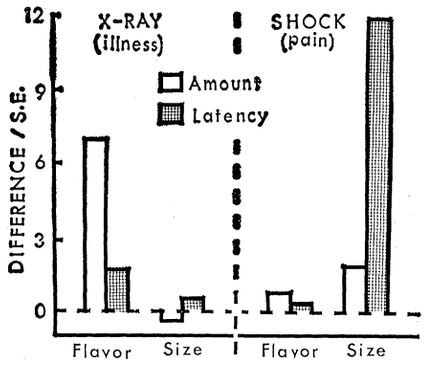

Fig. 1. Relative effectiveness of two attributes of food pellets (size or flavor) to act as cues after conditional pairing with two forms of noxious reinforcement (shock or x-ray) in four groups of rats. The mean difference between measures obtained with conditional and nonconditional forms of the food is scaled in terms of the standard error of that difference. (One S.E. is approximately 0.7 g or 8.2 seconds.) Amount reflects depressed consumption, and latency reflects hesitation before eating caused by the given cue on tests in absence of the reinforcer.

paws. Latency to begin eating was a more effective measure than amount eaten since every rat in the size-shock group hesitated much longer before eating the conditional size food; but once they commenced eating they continued to do so. However, the flavor of the pellet did not acquire significant CS properties when combined with the pain of shock. By contrast to the flavor-x-ray group, no animals in the flavor-shock group showed a decrease in preference for the shocked flavor even though the rats were shocked immediately after they began eating the flavored pellet (Fig. 1).

These data indicate: (i) that both the size cues and the taste cues were discriminable, (ii) that both x-ray and shock disrupted eating behavior, but (iii) that learning occurred only for certain stimulus combinations. Apparently, pairing a perceptible cue with an effective reinforcer does not insure effective associative learning; the cue must be "appropriate" for the consequences that ensue.

Since flavor is closely related to the chemical composition of food, natural selection would favor associative mechanisms relating flavor to the aftereffects of ingestion. The rat has such specialization in its anatomical structure, in gustatory receptors which sample food before it is incorporated by the internal viscera. Both the gustatory and visceral receptors send fibers that converge in the nucleus of the fasciculus solitarius (*4*). Other sensory systems do not send fibers directly to this nucleus; thus the neural organization reflects the propensity of the animal to associate flavor cues (but not size cues) with a subsequent malaise that is internally referred. When the consequences are beneficial (corrected vitamin deficit), the animal exhibits an increased preference for the flavor, and these shifts in preferences occur even though the subsequent reinforcement is delayed for hours (*5*).

Our evidence points toward a similar relation between the telereceptors (vision, audition) and cutaneous receptors. Externally referred sights and sounds are readily conditioned to the peripheral pain of shock. Although the mechanisms are considerably more complex, these systems also appear closely related both behaviorally and neurologically. When the consequence of eating is immediate peripheral pain, the animal exhibits fear responses (hesitation, jumping, squealing) to the nongustatory attributes of the food. But it does not display a reduced preference for either the gustatory or nongustatory attributes of the food. At higher intensities, both telereceptive and cutaneous cues produce similar orienting and startle reactions. These afferents may converge subcortically also probably at the level of the posterior thalamus (*6*). The probability of establishing associative learning depends in part on central integration of the particular afferent channels through which the conditionally paired stimuli are presented.

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Macrophage Spreading: Inhibition in Delayed Hypersensitivity

Abstract. *The capacity of peritoneal macrophages to spread was studied with cells of mice infected with* Listeria monocytogenes *and with cells of guinea pigs sensitized with BCG (bacille Calmette Guérin) vaccine or immunized with ovalbumin. In macrophages taken from animals having delayed hypersensitivity, this ability was markedly decreased by the presence of specific antigen for less than 1 hour. Such an effect was not observed in guinea pigs having only circulating antibodies.*

Rich and Lewis (*1*) showed that migration of cells from animals having delayed hypersensitivity to tuberculin could be inhibited by the presence of specific antigen. This basic fact has been extended to methods allowing in vitro quantitation of the inhibition of macrophage migration in systems specific for delayed hypersensitivity (*2*). Macrophages from animals treated with glucocorticoids lose their migratory capability, and, at the same time, such macrophages do not spread on glass surfaces (*3*). The absence of migration of such cells might reflect their inability to spread; the phenomenon of spreading inhibition might explain the macrophage-migration inhibition observed in delayed hypersensitivity. Experiments were therefore made to investigate the spreading ability of macrophages from animals exhibiting delayed hypersensitivities of the kind occurring in mice infected with *Listeria monocytogenes* (*4*) or in guinea pigs given BCG (bacille Calmette Guérin) vaccine. *Listeria monocytogenes* was grown in a medium of casein hydrolyzate (4 percent), NaCl (0.5 percent), and dextrose (0.2 percent) at *p*H 7.0; the culture supernatant was dialyzed and lyophilized as test material.

Female mice (N.C.S.) 4 to 5 weeks old (19 to 25 g) were injected intraperitoneally either with 7.5 to 9.5×10^4 living *Listeria monocytogenes* or with saline (controls). Peritoneal cells were collected 4 to 10 days later (*5*), the peritoneal cavities being washed with 5 ml of medium 199 containing 20 mg of bovine serum albumin (BSA) and 5 units of heparin per milliliter. The washings yielded 4.5 to 6.5×10^5 macrophages and 2.4 to 3×10^5 lymphocytes per milliliter. The cells were incubated in polyethylene tubes at 37°C for 30 minutes in the presence of

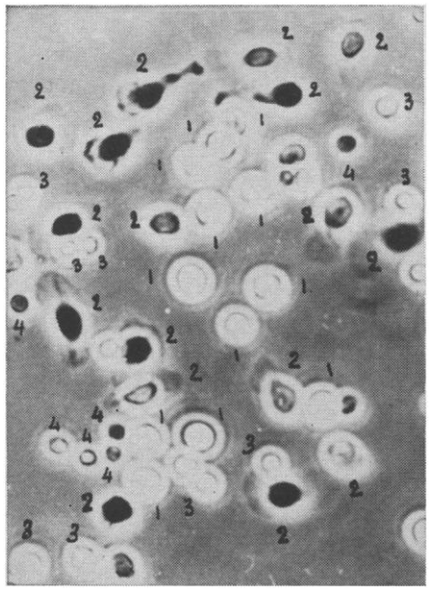

Fig. 1. Peritoneal washings taken from a normal mouse and photographed after incubation at 37°C for 30 minutes. Key: 1, macrophages; 2, spreading macrophages; 3, lymphocytes; 4, erythrocytes.

1 mg of the *L. monocytogenes* culture supernatant preparation. After being gently shaken, the cells were introduced into a Thoma counting chamber and incubated in a moist chamber at 37°C. The cells were examined 30 minutes later with a Leitz Ortholux phase microscope (Heine condenser and P.V. 25/0.50 lens) and photographed. With this technique (Fig. 1), macrophages were considered to have spread only when cytoplasm was clearly visible and showed an irregular border, and, at the same time, these macrophages had become darker. Care was taken to distinguish between these and dead macrophages that occurred occasionally. Although dead cells are also darker, they fail to exhibit cytoplasmic spreading and quite often show bleb-like vesicles.

Table 1. Macrophage-spreading (MSP) in tuberculin-sensitive and normal guinea pigs. I.U., international unit.

Tuberculin added to cell suspension (I.U./ml)	MSP	
	Tuberculin-sensitive animals	Normal
Skin-tested with 10 I.U. of tuberculin		
10	20	105
5000	45	84
500	21	104
50	35	86
5	40	83
500	23	173
5	53	132
0.5	63	141
0.05	50	118
Skin-tested with 50 I.U. of tuberculin		
500	78	45
500	63	
500	27	104
500	133	108
500	44	68
500	64	117
Average MSP for 500 I.U./ml		
500	57	103

The macrophage spreading percentage (MSP) was taken as the ratio of average percentage of cells spreading with antigen times 100 to the average percentage of cells spreading in absence of antigen. In three experiments with four mice infected with *Listeria* and four normal mice, the addition of *Listeria* supernatant preparation to the peritoneal cells of mice sensitized to *Listeria* caused remarkably reduced spreading of macrophages (average MSP as 51, 33, and 29 percent with antigen present) as compared with those of normal mice (95, 93, and 92 percent under similar conditions).

Similar experiments were made with peritoneal macrophage preparations from two random-bred strains (Hartley and "Pasteur Institute") of male and female guinea pigs. The animals were sensitized to tuberculin by injection of 0.1 ml of Freund's adjuvant containing 0.3 mg of BCG per milliliter into each footpad and 0.2 ml subcutaneously into the nuchal area. After 3 weeks or more, these animals were tested intracutaneously with 10 or 50 units of tuberculin (I.P. 48, of the Institut Pasteur). The size of the skin lesions was recorded at 24 and 48 hours. For comparison, guinea pigs were immunized with hen ovalbumin by initial intravenous injection of 2.1 mg in saline and intramuscular injections of 2.1 mg made after 1 and 2 weeks. Cells were taken 1 week later. The ovalbumin-immunized guinea pigs and some of the normal ones were tested with tuberculin 24 hours before they were used as cell donors.

Guinea pigs were exsanguinated by severance of cervical vessels, the abdominal skin was reflected, and 10 ml of washing fluid (Parker medium 199) was injected intraabdominally. The injected liquid was withdrawn with a syringe and No. 18 needle, care being taken to avoid contamination with erythrocytes. The average yield was 1.4×10^6 macrophages per milliliter and 0.6×10^6 lymphocytes, with marked individual variations. Each peritoneal cell suspension was tested by mixing 0.9 ml with (i) 0.1 ml of medium 199, (ii) 0.1 ml of medium 199 containing tuberculin in varying doses (Table 1), or sometimes (iii) with 0.1 ml of medium 199 containing ovalbumin. The various mixtures were handled in the way used with mouse cells.

When macrophages from hypersensitive guinea pigs were subjected to specific antigen for 30 minutes (Table 1), the spreading ability was decreased. Thus, in tuberculin-sensitive animals only 20 percent of macrophages usually spread in the presence of tuberculin; by contrast, 45 percent of macrophages spread upon incubation in medium alone, and no marked inhibition occurred when ovalbumin was added as nonspecific antigen. With different concentrations of tuberculin, MSP values were considerably lower with the cells of sensitive than with those of normal animals. Yet some aberrant reactions were observed. Thus, one of the sensitive guinea pigs showed no macrophage spreading inhibition (MSI) in the presence of tuberculin. This animal was cachetic and ill-looking at the time of peritoneal cell harvesting. On the other hand, cells from two of the control animals with a just-perceivable cutaneous reactivity to tuberculin exhibited a marked MSI in the presence of tuberculin. Marked individual variations were observed.

Guinea pigs that had been immunized to ovalbumin and that were producing circulating antibodies (PCA titer of 1 : 400 and 1 : 800, respectively) showed no MSI in the presence of 28 μg/ml of ovalbumin.

It is difficult to relate the inhibition of spreading and the inhibition of macrophage migration to a precise alteration either of the cell surface, or the cell metabolism, or both. However, our initial hypothesis was confirmed, namely, that MSI in the presence of specific antigen occurs under the same conditions required for inhibition of macrophage migration. Only a small amount of the material and a short time are needed to carry out this MSI test. This technique may be useful as a simple test for measuring delayed hypersensitivity in vitro.

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