Since Ehrlich and Morgenroth (5) first published their famous \textit{horror autotoxicus} concept, hundreds of observations have been made supporting the idea that the formation of deleterious antibodies against an organism's own antigen is an exceptional event, if possible at all. Investigations on human ABO blood groups have furnished the best known example of obedience to the biological principle of \textit{horror autotoxicus}. According to Landsteiner's law (13), every human being produces antibodies against A and/or B antigens whenever they are absent from the red cells—but autoantibodies directed against the antigens present on the individual's own erythrocytes never occur. This and other observations led many investigators to believe that the inability of autoantibody formation is a genetically established characteristic of every living organism. According to Furuhata (8), the presence or absence of natural blood group antibodies is genetically determined in a fashion similar to the blood group antigens. The observation of Owen (18) on cattle chimeras opened up a new era of experimental approach to the problem of \textit{horror autotoxicus}. His basic observations were confirmed in certain instances of human chimeras.

The biological importance of Owen's observations was recognized by Burnet and Fenner (3), who came to the conclusion that the \textit{horror autotoxicus} principle does not depend upon a genetical mechanism but may be acquired during ontogenesis. This new concept was proved to be correct by the basic experiments on immune tolerance performed by Medawar, Billingham, and Brent (see recent review paper by Medawar [16]). The fact that the fetus elaborates the mysterious ability to distinguish between an organism's own and foreign antigens is no longer questioned. According to the clonal selection theory of Burnet (1), clones which would otherwise be capable of producing antibodies are destroyed by contact with the corresponding antigens when exposed during the early stage of embryonic life.

Burnet (1, 2) went further, then, and tried to explain the problem of autoimmune diseases in terms of his clonal selection theory. However, for us, the entire field of autoimmune diseases has not been sufficiently clarified to properly evaluate the application and advantages of Dr. Burnet's theories in this particular field. To begin with, we do not know which human diseases are really caused by autoimmune mechanisms. We agree that some indication for auto sensitization is given by the presence of humoral autoantibodies. However, the presence of autoantibodies by no means proves the autoimmune character of a disease. Syphilis, for instance, is accompanied by antibodies reacting with the patient's own lipides, and if one did not know the causative agent of syphilis and did not have considerable information about this disease, it might easily be assumed by some enthusiasts that the pathogenesis of syphilis is connected with the production of autoantibodies. (By the way, I am not at all sure that certain symptoms of this disease could not be explained on that basis.)

Thirty-five years ago we (34) started our studies on tissue specificity with the intravenous injection into rabbits of foreign brain suspensions, such as bovine brain. In such brain antisera antibodies were found which were clearly organ-specific, inasmuch as they reacted with the brain extracts not only of the species used for immunization but also with the brain extracts of all mammals, and even of fish and birds. The intravenous injection of \textit{rabbits} with \textit{rabbit} brain suspensions, on the other hand, did not elicit antibody formation. Later these investigations were extended by Rivers and Schwentker (20) in the United States. These authors gave multiple intramuscular injections of foreign brain extracts to monkeys, and encephalomyelitis accompanied by myelin de-
denaturation of the material, when incorporated into an antibody-producing animal itself. However, isoimmunization with antigens originating not only from other animals of the given species but also from antigens in the antibody-producing animal. In our experiments in guinea pigs and dogs have not been emphasized that merely the production of circulating antibodies as elicited by the more “classical” methods of subcutaneous or intravenous injection is not in itself indicative of nor even essential for the occurrence of encephalomyelitis. The additional presence of acid-fast bacilli in complete Freund adjuvants serves to point out the significant role played by delayed hypersensitivity in the pathogenesis of this experimental disease.

The experimental approach to the production of thyroiditis opened up some new problems in autoimmune diseases (22, 31, 33). Extracts of pooled rabbit thyroid glands, prepared carefully to avoid denaturation of the material, when incorporated into Freund adjuvants and injected into rabbits, elicited circulating antibodies of outstanding specificity for the thyroid. Isoimmunization with thyroid extract was frequently accompanied by histological changes in the thyroids of such rabbits: infiltrations by lymphocytes and plasma cells resembling in many respects chronic thyroiditis in man, referred to by some authors as Hashimoto’s Disease. Both the appearance of circulating thyroid antibodies and the pathological changes in the thyroid gland were a great surprise to us; we did not expect either.

The term “isoimmunization” requires definition, especially because of the somewhat different terminology used in transplantation: Isoimmunization implies any immunization in which an animal is given injections of antigenic material originating from the same species but not from the animal’s own body. Isoimmunization may result in the production of isoantibodies—i.e., antibodies reacting with antigens occurring within the same species but not within the body of the antibody-producing animal itself. However, isoimmunization may also elicit autoantibody production, i.e., production of antibodies which react with antigens originating not only from other animals of the given species but also with antigens present in the antibody-producing animal. In our experiment, isoimmunization with thyroid glands resulted in autoantibody production; the antibodies produced by the animals under investigation reacted not only with the thyroid extracts of all individual animals of the species tested but even with extracts of the thyroid glands of the antibody-producing animal itself. Additional evidence for the autoantibody character of this type of thyroid antibody was procured by immunization of rabbits or dogs with thyroid extracts obtained from their own thyroid glands removed by surgical procedure (31).

Thyroglobulin is probably the leading antigen in experimental thyroiditis and one of the important antigens in human thyroiditis. There seems to be no doubt that thyroglobulin is endowed with autoantigenicity.

Entirely different results were obtained in the study on antigenic structures of pancreas (21, 32). From the concept developed in the experimental thyroiditis studies, attempts were made to produce experimental pancreatitis. Such an attempt seemed justifiable by the preceding recognition of an organ-specific pancreas globulin demonstrable by heteroimmunization. Isoimmunization of rabbits with pooled rabbit pancreas extracts also elicited the appearance of pancreas-specific antibodies. They were readily demonstrable by complement fixation and precipitation reactions when tested against pooled rabbit pancreas extracts.

However, when individual extracts instead of pooled extracts were tested, it became apparent that we were dealing with isoantibodies and not with autoantibodies. We based this statement on the observation that only certain and not all individual pancreas extracts reacted with certain pancreas antisera. A pancreas antiserum never gave a positive reaction with an extract of the animal’s own pancreas (Table 1). In addition, no histological changes could be found in the pancreas of these rabbits. It was felt, then, that we could postulate the existence of at least three or four pancreas-specific isoantigens in the rabbit, and I have to underline rabbit because similar experiments in guinea pigs and dogs have not been successful as yet.

Another organ of considerable interest in relation to autoimmunization is the adrenal gland. About 30 years ago organ-specific structures were found in the adrenal gland by means of antisera obtained by heteroimmunization (29). Colover and Glynn (4) and recently Steiner, Langer, Schatz, and Volpe (26) observed adrenalitis following immunization of guinea pigs with guinea pig adrenals. No serological investigations accompanied these reports. In collaboration with Dr. Milgrom,
the problems of hetero-, iso-, and auto-immunization with adrenal glands were approached in a similar manner, as was done with the thyroid and pancreas. Preliminary results were presented at the Federation meetings in Chicago in 1960 (30), and detailed manuscripts have now been submitted for publication. Briefly, it is possible to demonstrate adrenal-specific antigens by heteroimmunization. No histological changes were found in the adrenal glands of rabbits exposed to heteroimmunization with beef adrenals. On the other hand, isoimmunization with adrenal material carried out in rabbits and guinea pigs elicited the production of an antibody against a thermolabile constituent of adrenals, probably associated with the cortex.

The interesting facts about the active immunization of experimental animals with adrenal extracts of their own species were (a) antibodies were elicited which were highly specific for adrenal glands; (b) antisera obtained in the rabbit cross-reacted with the adrenal extracts of other species whereas the corresponding guinea pig antisera were species-limited, at least with the antisera available thus far; (c) adrenal antibodies reacted not only with pooled adrenal extracts but also with all individual adrenal extracts examined, including extracts of the adrenal glands obtained from the antibody-producing animal; (d) in a few instances it was possible to surgically remove one adrenal gland and to immunize the animal with extracts of its own gland. Here, too, adrenal antibodies were obtained. Therefore, as in the case of the thyroid gland, it is possible to produce autoantibodies against normal constituents of the adrenal gland. These autoantibodies are not necessarily removed by the normal tissue but may remain demonstrable in the circulation at least during certain periods of active immunization.

I hasten to add that we also observed histological changes in adrenal glands consisting of lymphocytic infiltrations, though these lesions were not as impressive as those Dr. Colover has seen. However, we found similar infiltrations in the adrenal glands of animals immunized with other organs—

| TABLE 1 |
| COMPLEMENT FIXATION TEST |
| Reaction of Rabbit Pancreas Antisera with Extracts of Individual Pancreas |

<table>
<thead>
<tr>
<th>Rabbit Pancreas Antisera</th>
<th>Individual Rabbit Pancreas Extracts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RABBIT PANCREAS</td>
<td>776 777 778 779 780 781 782 784 Pool</td>
</tr>
<tr>
<td>ANTISERA 776</td>
<td>--    --    --    --    --    --    --    --</td>
</tr>
<tr>
<td>777</td>
<td>--    ++    --    --    --    --    --    --</td>
</tr>
<tr>
<td>778</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
</tr>
<tr>
<td>779</td>
<td>++    --    --    --    --    --    --    --</td>
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<tr>
<td>780</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
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<td>781</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
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<td>782</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
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<tr>
<td>783</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
</tr>
<tr>
<td>784</td>
<td>++    ++    ++    ++    ++    ++    ++    ++</td>
</tr>
<tr>
<td>Normal rabbit serum</td>
<td>--    --    --    --    --    --    --    --</td>
</tr>
</tbody>
</table>

The plus signs represent the greatest dilution of antigen which gave complete inhibition of hemolysis. Readings taken after 40 min. at 37°C.

-- = No reaction with any dilution of antigen.
++ = Reaction with 1000-2500 antigen dilution.
+++ = Reaction with 2500-6500 antigen dilution.

* Protein concentrations: 776=1.35%, 777=0.77%, 778=1.53%, 779=0.94%, 780=0.86%, 781=1.58%, 782=1.11%, 783=0.86%, Pool=1.88%.
Our investigations on the possible autoantigenicity of the body’s own constituents were guided in the past by the search for the demonstration of organ-specific structures occurring within certain tissues. In addition to the examples mentioned, there are others which belong to this group, such as the lens of the eye (9, 10), the casein (14), spermatozoa (7), and perhaps a few others. One might possibly correlate autoantigenicity in this group of antigens with the relatively late appearance of organ-specific structures in embryonic life, or one might blame the particular anatomical position for separating the organ-specific structures involved from the antibody-producing cellular apparatus. At no time during our investigations have we stated that the mere demonstration of circulating antibodies would mean that they were the cause per se of the histological changes. On the contrary, we felt (33) that certain criteria have to be established in order to relate specific pathological findings to autosensitization. They are: (a) the direct demonstration of specific antibodies of the circulating type, or the demonstration by indirect means of the cell-bound type of antibody; (b) the recognition or isolation of the specific antigen against which this antibody is directed; (c) the production of antibodies against the same type of antigen in experimental animals; (d) the appearance of pathological changes in the corresponding tissues of an actively sensitized experimental animal that are basically similar to those in the human disease. We still believe that the animal experiment is most important and that it is an almost irreplaceable link in the chain of evidence for the autoimmune character of a human disease. Therefore, at this time we would be inclined to consider as human autoimmune diseases: chronic nonspecific thyroiditis, encephalitis following anti-rabies vaccination with Pasteur vaccine, and certain forms of aspermatogenesis. All these three diseases have a counterpart in animal experimentation, and the target tissues are known to be endowed with clear-cut organ specificity.

If I understand correctly, Dr. Burnet might not consider chronic thyroiditis (nor probably any of the above-mentioned conditions) as a true autoimmune disease. According to Dr. Burnet, thyroid autoantibodies may be quite easily formed because the organism fails to develop a self-recognition mechanism to antigens which normally do not reach the circulation. The appearance of pathological lesions in the thyroid gland would depend on the “weakness of immunologic homeostasis.” In contrast, Dr. Burnet refers to a second group of diseases, namely: systemic lupus erythematosus, hemolytic anemia, and rheumatoid arthritis, as...
true autoimmune diseases. He believes that in these diseases the autoantibody is directed against common body components, and explains the production of antibodies by the appearance of "forbidden clones."

We would not argue with Dr. Burnet regarding the interpretation of the first group of diseases. As a matter of fact, we would consider his interpretation as very close to ours, though using different terms. However, we would like to express some hesitation about classifying the second group of maladies as autoimmune. Lupus erythematosus, hemolytic anemia, and rheumatoid arthritis may be termed autoimmune diseases only on the basis of serological findings as yet not too well defined or understood. None of these diseases could be successfully reproduced in experimental animals.

It is possible that the serum factors observed in these diseases are really autoantibodies, but we would like to see more proof for this contention. For example, the rheumatoid factor cannot be considered an autoantibody without serious reservations. Rheumatoid sera react with \( \gamma \)-globulin of rabbit and human origin. There is an increasing body of evidence showing that the reaction of the rheumatoid factor with human \( \gamma \)-globulin is not that of a "true" autoantibody. We refer, for instance, to Steinberg's experiment (25) in which the serum of a white man suffering from rheumatoid arthritis reacted with the serum \( \gamma \)-globulin of certain Negroes but not with the \( \gamma \)-globulin of Caucasian origin. The rheumatoid factor found in the serum of a white patient reacting with rabbit \( \gamma \)-globulin and Negro \( \gamma \)-globulin would lead us to assume that the patient produced hetero- and iso-immunization in rabbits (21). We believe, at least to this moment, that the antigens responsible for the stimulation of pancreas antibodies were iso- and not auto-antigens.

Another doubt we have regarding the autoimmune character of the diseases under discussion is the irregular appearance of the antibodies involved. There is hardly any relation between the clinical course of acquired hemolytic anemia and the presence of antibodies; in many cases of this disease no antibodies are found at all. Similarly, juvenile rheumatoid arthritis and rheumatoid arthritis in agammaglobulinemia patients are not accompanied by the rheumatoid factor. On the other hand, this factor is also to be found in other human diseases.

Because of these reasons we would ask ourselves whether the serological findings accompanying lupus erythematosus, hemolytic anemia, and rheumatoid arthritis are really in cause-effect relation-

<table>
<thead>
<tr>
<th>TABLE 2 NONREACTIVITY OF AUTOIMMUNE SERA</th>
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<tr>
<td>From Table by Mackay and Larkin in Clinical Science, Vol. 18, No. 3, Aug., 1959</td>
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</tbody>
</table>

<table>
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<tr>
<th>Antigens</th>
<th>JAM</th>
<th>BEL</th>
<th>MAG</th>
<th>MCC</th>
<th>VAN</th>
<th>STE</th>
</tr>
</thead>
<tbody>
<tr>
<td>s = spleen; l = liver; k = kidney; c = colon.</td>
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<td>† . . . means not tested.</td>
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<tr>
<td>N refers to tissue antigens obtained from necropsy subjects dying of unrelated diseases.</td>
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<tr>
<th>Reaction of Sera and Antigens of Cases 1-6</th>
</tr>
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<tbody>
<tr>
<td>8000</td>
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<tr>
<td>64</td>
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<table>
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<tr>
<th>Cases</th>
<th>Reaction</th>
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<td>1-6</td>
<td>8000-39000</td>
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ship, or whether they are just sequela of some pathological events and/or therapeutical measures.

One should not underestimate the difficulties of recognizing an autoantibody by the test performed in vitro with the patient's serum: (a) In using antigenic material procured from other human beings one always runs the risk of mistaking isoantibodies for autoantibodies. For instance, posttransfusion isoantibodies frequently have been erroneously termed autoantibodies directed against erythrocytes, leukocytes, and platelets. (b) Antigenic material originating from the patient's own body is rarely obtained before the onset of the disease; employing biopsy and post mortem specimens, one may be dealing with tissues altered by the morbid process. (c) The extraction and isolation procedures may be responsible for antigenic alterations. (For example, isolated and partially denatured human γ-globulin gives a reaction with rheumatoid sera quite different from that of the unaltered γ-globulin present in the serum.)

Instead of believing in "forbidden clones" which may produce autoantibodies against common antigens, even against those present in the circulation, we would prefer to believe—at least until more experimental data are produced—that antibodies or antibody-like substances encountered in the three diseases under consideration are not true autoantibodies. In some instances blood transfusions may be blamed for the stimulation of antibody production. In other instances the stimulus is unknown and in the absence of adequate experimental data may be only subject to speculation. Here we are attracted by the idea that some morbid processes may affect the antigenic structure of some tissue and serum components. The altered antigens, no longer self, may stimulate the production of antibodies which could easily be misinterpreted as autoantibodies. The need for a clear definition of the term "autoantibody" and for agreement regarding this definition has become essential.

What, then, does the problem of autoantigenicity contribute to the problem of cancer immunology, in diagnosis and prevention? Obviously, the occurrence of antigens specific for cancer cells but not found outside in other normal tissues would constitute a challenge to isoinmunization and possibly to autoimmunization. A successful attempt to immunize against cancer, then, depends upon the occurrence of antigens which the body would refuse to recognize as self. These antigens could be cell constituents present in the cancer but not in the normal tissues, or they could even be viruses or virus-like substances not normally present in the cells, or at least not present in a way in which the body would consider them as its own and therefore would refuse to be immunized against them.

Our studies on organ specificity, begun a long time ago in Heidelberg, were undertaken with the hope at that time of finding models among normal tissue constituents which eventually could be applicable to cancer. The fact that we have been working more on the models than on the problem of cancer immunology probably is due to the fact that unexpected biological phenomena were encountered which elicited our curiosity. However, the time certainly has come now to apply more extensively what we have learned of iso- and autoantigens to malignancies. It is possible that antigenic constituents of some form of malignancies fall into a group of auto-antigens similar to thyroid antigens, and therefore they would be suitable for serological study. On the other hand, it might be necessary to learn more about the breakdown of what Burnet calls "immunological homeostasis," which he defines as "some mechanism by which immunological activity against body components is prevented." Several technics are already known which might possibly accomplish this goal.

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The Question of Self-Recognition by the Host and Problems of Autoantibodies and Their Specificity

Ernest Witebsky


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