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13. It is possible that what appears as an inhibitory zone, rapidly established around a proheterocyst (*9*) may actually be the outcome of competition between two or more smaller daughter cells, the first generally being the successful contender. There is, in fact, no sharp distinction between competitive and inhibitory zone mechanisms [see our model in (*12*)].
14. We thank P. Lawrence for helpful comments.
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Mutagenicity of Malonaldehyde, a Decomposition Product of Peroxidized Polyunsaturated Fatty Acids

Abstract. *Incubation of histidine requiring auxotrophs of the bacterium* Salmonella typhimurium *with malonaldehyde, a three-carbon dialdehyde, produced an increased number of revertants in specific strains. Mutagenesis was only observed in frameshift mutants with normal excision repair and did not occur in those base-pair substitution mutants tested. The results are consistent with the cross-linking of bacterial DNA by malonaldehyde leading to mutagenesis expressed through the error-prone repair system.*

A possible role for membrane lipid peroxidation in carcinogenesis and mutagenesis has been indirectly suggested by the ability of antioxidants, including vitamin E, to protect against experimental carcinogenesis (*1*), although other studies have failed to demonstrate such an effect (*2*). Similarly, there is evidence both supporting (*3*) and opposing (*4*) the contention that diets high in polyunsaturated fatty acids lead to a higher incidence of cancer. The relationship of lipid peroxidation to carcinogenesis and mutagenesis therefore remains tenuous, particularly in the absence of a clear-cut mechanism by which the breakdown of cell membrane unsaturated fatty acid might damage genetic material.

One potential intermediary in such a process is malonaldehyde, a three-carbon dialdehyde ($OHC\text{-}CH_2\text{-}CHO$) produced during the oxidative decomposition of polyunsaturated fatty acids. Malonaldehyde is formed during the metabolism of certain hydrocarbon carcinogens (*5*), has been reported to initiate skin carcinogenesis in mice (*6*), and will cross-link the amino groups of DNA in solution, presumably through the formation of Schiff bases (*7*). Although this latter process has a low *p*H optimum, the recent observation in this laboratory (*8*) indicating that malonaldehyde cross-links of aminolipid are formed as a consequence of red cell lipid peroxidation suggests that the cross-linking of amino groups by malonaldehyde does occur in vivo in humans with normal vitamin E levels.

The present study utilizes a microbial mutagenesis assay system to evaluate the possibility that this lipid peroxide decomposition product is capable of inducing mutation. Recent studies utilizing similar systems suggest that there is a high positive correlation between carcinogenic and mutagenic activity (*9*). The 12 *Salmonella typhimurium* strains used (kindly provided by Dr. B. N. Ames) are shown in Table 1. The mutations involved in these strains have been fully described elsewhere (*10*). Briefly, the bacteria are all histidine-requiring auxotrophs, each containing a mutation on one of four histidine loci. One set of mutants has the normal polysaccharide coat as well as a normal excision repair system; the second set has a deep rough mutation (rfa) in the cell wall constituents and normal repair; and the last set of mutants has both the rfa mutation and a deletion through the uvrB gene which eliminates normal excision repair. Three of the strains (hisG46, TA1975, TA1535) have been used to detect mutagens causing base-pair substitutions, while the remaining nine strains have been used to detect various kinds of frameshift mutagens.

Mutagenic activity was determined by the standard spot tests and the quantitative soft agar techniques as described by Ames *et al.* (*10*). Malonaldehyde was prepared from malonaldehyde bis-(dimethylacetal) by shaking with Dowex 50 (X2) in the H^+ form.

The mutagenic activity of malonaldehyde on the various tester strains is shown in Table 2. The results indicate that malonaldehyde is mutagenically active, but only on frameshift mutants with normal excision repair: the hisD3052 mutant is the most sensitive strain, followed by the hisC207 strain and the hisC3076 strain. None of the base-pair substitution mutants tested thus far has shown any increase in revertant frequency.

The pattern of mutagenic specificity demonstrated by malonaldehyde is similar to the mutagenic activity of Mitomycin C, a DNA cross-linking agent (*11*). This carcinogenic (*12*), bifunctional antibiotic requires the participation of excision repair processes to show mutagenic activity (*13*). It has been concluded that mutations produced by Mitomycin C are due to errors in repair by the error-prone repair system as a result of excision repair–induced DNA damage (*13*). Malonaldehyde, which is also a cross-linking agent, can alter DNA as indicated by the formation of fluorescent

Table 1. Strains used for mutagen testing.

Histidine mutation in strains				Additional mutations	
G46	C207	C3076	D3052	Repair	Cell wall
hisG46	hisC207	hisC3076	hisD3052	+	+
TA1975	TA1976	TA1977	TA1978	+	rfa
TA1535	TA1536	TA1537	TA1538	uvrB	rfa

Table 2. Mutagenicity of malonaldehyde on tester strains.

Strain	Average number of revertants per plate*				Additional mutations	
	Spot test		Soft-agar test			
	Control	MDA†	Control	MDA†	Repair	Cell wall
hisG46	19	21	8	12	+	+
TA1975	15	19	4	3	+	rfa
TA1535	9	11	13	16	uvrB	rfa
hisC207	22	62	21	72	+	+
TA1976	23	48	18	57	+	rfa
TA1536	2	2	8	8	uvrB	rfa
hisC3076	41	103	47	118	+	+
TA1977	34	74	40	95	+	rfa
TA1537	22	21	87	88	uvrB	rfa
hisD3052	38	285	28	164	+	+
TA1978	45	337	35	161	+	rfa
TA1538	61	78	39	57	uvrB	rfa

*Numbers represent averages from duplicate plates. †Ten millimoles of malonaldehyde in 0.1 ml of distilled water was applied to sterile filter disks (12.7 mm in diameter) placed on the center of seeded petri plates (for spot test) or mixed with cells in 2.5 ml of top agar (soft-agar test). Plates were counted after 2 to 5 days of incubation at 37°C. All numbers presented are counts on day 5.

products and the modification of thermal denaturation profiles and chromatographic behavior (*7*). Agents that alter DNA structure are known to elicit the functioning of bacterial repair processes (*14*). Possibly pertinent is the observation that malonaldehyde is reported to be particularly prone to react with guanine and cytidine (*7*) and that the frameshift revertants of the hisD3052 mutation observed in the present study appear to be expressed as a result of deletions of GC base-pairs (*15*).

The relationship between the present findings and human carcinogenesis or mutagenesis possibly associated with lipid peroxidation or the ingestion of a diet high in polyunsaturated fatty acids remains conjectural. In this respect it should be noted that the frameshift mutants utilized in our studies are particularly sensitive to mutagenesis by a number of polycyclic carcinogens (*16*), at least some of which are also associated with the production of lipid peroxidation during their metabolism (*5*). An additional area of possible pertinence, and the original impetus for this study, concerns the observation of chromosomal aberrations in the circulating lymphocytes of man and Chinese hamsters following inhalation of relatively low levels of ozone (*17*), an air pollutant known to be mutagenic in animals and bacteria (*18*). It is unlikely that this extremely reactive oxidant gas, which produces lung lipid peroxidation (*19*), can itself reach directly into the nucleus of circulating lymphocytes. As malonaldehyde is relatively polar it conceivably could penetrate into chromosomal material following membrane lipid peroxidation produced by ozone or other exogenous agents. In addition, a malonaldehyde-like compound has been reported to be formed during x-irradiation of DNA itself (*20*). Further study is required to ascertain the relationship of lipid peroxidation and malonaldehyde formation to human carcinogenesis and mutagenesis.

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A Serum Protein Associated with Chromatin of Cultured Fibroblasts

Abstract. *Antibodies to chromatin proteins from human and mouse fibroblasts which have been cultured for more than 25 generations with heterologous serum show specificity for a homologous α-serum protein. These results indicate that among the chromatin-associated proteins there is one (or more) which has extensive structural similarity to a serum protein. This is the first direct evidence that a serumlike protein or proteins could be chromatin associated in vivo, as has been suggested by experiments showing in vitro interaction between DNA and certain serum proteins.*

In bacteria, gene regulator proteins bind to specific sites of DNA. This suggests that in eukaryotes also, the capacity to bind DNA may be an important property of the proteins involved in genomic functions. For this reason a large number of nuclear and cytoplasmic proteins with the ability to bind to DNA have been extensively studied in multicellular organisms (*1–3*).

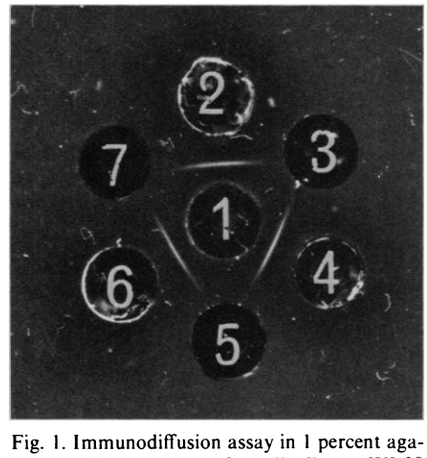

Fig. 1. Immunodiffusion assay in 1 percent agarose. *1*, Human serum; *2*, antibodies to WI-38 chromatin; *3*, mouse serum; *4*, as in *2* after adsorption by calf serum; *5*, as in *2* after adsorption by WI-38 chromatin; *6*, as in *2* after adsorption by 3T6 chromatin; *7*, preimmune serum. Chromatin and chromatin fractions were prepared as previously described (*7, 12*). Antibody adsorptions were performed mixing 1 ml of antiserum with 2 mg of DNA as chromatin in tris buffer, *p*H 8.2, 0.15*M* NaCl; or with 2 ml of serum. The antigen and antiserum mixture was incubated for 2 hours at 37°C and 16 hours at 4°C. At the end of the incubation the mixtures were centrifuged at 30,000*g* for 20 minutes. The supernatants were used for the immunodiffusion assay. The final antiserum dilution was 1 : 4. The dilution of serum used as antigen was 1 : 8.

It has been reported that mammalian serums and amniotic and cerebrospinal fluids also contain proteins capable of reacting with DNA in vitro (*4–6*). Kubinski and Javid (*5*) showed an increase in the concentration of such DNA-binding proteins in cerebrospinal fluid of patients with certain diseases of the central nervous system. More recently, Hoch *et al.* (*6*) showed that human serum contains a group of proteins with affinity for DNA and that the serum of patients with malignant disorders contains a higher concentration of such proteins. These authors suggested that these proteins could be bound to DNA in vivo and may be involved in genomic function. However, although the affinity of proteins for DNA may be related to their biological function, there is no direct evidence that serum proteins which bind to DNA in vitro are also bound to DNA in vivo.

In this report we provide evidence that serum proteins could be DNA associated in vivo. Using antibodies to chromatin proteins, we have found that a serum protein has extensive structural similarity to nonhistone chromosomal proteins.

Antibodies to chromatin proteins were obtained by injecting rabbits with chromatin preparations from 3T6 mouse or WI-38 human fibroblasts as previously reported (*7*). In the immunodiffusion assay we observed only a single precipitin line between each of our antiserums (five preparations for 3T6 chromatin and eight preparations