Table 1. The A and B protein activities of lactose synthetase in mammary tissue of immature rats, immature rats treated with perphenazine, and lactating rats. The results are expressed in nanomoles per minute per milligram of protein (mean ± standard error of the mean). Five animals were used for each experiment.

UDP-galactose utilized	
A activity	B activity
Immature	
0.043 ± 0.015	0.014 ± 0.004
Immature, 4 days after perphenazine	
0.054 ± 0.014	0.086 ± 0.040
Lactating	
0.97 ± 0.26	2.38 ± 0.15

glands and the high activities in the lactating glands are consistent with previous observations (*4*). The negligible response of enzyme activity to perphenazine administration is surprising in that during this 4-day period there is unequivocal histologic evidence of differentiation and increased protein and lipid synthesis in the mammary glands (*6*).

In contrast to the results noted above, the level of A protein activity in the tumors was high relative to that in the mammary glands of untreated rats (Table 2). The enzyme level was further increased after administration of estradiol, prolactin, or perphenazine. It is not known whether the estradiol acted directly on the tumor tissue or indirectly by stimulating pituitary prolactin release.

The B protein activity in the tumors from untreated rats was also very high relative to the activity in the untreated mammary glands (Table 2). Perphenazine or prolactin further stimulated B protein activity to levels found only in adult lactating mammary glands. Adrenocorticotropin, bovine growth hormone, or progesterone did not influence the level of A or B protein activity in the tumors.

It seems probable that in these experiments the virgin mammary tissue was not sufficiently differentiated to respond to the 4-day prolactin stimulus by synthesizing lactose. A period of intense hormonal stimulation, such as pregnancy, is required for complete differentiation of these cells. Hence, the experiments on the mammary carcinoma cells are of particular interest because, even in immature unstimulated rats, the tumors synthesize appreciable quantities of this unique mammary gland enzyme, lactose synthetase. The relatively high activities may be the result of normal endogenous prolactin secretion since they are further increased by exogenous prolactin or by the perphenazine-induced release of endogenous prolactin.

This tumor cell can be thought of as being in a functional state of differentiation comparable to the normal mammary cell in late pregnancy or early lactation with respect to A and B protein synthesis. Enzymes earlier in the biosynthesis pathway of lactose (hexokinase, uridine diphosphate galactose-4-epimerase, and uridine diphosphate glucose pyrophosphorylase) have been measured in carcinogen-induced mammary tumors (*7*). The activities of these enzymes are also comparable to those in normal glands in late pregnancy or early lactation.

Although the biochemical abnormalities responsible for sustained growth in cancer cells have not been identified, the R3230AC cell demonstrates certain features of hormone-dependent control of growth. If estrogen is administered to the tumor-bearing rat or if a prolactin-secreting pituitary is transplanted into these animals, the rate of tumor growth is markedly reduced in spite of increased protein and lipid synthesis. Also, if normal endogenous amounts of pituitary hormones are eliminated by hypophysectomy, tumor growth as well as specific protein and lipid synthesis is inhibited (*8*).

The ability of the R3230AC tumor cell to retain these certain, very specialized, differentiated characteristics (A and B protein synthesis and regulation) in addition to hormone-dependent growth regulation provides an experimental model of the mechanism of hormonal regulation of growth and differentiation of mammary carcinoma cells.

WILLIAM L. MCGUIRE*
Endocrinology Branch,
National Cancer Institute,
Bethesda, Maryland 20014

Table 2. Effect of hormone treatments on A and B protein activity in R3230AC mammary carcinoma. The rats received the indicated treatments subcutaneously on days 1 and 2 and were killed on day 3. The results (nanomoles per minute per milligram of protein) are expressed as the mean of five separate tumors ± standard error of the mean.

Treatment	Dose	UDP-galactose utilized	
		A protein	B protein
Saline alone		0.127 ± 0.017	0.675 ± 0.022
Adrenocorticotropin	5 units	0.159 ± 0.009	0.562 ± 0.045
Bovine growth hormone	1 mg	0.131 ± 0.022	0.542 ± 0.032
Estradiol	20 μg	0.200 ± 0.015	0.662 ± 0.020
Progesterone	4 mg	0.128 ± 0.016	0.470 ± 0.075
Ovine prolactin	1 mg	0.213 ± 0.018	1.440 ± 0.209
Perphenazine	0.5 mg	0.336 ± 0.047	1.150 ± 0.130

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* Present address: University of Texas Medical School, 7703 Floyd Curl Drive, San Antonio 78229.

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Lymphocyte Stimulation: Transfer of Cellular Hypersensitivity to Antigen in vitro

Abstract. *Tuberculin-sensitive human lymphocytes cultured for 36 hours with tuberculin elaborate a soluble material which causes nonsensitive lymphocytes to respond to tuberculin in vitro by transformation and proliferation.*

When lymphocytes from an individual with delayed hypersensitivity to an antigen are placed in short-term tissue culture with that antigen, they transform into blastlike cells, synthesize DNA, and divide (*1*). A small percentage of peripheral blood lymphocytes initially responds to such antigenic stimulation and, after approximately 48 hours, undergoes a proliferative response for several generations (*2*). Cells arising from this proliferation account for the majority of the lymphoblasts present when cultures are examined on days 5 to 7 (*2*). In a population containing sensitive cells, the recruitment of lymphocytes not themselves initially sensitive to antigen might serve, particularly during the first 2 days of culture, as an additional mechanism to enlarge this hypersensitive response of lymphocytes. There is evidence that this response to antigen in vitro parallels and has several characteristics of delayed hypersensitivity in vivo (*3*).

Earlier experiments showed that cell-free supernatants prepared by incubating sensitive leukocytes with specific antigen would confer on nonsensitive individuals the ability to manifest delayed hypersensitivity to that antigen (*4*). If leukocytes from a donor sensitive to both tuberculin and diphtheria toxoid were incubated with tuberculin purified protein derivative (PPD), the resultant supernatant could transfer delayed hypersensitivity only for tuberculin. The cells now could transfer sensitivity only for toxoid, the antigen to which they had not been exposed (see *4*).

We now report on a possible in vitro model for that in vivo transfer system. Supernatants prepared from sensitive human leukocytes cultured with tuberculin PPD activate lymphocytes from nonsensitive individuals so that the lymphocytes now respond to antigen by transformation and proliferation.

Suspensions of peripheral blood leukocytes, containing 50 to 80 percent lymphocytes in 18 percent autologous plasma media, were prepared from donors with either a markedly positive delayed hypersensitivity skin test to 0.02 μg of PPD (sensitive cells) or with no mark whatsoever after a skin test with 5.0 μg of PPD (nonsensitive cells). Supernatants were taken from cultures of 0.6 to 1.2×10^6 cells per milliliter at 36 hours, a time when no antigen-stimulated cell division has been observed. These supernatants were assayed by incubation at a final 1:4 dilution with cultures of 0.6×10^6 nonsensitive cells per milliliter. Cultures were examined after 6 days by counting the percentage of transformed cells on May-Grunwald-Giemsa stained smears, by radioautography, or by measuring the incorporation of C^{14}-thymidine as determined by liquid-scintillation counting. The results of each method of examination were in good agreement.

When supernatants from stimulated cells, prepared by incubating sensitive cells with 0.6 μg of PPD per milliliter for 36 hours, were placed on nonsensitive cells, they induced a 4- to 25-fold increase in thymidine incorporation compared to that induced with PPD alone or with PPD in combination with supernatants from sensitive cells cultured without PPD (Fig. 1). Antigen is necessary for the production of stimulatory activity by sensitive cells, is carried over into the assay cultures of nonsensitive cells, and is adjusted to the same final concentration in all nonsensitive cultures, except as indicated. In the control experiments neither antigen alone, nor antigen added to unstimulated supernatant after separation from the sensitive cells, nor supernatant from nonsensitive cells cultured with PPD, leads to a response. The results of this basic experiment have been successfully reproduced 28 times with 13 combinations of sensitive donor and nonsensitive recipient cells.

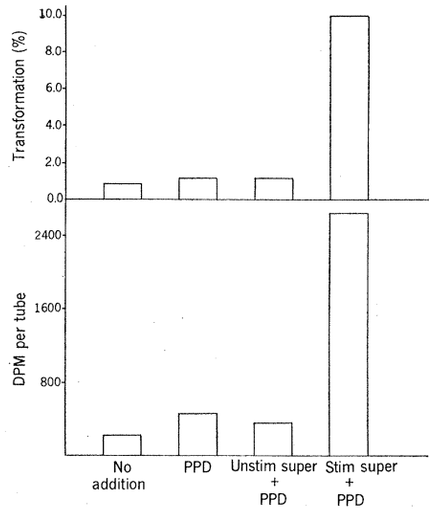

Fig. 1. Response of nonsensitive lymphocytes to cell-free supernatants from sensitive leukocytes. Above, percentage of blast transformation; below, incorporation per culture of thymidine-2-C^{14} into DNA as assayed by liquid-scintillation counting. Each value is the mean of three cultures. Final concentration of PPD adjusted to 0.8 μg/ml in all tubes, except those marked "No addition." Cultures were examined on day 6.

To judge whether the nonsensitive lymphocytes are responding to the antigen carried over in the supernatant or directly to the material released into the media by the antigen-stimulated sensitive cells, antigen must be removed from the active supernatants. At present we have not yet achieved a complete separation. However, the addition of more PPD to an active supernatant containing PPD induces an increased response above that due to the supernatant with its residual antigen. This suggests that, in the presence of this supernatant activity, the nonsensitive cells are responding to antigen.

The amount of PPD present in the active supernatant can be reduced considerably if, in the production of the activity, the sensitive cells are exposed to PPD for only 1 hour, then washed, and allowed to continue in culture without additional antigen. Media removed at 36 hours from such cells contains marked supernatant activity but much less antigen. These supernatants containing little residual PPD directly induce a small response by nonsensitive cells. However, the addition of increasing amounts of PPD to nonsensitive cells in the presence of these supernatants results in further increments of lymphocyte transformation (Fig. 2). This increased proliferative response with increasing concentrations of antigen is similar to that observed with the culture in vitro of natively sensitive lymphocytes. Other experiments have demonstrated that supernatants retain full activity after dialysis and after centrifugation at 100,000*g* for 1½ hours. The activity is destroyed by heating at 56°C for ½ hour.

Since these experiments involve the addition of medium from the leukocyte culture of one individual to the leukocyte culture of another individual, any direct stimulation might be due to transplantation antigens. In experiments by others a sedimentable material, considered to be a form of transplantation antigen, which is capable of stimulating individual lymphocyte cultures is found in the media of mixed leukocyte cultures from genetically different donors (*5*). A small amount of soluble transplantation antigen can be released from cell membranes by sonication of spleen cells (*6*). This material will stimulate allogeneic lymphocyte cultures (*7*). However, we feel that the stimulation produced by our supernatants is not due to transplantation antigens be-

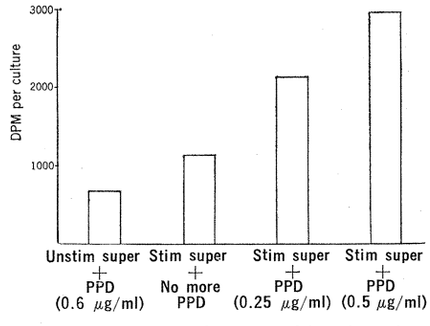

Fig. 2. Response of nonsensitive lymphocytes to 36-hour cell-free supernatants from sensitive leukocytes. Stimulated supernatants were prepared by exposing sensitive cells to PPD (0.25 μg/ml) for 1 hour, washing, and culturing without additional antigen for 36 hours. The amount (0.6 μg/ml) of PPD added as a control to the unstimulated supernatant after separation from the sensitive cells is greater than the maximum possible amount of PPD in any of the other assay cultures, even if residual antigen in the stimulated supernatant were assumed to be all of that initially added to the sensitive cells, that is, 0.25 μg/ml.

cause (i) unstimulated sensitive cell cultures produce no supernatant activity, (ii) none of the active material in the supernatant is sedimentable at 100,000*g*, (iii) the amount of direct stimulation of nonsensitive cells by a supernatant is decreased greatly in experiments where residual antigen is decreased even though the sensitive cells themselves undergo a vigorous proliferative response, and (iv) the active substance causes nonsensitive cells to respond to additional tuberculin.

It is possible that some of the different biological activities which have been detected in the media of sensitive leukocytes exposed to antigen in vitro may be due to the same material. However, in addition to its property of activating nonsensitive lymphocytes to respond to antigen, the heat lability and nondialyzability of our material distinguish it from many of these activities. For example, the inhibition of the migration of guinea pig peritoneal macrophages, an in vitro correlate of delayed hypersensitivity, is mediated by a heat-stable factor produced by lymphocytes upon exposure to specific antigen (*8*). The media of human lymphocyte cultures stimulated by phytohemagglutinin is cytotoxic for many tissue culture lines; the activity is heat-stable at 56°C and labile at 100°C (*9*). Cytotoxic activity is also found in the media of sensitive lymphocytes sitmulated by PPD (*10*). The physical properties of our lymphocyte-activating material differ from the heat-stable, dialyzable polynucleotides released from sensitive spleen cells incubated with specific antigen. These polynucleotides, when subsequently injected in vivo as an adjuvant with the specific or an indifferent antigen, cause transiently larger numbers of mouse spleen plaque-forming cells during the first few days after antigen injection (*11*).

Our studies parallel the earlier experiments in vivo which demonstrated that supernatants prepared from sensitive leukocytes incubated with specific antigen will transfer delayed hypersensitivity to previously nonsensitive individuals (*4*). Only 1/500th the number of cells per milliliter in the studies in vivo were used in our system in vitro for the preparation of supernatants. The supernatants were active in vivo after 1 hour of incubation of cells with antigen (*4*); in our system this smaller number of cells must be incubated with antigen for 36 hours for the production of optimum activity in the supernatant. The biochemical relation between the transfer factor released by antigen from sensitive cells and the dialyzable transfer factor prepared by the mechanical disruption of sensitive leukocytes is still unknown (*12*). Dialyzable transfer factor has been shown to induce nonsensitive cells to respond to tuberculin to a much smaller degree than that obtained with active supernatants (*13*).

Although the production of activity in the supernatant is immunologically specific and although in the presence of such activity nonsensitive lymphocytes will respond to additional antigen, it is not yet known whether the action on the nonsensitive cells in vitro is also immunologically specific.

FRED T. VALENTINE
H. SHERWOOD LAWRENCE
Infectious Disease and Immunology Division, Department of Medicine, New York University School of Medicine, New York 10016

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Neutron Diffraction of Cell Membranes (Myelin)

Abstract. *Small-angle neutron diffraction (wavelength 4.05 angstroms) of human and rabbit sciatic nerve has been carried out by means of the Brookhaven high flux beam reactor with an automated slit camera. Most of the free water of the nerves was substituted in order to minimize incoherent scatter of hydrogen atoms. The differences in amplitude and phase shifts between neutrons and x-rays resulted in a neutron diffraction pattern that was completely different from the x-ray pattern. The neutron pattern consisted of a single peak of about 89-angstrom spacing in the region examined (up to 6-angstrom spacing). The strong third, fourth, and fifth order reflections (about 60, 45, and 36 angstroms) seen in the x-ray pattern were suppressed. The neutron data indicated a strong scattering from one portion of the membrane.*

The recent availability of high flux neutron reactor sources makes it practical to examine biological specimens only 1 to 2 mm thick. X-ray diffraction estimates the electron density distribution of the specimen, whereas neutron diffraction measures the distribution of those atomic nuclei that have a large cross section for elastic coherent scattering (*1–3*). Combination of x-ray and neutron diffraction data can be expected to aid considerably in the structure analysis of biological materials.

The very low level of radiation damage associated with neutron diffraction (as a result of the low quantum energy of each neutron) is an important advantage for biological work. However, a severe disadvantage is the high level of incoherent neutron scattering of hydrogen. In many cases, this will require that D_2O be substituted for the free water of the biological specimen.

We have studied the neutron diffraction of human and rabbit sciatic nerve, using the high flux beam reactor of the Brookhaven National Laboratory and a small-angle neutron diffraction camera designed and built by Dr. Nathans. The general arrangement is shown in Fig. 1. Neutrons from the reactor were