Discussion Following Dr. Eisen's Paper
PREPARED BY MERRILL W. CHASE, CHAIRMAN

[Dr. Lawrence then spoke on the topic of delayed-type allergies, homograft sensitivity, and autoimmune disease as expressions of the immunological origins and consequences of genetic individuality.]

DR. LAWRENCE: "Any view of neoplastic growth occurring as a consequence of a central or peripheral failure of host immune mechanisms involves the possibility that tumor cells have acquired an antigenically foreign aspect as viewed by the host's self-recognition system. This possibility, however attractive and proximate, remains an assumption awaiting experimental definition.

"Nevertheless, it is an immunological fact of life that each individual of mammalian species does indeed learn to recognize his own cells and cellular constituents during embryonic development (The Production of Antibodies [Melbourne: Macmillan]; Phil. Trans., s.B, 239:357, 1956). In the usual course of events, such self-recognition precludes the biological paradox of the host's undertaking an immune response against his own tissues. That this guarantee does not always function to safeguard against autoimmune reactions has been well documented. Of surpassing interest to this symposium is the hope that there may be at hand, or may be fashioned, means to inaugurate in the host a highly specific autoimmune reaction directed exclusively at the tumor he is harboring."

"Unhappily, there are no technical methods presently applicable to this end; indeed, there is some doubt whether the question itself is a valid one. However, in this restrained climate, if one is willing to regard a tumor as an immunologically privileged type of homograft from the host's viewpoint, there may be relevance in the discussion to follow.

"As an outgrowth of Chase's observation in animals (Proc. Soc. Exp. Biol. & Med., 59:134, 1945), there has been found in extracts of human leukocytes obtained from donors with hypersensitivity of the delayed tuberculin type a factor or factors which, when injected into a nonsensitive recipient, causes the latter to respond as the donor to the specific delayed hypersensitivity transferred. The altered reactivity induced in the recipient by this means is prompt, widespread, and enduring. The agent involved in the transference of this biological function has been termed "transfer factor" with the realization that one or more factors may be involved in the transaction. Transfer factor, in its immediate effects, leads to the tissue damage seen in delayed types of hypersensitivity, serving here as the effector reagent, and, in its enduring effects, functions as the machinery which continues to cause the recipient to behave as the donor for prolonged periods (Cellular and Humoral Aspects of the Hypersensitive States [New York: Hoeber], p. 279). It has served to transfer delayed hypersensitivity to a variety of bacterial (tuberculin, streptococcal proteins, diphtheria toxoid), fungal (coccidioidin), denatured human serum protein (J. Exp. Med., 113:1029, 1959), and tissue (skin homograft) antigens (J. Clin. Investigation, 39:185, 1960) in humans. Some of the properties of transfer factor, insofar as they are currently known, are set forth in Table 1 (Cellular Aspects of Immunity, Ciba Symposium, p. 243).

"In this perspective, transfer factor may be looked upon as the common effector pathway of the delayed type of altered tissue reactivity, whether this event is initiated by bacterial cells, fungal cells, or cells of yet another type such as those found in tissue homografts.

"Elsewhere we have proposed the notion that the tissue damage incident to delayed bacterial hypersensitivity, to tissue homograft sensitivity, and to autoimmune disease are variant expressions of one immunological mechanism. We have pictured the event of tissue damage as resulting from the interaction of specifically sensitized cell populations bearing transfer factor with other tissue cell populations—foreign or altered—within the same host (Cellular Aspects of Immunity; Physiol. Rev., 39:811, 1959; Ann. Rev. Med., 11:207, 1960).

"This postulate accepts the experimental fact that staunch and enduring delayed hypersensitivity occurs in nature following infection by bacteria come to reside within phagocytic cells of the host for prolonged periods of time; following infection by viral agents with characteristically
obligatory intracellular parasitism; or following contact with simple chemicals, which in order to function as delayed allergens are obliged to form a complex with the host's cells (Am. J. Med., 20:425, 1956). The postulate also accepts the biological implications of Billingham, Brent, and Medawar's demonstration of actively acquired tolerance (Phil. Trans., s.B, 239:357, 1956).

"The first assumption to be made suggests that the inducing agent for delayed hypersensitive states (bacteria, virus, simple chemical) in consequence of prolonged intracellular residence results in the formation of an intimate complex between the inducing agent and the cellular constituents of the host. That virus particles, in usurping control of the machinery of the cell to turn it to their own devices, are in a position to form such a complex is not at all incomprehensible. That simple chemicals engaged in the induction of delayed sensitivity must form such a complex has been shown by Eisen (J. Exp. Med., 95:473, 1952).

"It is postulated that macrophages ordinarily recognized as "self," will, when dying or dead, be phagocytosed by other reticuloendothelial elements and may now be recognized as slightly or wholly foreign. Similarly, living cells may be now recognized as different depending upon the extent to which the inducing agent ("x") has altered the tissue antigens. Implicit in the recognition of such alien cells is an immune response undertaken by the host, not against "self" alone or against "x" alone, but against the complex "self + x," wherever and whenever the inducing agent or its products are in combination with the host's tissues.

Considerable experimental evidence has confirmed the role of transfer factor as the effector reagent for the delayed type of allergic inflammatory response and the instrument of tissue damage in the ordinary context of that response (Cellular and Humoral Aspects of the Hypersensitive States, p. 279; Cellular Aspects of Immunity, p. 243). The postulate we have considered would suggest that transfer factor may now be viewed as an immune factor directed not against tuberculin alone, for example, or against "self" alone, but against "self + tuberculin" in combination.

"This would lead to the interpretation that the patient with caseating cavitary pulmonary tuberculosis, in attempting to reject the tubercle bacillus, has been caused inadvertently to reject parts of his own pulmonary tissue as if it were indeed foreign or a homograft. The postulate would also view the injection of tuberculin intradermally into sensitive individuals as a device for coating epidermal cells, ordinarily recognized as "self," with the appropriate "x" material resulting in conversion to a local group of "self + x" cells. The latter cells would interact with sensitized leucocytes bearing transfer factor. The resultant in-

### TABLE 1

<table>
<thead>
<tr>
<th>Biological</th>
<th>Biochemical TF unaffected by:</th>
<th>Immuno Biological</th>
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<tbody>
<tr>
<td>Endows recipient with specific sensitivity of donor.</td>
<td>25° or 37°—6 hrs.</td>
<td>Interacts with but is not neutralized by antigen.</td>
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<tr>
<td>Sensitivity is systemic.</td>
<td>Distilled water lysis.</td>
<td>White blood cells (WBC) desensitized by antigen.</td>
</tr>
<tr>
<td>Onset early (hrs.).</td>
<td>Freeze-thaw 10 cycles.</td>
<td>Neg. WBC+antigen—no transfer.</td>
</tr>
<tr>
<td>Duration long (mos.-year).</td>
<td>Deep freeze—5 months.</td>
<td>No detectable antibody (AB) in donor WBC extract.</td>
</tr>
<tr>
<td>Minute dosage WBC effective.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As little as 0.01 ml.—local transfer.</td>
<td>DNAse</td>
<td>Not active sensitization—early onset.</td>
</tr>
<tr>
<td>As little as 0.1 ml.—systemic transfer.</td>
<td>RNAse</td>
<td>Not passive sensitization—long duration.</td>
</tr>
<tr>
<td>Capacity for transfer depends on degree donor sensitivity and dosage WBC used.</td>
<td>(DNAse + trypsin)</td>
<td>Repeated test with antigen may increase intensity and duration of transferred sensitivity—yet is not necessarily its cause.</td>
</tr>
<tr>
<td>Negative donors incapable.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracts or cell-free supernatants as effective as viable cells.</td>
<td></td>
<td></td>
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<tr>
<td>Does not cross species barrier.</td>
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flamatory response, although truly a "tuberculin reaction," would really represent a local type of homograft rejection reaction undertaken by the host against such coated epidermal cells which he has come to regard as foreign.

"The postulate would therefore regard delayed allergy of the tuberculin type—in its induction, manifestations, and transfer—as a quantitatively less intense but qualitatively similar variant of homograft sensitivity. The intensity and tempo of classical homograft rejection reactions are known to be conditioned by the genetic disparity of donor and recipient of tissues; the intensity and tempo of delayed type hypersensitivity reactions, viewed as a type of homograft rejection phenomenon, are postulated as being conditioned by the degree to which the inducing agent (x) has altered the tissue antigens or genetic individuality of host cells (self).

"In the spectrum of autoimmune diseases, it suggests a role for unknown inducing agents for entering into complexes with the "self" of the respective tissues involved. For example, during the particular viral infection or immunizing procedure which precedes the development of post-infectious "allergic" encephalomyelitis in man the virus may function as "x" to complex the "self" of nervous tissue. The resulting disease could be an expression of the host's attempt to reject parts of his brain as if it were a homograft. In the laboratory animal, injection of bits of brain mixed with Freund's adjuvant function as a much more efficient complexing agent than the viral infection, insuring the development of experimental allergic encephalomyelitis.

"In other diseases of this general category it is suggested that the serum factors detected (thyroglobulin antibody, L-E factor, rheumatoid factor) are signals that an earlier immunological event has occurred, rather than the instruments of tissue destruction observed. We would regard that earlier immunological event to be a cell-to-cell interaction of the type described above, initiated by as yet unknown inducing agents."

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Dr. Toolan: "Some time ago we were interested in skin homografts in rabbits and wanted to learn how long we could prolong the life of these grafts if the hosts were given cortisone. We found that, if we adjusted the cortisone properly to the particular rabbit, we were able to keep the grafts alive for 6 months or longer. At the end of this time, if cortisone was discontinued and the rabbits were put on ACTH, their grafts were rejected, but not in the fashion that one would see in normal animals. It took about a month, whereas ordinary grafts are rejected in 7 days.

"Consequently, we felt it was possible that we had here a slow-motion picture of graft rejection that allowed us to see some events otherwise kaleidoscoped in animals not treated with cortisone. Instead of seeing an overwhelming inflammatory picture, what we saw within the first 5 days after cessation of cortisone was hyalinization of the ground substance in the graft itself and in host tissues immediately adjacent. No invading host cells were present at all. Meanwhile, the epidermal cells of the graft were in excellent condition and to all appearances healthy.

"About the 7th–8th day there was a tremendous influx of polymorphonuclear leukocytes into all the vessels of the graft. One must keep in mind the fact that this picture was not one of primary inflammation; it was something occurring 6 months after the grafts had been placed on their hosts.

"The leukocytes next migrated through the tissues and went out through the epidermis, without apparently—at this stage—damaging the epidermis in any way. They then formed a layer on the surface of the epidermis, producing the crust one often sees on sloughing grafts.

"Not all the leukocytes migrated immediately through the epidermis. A considerable number of them lined up and became flattened along the basal edge of the epidermis. They then became swollen and lost some of their granules into the surrounding tissues. We can only speculate what the polymorphonuclear leukocytes were doing. Possibly they were concerned in some phenomenon of sensitization.

"After all these events had occurred, the lymphocytes appeared for the first time. When they arrived they, too, attached themselves to the basal edge of the epidermis. They then became swollen and lost some of their granules into the surrounding tissues. We can only speculate what the polymorphonuclear leukocytes were doing. Possibly they were concerned in some phenomenon of sensitization.

"What the particular sequence of events may mean, I cannot say. It may be the sum of several immunological phenomena in which the lymphocytes play a conspicuous and final role. One should not forget, however, that events occurring in the ground substance may be initial to the whole procedure."

Dr. Frank J. Dixon asked how long it took for the lymphocytes to appear in the skin site after the cortisone was stopped, and how this ap-
pearance relates to the circulating lymphocytes. Were the latter depressed, or did they return shortly after the cortisone was stopped?

Dr. Toolan replied that it took about 10 days for the lymphocytes to appear in the skin graft. The peripheral lymphocytes did not show particular depression. She felt that a very important role had to be assigned to a competent ground substance, a conclusion she had reached also from other observations.

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[The topic was continued in an evening session. Chairman Chase introduced Dr. Pensky at this point, to speak on the effect of C'1 esterase inhibitor of human serum on various immune systems requiring complement.]

Dr. Pensky: "A protein from human serum which specifically inhibits the enzyme activity of C'1-esterase has been purified approximately 500-fold by ion-exchange chromatography. This inhibitor instantaneously and stoichiometrically inhibits the esterolysis of certain synthetic amino acid esters, such as N-acetyl-L-tyrosine ethyl ester and p-toluenesulfonylarginine methyl ester by C'1-esterase, of which human and guinea pig C'1 is the enzymatically inactive precursor.

"The action of the C'1-esterase inhibitor has been studied in four separate immune systems involving the action of complement. These were (a) complement fixation, with pneumococcal S III-rabbit anti-S III serum, (b) immune cytotoxicity, with human amnion cells and specific rabbit antiserum, (c) immune human hemolysis (Donath-Landsteiner reaction) involving human erythrocytes and human erythrocyte antibody, and (d) immune hemolysis of sheep erythrocytes, sensitized with rabbit antibody, by human complement.

"All systems involve the action of complement, and in each the action of complement could be blocked by C'1-esterase inhibitor at an early stage involving the participation of C'1 or C'1-esterase. The inhibition of hemolysis or of cytotoxicity could in all instances be reversed by adding sufficient C'1-esterase to the system to bind all the esterase inhibitor present. The inhibitor appears to be a heat-labile a-2 globulin which is rapidly inactivated below pH 5.5 and is not identical with serum trypsin or plasmin inhibitor. This material shows promise as a tool in the investigation of immune and cytotoxic phenomena in which complement is or is suspected to be involved."
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