type during the period of enzyme synthesis (*3*).

Ovulation was induced in mature XO and XX females by the intraperitoneal administration of 5 international units (I.U.) of pregnant mare serum gonadotropin (Equinex, Ayerst); 48 hours later 5 I.U. human chorionic gonadotropin (A.P.L., Ayerst) was administered intraperitoneally. The mice were killed 24 hours after the second injection, and the oviducts were removed. The ova were collected from the oviducts, and the cumulus cells were stripped off by brief exposure to hyaluronidase (Wydase, Wyeth). The ova were washed three times in Krebs-Ringer bicarbonate with 0.1 percent bovine serum albumin and then assayed for G6PD or LDH activity (*3*). Both assays were carried out at 37°C in total volumes of 0.12 ml with a Gilford 2000 recording spectrophotometer. Two to four ova were used for each G6PD assay and one for each LDH assay. Oocytes were obtained from a total of 6 XX (*Ta*/+) and 7 XO (4 *Ta*/O, 3 +/O) females. In nearly all instances, fewer usable ova were obtained from XO (mean 5.6) than from XX females (mean 13.8).

In each separate experiment, as well as in the combined results, the mean G6PD activity in XO oocytes was half of that in XX oocytes (Table 1). This is what had been predicted, since XO oocytes have half as many X chromosomes as XX oocytes. The G6PD activities in the two different genotypes of XO, *Ta*/O and +/O, did not differ, an indication that the results were not influenced by the presence of the tabby mutation. By contrast to G6PD, the amounts of autosomally controlled LDH in the XO and XX oocytes did not differ significantly. Therefore, the G6PD results cannot be attributed to some general metabolic abnormality of XO ova. Furthermore, follicular and other ovarian stromal cells all manifest X-chromosomal inactivation and have only one functioning X chromosome in both XO and XX animals (*2*). Therefore the enzyme must be synthesized within the egg, and the difference in G6PD activities must reflect an intrinsic difference in the oocyte—the difference being the number of active X chromosomes.

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Leukotactic Factor Produced by Sensitized Lymphocytes

Abstract. *Lymph node lymphocytes obtained from guinea pigs exhibiting delayed hypersensitivity are stimulated in vitro by specific antigen to produce a soluble factor that is chemotactic in vitro for mononuclear macrophages. The material is nondialyzable, relatively heat stable, and elutes from Sephadex G-100 in the fraction containing molecules smaller than immunoglobulins.*

In recent years, several aspects of the mechanism of delayed hypersensitivity have been delineated with the use of an in vitro assay for cellular hypersensitivity which features the inhibition of random cell migration from capillary tubes (*1*). Lymphocytes from guinea pigs exhibiting delayed hypersensitivity produce, during incubation with specific antigen, a soluble material that inhibits the random migration of normal macrophages in vitro (*2–4*). This factor elutes on gel filtration with materials the size of albumin and has been termed migration inhibitory factor (MIF) (*5–7*). In the same system, only a few sensitized lymphocytes need be present to affect the behavior of a large number of nonsensitized macrophages (*2*, *8*). Further, studies made with the use of passive transfer of delayed hypersensitivity have demonstrated that the majority of cells present in a delayed skin lesion are nonsensitized cells of host origin, only a few being specifically sensitive to antigen (*9*). Therefore, it was of interest to determine whether sensitized lymphocytes stimulated by antigen would produce a soluble chemotactic factor (or factors) that would attract large numbers of mononuclear cells; the presence of such a factor (or factors) might well explain the predominance of nonsensitive mononuclear cells in the delayed skin reaction.

Lymph node lymphocytes obtained from guinea pigs previously injected with *o*-chlorobenzoyl chloride conjugated to bovine gamma globulin (OCBC-BGG) (*10*) in complete adjuvant (*11*) were placed in culture medium (*12*) at a cell concentration of 2.4×10^7 per milliliter, as described previously (*3*, *5*). The specific antigen, OCBC-BGG, was added to one group of cultures to a final concentration of 100 μg/ml; the control group received an equal volume of physiologic saline. The cultures were gassed with 5 percent CO_2 and air to *p*H of 7.5 and incubated for 24 hours at 37°C; the medium was then centrifuged at 17,300 *g* for 20 minutes, and the supernatant was collected. Samples of the supernatants obtained from the control and antigen-stimulated lymphocytes were supplemented with normal guinea pig serum (15 percent final volume) and were assayed for MIF activity on normal peritoneal exudate cells as previously described (*3*). Chemotactic testing was carried out within 48 hours with material kept at 5°C or frozen in dry ice (freezing and thawing one time had little effect on the chemotactic activity of a given preparation).

Chemotactic studies were performed with rabbit or guinea pig mononuclear cells from an exudate induced 4 days earlier by the intraperitoneal injection of sterile mineral oil (*13*). Only results obtained with rabbit mononuclear cells are reported here, although guinea pig cells responded in essentially the

same manner. The method of the chemotactic assay was the micropore filter technique (*14*) that is described in detail elsewhere (*15*). Chemotaxis chambers contain an upper and lower compartment separated by a micropore filter (pore size, 5 μm; type, SMWPO2500) (*16*). The cell suspension, at a concentration of 2.5×10^6 cells per milliliter, was made up in Medium 199 (*17*) with 10 percent rabbit serum and was placed in the upper compartment of each chamber. The lower compartment contained the test material diluted to 1.0 ml in Medium 199. Chemotactic activity was assessed by counting, in five randomly selected high-power fields, the number of cells that had migrated toward the lower compartment; total cell counts in excess of 30 were rarely encountered in negative controls. When a positive response occurred, the counts were usually at least fivefold greater (Table 1). The antigen was also assayed as one of the controls in each experiment and was not by itself chemotactic.

Culture fluids from sensitized lymphocytes that had been incubated in the presence of antigen were chemotactic for mononuclear cells (Table 1). The activity was dose-dependent on the amount of culture fluid tested. To date, a total of four experiments have been performed with similar results.

To assess the requirement for antigen in the appearance of chemotactic activity in culture fluids, OCBC-BGG was added in two experiments to supernatants from sensitized cells only after the incubation period had terminated and the cells had been removed (Table 1). The mere presence of antigen in these supernatants was not associated with chemotactic activity—antigen had to be present during the period of incubation with lymphocytes to stimulate the production of chemotactically active material.

The production of chemotactic activity was found to be immunologically specific. Lymphocytes from guinea pigs sensitive to OCBC-BGG were incubated with OCBC-BGG, with ovalbumin (an unrelated antigen), and with saline. The MIF and chemotactic activity were found only in the supernatants from lymphocytes incubated with OCBC-BGG, emphasizing that the requirements for specific antigen in the production of chemotactic activity was similar to this requirement for generation of MIF activity.

Table 1. Chemotactic activity for mononuclear cells in various sensitized lymphocyte fluids Values represent counts of migrated cells in five fields (high-power objective used).

Culture fluid (code No.)	Amount tested (μl)	Chemotactic activity of culture fluids	
		Cultured without antigen	Cultured with antigen
278, 279*	50	0	50
	100	12	74
	200	18	100
286, 287*	100	6†	39
	200	45†	145
	300	35†	195
290, 291*	200	20	180
295, 296*	100	0†	15
	200	0†	120
	200 (56°C)‡		100

* Nos. 279, 287, 291, and 296 were the supernatants from sensitized lymphocytes incubated with antigen; Nos. 278, 286, 290, and 295 were their respective controls, incubated in absence of antigen. † For control purposes, appropriate amounts of OCBC-BGG were added to culture fluids at the time of chemotactic testing to simulate antigen concentrations in stimulated culture fluids. ‡ This sample was heated at 56°C for ½ hour.

In order to assess its true chemotactic nature, 100 μl of culture fluid, preparation No. 279, was placed in both upper (containing cells) and lower (containing test material) compartments of a chamber. In contrast to the count of 74 (Table 1) when fluid No. 279 was present only in the lower compartment, the simultaneous addition of this fluid to the upper compartment resulted in a count of 20, which was background activity. This indicates that, like a chemotactic factor, the culture fluid would cause a cell response only if present in higher concentration in the lower compartment. The addition of 100 μl of No. 279 fluid to the upper compartment of a chamber only resulted in a background count of 18, indicating that nonspecific migration of cells was not stimulated by this preparation.

The chemotactic factor was relatively heat stable with little reduction after heating at 56°C for 30 minutes (Table 1); MIF activity is also present after heating to 56°C for 30 minutes (*3*). In another experiment (*18*), chemotactic activity was found in culture fluids of lymphoid cells incubated with the antigen dinitrophenyl–bovine gamma globulin to which they had been sensitized, which showed that the production of a chemotactic factor by sensitized mononuclear cells was not a unique feature of OCBC-BGG.

In two experiments, supernatants from antigen-stimulated lymphocytes and from control cultures were concentrated by negative pressure dialysis and fractionated on Sephadex G-100. Fractions containing excluded material estimated to be of relatively large molecular weight (as shown by the use of immunoglobulins G and M as protein markers) and the retarded fractions containing the smaller molecular weight proteins (similar to bovine albumin) were pooled and concentrated by vacuum dialysis. When these were assayed for their respective biological activities, both MIF and chemotactic activity were present in the latter fraction that contained smaller molecular weight proteins, whereas neither activity was found in the first fraction. The ultracentrifugation of an MIF-rich preparation (No. 279) in a sucrose density gradient revealed the presence of chemotactic activity between the markers bovine serum albumin and rabbit immunoglobulin G. This finding was consistent with the results of Sephadex chromatography. These preliminary studies show that the biological activities of chemotaxis and MIF have not been dissociated.

In addition to producing MIF and a leukotactic factor after stimulation with antigen, sensitized lymphocytes produce soluble materials with a variety of biological functions, dependent upon the method of assay. Such materials have been reported to produce delayed-type hypersensitivity lesions when injected intradermally (*6*), to have interferon-like activity (*19*), to be cytotoxic to certain cells in culture (*20*), to stimualte normal lymphocytes to divide and transform (*21*), and to contain newly formed antibodies. Until the active principles in these various systems have been clearly defined in physical-chemical terms, it will not be possible to determine how many different types of molecules are produced by sensitized lymphocytes when stimulated by specific antigen and whether a given factor will have more than one biologic activity.

The data from the in vitro experiments described in this report suggest an explanation for certain in vivo events found in delayed hypersensitivity reactions. The elaboration of a chemotactic factor by a few sensitized lymphocytes following interaction with an intradermally injected antigen could be responsible for the accumulation of a large number of nonsensitized mononuclear cells seen at the site of such

reactions. This explanation would be consistent with radioautographic data of delayed hypersensitivity of the skin (*9*) and with the findings that MIF-rich supernatants, when injected into the skin, produce an infiltrate of mononuclear cells (*6*). It is also possible that the mononuclear cells, having reached the site to which they have been attracted, are prevented from leaving that site by the action of MIF. The possibility that supernatants from antigen-stimulated lymphocytes are chemotactic for other types of leukocytes and the relation of the chemotactic factor to MIF and to other known factors that are chemotactic for mononuclear cells remain unanswered questions.

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Stimulus Properties of Reinforcing Brain Shock

Abstract. *Rats easily discriminate between two types of subcortical brain shock which differ in reinforcing properties. When both stimuli are either neutral or positively reinforcing subjects have difficulty in responding differentially to the two types of electrical stimulation of the brain. Possible implications for a theory concerning a generalized or diffuse reinforcement system are discussed.*

The perceptual properties of intracranial reinforcement are an almost completely neglected aspect of the phenomenon of self-stimulation. Rewarding septal and hypothalamic brain shock does not differ markedly (if at all) from auditory signals when used as conditioned stimuli in experiments on avoidance learning in a shuttle box (*1*); thus it is reasonable to assume that rewarding the electrical stimulation to the brain (ESB) has stimulus or cue properties. As a next logical step, it seems important to compare the stimulus characteristics of the reinforcement produced by electrical stimulation of various brain sites.

In self-stimulation experiments we may be tapping the neural substrate of conventional biological drive and reward (*2*). For example, several brain sites producing rewarding effects when stimulated also elicit "stimulus-bound" eating, drinking, or copulation (*3*). Furthermore, self-stimulation rates in certain areas appear to be modulated by specific drive and hormonal conditions (*4*). Self-stimulation at various anatomical points may involve the activation of several *different* reward systems. Therefore, one might assume that animals readily discriminate among different types of rewarding ESB just as they discriminate among conventional rewards.

In our experiment we attempted to establish rewarding brain shock in one locus as a discriminative stimulus (S^D) for response emission in a task motivated by hunger and rewarded by food. Reinforcing ESB in another locus served as S^A, indicating to the animal that the food reward was not available.

Twelve male albino rats were implanted with two bipolar stimulating electrodes and were tested for self-stimulation at both sites. In all subjects, one electrode was aimed at the diagonal band of Broca, and the other was placed in either the medial forebrain bundle-lateral hypothalamus or in the ventral tegmental nucleus (Tsai), as described elsewhere (*5*). In three subjects, both electrodes produced positively reinforcing effects (+,+); in three other rats both electrodes were "neutral" (0,0); and in the remaining six, one of the electrodes was reinforcing while a second electrode was "neutral" (+,0). In the self-stimulation tests and in all subsequent phases of the experiment, both electrodes were stimulated at the same intensity.

After the self-stimulation tests, subjects were placed on a 23-hour schedule of food deprivation and were trained to press a lever to obtain food pellets (45 mg). Once this response was learned the rats were gradually placed on a reinforcement schedule of 11 responses for one reinforcement (FR 11 : 1), and several daily 10-minute sessions were given until response rates stabilized. Six additional 10-minute sessions were then given during which subjects continued to respond for food on the FR 11 : 1 schedule, except that noncontingent, pulsing brain shock was delivered through alternate electrodes throughout the session. The electrical stimulation to the brain was 60 hz, pulsed 0.3 seconds on, 3.0 seconds off, at the intensity determined for each subject during the earlier self-stimulation tests. The stimulation was delivered in 1-minute trains through one electrode at a time, according to a predetermined random order. Thus, on each of 6 days, the rats received five 1-minute trains of brain stimulation through each electrode while working for food on the FR 11 : 1 schedule. This phase of training was included because previous studies had indicated that noncontingent ESB frequently interferes with ongoing behavior. During this phase stable base rates of responding were established for assessing later behavior.

During the final phase of the experiment, discrimination training, subjects