30 minutes of exposure to these fluids. Apparently there is no time delay in either the preference for the S+G solution or the high rate of consumption. To compare preference differences between thirsty and nonthirsty animals, six comparable male animals treated identically, except that they were deprived of water for 48 hours prior to the test, consumed approximately equal quantities (9.5 ml) of the 0.125 percent S+G solution and tap water during the first 30 minutes. To the extent that deprivation-induced thirst simulates diuresis-induced thirst, these findings would argue against any explanation based upon a diuresis-induced thirst, as animals have different preferences when thirsty and when allowed water ad libitum.

The results indicate that a large part of the unique motivating properties of the S+G solution can be explained by the fact that the combination of saccharin and a relatively small amount of glucose produces a mixture that is very palatable, and possesses a minimum of postingestional inhibiting factors. A 0.25 percent saccharin solution is very sweet, but also is judged by most people to have a bitter "off-taste." A glucose solution equal in sweetness to the saccharin solution would be above a 60 percent solution. It is known that animals cannot consume much of such a high concentration of sugar during a 24-hour period, although they exhibit a preference for such a concentration in brief exposure tests. The addition of a small amount of glucose to a very sweet saccharin solution may eliminate the bitter taste component without appreciably adding to the postingestional inhibiting factors such as caloric intake and osmolarity.

ELLIOT S. VALENSTEIN
VERNE C. COX
JAN W. KAKOLEWSKI
Department of Psychophysiology-Neurophysiology, Fels Research Institute, Yellow Springs, Ohio

References and Notes

1. P. T. Young and J. T. Greene, *J. Comp. Physiol. Psychol.* **46**, 288 (1953).
2. C. Pfaffmann, in *Psychology: A Study of a Science, Study II*, S. Koch, Ed. (McGraw-Hill, New York, 1962).
3. E. Stellar, in American Physiological Society, *Handbook of Physiology*, J. Field, Ed., section 1, *Neurophysiology*, vol. 3 (Williams and Wilkins, Baltimore, 1960).
4. The impressive amount of the saccharin + glucose mixture that could be consumed was further demonstrated with five naive male animals (average weight 330 g, age 90 days) that were completely deprived of food. Over 8 days the average daily consumption of the 0.125 percent S+G solution increased from 148 ml to 343 ml.
5. E. S. Valenstein, J. W. Kakolewski, V. C. Cox, *Science* **156**, 942 (1967).
6. ———, unpublished data.
7. ———, *Science* **156**, 942 (1967); E. S. Valenstein, V. C. Cox, J. W. Kakolewski, *Psychol. Rep.*, in press.
8. E. S. Valenstein, J. W. Kakolewski, V. C. Cox, *Science* **156**, 942 (1967).
9. Supported by NIH research grant M-4529, NIH career development award MH-4947, and NASA research grant NsG-437. We are pleased to acknowledge the assistance of Antioch College students: Reed Elliott, Pegene McPhaden, and Kurt Wallen.

25 May 1967

Leukotactic Factor Elaborated by Mixtures of Genetically Dissimilar Cells

Abstract. *Mixtures of immunologically competent cells, from genetically dissimilar donors from inbred strains of mice, rats, or Syrian hamsters, cultivated in vitro elaborate into the medium a factor capable of attracting polymorphonuclear cells when it is injected into the skin of irradiated hamsters. Supernatant medium of cultured node cells from genetically identical donors contains no leukotactic activity. The degree of the cell accumulation seemed to parallel closely the degree of histoincompatibility.*

The role played by lymphoid cells in homograft reactions has been considered both in its immunological (*1*) and possibly nonimmunological aspects (*2*). When immunologically competent cells, like lymph node cells, from two strains of inbred animals of a given species are mixed in vitro and inoculated in the skin of lethally irradiated hamsters, delayed inflammatory reactions develop (*3*). These are absent when the components of the cell mixtures are injected separately, or when cells unable to react with each other for genetic reasons are mixed and inoculated. Irradiated hamsters might therefore be used for the study of cellular mechanisms in homograft reactions. In this model, the cutaneous inflammatory reactions are of equal intensity whether lymphoid cells from specifically sensitized animals are mixed with cells of donor-type origin, or with mixtures of lymphoid cells from normal, immunogenetically dissimilar animals. Furthermore, the reactions are independent of the species used, since mixtures of genetically incompatible lymphoid cells from inbred strains of mice, rats, guinea pigs, Syrian hamsters, or even mixtures of blood lymphocytes from two unrelated human individuals, may provoke reactions. Finally, the intensity of the reactions seems to reflect the degree of histocompatibility differences existing between the cells used in the mixtures. Thus these inflammatory reactions are seemingly an expression of cellular homograft reactivity developing in the integument of irradiated animals serving as immunologically neutralized vehicles. Their nature is, however, completely unknown.

An indication of the cellular events was obtained by impression smears prepared from skin reactions. These showed a striking accumulation of polymorphonuclear (PMN) cells at sites where node cell mixtures were injected, but not at sites where the components of the mixtures were injected separately.

This finding suggested that the interaction of genetically incompatible node cells in the skin of irradiated hamsters might lead to the formation of a leukotactically active mediator. To test this hypothesis, immunologically competent cells were confronted in vitro with cells carrying alien transplantation antigens, and the culture fluid was tested for a leukotactic factor.

Lymph nodes from animals of various inbred strains of mice, rats, or Syrian hamsters were harvested, and viable suspensions of node cells were prepared in tissue culture medium 199 (TCM 199). The medium contained 5 percent heat-inactivated calf serum, 100 international units of penicillin per milliliter and 50 μg of streptomycin per milliliter. Portions of 10×10^6 node cells from each of the donors were mixed and cultivated in plastic culture dishes (Falcon, 60 × 15 mm) in 5 ml TCM 199 for 48 hours at 37°C in a humid atmosphere of 95 percent air to 5 percent CO_2. Controls were provided by cultivating 20×10^6 node cells from one or from the other of the donors, or by incubating TCM 199 without cells under identical conditions. At the end of the incubation period, culture fluids were harvested and centrifuged (450 g for 10 minutes). The cell-free supernatants were concentrated to one-tenth their volume by lyophilization and reconstitution with physiological saline and dialyzed overnight against 1/15 molar phosphate buffer (*p*H 7.8) in the cold. Volumes of 0.1 ml were then inoculated with 28-gauge hypodermic needles in

the close-clipped skin of 8- to 12-week-old MHA hamsters, which had been irradiated with 1500 roentgens 28 hours previously (*3*). Intracutaneous inoculations of experimental and control supernatants were distributed randomly over the dorsal surface of several hamster hosts.

The intracutaneous injection of supernatants from cultivated mixtures of incompatible node cells consistently provoked severe inflammatory reactions (Table 1). When scored against an arbitrary scale (*4*) they reached +++ to ++++. The inoculation of control supernatants elicited only minor reactions. The intensity of cutaneous reactions provoked by culture fluids of mixed cells decreased with increasing genetic similarity of the cultivated node cells. Cutaneous responses could be elicited by means of supernatants from two types of node cell cultures: those composed of cells taken from animals specifically sensitized by rejection of skin homografts and donor-type cells and, more importantly, also by those made up of cells obtained from normal individuals of two inbred strains of the same species. Skin reactivity attained maximal intensity 24 hours after inoculation of culture fluids and waned rapidly thereafter.

Since irradiated hamsters, when used as hosts, are severely depleted of circulating lymphocytes but contain 1 to 2 million granulocytes per milliliter of blood (*3*), it is reasonable to assume that these cutaneous reactions are the result of granulocyte accumulations. That this was indeed the case could be shown by means of imprints. These revealed remarkable accumulations of PMN cells.

To determine the degree of cellular accumulations, skin reactions were excised 24 hours after inoculation of culture fluids. The tissue was trypsinized (*5*), and the number of PMN cells released was counted.

Supernatants of cultivated mixtures of node cells from genetically dissimilar donors of all three species tested contained a factor capable of attracting considerable numbers of PMN cells (Table 1). In contrast, culture fluids of genetically compatible node cells revealed little if any leukotactic activity. Characteristically, the number of PMN cells counted in these control reactions was of magnitude similar to that observed in reactions elicited by injection of incubated TCM 199.

The results obtained with mice are of particular interest, since certain strain combinations represent various degrees of histoincompatibility. Mixtures of node cells from mouse strains differing at the strong H-2 locus in addition to non-H-2 histocompatibility loci (CBA + C57BL/6) seemed to elaborate more leukotactic factor than cell mixtures identical at the H-2 locus (CBA + C3H). Still smaller amounts of this factor were produced by cells differing at the relatively weak Y locus only (C57BL/6 ♀ + C57BL/6 ♂).

Table 1. Accumulation of PMN cells in cutaneous reactions provoked by the injection of culture fluids of genetically incompatible or compatible lymph node cells cultivated in vitro for 48 hours.

Source of culture fluid from node cells	Skin sites tested (No.)	Reaction score (mean)	10^6 PMN cells per skin site (mean ±S.E.)
Mouse			
CBA + C57BL/6	49	+++	2.72 ± .13
CBA + C3H	18	++	1.32 ± .11
C57BL/6 ♀ + C57BL/6 ♂	29	+	0.76 ± .08
CBA	60	±	.30 ± .14
C3H	18	±	.20 ± .07
C57BL/6 ♀	48	±	.28 ± .04
C57BL/6 ♂	52	±	.23 ± .02
Rat			
DA + Lewis	20	+++	2.74 ± .26
DA + (Lewis × DA)F1	16	+++	2.15 ± .28
DA + Lewis T*	24	+++	1.46 ± .26
DA T + Lewis T	24	±	0.33 ± .01
DA	16	±	.37 ± .09
Lewis	20	±	.50 ± .07
(Lewis × DA)F1	16	±	.25 ± .05
DA T	20	±	.30 ± .07
Lewis T	24	±	.30 ± .07
Hamster			
CB + MHA	24	+++	2.00 ± .15
CB + LSH	24	+++	1.50 ± .14
MHA + LSH	24	±	0.38 ± .10
CB	24	±	.30 ± .07
MHA	24	±	.20 ± .04
LSH	24	±	.26 ± .04
TCM 199			
	104	±	.28 ± .02

* T, thymocytes.

Similarly, the supernatants of cultivated node cell mixtures from hamster strains MHA and LSH, known to differ only by trivial isoantigenic differences (*7*), likewise contained only insignificant amounts of leukotactic activity. On the other hand, supernatants of cultivated node cell mixtures from hamster strains CB and MHA, or from hamster strains CB and LSH, both provoked considerable accumulation of PMN cells. These latter strain combinations differ by major histocompatibility loci (*7*).

Kinetics of liberation of the leukotactic factor were studied with mixtures of node cells (from Lewis and DA rats) cultivated mixed or singly for varying periods of time. Whereas the cultivation of mixtures for only 15 minutes (most of the cells settled to the bottom of the dish in this time) did not result in the production of detectable amounts of the leukotactic factor, appreciable activity was present within 2 hours. Activity appeared to be at its peak after 4 to 8 hours of cultivation.

The speed with which competent cells seem to respond to foreign antigens raises the question of the immunological basis of the phenomenon. To answer this question I used rat thymocytes (from DA and Lewis rats) which, unlike lymph node cells, fail to provoke reactions when mixed and inoculated into the skin of irradiated hamsters (*3*), a confirmation of earlier observations that this cell type lacks immunological competence (*6*). Consequently, if the leukotactic factor were synthesized as a result of an immunological process, cultivation of mixtures of the rat node cells should lead to its production, whereas that of DA and Lewis rat thymocytes should not. Results fully confirmed the expectation (Table 1).

With the exception of mixtures composed of female and male node cells from the C57BL/6 mouse strain, the cellular interactions described so far are of a two-way nature, because both components are immunologically competent node cells. To test whether immunological one-way reactions would also result in the production of leukotactic activity, DA rat node cells were cultivated either with (Lewis × DA) F1 rat node cells or with Lewis rat thymocytes. The latter cell types both carry Lewis isoantigens but are incapable of reacting against DA rat node cells. Culture fluids from these cell mixtures elicited cutaneous reactions of similar intensity which contained much the same number of PMN cells as immunological two-way reactions (Table 1).

These results suggest that the confrontation of genetically dissimilar cells with transplantation antigens lead to the elaboration of a leukotactic factor. This factor appears to be specifically granulotactic, for skin reactions elicited by inoculation of culture fluid into normal, that is, nonirradiated MHA hamster hosts, contained predominantly PMN cells. It is tempting to assume that the same factor is responsible for the accumulation of PMN cells in cutaneous inflammatory homograft reactions (*3*).

The findings suggest that the elaboration of leukotactic factor depends on at least two conditions: immunological competence and presence of isoantigenic differences. If, as in the case of rat thymocytes, the cultivated cells lack immunological competence, discernible

amounts of leukotactic factor will not be detected, even though the cells presumably differ by strong isoantigens. If, on the other hand, differences in isoantigenicity are absent or minor, little or no leukotactic activity will be elaborated, despite the fact that both components of the cell mixture are competent. However, to produce the factor, the components of cultivated cell mixtures need not both be immunologically competent. Mixtures composed of node cells and cells carrying foreign transplantation antigens will be just as effective. The results would also indicate that competent cells recognize isoantigenic differences within a surprisingly short period of time. Since the intensity of leukocytic infiltration reflects degrees of immunogenetic differences, this should provide a means for quantitative measurement of histocompatibility.

Although final judgment on the nature of the cellular interaction leading to the production of leukotactic factor would be premature, the available evidence indicates that it is an immunologically specific process. While the results offer a possible explanation of the nature of skin reactions in lethally irradiated hamsters after inoculation of genetically dissimilar cells, their more general implications for homograft reaction and cellular hypersensitivity remain to be elucidated.

H. RAMSEIER

Department of Medical Microbiology, University of Zürich, 8006 Zürich, Switzerland

References and Notes

1. R. E. Billingham and W. K. Silvers, *Annu. Rev. Microbiol.* **17**, 531 (1963); J. L. Gowans and D. D. McGregor, *Progr. Allergy* **9**, 1 (1965); L. Brent and P. B. Medawar, *Brit. Med. Bull.* **23**, 55 (1967).
2. G. Möller and E. Möller, *Nature* **208**, 260 (1965); G. Möller, V. Beckman, G. Lundgren, *ibid.* **212**, 1203 (1966).
3. H. Ramseier and J. W. Streilein, *Lancet* **1965-I**, 622 (1965).
4. H. Ramseier and R. E. Billingham, *J. Exp. Med.* **123**, 629 (1966).
5. Four identical skin sites, each measuring 1 cm in diameter, were cut into small fragments and trypsinized in 10 ml of 0.25 percent trypsin (Difco) at room temperature. Samples were removed after 10, 15, and 20 minutes with leukocyte pipettes and 2 percent acetic acid as a diluent. Samples were counted in a hemocytometer by phase-contrast microscopy. The mean of three counts was used to estimate the number of PMN cells present per skin reaction.
6. R. E. Billingham *et al. J. Nat. Cancer Inst.* **28**, 365 (1962); R. E. Billingham and W. K. Silvers, in *The Thymus*, V. Defendi and D. Metcalf, Eds. (Wistar Institute Press, Philadelphia, 1964), p. 41.
7. R. E. Billingham, G. H. Sawchuck, W. K. Silvers, *Proc. Nat. Acad. Sci. U.S.* **46**, 1079 (1960).
8. Supported in part by grant No. 3887 of the Swiss National Foundation for Scientific Research and by a Funds for Research in Allergy. I thank Miss M. Leuenberger for technical assistance and Prof. J. Lindenmann for helpful suggestions and criticism.

8 May 1967

Subcellular Structure of Endosperm Protein in High-Lysine and Normal Corn

Abstract. *Optical microscopy shows that the protein network in endosperm cells of normal corn is composed of an amorphous matrix in which granules averaging about 2 microns in diameter are embedded. That these granules are rich in zein is demonstrated by their solubility in 80 percent ethanol. High-lysine corn, with submicroscopic granules clearly resolved only in the electron microscope, has a much lower content of zein than normal corn. The small size of subcellular protein granules in high-lysine maize as compared with normal corn correlates with the reported difference in zein content of the two types of corn.*

A marked change in the composition of endosperm protein of maize was first revealed by Mertz *et al.* (*1*). They discovered that the protein in a strain of floury maize, homozygous for the mutant gene *opaque-2* (o_2), contained about twice as much lysine as normal maize and only one-third as much zein (*1*).

To establish a structural basis for this change in protein composition, we compared the subcellular structure of the endosperm of high-lysine mutants with that of normal corn. Microscopically, cells of the maize endosperm show starch granules embedded in a protein network. If the starch is digested with α-amylase, the protein network can be exposed for microscopic examination. In normal corn, this network consists of a matrix protein in which globular protein granules, a little less than 2 μ in diameter (average), are embedded (Fig. 1). These granules may appear as strings of beads.

Unlike normal corn, endosperm cells of the high-lysine mutant o_2 gave little evidence of protein granules when examined with a light microscope. However, the electron microscope showed that granules were actually present in the matrix protein (Fig. 2); their diameter is approximately 1/20 that found in normal corn.

The proteinaceous character of the network was demonstrated by digestion with the protease, pronase; both components, the granules and matrix, were solubilized. A positive staining reaction indicative of protein was obtained in both components on treatment with ninhydrin-Schiff or chloramine-T Schiff reagent (*2*). Treatment with 80 percent ethanol dissolved the granules and left the matrix protein intact. In view of the fact that zein is defined as the alcohol-soluble fraction of corn endosperm, the effect from treatment with ethanol provided direct evidence that the granules were the primary site of zein deposition in corn. Duvick (*3*) had previously suggested

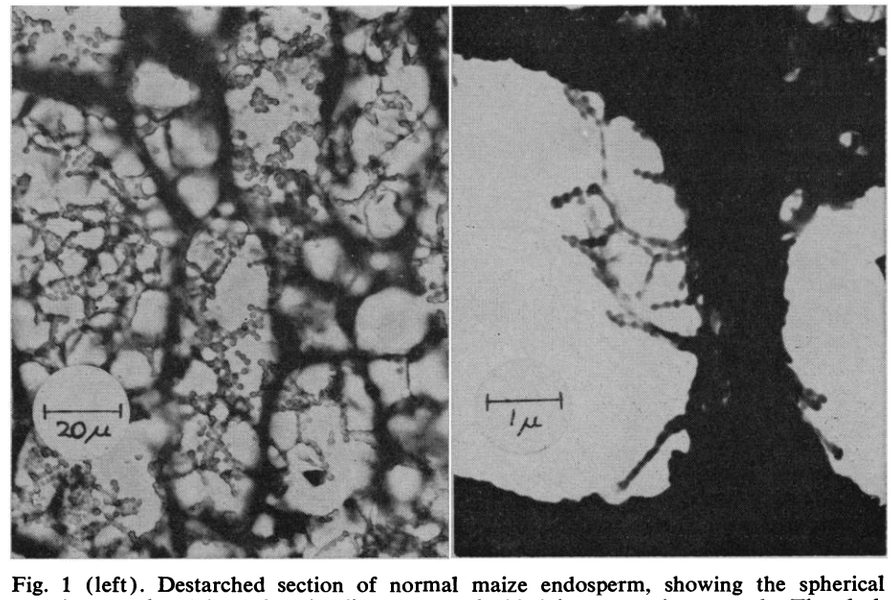

Fig. 1 (left). Destarched section of normal maize endosperm, showing the spherical protein granules (about 2 μ in diameter) embedded in a protein network. The dark lines are cell walls. Fig. 2 (right). Destarched *opaque-2* maize endosperm, showing spherical protein granules in beadlike arrangement (average diameter 0.1 μ).