ascending from the pontine level through the midbrain tegmentum could be channeled in part to the hypothalamus, either by way of relays in the paramedian mesencephalic cell groups (*17*), or by way of a recently described, more lateral mesencephalohypothalamic route (*18*). The terminal degeneration in the lingual area of the thalamus after a lesion in the parabrachial nuclei is not as intense as the degeneration which results more laterally in the ventrobasal thalamic nuclei from a lesion in the principal trigeminal nucleus. This suggests that additional synaptic relays may intervene between the pontine taste area and the thalamus. Since the central tegmental pathways project both dorsally and ventrally in the diencephalon, polysynaptic gustatory fibers in these pathways provide a potential route over which taste information could reach the hypothalamus.

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7. The electrode was lowered in 50- or 100-μm steps until a response to tongue stimulation was encountered, or until the electrode reached a point 8.5 mm below the skull surface. No more than four penetrations were made in any preparation used for studies of degeneration. In several cases only one penetration was necessary in order to localize a gustatory response. One control animal was prepared with six penetrations through the cerebellum into the medulla without lesions. No degeneration entering nonvestibular portions of the brainstem was seen.
8. Aside from 0.25*M* NaCl and distilled water, the taste stimuli were 0.5*M* sucrose, 0.001*M* quinine hydrochloride, and 0.003*M* hydrochloric acid. Although the responses to the various stimuli differed from one placement to the next, no differences were obvious in the anatomical results.
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Lymphocyte DNA Synthesis Inhibition

Abstract. *A specific endogenous inhibitor for lymphocyte DNA synthesis that can be isolated from the lymphoid system and which is probably cell specific is described. The inhibitor is thermolabile, is destroyed by trypsin, and has a mass of about 30,000 to 50,000 daltons.*

Bullough and Laurence proposed that epithelial cells make a specific inhibitor of the mitosis of the cell (*1*). Subsequent studies by others have indicated that these specific endogenous mitotic inhibitors might also be obtained from melanocytes (*2*), granulocytes (*3*), kidney cells (*4*), and cells of the lens (*5*) for each of these cell types, respectively. Moorhead *et al.* presented evidence that aqueous extracts of the lymph nodes from pigs can inhibit the transformation of human lymphocytes induced by phytohemagglutinin (PHA) (*6*). We now report that the lymphoid system contains noncytotoxic specific inhibitors of the incorporation of tritiated thymidine into human lymphocytes stimulated by PHA, by mixed leukocyte cultures, and by leukocytes derived from patients with lymphocytic leukemia.

Leukocytes were obtained from normal human donors and grown in medium 199 (10^6 cells per 2 ml) supplemented with 2 m*M* glutamine and 20 percent autologous human plasma; the medium also contained penicillin and streptomycin. These cultures were incubated in triplicate with PHA (PHA-p, Difco) for 66 hours at 37°C. Then 1 μc of [^{3}H]thymidine was added to the culture medium, and the cells were incubated for six more hours. The cultures were then washed once with cold saline and precipitated with 5 percent trichloroacetic acid (TCA); the precipitate was washed three times with 5 percent TCA, and then solubilized with 0.5 ml of NCS (Nuclear-Chicago) solubilizer; the radioactivity was determined in a liquid scintillation counter. The cytology and viability of the cultures was ascertained, before TCA precipitation, with vital staining (trypan blue, 0.4 percent).

Rat lymph nodes and spleen, and calf thymus and spleen, were homogenized at 4°C in 0.15*M* NaCl (10 ml/g) and homogenized by sonication for 1 minute and then centrifuged at 10,000*g* and 4°C for 1 hour. This supernatant was removed and dialyzed against 200 volumes of distilled water for 48 hours at 4°C. The precipitated euglobulins were removed by centrifugation, and the supernatant was lyophilized. This is the S_1 fraction.

Portions (1.0 mg) of S_1 derived from calf spleen and thymus and rat spleen and lymph nodes were incubated with normal human leukocyte cultures for 72 hours, and all inhibited approximately 60 to 100 percent of the [^{3}H]thymidine incorporation by lymphocytes that had been stimulated by two different batches of PHA (Table 1).

Inbred rats (Fisher) were the source of the lymph node and spleen S_1 extracts. These extracts inhibited PHA-induced uptake of [^{3}H]thymidine by cultured syngenic rat lymphocytes and cultured human lymphocytes. Thus, it would appear that the suppression of PHA-induced transformation is not due to xenogenic differences between the donors of the lymphoid extract and of the cultured lymphocytes.

Similarly prepared extracts of rat muscle and of WI-38 fibroblasts did not inhibit PHA-stimulated incorporation of [^{3}H]thymidine into lymphocytes.

Table 1. Inhibition of tritiated thymidine incorporation into transformed human lymphocytes stimulated by two different preparations of PHA by various lymphoid extracts. The cytotoxicity of the extracts is expressed as 0 if all cells were viable and there was no apparent loss in numbers; as ± if a reduced number of viable cells remained; and as + if few if any viable cells remained; M.W., molecular weight.

Test substance	PHA-I*			PHA-II*		
	Count/min	Inhibition (%)	Cytotoxicity	Incorporation (count/min)	Inhibition (%)	Cytotoxicity
	Control (PHA)					
	68,000	0	0	110,000	0	0
	Fractions (rat)					
Spleen	8,250	88	±	22,000	88	±
Lymph node	5,400	92	0	11,000	90	0
Muscle	65,500	0	0	112,000	0	0
	Fractions (calf)					
Thymus	27,250	60	0	35,250	68	0
Spleen	6,800	90	±	6,600	94	±
	Subfractions (spleen)					
100,000 M.W.	100	99+	+	150	99+	+
50,000 to 100,000 M.W.	300	99+	+	350	99+	+
30,000 to 50,000 M.W.	3,400	95	0	5,400	95	0
10,000 to 30,000 M.W.	47,800	30	0	83,000	25	0
Muscle	69,000	0	0	106,000	0	0

* Each figure represents the mean of at least three replicates.

The S_1 fractions from both calf thymus and spleen were also subjected to molecular sieving chromatography on both Sephadex G-75 and Bio-Rad 100 gels, but no resolution of the inhibitory activity was obtained.

The dissolved S_1 fractions of calf spleen and thymus were precipitated with increasing concentrations of ammonium sulfate; the resulting precipitates and the final supernatant (65 percent saturation) were separately dialyzed against distilled water. These fractions caused less than 20 percent inhibition of PHA-induced synthesis of DNA in lymphocytes.

The S_1 fraction from calf spleen and thymus was subjected to molecular sieving via membrane filtration (Amicon Corporation), a process that yielded fractions with molecules larger than 100,000 daltons; fractions between 50,000 and 100,000 daltons; fractions between 30,000 and 50,000 daltons; and fractions between 10,000 and 30,000 daltons (*7*). The inhibitory capacity of these "sieved" subfractions (0.25 mg, dry weight, per milliliter of the cultured cells) of S_1 from calf spleen were tested. The fraction larger than 100,000 daltons was cytotoxic, as judged from the trypan blue exclusion test and from cell counts performed at the time of harvesting. The subfraction between 50,000 and 100,000 daltons contained some cytotoxic materials, but that between 30,000 and 50,000 daltons showed no cytotoxicity. This fraction between 30,000 and 50,000 daltons inhibited nearly all of the [^{3}H]thymidine uptake (Table 1).

Cells were incubated for 6 hours with [^{14}C]phenylalanine rather than with [^{3}H]thymidine. Such studies, along with others in which [^{3}H]uridine was used, indicated that the incubation of human lymphocytes with the sieved extract (30,000 to 50,000 daltons) of calf spleen S_1 had no effect on the incorporation of RNA precursor or protein precursor into these cells during this period of exposure to the lymphoid system extract. These data also supported the finding that this subfraction was not cytotoxic but was specifically inhibiting tritiated thymidine incorporation into lymphocyte DNA.

The sieved extract of calf spleen was also incubated with WI-38 fibroblasts and HeLa cells in vitro. The population doubling times of WI-38 fibroblasts were 31 to 34 hours in the presence or absence of the lymphoid extract. Similarly, incorporation of [^{3}H]thymidine by HeLa cells was not inhibited by the addition of sieved extract to the culture medium. These studies suggest that this lymphoid extract is a specific inhibitor of [^{3}H]thymidine incorporation by lymphocytes.

Solutions of the sieved extract of calf spleen lost their inhibitory activity toward PHA-stimulated [^{3}H]thymidine uptake in lymphocytes by heating at 55°C for 30 minutes, or by exposing them to trypsin for 1 hour at 25°C or by storage in the dry state for 1 month at 4°C.

The effects of crude thymus S_1 (0.50 mg/ml) on the incorporation of [^{3}H]-thymidine by one-way mixed leukocyte cultures (MLC) were determined. Leukocytes from two normal, unrelated donors were obtained and divided into two portions; one of these portions of each individual's leukocytes received 4000 roentgens of x-ray, a dose which blocks their proliferating capacity but allows them to remain stimulatory. Suspensions of irradiated cells (5×10^5 cells in 1 ml) from donor "A" were mixed with an equal number of unirradiated cells from donor "B"; similarly irradiated cells from donor "B" were mixed with unirradiated cells from donor "A." Control cultures consisted of equal numbers of autologous irradiated and unirradiated cells.

After 7 days in medium 199 and 20 percent fetal calf serum, 2 μc of [^{3}H]thymidine was added to each culture and, after 6 hours of further incubation, the radioactivity was determined in the usual manner. The addition of crude thymus S_1 reduced the [^{3}H]thymidine uptake in these MLC's by 41 percent for donor "A" and by 45 percent for donor "B," with no apparent cytotoxicity as judged from vital dye staining and cytological characteristics.

Cells were also obtained from long-term cultures derived from the blood of two juvenile patients with lymphocytic leukemia. After being incubated for 6 hours with 1 μc of [^{3}H]thymidine, the cells incorporated approximately 20,000 count/min per 10^6 cells. When 0.5 mg of the sieved extract was added to the 2 ml of cultured cells, the total number of counts incorporated by these leukemic leukocytes was reduced to approximately 1000 count/min per 10^6 cells. Cytologic examination of these cells after they were exposed to the sieved extract from calf spleen did not reveal any apparent cytoxicity. Furthermore, these cells could incorporate equivalent amounts of [^{14}C]-phenylalanine in the presence or absence of the sieved extract from calf spleen.

Thus, it would appear that [^{3}H]thymidine incorporation by cultured leukocytes from leukemia patients or by human lymphocytes stimulated either by PHA or by allogenic leukocytes could be inhibited by the addition to these cells in culture of a partially purified extract of the lymphoid system of the cow. Further, this inhibition was apparently not associated with either cytotoxicity or species specificity of the extract tissues and was specific for cell type. The active component of the ex-

tract appears to be a macromolecule with a mass of approximately 30,000 to 50,000 daltons; it is thermolabile and destroyed by trypsin. It is apparently not stable on long-term storage, even in the cold.

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Talc-Treated Rice and Japanese Stomach Cancer

Abstract. *The Japanese prefer talc-dusted rice in their diet. The incidence of stomach cancer in Japan is unusually high. Most talc has some asbestos contaminants. Epidemiologic evidence is presented that the asbestos-contaminated talc on rice in the diet is the carcinogen or cocarcinogen responsible for the high incidence of Japanese stomach cancer.*

The high incidence of stomach cancer in Japanese men represents a challenging problem in cancer epidemiology. Study of age-adjusted rates of mortality from stomach cancer in 24 countries showed that from 1962 to 1963 Japanese men had the highest rate for all nations; the rate, 67.96 per 100,000 in the male population, was seven times that for men in the United States (*1*). Since talc frequently contains asbestos (*2*), and since workmen exposed to asbestos show a marked increase in gastrointestinal cancer, especially stomach cancer (*3*), the hypothesis that the increased rate of cancer of the stomach in Japan is due to ingestion of rice contaminated with asbestos seems attractive and has been recently suggested in a brief note (*4*). This report presents evidence for this relationship at somewhat greater length.

Rice grown in California and meant for the American consumer is milled mechanically. Rice is prepared differently for the Japanese consumer. It is milled and treated with glucose, and then talc is added. The talc is held to the surface of the grain and is said to preserve its flavor better. Japanese consider this rice more tasty, both here and in Japan. Two percent of all talc produced in California from 1959 to 1963 was used as a rice additive, primarily for export to Japan (*5*). Rice containers in Los Angeles markets intended for the Japanese customer usually bear the legend: "Coated with glucose and talc." Optical microscopy of the ash of such store-bought rice showed 3.7×10^6 asbestos-form fibers per gram (Fig. 1).

Talc, the mineral, is a hydrous silicate of magnesium. So are some forms of asbestos. The difference between these two minerals is not in their chemical compositions, but rather in their structure, asbestos being fibrous and mineral, talc being flaky or granular. However, commercial talc is composed not only of the pure mineral talc, but also of fibrous silicates, which are in part classifiable as asbestos. There is no firm mineralogic definition of commercial talc, and in most areas the mineral talc forms much less than half of commercial talc. The composition of commercial talc varies with the deposit. Vermont mines produce a platy or granular type of mineral relatively free of asbestos minerals (*6*). New York and California talcs contain more fibrous silicates, including anthophyllite and tremolite (*2*, *5*).

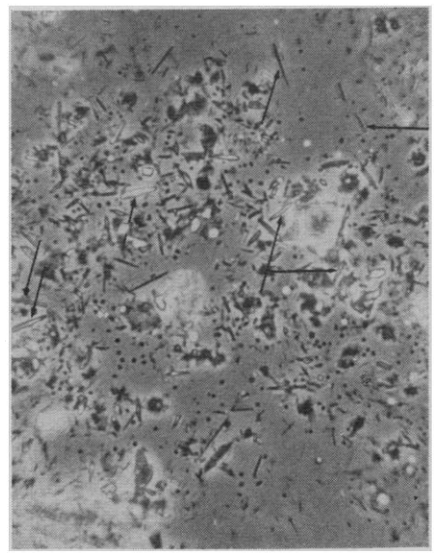

Fig. 1. Photomicrograph of the ash of a specimen of rice intended for Japanese consumption and purchased in a Los Angeles market. Arrows indicate asbestos-form fibers. The specimen contained 3.7×10^6 such fibers per gram.

Respired asbestos and talc dust cause similar disorders in workmen. Older talc workers show more than four times the expected incidence of lung and pleural cancer (*7*). Talc or talcose minerals, including contaminating asbestos, are apparently the responsible carcinogens since, where confirmatory evidence could be obtained, all those talc workers with pleural and lung cancer also had talc pneumoconiosis. Autopsies on six talc workers with talc pneumoconiosis showed the presence of the characteristic "asbestos body" in the lungs of every one (*8*). When talc is examined by phase contrast microscopy, from 8 to 30 percent of the particles are fibrous silicates. Identifiable tremolite, anthophyllite, and chrysotile are also seen (*9*). The large talc deposits of New York and California regularly contain the amphibole asbestos minerals, tremolite and anthophyllite. These are normal ingredients of commercial talcs from these areas. Chrysotile asbestos occurs in veins in talcose mineral deposits and may be then mined with the talc and create a contaminant (*9*).

Asbestos is an established carcinogen. Not only does it cause lung cancer, but it is associated with a substantial increase of morbidity from stomach cancer (*3*). Although chrysotile is the most important commercial fiber of asbestos, there is no reason to regard the amphibole asbestos minerals as safe. To the contrary in one study an amphibole asbestos mineral predominated in asbestos-bodies in the sputum of a group of men exposed to asbestos and having a high incidence of mesotheliomata (*10*). The fact that talc workers exposed to fibrous talc, which contains amphibole asbestos, develop more pulmonary injury than do workers exposed only to talcs relatively free of fibrous silicates or amphiboles (*11*), indicates the pathogenicity of amphibole asbestos.

The Japanese preference for talc-coated rice means that rice eaters of that nation are exposed to the hazard of ingestion of amphibole asbestos, as