assured to-day than when he wrote, a year ago. We may be certain that nobody sees more clearly than he that the threat of final disaster to all man's hopes and achievements will not be forever averted, if the possibility of the "monstrous perversion" of science is allowed to remain and to continue its evil growth. Even in the past year our enemies have thrown a new and vivid light on future possibilities, by the new weapons which science has enabled them to put on trial for our destruction. Though a people's unflinching courage and an answering effort of science and organization, together with the progress of the Allied armies over the launching areas, have given us confidence that flying bombs and the like will not affect the issue of this war, the warning which they give, as to what the future might hold, is not the less clear. The writing on the wall must be plain for all to read. If, when the memories of the present war begin to fade, the world should allow science again to be exploited by a nation grasping at predominance by conquest, science will no longer be invoked only as an aid to what valor can achieve by land, sea or air, but as an agent, in itself, of blind annihilation at an ever lengthening range. When we men of science regain that freedom, for the ultimate preservation of which we have loyally accepted, through these tragic years, the bonds of secrecy and submission to authority, we can not put aside with these our proper share in the new responsibility for the future of mankind, which this war's experiences have laid upon the men of good-will in all nations. It is true, indeed, that neither the present abuse of science, nor any possibility of final disaster to civilization, which might come of a future perversion of its powers, can be charged as a fault to science itself; no more, indeed, than we could properly charge to religion, as such, the wars which once devastated much of Europe in its name. But we men of science can not escape from our growing share in the responsibility, in "the greater task," as Mr. Churchill has written, "of directing knowledge lastingly towards the purposes of peace and human good." No man of science has the right to prescribe for another his interpretation in detail of that duty; but there is one aim which may unite us, perhaps for the most effective action within our common grasp, and one which is worthy of all our common influence and effort. Let me quote again from Mr. Churchill's letter: "in this task," he writes, "the scientists of the world, united by the bond of a single purpose which overrides all bounds of race and language, can play a leading and inspiring part." To build anew, and on a firm and broadening foundation, a world community in science, is surely an aim worthy of our utmost effort and devotion; but there can be no swerving from the present duty, and the call on science by war may yet be sterner, before we have won the freedom thus to work for the future of the world.

THE SITE OF ANTIBODY FORMATION*

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EVOLUTION in science is brought about by the discovery of facts and the elaboration of patterns into which these facts will fit. The earliest facts about the formation of antibodies discovered by Metschnikoff and others fitted well into the pattern of the reticulo-endothelial theory of antibody production. However, in recent years additional facts have been brought to light which are difficult to reconcile with this theory,[1, 2, 3, 4] and we have recently presented new facts which seem to be inconsistent with the old concept.[5, 6] The latter lend themselves to the elaboration of a theory which is consistent not only with the old facts but also with the new ones. This new pattern of antibody formation is the subject of the present presentation.

THE RETICULO-ENDOTHELIAL THEORY OF ANTIBODY FORMATION

The reticulo-endothelial theory of antibody formation was widely accepted, undoubtedly because it seemed plausible that the cells which phagocytose and destroy bacteria should also be concerned with the synthesis of antibodies.[7] "It is chiefly this phagocytosis of formed antigens (erythrocytes, bacteria) which has directed the attention to the reticulo-endothelium as the possible source of the antibodies."[8]

There are two important arguments which have been

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1 L. Hektoen, *Jour. Infect Dis.*, 17: 415, 1915.

2 J. B. Murphy and E. Sturm, *Jour. Exp. Med.*, 41: 245, 1925.

3 W. E. Ehrich and W. Voigt, *Beitr. Path. Anat.*, 93: 348, 1934.

4 A. R. Rich, M. R. Lewis and M. M. Wintrobe, *Bull. Johns Hopkins Hosp.*, 65: 311, 1939.

5 W. E. Ehrich and T. N. Harris, *Jour. Exp. Med.*, 76: 335, 1942.

6 T. N. Harris, E. Grimm, E. Mertens and W. E. Ehrich, *Jour. Exp. Med.*, in press.

7 Annotations, *Lancet*, 1: 654, 1943.

8 R. H. Jaffe, "The Reticulo-Endothelial System." Handbook of Hematology, Vol. II. New York: Hoeber. 1938.

advanced in support of this theory and which enjoy general consideration. One of these is based on the observation that the output of antibodies may be depressed through blockage of the reticulo-endothelium with phagocytotic material such as iron-sugar, india ink, trypan blue or collargol. In interpreting this finding, it has been argued that the "blockade" acts by interfering with the phagocytic and digestive function of the macrophage, that this cell while engaged in digesting one colloid, could not well take care of another, and therefore could not produce antibodies.

The phagocytic and digestive function of the macrophage can not be questioned. But have we any proof for the contention that this function results in antibody formation? Suppose that Bunting[9, 10] was right when he postulated that the micro- and macrophages merely destroyed living organisms, but were not instrumental in combating toxins or in manufacturing antitoxins which he thought were produced by the lymphocyte. Or let us consider a modification of Bunting's theory, namely, that the micro- and macrophages merely break down formed antigen and thus prepare it for proper utilization by the lymphocyte or similar cells which can take up only dissolved material. If these or similar views were correct, the results of the blockade experiments could be interpreted differently. They could mean that blocked reticulo-endothelium could not prepare formed antigen for proper utilization by the antibody-forming cell. They could signify as well that the stimulated macrophages ingested and destroyed the antigen so rapidly[10] that its effective contact with the antibody forming cell was greatly reduced. That the latter explanation should be considered is borne out by recent experiences with so-called adjuvants, *i.e.,* oils which, when injected with the antigen, prolong antibody formation (Freund and Bonanto[11]). There is evidence to believe that this prolongation may be due to retention of antigen which, with the oil, is taken up by the macrophages and, because of the oil, is slowly broken down resulting in slow release and therefore more effective contact with the antibody-forming cell.

The other important argument in support of the reticulo-endothelial theory to be discussed here is the one which was recently advanced by Sabin.[12] Using a dye-protein, this author noted that after phagocytosis by the macrophage some of the dye was removed from each dye-protein aggregate, and after removal of the dye the protein particles were no longer visible. This was interpreted to mean "that the protein has been rendered into soluble form and passed into the cytoplasm." She further noted shedding of cytoplasm by the macrophage, which was "coincident with the time when the dye-protein is no longer visible within these cells, and when there are antibodies in the serum." This was believed to be an anatomical expression of synthesis of antibody within the cytoplasm of the macrophage and expulsion by the cell of the finished product.

Although Sabin's theory has attracted the attention of many investigators, it should be clear that the facts upon which it was based were not new and may be interpreted differently. It should be noted, for example, that she did not use the dye-protein in the soluble form in which it had been prepared; but that she first made an alum precipitate, the particles of which were large enough to be readily visible. She thus produced aggregates resembling bacteria or other formed antigens, which were so large that they had to be engulfed by the micro- and macrophages, and split by the enzymes of these cells before the original character of the antigen was regained.

The process observed by Sabin is obviously the same as the phagocytosis and digestion of gram-positive or acid-fast bacteria which, since the days of Metschnikoff, have been seen by numerous investigators. What was observed was splitting of raw material; the synthesis of antibodies within the phagocytic cell was not seen but merely deduced. It is equally possible that the products which were expelled from the cytoplasm of this cell were split products of the alum precipitate, rather than synthesized antibody.

The shedding of cytoplasm was stated to be a characteristic of macrophages. Such shedding has, however, been described and illustrated as a characteristic of lymphocytes[13] and of myeloblasts and promyelocytes.[14] It is generally known in thrombocytopoiesis.[15] Moreover: "It was striking that it was not these (macrophages showing digestion) that presented the shedding phenomenon but rather those without visible dye-protein particles."[12] Also: "It should be made clear that this process of shedding has not been observed within the living animal, but only in living cells removed from the animal. When the omentum or a drop of peritoneal fluid is first mounted on a slide, all the cells are rounded, but on standing for a short time the reaction of the shedding begins."[12] Similar phenomena are readily observed in supravital preparations of lymphocytes preceding their disintegration.

The relevance of this discussion is best shown by

[9] C. H. Bunting, *Wisconsin Med. Jour.*, 24: 305, 1925.
[10] C. H. Bunting, "The Polymorphonuclear Neutrophile Leucocyte." Handbook of Hematology, Vol. I. New York: Hoeber. 1938.
[11] J. Freund and M. V. Bonanto, *Jour. Immunol.*, 48: 325, 1944.
[12] F. R. Sabin, *Jour. Exp. Med.*, 70: 67, 1939.
[13] H. Downey and F. Weidenreich, *Arch. Mikr. Anat.*, 80: 306, 1912.
[14] R. Schuster, *Folia Haemat.*, 63: 382, 1940.
[15] M. Watzka, *Zeitsch. Mikr. Anat. Forsch.*, 41: 498, 1937.

Sabin's rather casual observation that the dye-protein combination was engulfed and digested by the polymorphonuclear leucocytes as well as by the macrophages. But here the observed fact is interpreted in a different way: the granulocytes "play a role in bringing the antigen into the macrophages." This view will not be shared by many in the light of our knowledge of the numerous and vigorous enzyme systems contained in these cells.

We may say, then, that the observations of Sabin as well as the blockade experiments have added little to what was known to Metschnikoff 60 years ago. It has been merely shown once more that both the micro- and macrophages engulf and digest formed antigenic material and it has been revealed that proper blockade of the reticulo-endothelial system may interfere with this process. The products of digestion of the macrophage, however, have not been identified. Indeed, there is no evidence to show that the antibodies are products of this digestion.

The Lymphocytic Theory of Antibody Formation

Paralleling the reticulo-endothelial theory but obscured by it, a lymphocytic theory of antibody formation has long existed. Bunting[9, 10] has always maintained that antibodies are formed by the lymphocyte rather than the granulocyte or the macrophage. "The toxins (antigens) are apparently affixed by the lymphoid cells. If in great intensity the toxins cause necrosis of lymphocytes; if in proper dilution, one finds not necrosis, but stimulation and proliferation, with the production of antibodies. I realize that all will not agree with me that the lymphocytic series of cells produces the antibodies, yet all the pathological evidence I can obtain inclines me to that view."[9] That the polymorphonuclear leucocyte "does not play a part in antitoxic immunity seems to be indicated by a series of clinical observations which have been summarized in a general pathological law to the effect that no disease which runs its course with a neutrophil leucocytosis is followed by a lasting immunity."[10] Moreover, it was found that a high monocyte-lymphocyte ratio indicated susceptibility to tuberculosis, while a high lymphocyte-monocyte ratio suggested resistance.[16] "Considerable evidence both from infections in human patients and in animals suggest that the percentage of lymphocytes gives an index of the hosts' resistance, particularly in chronic diseases where a relative or absolute lymphocytosis is often associated with repair and recovery."[17]

These and similar clinical and pathological observations received experimental support first by Hektoen,[1] who showed that white rats exposed to x-rays revealed a decrease in hemolysin production, and that this was accompanied by a simultaneous reduction in the quantity of lymphatic tissue and bone marrow and in the number of circulating lymphocytes.

The observations of Hektoen were extended by Murphy and Sturm,[2] who exposed rabbits to x-rays of sufficient intensity to reduce the amount of their lymphoid tissue without damage to their bone marrow, and showed that a definite deficiency in the production of precipitins, bacterial agglutinins and protective antibodies resulted. On the other hand, rabbits which they subjected to an exposure of dry heat in amounts sufficient to increase the activity of the lymphatic tissue developed antibodies in larger quantity than did untreated animals. The effect of the x-rays on antibody formation could possibly be explained by blockage of the reticulo-endothelium, which was found to be engorged with the remains of lymphocytes. However, the response to heat could not be similarly explained because there was no evidence that dry heat had the slightest effect on macrophages.

Meanwhile, other facts were discovered which were difficult to reconcile with the reticulo-endothelial theory. It was observed, for instance, that after doses of staphylococcus vaccine large enough to stimulate marked proliferation of reticulo-endothelial elements the antibody titer remained low, whereas with small doses which did not produce visible proliferation of these cells high titers were obtained.[3] In the same experiments, the rise in antibody titer in the serum was found to parallel the activity of the Malpighian bodies of the spleen,[3] the cells of which were found to be lymphoid cells rather than histiocytes.[4]

The lymphocytic theory of antibody formation received new impetus through the experiments of McMaster and Hudack,[18] who showed conclusively that antibodies may be formed in lymph nodes. If two different antigens were injected, one into each ear of mice, the corresponding antibody appeared first in the lymph node of the same side. Fixation in the injected tissue or its regional lymph node as discussed by Menkin[19] was thus ruled out.

The observations of McMaster and Hudack were extended by our experiments[5] which showed that the cellular response within the lymph node during antibody formation is chiefly lymphocytic. The reticulo-endothelium in these experiments seemed to react independently. When antigens were injected into the pad of the hind foot of the rabbit, antibodies first appeared 2 to 4 days after the injection in the lymph

[16] C. A. Doan and F. R. Sabin, *Jour. Exp. Med.*, 52: 113, 1930.

[17] W. H. Taliaferro and C. Kluever, *Jour. Infect. Dis.*, 67: 121, 1940.

[18] P. D. McMaster and S. S. Hudack, *Jour. Exp. Med.*, 61: 783, 1935.

[19] V. Menkin, "Dynamics of Inflammation." New York: The Macmillan Company. 1940.

draining the popliteal lymph node (the only node regional to the site of injection). They reached their highest titer after six days. In all experiments it was found that the antibody titer was higher in the efferent lymph; in some cases the concentration was 100 times that found in the lymph of the afferent vessel. The production of antibody in the popliteal lymph node was preceded and accompanied by a rise in the output of lymphocytes in the efferent lymph which ranged from 15,000 to 20,000 per cu.mm. to 60,000 to 80,000 per cu.mm. or more. At the same time hyperplasia of the lymphatic tissue within the node occurred resulting in some experiments in a weight increase of the node from 0.2 gm to 1.0 gm or more. These observations lent little support to the idea that antibodies are direct products of reticulo-endothelial cells. The latter concept, in fact, is hardly consistent with the complex chain of events in the lymph node during the formation of antibodies as we have described it.

In a recent paper[6] we now have shown that, during antibody formation in the popliteal lymph node of rabbits, the lymphocytes in the efferent lymph vessels contain antibodies in a much higher concentration than the surrounding lymph. The ratio of titers amounted to from 8 to 16 in many instances. This observation seems to offer only two possible interpretations, that the lymphocyte either absorbs or adsorbs, or produces antibodies. Various *in vivo* and *in vitro* experiments[6] failed to show absorption or adsorption of antibody by the lymphocyte; nor was this idea supported by the observations of McMaster and Hudack[18] or our previous experiments.[5] On the other hand, it was noted that the ratio between lymphocyte titer and lymph plasma titer was greatest on the fifth day of the experiment, which was the time of greatest rate of antibody formation in the lymph node. The average ratios of titers fell from 7 on the fifth day to 2.3 on the seventh day, in sheep erythrocyte experiments, and from 5 on the fifth day to 3 on the seventh day, in typhoid vaccine experiments. This observation is consistent with a primary appearance of antibodies within, or on the surface of, the lymphocyte, and inconsistent with what would be expected if absorption or adsorption took place.

It is true that the lymphocyte is a somewhat prosaic cell with no particularly striking morphologic characteristics. If stained with routine stains, the cytoplasm seems to be singularly undifferentiated and unspecialized, especially when compared with that of the other white cells of the blood.[20] However, when studied while living it shows many interesting features, among which the refractile bodies of Gall[21] are the most intriguing. Moreover, lymphocytes contain a wealth of enzyme systems, which have been recently discussed by Barnes.[22]

It is also true that the lymphocyte does not phagocytose and therefore can not absorb corpuscular matter. But who can deny that it has the faculty of absorbing or adsorbing dissolved antigens or split products of particulate antigenic substances? In fact, if our reasoning is correct, the lymphocyte goes into action only after the raw material, *i.e.*, bacteria or other formed antigens, have been properly prepared by the action of micro- or macrophages. It seems that the polymorphonuclear leucocyte and the macrophage as well as the lymphocyte may be instrumental in antibody production. It may be through the cooperation of all these elements that antibodies are produced. If this concept is correct, it becomes clear why blockage of the reticulo-endothelial system may or may not interfere with antibody formation. It is also obvious why the destruction of lymphocytes by x-ray produces a reduction in antibody formation and why the stimulation of lymphocytopoiesis by dry heat induces an increase.

SUMMARY AND CONCLUSIONS

An attempt has been made to show that facts previously regarded as evidence for the reticulo-endothelial theory of antibody formation may be interpreted differently. Moreover, there are recent observations which are difficult to reconcile, if not inconsistent, with this pattern. However, the new observations as well as the old facts seem to fit into another theory of antibody formation in which the lymphocyte and possibly the granulocyte as well as the macrophage play an essential role. This theory is consistent also with the present concepts of the chemical reactions involved in antibody formation.[23, 24]

OBITUARY

CHARLES LE ROY GIBSON

CHARLES LE ROY GIBSON, associate professor of chemistry at the University of New Mexico, died at his home in Albuquerque on December 8, 1944.

Dr. Gibson was born at Clovis, New Mexico, on February 19, 1911, where his father was an official of the A. T. & S. F. Railway. He received his secondary education in the Belen, New Mexico, high school. During his high-school days, following a trip of the Belen high school football team, on which he

[20] C. K. Drinker and J. M. Yoffey, "Lymphatics, Lymph and Lymphoid Tissue." Cambridge: Harvard University Press. 1941.
[21] E. A. Gall, *Amer. Jour. Med. Sci.*, 191: 380, 1936.
[22] J. M. Barnes, *Brit. Jour. Exp. Path.*, 21: 264, 1940.
[23] S. Mudd, *Jour. Immunol.*, 23: 423, 1932.
[24] L. Pauling, D. H. Campbell and D. Pressman, *Physiol. Rev.*, 23: 203, 1943.