Since hematologists agree that the percentage distribution of the different cell types is of paramount importance in leukocytic counts it would seem that the contents of Chapters 9 and 10 could be condensed to advantage. It is regrettable that Dr. Muller has not stressed the fact that a leukocytic count contains at least six variables and that it is the proportional relationship of the variables that constitutes the significance of any leukocytic count.

The chart shown on page 163 presents an interesting theoretical concept. It can not be accepted as factual for two reasons—first, it is extremely difficult to determine when a tuberculous individual has eradicated the tubercle bacillus from his system; second, to assume that a tuberculous infection may be "healed" because leukocytic counts remain within normal limits is unwarranted.

The occurrence of anemia in tuberculous patients is generally recognized and the need to determine the type of anemia is evident. Dr. Muller's presentation of this subject is valuable. A condensation of the text would enhance its value.

The sixty-page dissertation on the sedimentation rate of erythrocytes could be condensed considerably, thus emphasizing the salient facts. The correction table devised by Dr. Muller undoubtedly permits a more precise determination of the "rate of sedimentation." From a practical point of view the necessity for such precision may be debated. To the clinician the thing of importance is whether the rate is abnormal and, if so, how far it deviates from normal. In this respect the results should not be interpreted too finely—a difference of 0.5 mm per minute would be significant, whereas a difference of 0.2 mm would be equivocal. In a routine diagnostic service a single reading at one hour and a determination of the percentage of sedimentation based upon the plasma volume will give the clinician the essential facts, although admittedly this method does not guarantee the mathematical accuracy of Dr. Muller's method. Dr. Muller might have added to her requirements a uniform temperature for all sedimentation tests since temperature does affect the test to some extent.

In Part 4 Dr. Muller demonstrates that hematological and clinical findings often disagree, especially in individuals who seem to be progressing satisfactorily. It is where such disagreements occur that the hematological findings are of especial value. That the leukocytic reaction and the sedimentation rate are two independent phenomena which often disagree is also clearly demonstrated. This portion of the monograph would be improved greatly by a reduction in the number of tables and graphs. In some instances the number of patients and of serial tests is too small to carry much weight.

On page 311 Dr. Muller states: "The index is unfavorable in 37.8 per cent. of the examinations in Group A; as seen above these cases were clinically quiescent most of the time. Since there is an incidence of lymphopenia in only 14.1 per cent., and of neutrophilia in only 7.4 per cent., *the unfavorable index is obtained by the interpretation of essentially normal values.*" This interpretation is based upon the arbitrary normal values proposed by Dr. Muller, not upon the normal values which I reported in the *American Journal of Medical Sciences* in January, 1929. It is impossible to obtain an unfavorable index from normal leukocytic counts if the normal values I have determined are used. Furthermore, unless such normal values are acceptable the index values are not valid.

The reviewer is impressed by the fact that Dr. Muller considers changes within a rather narrow zone in Schilling counts and in sedimentation rates as significant, whereas rather broad changes must be present in a differential percentage before any interpretation is allowed. This is a common error among physicians and is due apparently to the difficulty of appreciating proportional changes between several variables. The entire purpose of the leukocytic index, as reported by the reviewer, was to demonstrate that proportionally the several variables could be abnormal in a leukocytic count that would ordinarily be accepted as normal.

Part 6 is well done. Dr. Muller could have insisted, with profit, on the counting of 400 cells to obtain a differential percentage to make the determination reliable statistically when different counts are to be compared. A reliable count can be obtained from a single well-made blood smear.

The general impression of the reviewer is that a much more concise monograph on this important subject would have evolved had the author had a much longer and broader experience in the field of tuberculosis.

E. M. MEDLAR

SPECIAL ARTICLES

AN EXPERIMENTAL TEST OF THE FRAMEWORK THEORY OF ANTIGEN-ANTIBODY PRECIPITATION

THE framework theory (lattice theory) of serological precipitation and agglutination, first proposed by Marrack,[1] has not been accepted by all investiga-

[1] J. R. Marrack, "The Chemistry of Antigens and Antibodies," Report No. 194 of the Medical Research Council,

tors in the field, although it is supported more or less strongly by a considerable body of evidence.[1,2,3,4] We have now carried out some experiments which correspond so well in their results with the predictions of this theory as to leave little doubt of its correctness.

We have synthesized a substance which gives a specific precipitate with a mixture of two different antisera, but gives no precipitate with either antiserum alone. The substance contains two different haptenic groups, R and X, to which the two antisera are homologous: the anti-R serum was made by injecting rabbits with azoprotein containing R groups, and the anti-X serum by injecting with azoprotein containing X groups. The R and X groups were respectively the *p*-azophenylarsonic acid group and the *p*-azobenzoic acid group, and the RX substance used in most of our work was 1-amino-2-*p*-(*p*-azophenylazo)phenylarsonic acid-3,6-disulfonic acid-7-*p*-(*p*-azophenylazo)benzoic acid-8-hydroxynaphthalene. Similar results were also obtained with 1,8-dihydroxy-2-*p*-azophenylarsonic acid-3,6-disulfonic acid-7-*p*-(*p*-azophenylazo)benzoic acid-naphthalene.

The experimental results show that in the formation of the precipitate both of the two haptenic groups of the molecule enter into specific reaction, the R group with an anti-R antibody molecule and the X group with an anti-X antibody molecule. This is shown by the fact that the RX substance precipitates only with a mixture of the two antisera, and not with either one alone; the explanation of the failure of precipitation with only one antiserum given by the framework theory is that with respect to either antiserum the RX molecule is only monohaptenic and hence only univalent, and so can not act as the link between antibody molecules in the formation of a framework.

With the effective bivalence of the precipitating antigen thus proved, knowledge of the antibody-antigen molecular ratio for the precipitate provides the value of the average valence of the antibody molecules. The molecular ratio was found by analysis to be 0.7, which corresponds to $2/0.7 = 2.8$ for the average antibody valence.[5] If the antibody were univalent the molecular ratio would have the value 2, which is far greater than the experimental value. Further evidence for the effective multivalence of antibody is provided by the observation that the RX precipitate is soluble in excess of the mixed antisera; this solubility can not be explained on the basis of univalent antibody.

A detailed account of this work will be published in the *Journal of the American Chemical Society.*

LINUS PAULING
DAVID PRESSMAN
DAN H. CAMPBELL

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,
CALIFORNIA INSTITUTE OF TECHNOLOGY

His Majesty's Stationery Office, London, 1934; Second Edition, Report No. 230, 1938.

[2] M. Heidelberger and F. E. Kendall, *Jour. Exptl. Med.*, 61: 559, 563, 1935; 62: 467, 697, 1935; M. Heidelberger, *Chem. Rev.*, 24: 323, 1939.

[3] L. Pauling, *Jour. Am. Chem. Soc.*, 62: 2643, 1940.

[4] L. Pauling, David Pressman, Dan H. Campbell and collaborators, *ibid.*, 64: 2994, 3003, 3010, 3015, 1942; 65: 728, 1943.

[5] Similar values of the antibody-antigen molecular ratio have been previously reported (footnote 4) for precipitates of anti-R antisera and simple substances containing two or more R haptenic groups.

THE PRODUCTION OF MULTIPOLAR MITOSES IN NORMAL EMBRYONIC CHICK CELLS

THE sporadic occurrence of an abnormal type of cell division, namely, multipolar mitosis, in normal somatic cells remains unexplained. Kemp[1] reported finding a single tripolar mitosis in approximately 10,000 dividing cells in tissue cultures of tissues from normal chick embryos and adult fowls. A later study[2] of dividing cells from a double monster (Cephalopagus) revealed five triasters among 500 mitotic cells. The fact that multipolar mitoses with resultant unbalance of chromosome number in daughter cells occur during the growth of certain types of malignant cells is too well known to be emphasized.[3, 4, 5, 6] However, the significance of their occurrence in relation to malignancy has not yet been established. Attempts to produce this type of cell abnormality in normal somatic cells have met with varied degrees of success.[7, 8, 9, 10]

In the present experiments 4 series of cultures (total 92) of eight-day normal embryonic chick heart muscle were made by the usual hanging drop technique. A total of 12 to 14 chick hearts were used. The culture medium consisted of 1 drop of fowl plasma (dil. 1:1 with Tyrode solution) and 2 drops of a mixture of embryonic juice from 8- and 11-day chick embryos (dil. 1:1 with Tyrode solution). The

[1] T. Kemp, *Zeit. f. Zellforsch. u. mikrosk. Anat.*, 11: 429, 1930.

[2] T. Kemp and J. Engelbreth-Holm, *Arch. f. exp. Zellforsch.*, 10: 117, 1931.

[3] Th. Boveri, "Zur Frage der Entstehung der malignen Tumoren." Jena, 1914.

[4] M. Levine, *Jour. Cancer Research*, 14: 400, 1931.

[5] M. R. Lewis and L. C. Strong, *Am. Jour. of Cancer*, 20: 72, 1934.

[6] J. Maurer, *Arch. f. exp. Zellforsch.*, 21: 191, 1938.

[7] W. G. Whitman, *Am. Jour. of Cancer*, 17: 932, 1933.

[8] E. Marie Hearne Creech, *Am. Jour. of Cancer*, 35: 191, 1939.

[9] W. R. Earle and Carl Voegtlin, *Public Health Reports*, 55: 303, 1940.

[10] E. F. Stilwell, *Anat. Rec.*, 76: 205, 1940, and 84: 193, 1942.