ponents are important in the early modification of biochemicals. Nevertheless, biological activity probably determines the overall organic content in the sediments.

The same pattern was found in a second algal mat from the middle Laguna Madre. This mat was much larger in area than the first, but the layered structure was not as well developed. Preliminary data from a sediment core show the early disappearance of highly unsaturated acids (Table 1). However, oleic acid has not fallen to the very low level of the algal mat, although the sediment is much older. This may be due to the very different type of environment.

A detailed study of the geochemistry of fatty acids in recent sediments is being carried out in this laboratory. A more complete report will be published later.

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Circulating DNA as a Possible Factor in Oncogenesis

Abstract. *Infectious DNA from the tumor-inducing polyoma virus and pneumococcal-transforming DNA can be recovered from the blood of mice in biologically active form after intraperitoneal injection. Polyoma DNA appeared to undergo less inactivation than did transforming DNA. In light of these observations, the metastatic spread of cancer may possibly be favored by circulation of tumorigenic DNA in the blood stream.*

Tumors frequently develop at places remote from the site of inoculation when infectious DNA (*1*) isolated from the tumor-inducing polyoma virus (*2*) is injected into newborn and adult hamsters (*3*). This suggests that tumorigenic DNA may be transported by way of the blood or the lymphatic system and later penetrate cells of various tissues. Mammalian cells in vitro (*4*), and possibly in vivo (*5*), can take up DNA. However, it is so far unknown whether biologically active DNA can appear and persist in the blood stream after its injection elsewhere in the body. Accordingly, DNA prepared from polyoma virus, pneumococcus, L1210 leukemia cells, or Ehrlich ascites cells, was administered to mice, and its appearance in the blood was studied.

Infectious DNA was isolated by a trichloroacetate modification (*6*) of the phenol procedure (*1*) from a stock of polyoma virus prepared from infected mouse kidney cells (*7*). The infectious titer of the DNA, determined by plaque assay (*6, 8*) in the presence of mouse plasma, was about 2.8×10^4 plaque-forming units (PFU) per milliliter. Pneumococcal-transforming DNA (*9*) was prepared according to the procedure of Hotchkiss (*9*). Transformation assays for the streptomycin marker were performed with the R36A receptor strain of pneumococcus with antiserum to the rough strain of pneumococcus (*10*). Tritium-labeled DNA (specific radioactivity, 0.5 μc/mg) was isolated by the detergent technique from L1210 ascites leukemia cells grown in BDF_1 mice which had received thymidine-^{3}H intraperitoneally (Schwarz BioResearch, Inc.) (*11*). DNA labeled with ^{32}P was similarly isolated from Ehrlich ascites carcinoma cells (5 days old) grown in mice of the A-strain, 24 hours after an injection of 20 μc $Na_2H^{32}PO_4$ (Commissariat de l'Energie Atomique, France).

Male Swiss mice (about 25 g) were injected with DNA either intraperitoneally or intravenously (Tables 1 to 3). Blood, collected by heart puncture with syringes containing saline-citrate, was immediately centrifuged to obtain plasma. After being washed, the separated blood cells were resuspended in a volume of saline equal to that of the blood withdrawn.

To learn the extent of its appearance and possible breakdown in the blood, DNA-^{32}P was injected intraperitoneally into mice, and the fractions of the plasma soluble and insoluble in perchloric acid (*12*) were examined. The relative radioactivities of these fractions over a 2-hour period (Fig. 1) indicate that there was a rapid and considerable transfer of radioactivity from the peritoneal cavity to the blood stream. The DNA did not break down

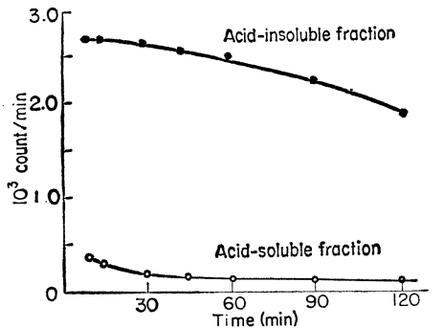

Fig. 1. Changes in plasma of mice after intraperitoneal injection with 0.2 mg of DNA-^{32}P (5×10^4 count/min mg^{-1}) isolated from mouse ascites cells. Each point, which represents the average of data for eight mice, is calculated for the total blood plasma which is taken as 1.3 ml for a mouse weighing 30 g.

extensively in the blood since only small amounts of acid-soluble material appeared in the plasma during a 2-hour period in which the bulk of the radioactivity remained in an acid-insoluble form, and at an almost constant level.

To estimate the relative distribution of DNA, or fragments thereof, between the blood cells and plasma, DNA-^{3}H (from L1210 ascites leukemia cells) was injected, and radioactivities of these two blood fractions were determined over a 45-minute period (Table 1). When the DNA was given intravenously, about half of its radioactivity could be demonstrated in the blood; roughly twice as much was associated with the cellular elements as was present in the plasma. On the other hand, roughly one-fourth of the radioactivity of DNA injected intraperitoneally was found in the blood, and the amount of label associated with the cells was about three times that present in the plasma.

Although the form or forms in which the intraperitoneally injected DNA-^{3}H circulated in the blood plasma are not yet known precisely, it was considered, as a result of CsCl density-gradient centrifugation, to be macromolecular, 25 percent of the material having the buoyant density of the native and the remainder that of heat-denatured nucleic acid (*13*). The amount of radioactive substance in the blood remained constant for 45 minutes (Table 1) after the injection of DNA-^{3}H by either route.

The radioactivity remained at the same absolute concentration in the blood even when the amount of DNA-^{3}H injected intraperitoneally was varied over a 20-fold range (Table 2). The

radioactivity was the same in both plasma and serum. There would thus appear to be some physiological mechanism which can regulate the amount of DNA transferred from the peritoneal cavity to the blood stream.

When assessing the biological activity of pneumococcal-transforming DNA and polyoma virus DNA after injection into mice, it was important to know whether plasma exerted any effect in vitro on the activity of these nucleic acids. Plasma caused a 90 to 99 percent decrease in the transforming activity of pneumococcal DNA (Table 3) when this DNA was incubated for 10 minutes with plasma rather than with phosphate-buffered saline. Nuclease inactivation of *Hemophilus influenzae*–transforming DNA after incubation with normal human plasma has already been described (*14*). In sharp contrast, there was a two- to threefold increase in the plaque-forming activity of infectious polyoma virus DNA after incubation with plasma (*15*). The closed-ring structure of polyoma virus DNA (*16, 17*) may be responsible for its resistance to inactivation under these circumstances. Polyoma DNA is resistant to boiling (*18*), as is the infectious DNA from the tumorigenic papilloma virus (*19*). In fact, the infectivity of polyoma virus DNA increases about threefold after it is boiled in solution for about 15 minutes (*18*) and a similar resistance to treatment with alkali (*p*H 12.2) is also observed (*17*). Both these treatments cause a profound decrease in the activity of bacterial-transforming DNA (*20*).

Table 1. Appearance and persistence of radioactivity (count/min) in the blood after injection of DNA-^{3}H into mice.

Time after injection (min)	Intravenous*		Intraperitoneal*	
	Blood cells (count/min)†	Plasma (count/min)†	Blood cells (count/min)†	Plasma (count/min)†
5	1260	620	590	200
10	1190	530	592	180
15	1180	570	620	190
30	1330	516	650	180
45	1250	520	540	170

* Adult mice were injected intraperitoneally with 0.1 mg of DNA-^{3}H (prepared from L1210 ascites leukemia cells) dissolved in 0.2 ml of saline-citrate, or intravenously with 0.1 mg in 0.1 ml. Total radioactivity injected per mouse was 3300 count/min. The data are calculated for 1.5 ml of blood (or the plasma therefrom). † Data represent the averages of results from four experiments.

Table 2. Radioactivity in whole blood after intraperitoneal injection of varying amounts of DNA-^{3}H into mice.

Amount of DNA-^{3}H injected		Radioactivity (count/min) after injection	
μg	Count/min	10 min	45 min
25	825	560	570
50	1650	590	600
100	3300	620	750
400	13200	610	640

Table 3. Appearance and degree of infectious activity* of polyoma DNA (measured in plaque-forming units per milliliter) and transforming activity (in numbers of streptomycin-resistant cells per milliliter) of pneumococcal DNA after their injection into mice.

DNA incubated with		Route of administration			
		Intraperitoneal§		Intravenous§	
Plasma†	PBS‡	Blood cells	Plasma	Blood cells	Plasma
		Polyoma DNA activity in PFU/ml			
2.4×10^4	1.1×10^4	1×10^2	2×10^2	1×10^2	6×10^2
3.2×10^4	1.1×10^4	3×10^2	0	0	2×10^2
3.0×10^4	1.1×10^4	3×10^2	2×10^2	0	4×10^2
		0	0	0	1×10^2
		Pneumococcal DNA activity in No. streptomycin-resistant cells/ml			
1.6×10^6	1×10^3	0	1.6×10^3	0	0
7.8×10^6	1×10^3	0	1.6×10^3	0	0
5.4×10^6	1×10^3	0	3×10^3	0	0

* All activities were calculated for 1.0 ml out of a total volume of 2 ml of blood per mouse. † The DNA was added to freshly prepared plasma from nontreated mice. Several of the plaques induced by polyoma DNA were isolated. Their cytopathic effect on mouse embryo cells was demonstrated. The presence in these cells of hemagglutinins which reacted with guinea pig red blood cells and which could be inhibited by a rabbit antiserum to polyoma virus confirmed that the plaques were due to production of polyoma virus. ‡ Phosphate-buffered saline. § Ten minutes after Swiss mice were injected, the blod was withdrawn by heart puncture into 0.15*M* NaCl-0.015*M* Na citrate, and the plasma was prepared immediately.

The difference in the way the biological properties of these nucleic acids respond to inactivating treatments corresponds to what was seen when they were injected into mice and allowed to remain in the blood for 10 minutes. Whereas the pneumococcal-transforming DNA became almost completely inactivated, there was a significant residual infectivity in the blood after both intravenous and intraperitoneal injection of the polyoma DNA (Table 3). Between 2 and 8 percent of the injected polyoma DNA infectivity (in PFU) could be demonstrated in the plasma and washed blood cells. Under the experimental conditions employed, such an amount of polyoma DNA in the blood would amount roughly to 10^{10} molecules if the molecular weight is 3.0×10^6 (*21*). Since the biological activity of this material in the blood was still demonstrable by the plaque assay, it would be expected to reach appropriately competent cells and accordingly initiate its characteristic tumorigenic effect (*3, 22*).

The demonstration of infectivity in blood of mice after the intraperitoneal injection of polyoma viral DNA shows that tumor-inducing DNA can be transported in biologically active form from one part of the body to another. Although it is not yet clear whether normal or tumor cells can excrete or secrete DNA, or even, if they could, whether such nucleic acids would still retain biological activity, it is known that *Neisseria* and strains of *D. pneumoniae* do release biologically active DNA into their culture media (*23*). There is thus a precedent for anticipating that a similar phenomenon might possibly occur with mammalian cells. Release of DNA, or fragments thereof, may also be expected to follow the disintegration of cells in the necrotic parts of actively growing tumors in animals. If such nucleic acids, released in active form (cf. *24*), were to be taken up and circulated in the blood, they might behave as did the viral DNA described here, and thus they could possibly lead to a spread of the tumor. In these circumstances, treatment with antibodies which react with nucleic acids (*25*) might be investigated as a possible procedure to arrest metastasis.

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Myeloma Proteins and the Clinical Response to Melphalan Therapy

Abstract. *Objective improvement after therapy with melphalan occurred in all patients producing only Bence Jones kappa proteins, in half of the patients with myeloma serum proteins, and in none of those producing only Bence Jones lambda proteins.*

Melphalan, 3-{p-[bis(2-chloroethyl) amino]phenyl}-L-alanine hydrochloride, therapy induces objective improvements in about 50 percent of myeloma patients (*1*). The Myeloma Section of the Southwest Cancer Chemotherapy Study Group has analyzed (*2*) the responses of 132 myeloma patients treated with this drug. None of the following features correlated significantly with the response to therapy; age; sex; race; the interval between the onset of symptoms and initiation of melphalan therapy; the morphological classification of the predominant marrow plasma cell as mature, immature, or blast; or the presence or absence of Bence Jones protein (BJP) in the urine. However, objective improvement was more frequent in patients producing myeloma proteins containing kappa (κ) L-chains (*3*) than in those with lambda (λ) L-chains ($p < .01$). It is our purpose to compare two groups of myeloma patients divided on the basis of the antigenic type of L-chain contained in the myeloma protein (54κ, 37λ), to show the similarity of the two groups with respect to their status prior to therapy and the melphalan-induced leukopenia, and to show the marked difference in the incidence of objective improvement resulting from melphalan therapy.

Proteins produced by malignant plasma cells may be classified on the basis of structural and antigenic properties (*4*). The reduction of disulfide linkages with mercaptans and subsequent alkylation of the free -SH groups splits γ-globulin into L (light) and H (heavy) polypeptide chains. The BJP's present in the urine of some myeloma patients contain only L-chains with κ or λ antigenic determinants. Myeloma serum proteins combine one or the other type of L-chain with a gamma (γ) or an alpha (α) H-chain. The antigenic properties and structural features of the myeloma protein are stable, heritable characteristics of the plasma-cell tumor clone (*5*). Since the structural and antigenic properties of a myeloma protein are determined by the type of plasma cell affected by the malignant transformation, classifying patients with myeloma by the type of myeloma protein would yield a homogeneous grouping of patients for comparison of the clinical response to melphalan therapy.

All the available serum and urinary proteins of myeloma patients treated with melphalan were typed by immunoelectrophoresis with rabbit or goat antiserums to γ_2-globulin (IgG) and γ_1A-globulin (IgA) and rabbit antiserums to κ- and λ-BJP (*3*). In all the patients with myeloma proteins in both serum and urine, the antigenic type of L-chain in the serum protein was identical with that of the BJP. The IgG myelomas (*6*) were considered as one group. The records of the patients were reviewed to establish the status before treatment, the toxic effects of melphalan on leukocytes, and the occurrence of objective improvement (*7*).

Patients were classified as being objectively improved (responders) by the melphalan therapy if one or more of the following criteria were satisfied: (i) a decrease in the myeloma serum protein to 50 percent, or less, of the value before study; (ii) a decrease in the amount of urinary protein excreted per 24 hours to 50 percent, or less, of the value prior to study; (iii) an increase of 2.0 g percent, or more, in hemoglobin; (iv) shrinkage of palpable plasmacytomas of 50 percent or more; and (v) decrease in serum calcium from more than 6.0 to 5.0 meq/lit. or less.

The foregoing criteria were selected arbitrarily, but with the belief that the changes are probably clinically significant. The effect of melphalan therapy was not evaluated if the changes in the myeloma proteins in serum or urine were not determined, or if the patient died or was otherwise lost in less than 3 weeks.

Two different dosages of melphalan were used. Initially 0.2 mg/kg per day (schedule A) was administered until the leukocyte count fell below 3000/mm^3, or platelets fell below 100,000/mm^3. Because this dosage frequently caused severe leukopenia, we administered a dose of 0.5 to 2.0 mg/kg (in accordance with the patient's tolerance) over a 4-day period (schedule B). Doses causing moderate leukopenia were repeated as the marrow recovered, 6 to 10 weeks later.

Most of the patients were treated with schedule B, and all patients started on melphalan have continued to receive repeated courses of the drug at 6- to 10-week intervals (Table 1).

The better responses in the patients having κ L-chains in the myeloma protein were primarily due to the fact that all patients producing only a κ-BJP were objectively improved, whereas all those producing only a λ-BJP failed to respond. The differences between the incidence of response in the patients with γ_2 κ_2- as against those with γ_2 λ_2-