 to 35 μ; density, 1.05.
6. N. G. Anderson, *Exp. Cell Res.* **9**, 446 (1955).
7. Facilities provided by National Cancer Institute contract PH 43-65-641. We thank G. H. Weiss, N. G. Anderson, Sheldon Gottlieb, J. E. Verna, and Theresa Pretlow for interest and criticism; and Carol Fisher, Marcia Cleveland, and Clinton Thompson for technical assistance.

17 July 1968

Oncogenicity by Methyl Methanesulfonate in Male RF Mice

Abstract. *The incidences of lung tumors and thymic lymphomas were increased in young adult male RF mice receiving 30 milligrams of methyl methanesulfonate per kilogram of body weight daily in the drinking water throughout life. Differences in oncogenicity between treatment with methyl methanesulfonate and with dimethylnitrosamine or diethylnitrosamine suggest a qualitative difference between the site (or sites) of alkylation by methyl methanesulfonate and by dimethylnitrosamine or diethylnitrosamine within the nucleic acids.*

The mutagenic and carcinogenic action of alkylating agents has been partly attributed to alkylation of nucleic acids, which results in an altered cellular genome (*1, 2*). Diethylnitrosamine (DEN) and dimethylnitrosamine (DMN), potent carcinogens in various animal species (*3, 4*), alkylate nucleic acids, especially at the 7-position of guanine (*5*). Methyl methanesulfonate (MMS) is reportedly as effective as DMN in alkylating DNA and RNA (*2*); it also methylates at the 7-position of guanine in bacteriophages (*6*). No reports, however, incriminate MMS as a carcinogen; repeated doses have been given to male rats for study of the effect on fertility (*7, 8*), but even after prolonged treatment no tumors were observed (*9*). We now report demonstration of an oncogenic effect by MMS on lung and thymus in mice.

Sixty-three 11-weeks-old male mice from a noninbred subline of the RF/Un strain were given MMS (*8*) for life in drinking water at 20 mg/100 ml. Fresh drinking solutions were prepared daily, and the water consumption per cage (initially eight mice) was measured daily. Mice were weighed weekly, and the intake of MMS was calculated in milligrams per kilogram of body weight. Groups each of four mice were killed and necropsied after 3, 6, 9, and 12 months of treatment; the remainder were necropsied at death or killed when in a moribund condition, and tissues from all major organs were examined histologically. A group of 162 untreated RF male mice, handled similarly, served as controls.

Of the 47 treated mice allowed to die, 70.2 percent had primary pulmonary tumors, compared to 38.9 percent of 162 controls (Table 1). The mean age at death of mice with lung tumors was slightly less in the treated group than in the control group: 19.7 versus 22.0 months. In the 28 mice examined histologically, the incidence was 82 percent, suggesting that the overall incidence would have been even higher if lung tissue from all mice had been examined microscopically. The tumors were adenomas morphologically indistinguishable from those observed either of spontaneous origin or after treatment with DEN (*3*) or DMN (*4*).

Thymic lymphomas developed in 14.9 percent of the treated mice and in 3.7 percent of the controls (Table 1); these neoplasms originated in the thymus, infiltrated locally into the lungs and heart, and then spread throughout the body. The disease process appeared identical in treated and untreated mice, with mean ages at death of 17.3 and 15.4 months, respectively. Myeloid leukemia was not observed in the mice treated with MMS but was diagnosed in 4.3 percent of the controls. Other types of leukemias and lymphomas were predominantly reticulum-cell sarcomas in both treated and control mice, the overall incidences being 44.7 and 60.5 percent, respectively; the incidences were not different when adjusted for differences in survival time. Other pathological changes in the treated mice did not differ from those in the controls.

Mice receiving MMS showed no gain in weight, in contrast with the controls whose mean weight increased from 35 g at 11 weeks of age to 43 g at 43 weeks. Although the calculated daily dose levels of MMS were from five to 30 times the sublethal levels of DEN and DMN, respectively (*3, 4*), the mean survival time of treated mice (18.4 months) was not greatly different from that of the controls (20.5 months). On the basis of the mean survival time, the mean cumulative dose for all treated mice was 13,800 mg/kg—approximately 30 mg/kg daily.

Table 1. Observed incidences (percentages) of neoplasms in 47 male RF mice treated daily with methyl methanesulfonate (MMS) at 30 mg/kg in the drinking water; controls numbered 162. "Other" leukemias were primarily reticulum-cell sarcomas.

Item	Treatment	
	MMS	None
Tumor sites		
Lung	70.2	38.9
Liver	4.2	3.7
Stomach	2.1	0
Leukemias		
Thymic lymphoma	14.9	3.7
Myeloid	0	4.3
Other	44.7	60.5

Alkylation occurs primarily at the 7-position of guanine in both RNA and DNA after administration of MMS (*10*), as after DMN or DEN (*1, 2, 5*). The fact that levels of methylated guanine in rat kidney after administration of MMS are comparable to those following nitrosomethylurea or DMN (*11*) suggests that the precise location or grouping of the alkylation, along the nucleic acid chain, may be important. The significance of alkylation of proteins or of bases other than guanine in the oncogenic process is unknown; however, the 1- and 3-positions of the imidazole ring of histidine amino acid residue are principal sites of reaction with DMN (*12*), whereas MMS reacts with the sulfhydryl group of cysteine, with no detected reaction with the histidine side chain (*13*).

If one measures by induction of lung tumors, MMS is slightly more effective than DEN (*3*) but less so than DMN (*4*). While DEN and DMN fail to induce leukemia (*3, 4*), MMS is leukemogenic, resembling x-rays (*14, 15*) and nitrogen mustard (HN2), triethylene melamine, and myleran (*14–16*), all of which induce thymic lymphomas. The fact that MMS fails to reduce the latent period for lung tumors and thymic lymphomas suggests that carcinogen-screening programs require observation periods sufficiently long for detection of late-occurring as well as early tumors.

N. K. Clapp, A. W. Craig*
R. E. Toya, Sr.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

References and Notes

1. P. Brookes and P. D. Lawley, *Brit. Med. Bull.* **20**, 91 (1964); *J. Cellular Comp. Physiol.* **64**, 111 (1964).
2. P. N. Magee, in *Molekulare Biologie des malignen Wachstums*, H. Holzer and A. W. Holldorf, Eds. (Springer, Berlin, 1966), pp. 79–95.
3. N. K. Clapp and A. W. Craig, *J. Nat. Cancer Inst.* **39**, 903 (1967).

4. ———, R. E. Toya, Sr., *ibid.*, in press.
5. K. Y. Lee, W. Lijinsky, P. N. Magee, *ibid.* **32**, 65 (1964); P. N. Magee and K. Y. Lee, *Biochem. J.* **91**, 35 (1964).
6. P. Brookes and P. D. Lawley, *Biochem. J.* **89**, 138 (1963).
7. H. Jackson, B. W. Fox, A. W. Craig, *J. Reprod. Fertility* **2**, 447 (1961).
8. Synthesized in our laboratory. H. Jackson and A. W. Craig, *Ann. N.Y. Acad. Sci.*, in press.
9. A. W. Craig and H. Jackson, unpublished.
10. D. J. Pillinger, thesis, St. Andrew's University, Scotland (1961).
11. P. N. Magee, V. M. Craddock, P. F. Swann, in *Carcinogenesis, A Broad Critique* (Williams and Wilkins, Baltimore, 1967), pp. 421–39.
12. V. M. Craddock, *Biochem. J.* **94**, 323 (1965).
13. A. W. Craig, D. P. Pillinger, B. W. Fox, unpublished.
14. J. W. Conklin, A. C. Upton, K. W. Christenberry, T. P. McDonald, *Radiation Res.* **19**, 156 (1963).
15. A. C. Upton *et al.*, *Nat. Cancer Inst. Monograph 22* (1966), pp. 329–47.
16. A. C. Upton, F. F. Wolff, E. P. Sniffen, *Proc. Soc. Exp. Biol. Med.* **108**, 464 (1961).
17. Jointly sponsored under the NCI–AEC Carcinogenesis Program by PHS and AEC under contract with Union Carbide Corp.
* Present address: Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester 20, England.

24 July 1968

Migratory Animals as Dispersal Agents of Cultural Materials

Abstract. *Migratory animals (such as waterfowl, fish, whales, and seals) and large land animals (such as deer, elephant, bear, and cattle) that were hunted for food by aboriginal peoples may be struck, but not killed, by projectile weapons which remain imbedded in the body. Individuals which survive may be killed elsewhere, and the projectile weapon tips may be recovered. Numerous instances are cited. Such exotic artifacts can be mistakenly attributed to cultural contact between distant human groups unless archeologists are aware of this special kind of diffusion process.*

A possible means of noncultural diffusion of certain kinds of artifacts to distant areas is by migratory animals carrying such items imbedded in their bodies. A parallel in biology is the transport of viable algae and seeds by migratory water birds. Transport by floating objects (such as derelict boats, planks, or masts) with iron imbedded or attached, from which people with no experience working with metal secured iron, or dense stones used to make cutting tools and recovered by occupants of coral islands from the root net of drift trees (*1*), represent adventitious distributions which serve as a warning that the occurrence of all artifacts or materials of distant origin may not be taken as proof of contact through travel or trade between the areas and peoples concerned. Large mammals, not surprisingly, can receive a spear or dart which does not cause death and whose point (or at times the whole shaft) may remain imbedded and may be carried until the animal's death. Inferences on the migrations of oceanic whales and the existence of the Northeast Passage before it was traversed by Europeans were derived from harpoons found in whales' bodies in the 17th and 18th centuries by oceanic voyagers (*2*). Aboriginal harpoons found in whales' tissues were also recorded by 19th-century European sailors (*3*); in earlier times such spent weapon points recovered from killed or stranded whales could have served as models. The St. Lawrence Island Eskimos are reported to believe that weapon tips found in this way possess magical properties, and they keep the tips as charms of great value and potency (*4*). Harpoons are still being found in the bodies of stranded whales (*5*).

There have frequently been reported migratory waterfowl that carried weapons from the locality where they were hunted to distant places. A notable example is that of two honey buzzards (*Pernis apivorus*), shot in Finland in 1894 and 1900, each of which was found to have sticking in a wing a wooden arrow identifiable as having been made by the Pangwe tribe of southwestern Cameroon (*6*). Ducks, brants, and swans that carried Eskimo bone arrowheads have been killed in California, Indiana, North Carolina, and Nebraska (*7*). Eskimo toggle harpoons, such as the one found in an archeological site in Iowa (*8*), or some of the barbed bone projectile points from the exposed strands of the now-desiccating Pyramid Lake in Nevada (*9*) may have been introduced by migratory waterfowl wounded in Alaska.

Other animals which when killed and butchered have been found to bear imbedded weapon points are deer, grizzly bears, hogs, cattle, and seals (*10*). Among the most ancient finds of this sort are the weapon tips penetrating reindeer bones in the Upper Paleolithic (Magdalenian) site of Stellmoor, Germany (*11*), and seal skeletons from the Mesolithic period found on raised beaches in Finland (*12*).

Fish may serve as disseminators of items such as spear tips and hooks in much the same way that large sea and land mammals do (*13*). It has been suggested and discussed at length that the unusual C-shaped shell fishhooks of the Santa Barbara region may have been introduced there by live tuna migrating from the western Pacific and arriving on the California coast with such hooks snagged in their mouths (*14*). Fish are also recorded as carrying artifacts such as a knife and scissors in the stomach (*15*). An instance of a South American rhea with three stone artifacts (two axes and a hammer) as gizzard stones that were swallowed at some distance from where it was killed is parallel (*16*).

Live elephants also carry imbedded weapons that are found when they are killed and butchered. (*17*). Since these animals live as long as 100 years and are migratory over distances of many hundreds of miles, one wonders whether the mammoths of terminal Pleistocene times, found in North America associated with flint projectile points, might not be late-surviving, repeatedly attacked individuals that were carrying weapon tips acquired over a span of several scores of years in quite different localities. Several mammoth skeletons have been associated with numbers of flint projectile points (*18*), and the pieces in each of these lots vary somewhat in size, form, and material. Krieger (*19*) has called attention to the use of nonlocal stone for projectile points in some Early Man kill sites, and he suggests that this may indicate far-ranging procurement of implement material by the hunting group. This may be true, but the alternative possibility, that points made of flints which occur at some distance away may have been acquired from the bodies of killed animals, may be worth considering.

Paleo-Indians (also called Big Game Hunters) in the New World (*20*) are usually assumed to have been free-ranging, nomadic hunters, but they may, for all we know, have been seasonally settled foragers occupying specific territories. Petrographical and typological examination of the weapon points found associated in kill sites of the Big Game Hunters could lead to some understanding of the nature of their social groups, if it could be demonstrated that some of these weapons were foreign to the area in which they were found.

ROBERT F. HEIZER
Department of Anthropology, University of California, Berkeley

References and Notes

1. T. Rickard, *Geogr. Rev.* **24**, No. 4 (1934).
2. G. F. Muller, *Voyages from Asia to America* (London, 1761), p. xlii; J. Burney, *A Chronological History of North-Eastern Voyages of Discovery* (London, 1819), pp. 109–110; J. W. de Peyster, *The Dutch at the North Pole* (New York Historical Society, New York, 1857), p. 10; G. Steller, *Beschreibung von*