Hematology of Sprue

REPORT ON 100 CASES IN PUERTO RICO*

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EVER SINCE 1908, when the late Dr. B. K. Ashford[1] first called attention to its existence in this country, the sprue syndrome has occupied a position of distinction in the medical history of Puerto Rico, and has been the subject of various investigations. Among those contributions dealing with the hematology of sprue, the works of Serra[2] and J. Suárez[3] merit special attention, as detailed information is given concerning the blood picture in 45 and 70 cases of sprue, respectively. More recent papers by Castle and associates[4] and R. M. Suárez[5] also include blood findings in 92 and 35 cases. On the other hand, an excellent clinical and critical summary covering the literature[6] on sprue, dispenses with the blood picture in a few lines, thus indicating the necessity for more complete hematologic study of the blood levels which are believed to be indistinguishable from those found in Addisonian anemia of temperate climates. It seems, therefore, that a study of the peripheral blood picture in uncomplicated sprue would not only be a contribution to the clinical aspects of the syndrome as found in Puerto Rico, but would offer data for comparison to those investigators in northern latitudes who are familiar with the blood picture in Addison's anemia.

MATERIAL AND METHODS

The present work consists of an examination of the initial peripheral blood picture before treatment in individuals of both sexes suffering from sprue. There were 57 males and 43 females, ranging from 12 to 78 years of age, the mean age for the entire group being 40.14 years. There were 87 white and 13 colored individuals, among which two full-blooded negroes are included, the others in the colored group being mulattoes. The majority of the cases were observed either at the clinic or in the wards of the University Hospital of the School of Tropical Medicine, San Juan. As far as we know, 98 persons were natives of this Island, of either Spanish or colored extraction, and of the remaining two, one was a male Venezuelan mulatto and the other,

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a Continental American who had resided in Puerto Rico for several years before the onset of sprue.

Analysis of other clinical data and results of therapy in this group are to form the subject of a future communication.

The following examinations were made: Enumeration of erythrocytes, leukocytes and platelets, and differential white cell counts, including study of stained smear; estimation of hemoglobin and determination of the volume of packed red cells. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated. Hematologic studies were made from 3 cc. samples of venous blood within three hours after drawing without stasis, and rendered incoagulable by the addition of a mixture of dry potassium and ammonium oxalate (4 mgs. of the former and 6 mgs. of the latter per 1 cc. of blood). In some cases dry potassium oxalate (2 mgs. per 1 cc. of blood) was used. In these, to correct the shrinkage due to the oxalate employed, the volume of packed red cells was multiplied by the factor 1.09. The corpuscular counts were made by the usual technique with certified apparatus. The mean of two corpuscular counts and of two hemoglobin determinations was recorded for each case. Smears for differentials and examination of red and white cell morphology were prepared from capillary blood taken from finger and stained with Wright's, Jenner's or Giemsa's stain. From one to four hundred white cells were counted. A single Newcomer-Klett hemoglobinometer with a solid standard was employed so calibrated that 14.5 gms. of hemoglobin per 100 cc. of blood was equivalent to 100 per cent hemoglobin. The diluting fluid of Rees and Ecker was employed in counting the platelets, and the direct method was followed.

Wintrobe's hematocrit[7] was employed to determine the volume of packed red cells and the most recent morphological classification of the anemias[8] presented by this investigator was followed.

In order to ascertain the reliability of the data collected for obtaining significant constants, the results were subjected to statistical analysis. The arithmetic mean of the distribution, the Standard Deviation $\sigma = \sqrt{\frac{\Sigma(u - M)^2}{N}}$ and the Probable Error of the Mean[9] $PE_M = \pm \frac{0.6745\,\sigma}{\sqrt{N}}$ were calculated for the erythrocytes, hemoglobin (grams), volume of packed red cells, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and leukocytes.

RESULTS

Red Blood Cells and Hemoglobin: The mean erythrocyte count for the entire group was 2,510,000 ± 0.064 millions per cu.mm., the lowest and highest counts being 0.84 and 4.47 millions respectively. The mean hemoglobin for the group was 9.77 ± 0.23 grams (67.5%) and the figures ranged from 3.7 grams (26%) to 16.0 grams (110%).

Ninety-five individuals showed a red cell count below four millions, while in fifty-one only the hemoglobin fell below 10.2 grams (70%). None of the cases presented normal levels for red cells and hemoglobin.

A macrocytic type of enemia was found in ninety per cent of all cases; normocytic, in seven per cent; simple microcytic, in one per cent and hypochromic in two per cent.

The mean corpuscular volume for the group was 119.14 ± 1.45 cubic microns, which is characteristic of macrocytic anemia, the values ranging from 70 to 194; the mean corpuscular hemoglobin was 39.94 ± 0.61 micromicrograms, fluctuating between 19 and 61; the mean corpuscular hemoglobin concentration was 33.38 ± 0.38 per cent, values ranging from 15 to 45 per cent. The mean volume of packed red cells amounted to 28.89 ± 0.61 cc. with variations between 9.81 and 47.9 cc. per 100 cc. of blood.

Examination of stained smear revealed varying degrees of macrocytosis, anisocytosis being more frequently encountered and more marked than poikilocytosis. Anisocytosis was reported in fifty cases and poikilocytosis in forty-five. Hypochromia was mentioned in seventeen cases. Macrocytes appeared to be the predominating cell type (10-18 microns), but microcytes were often observed also (4-6 microns), though in smaller proportions. Bizarre figures were uncommon. Oval macrocytes and erythrocytes were present in most smears. Polychromatophilia, basophilic stippling, Howell-Jolly bodies and Cabot's ring-bodies were encountered in some instances.

Among the nucleated cells, megaloblasts were observed in several cases. As a rule, when the red cell count fell below two millions, nucleated cells were most numerous, the most predominating type being the pronormoblasts; erythroblasts and normoblasts were less in number.

White Blood Cells: In ninety-nine individuals the mean leukocyte count was 4,960 ± 0.144 cells, the values ranging from 950 to 9,800 cells per cu.mm. Leukopenia (below 5,000) was present in 56.5 per cent of the cases. In 43.5 per cent the white cells fell within normal variations. There were no counts of ten thousands or higher.

Differential leukocyte counts were performed in 82 cases. The mean

for juvenile neutrophiles (non-segmented) was 10.12 per cent with fluctuations between zero and forty-six per cent. The segmented neutrophiles presented a mean of 44.22 per cent with variations of seventeen and a half to seventy-eight per cent. The mean for eosinophiles was 6.34 per cent values, ranging from zero to forty-two per cent. The percentage of eosinophiles was not considered significant because of accompanying helminth infestations in some individuals. It is our impression, however, that in the absence of intestinal parasites or other contributing factors which are known to produce eosinophilia, the percentage of these cells is not increased above normal limits in sprue. The basophiles varied from zero to one and one-half per cent, no cells being found in 81 per cent of the cases. The lymphocytes ranged from 7 to 77 per cent, the mean being 36.40 per cent. The monocytes fluctuated from 0 to 10 per cent, with a mean of 3.01 per cent. In most instances the leukocytes appeared well stained with distinguishable features. A striking and frequent observation was the macrocytosis of all cell types, while anisocytosis, though present, was not marked.

A few promyelocytes and neutrophilic myelocytes were reported in two cases only. Some of these cells were intensely basophilic and it was at the time felt that it was very difficult to decide to which type a given cell belonged.

Neutrophilic metamyelocytes comprised by far the most numerous cells of the non-segmented group. Some of them showed vacuoles and the cytoplasm was basophilic. In others the nucleus occupied most of the cell, the cytoplasm being light blue and containing small basophilic granules. Giant staff cells with broad tortuous nuclei filling the greater part of the cell were not infrequently encountered in some cases. The nuclei of such cells was often vacuolated and at times pycnotic, and the cytoplasm contained large, round, lilac granules, but was not vacuolated. The basichromatin was coarse in some places but occasionally loose, with distinct oxychromatic spaces. Large, segmented, neutrophilic polymorphonuclear (16-23 microns) leukocytes (macropolycytes or pernicious anemia neutrophiles) containing 6-10 subdivisions of the nuclei and a more transparent cytoplasm than normal cells, were encountered in some instances. The nuclear chromation was frequently coarse with clear spaces among the interlacing strands. Vacuoles were but rarely found in the cytoplasm. Occasionally, large lilac granules were scattered among the clear cytoplasm. Anisocytosis of these cells was striking. These "pernicious anemia neutrophiles" have not been encountered as frequently as the nucleated red cells.

In some cases the lymphocytes were frequently unusually large,

sometimes as large as a normal polymorphonuclear leukocyte. The cytoplasm was scant, containing none or very few azurophilic granules. Clumping of the nuclear chromation was often seen, leaving clear spaces in the nucleus, but no characteristic fenestrations as found in infectious mononucleosis were encountered. Pycnotic nuclei without nucleoli were also observed. In only 34 per cent of the individuals studied did the leukocytes show the relative lymphocytosis which is often mentioned in the literature. Absolute lymphocytosis was present in 2.45 per cent of the cases.

The macrocytosis of the leukocytes and the presence of macropolycytes in the material presented, add further points of similarity to the peripheral blood picture of sprue and Addisonian anemia. To the best of our knowledge these observations have not been reported elsewhere.

No abnormal changes other than macrocytosis were encountered in the eosinophiles, basophiles and monocytes.

Reticulocytes: The mean for twenty-two cases was 1.8 per cent, values ranging from 0 to 12 per cent. The majority of the cases fell between 1 and 2 per cent or fraction thereof. The amount of reticulum was usually abundant and the cells seemed as a rule larger than the average red cell.

Platelets: Counts were performed in twenty-five individuals, the mean value being 142,000 platelets per cu.mm., while the range was 40,000 to 290,000. The majority of cases fell between 100,000 and 200,000. The cells appeared well stained and they were usually arranged in clumps or groups. No giant platelets were found and no megalokaryocytes were observed.

DISCUSSION

The close similarity of the peripheral blood picture in Addisonian pernicious anemia and in sprue has been well established by others[10, 11, 12]. Histopathological studies of the bone-marrow in sprue during exacerbation, remission and at post-mortem[13] have also revealed similar changes to those found in Addison's anemia. As stated above, the presence of macropolycytes and the macrocytosis of the leukocytes strengthen the similarity between the blood picture of sprue and Addisonian anemia. These considerations, in addition to the material presented in this report lead us to the belief that in the light of modern criteria for aplastic anemia, the anemia in sprue cannot be considered aplastic or unregenerative any more than the blood picture in Addison's anemia can be so labeled. Certainly, as is well known, the clinical picture is quite different in sprue and aplastic anemia. It may be argued,

however, that all the cellular elements in the peripheral blood are decreased in sprue—a characteristic of aplastic anemia; but the continued presence of reticulocytes, nucleated cells of the hemoglobiniferous series and polychromatophilia, even in the most advanced cases, is more the rule than the exception in the anemia of sprue. The term, aplastic, seems to have been originated by the early workers[14, 15, 16] who, from a study of the peripheral blood picture, were of the opinion that an aplastic type of anemia occurred in sprue. Later, as bone-marrow studies were made, the term was more properly used in connection with the pathological state of the marrow. We believe that its usage in relation to the peripheral blood picture in sprue is confusing and misleading. Moreover, the aplastic bone-marrow reported by some workers[14] has been considered a terminal stage seen at autopsy, and has not been demonstrated as existing throughout other marrow areas of the body. We concur with J. Suárez (*loc. cit.*) in that though aplasia of bone-marrow may be found in advanced cases, the clinical picture is entirely different from that of aplastic anemia, and during most of the natural history of sprue the bone-marrow is hyperplastic in the majority of cases. Furthermore, the bone-marrow aplasia, when present, does not seem to be permanent, but is usually susceptible to regeneration under adequate liver therapy. We may add that none of our cases presented a peripheral blood picture of so-called aplastic anemia. In the only fatal case of this series, who died during hospitalization, numerous normoblasts and several megaloblasts were observed in the peripheral blood on the day before death. Examination of bone-marrow from a rib and vertebra at autopsy (Dr. E. Koppisch) revealed intense hyperplasia.

Considering the entire group of cases as a unit, the mean increase of 10.12 per cent of the non-segmented granulocytes, the majority of which were the metamyelocytes, would indicate, according to the theory of the Schilling hemogram, a regenerative displacement of cells in a pathological blood picture, especially in septic diseases. But such does not seem plausible in sprue. However, the mean for segmented neutrophiles was 44.22 per cent; the addition of both mean counts amounted to 54 per cent which, after computing the absolute number of neutrophiles from the mean leukocyte count for the entire group, or 4,960, gives an absolute total mean of 2,678 cells that is well within the limits of normal. Moreover, myelocytes were but infrequently observed (2 cases) and the staff cells were not increased above normal figures. In those instances in which the total leukocyte count was low (below 5,000) an absolute reduction in the polymorphonuclear cells occurred.

Cooke[17] was the first to describe the large hypersegmented hyperpolymorphic neutrophiles in Addisonian pernicious anemia, which he called macropolycytes. The origin of these atypical cells was explained in the following hypothesis formulated by this investigator: First, there occurs a biochemical change in the blood plasma which causes these cells to age prematurely and become hypersegmented. Second, the mechanism by which neutrophiles are eliminated from the circulation may be altered, with the production of abnormally immature neutrophiles. Third, the polymorphonuclears may be inherently abnormal, owing to a defect in parent cells or to an altered cellular environment, which is responsible for the changes in the macropolycytes, as these cells disappear after the administration of liver, as well as those of the erythrocytic series. The observations of Tempka and Braun[18] followed closely those anticipated in Cooke's third hypothesis. The former investigators studied bone-marrow biopsies from cases of Addisonian anemia, finding alterations in the myeloblasts, metamyeloblasts, promyelocytes, myelocytes and metamyelocytes. The most characteristic change, however, was noted in the neutrophilic stab forms which contained tortuous, coarse nuclei occupying about two-thirds of the cytoplasm. These abnormal stab forms appeared to come directly from promyelocytes without passing through the intermediate myelocytic and metamyelocytic stages. These investigators were led to believe that there is a regenerative-degenerative change in the neutrophilic series in Addisonian anemia.

Jones,[19] working also with biopsy specimens and dry imprints from patients with Addisonian anemia during relapse, emphasized the marked anisocytosis and macrocytosis of the leukopoietic elements. He has demonstrated that the macropolycytes in Addison's anemia have a different life history from that of the neutrophiles in the normal bone-marrow. The neutrophilic series is affected to the extent that there is developing in the bone-marrow a pathologic series which gives rise to the abnormal macropolycytes of the peripheral blood as found in Addison's anemia.

Tempka and Braun (*loc. cit.*) believe further that there is a panmyelopathy of the marrow in Addisonian anemia, affecting the hemoglobin series, the neutrophiles, and producing impairment of the megakaryocytic system. The lymphocytes were also affected, being increased in number, and nuclear pyknosis was observed in some instances. In the present work mention has been made of the macrocytosis of these cells and pyknosis of the nucleus in the peripheral blood of pa-

TABLE I

Tabla I

Percentage distribution for erythrocytes, hemoglobin (grams—per cent), volume of packed red blood cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and leukocytes in 100 patients with sprue before treatment

Porcentaje de hematíes, hemoglobina (gramos %), hematíes apilados, media volumétrica globular, media hemoglobínica globular, media hemoglobínica de concentración y leucocitos, en un centenar de enfermos de esprú antes del tratamiento

Erythrocytes—millions per cu.mm.
Hematíes—millones, por mm.c.

0–.99	1–1.99	2–2.99	3–3.99	4–4.99
9	22	36	28	5

Hemoglobin—grams
Hemoglobina—gramos

2–4.9	5–6.9	7–8.9	9–10.9	11–12.9	13–14.9	15–16.9
9	15	16	20	20	16	4

Per cent
Porciento

20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–109	110–119
6	7	14	12	12	17	13	6	11	2

Volume of packed red cells—cc. per 100 cc. of blood. (Normal: 45–46 cc.)
Volumen de glóbulos apilados—cc. por 100 cc. de sangre (Valor normal: 45–46)

5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49
1	7	9	18	16	22	16	9	2

Mean corpuscular volume—cubic microns (Normal values: 80–94)
Media volumétrica globular—micras cúbicas (valores normales: 80–94)

70–79	80–89	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	>170
2	1	15	18	15	21	15	4	1	5	3

Mean corpuscular hemoglobin—micromicrograms (Normal values: 27–32)
Media hemoglobínica globular—micromicrogramos (valor normal: 27–32)

<27	27	28	29	30	31	32	>32
4	1	1	3	0	4	4	83

Mean corpuscular hemoglobin concentration—Per cent (Normal: 29–38)
Media hemoglobínica de concentración—porcentaje (valor normal. 29–38)

<29	29	30	31	32	33	34	35	36	37	38	>38
13	4	9	10	8	7	8	7	9	6	7	12

Leukocytes—thousands per cu.mm. (99 cases)
Leucocitos—millar por mm.c. (99 casos)

<1	1	2	3	4	5	6	7	8	9	>10
1	2	13	13	27	11	14	9	8	1	0

tients with sprue. This concept of panmyelopathy for pernicious anemia has been recently confirmed by Dameshek and Valentine[20] and by Jones (*loc. cit.*).

Although bone-marrow studies have not been made by us, we are inclined to accept, from the evidence presented on the study of the peripheral blood in sprue, the views of Tempka and Braun, Dameshek,

TABLE II

Tabla II

Comparison of blood values of 100 patients with sprue with those of ninety-two cases with sprue reported by Castle and associates (Percentage distribution of cases)

Comparación entre los valores sanguíneos de 100 enfermos de esprú y noventidós casos comunicados por Castle y colaboradores

Red blood cells—millions per cu.mm.
Hematies—millones, por mm.c.

	0–0.99	1–1.99	2–2.99	3–3.99	4–4.99
Castle....	9.8	41.3	33.7	14.1	1.1
R.R.M...	9	22	36	28	5

Mean corpuscular volume—cubic microns (Normal values: 80–94)
Media volumétrica globular—micras cúbicas (V.M.: 80–94)

	<80	80–89	90–99	100–109	110–119	120–129	130–139	140–149	>150
Castle....	2.2	1.1	6.6	12.1	21.9	18.7	16.5	12.1	8.8
R.R.M...	2.0	1	15	18	15	21	15	4	9

Mean corpuscular hemoglobin concentration—percentage (Normal: 29–38)
Media hemaglobinica de concentración. Porcentaje (normal 29–38)

	<29	29	30	31	32	33	34	>34	35	>35
Castle.....	2.2	2.2	7.7	9.9	15.4	27.4	15.4	19.3		
R.R.M.....	13	4	9	10	8	7	8		7	34

White blood cells—thousands per cu.mm. (99 cases)
Leucocitos. Millones por mm.c. (99 casos)

	<3	3–3.9	4–4.9	5–5.9	6–6.9	>7
Castle.....	18.5	42.4		23.9		15.2
R.R.M....	16	13	27	11	14	18

Valentine and Jones in explaining the observations included here concerning the presence of macropolycytes and other changes in the leukocytes. The presence of an increased number of metamyelocytes (10%), together with leukopenia observed in 56.5 per cent of the cases on the peripheral blood of sprue may seem indicative of an arrest in the maturation of the granulocytes as suggested by Davidson and Gulland[21] for Addisonian anemia. The assumption is made that the large forms of metamyelocytes and the staff cells observed by us in the peripheral blood may suggest monstrosities in cellular development occurring in the bone-marrow. The presence of macropolycytes is also considered evidence of cellular maldevelopment from the abnormal metamyelocytes of the marrow rather than of increased maturity as is currently believed. The apparent multiplicity of these nuclei may be due to the breaking up of the extremely large nucleus of the metamyelocytes as suggested by Dameshek and Valentine in Addisonian anemia.

From the study of the peripheral blood in one hundred cases of sprue, it is suggested that the blood picture in sprue reflects not alone a dis-

turbance of the red cells, but one of the white cells and platelets as well; that is, an involvement of the entire marrow rather than one of the hemoglobiniferous cells only. Further bone-marrow studies during various phases in the life history of sprue are needed to correlate the findings described in this investigation with those of a panmyelopathy of the marrow, the existence of which is assumed from the study of the peripheral blood. It is our belief that the peripheral blood picture in Addisonian anemia and in sprue are indistinguishable.

Table I presents the percentage distribution for erythrocytes, hemoglobin, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and leukocytes in the cases studied.

Table II gives a comparison of blood values in the present group with those of ninety-two individuals studied in Puerto Rico and reported by Castle and associates in 1935.

A table containing the hematologic data from each case discussed in this report has been prepared and is available, but lack of space does not permit its presentation.

SUMMARY

Hematologic studies were performed in one hundred individuals with sprue before treatment, fifty-seven of which patients were males and forty-three, females. The oldest was a white man of 78 years of age, and the youngest, a white girl of 12. The mean age for the series was 40.14 years. There were 87 white and 13 colored individuals, including two full-blooded negroes, while the others in the colored group were mulattoes.

Enumeration of erythrocytes, leukocytes, platelets, differential white cell counts and examination of stained smears were performed. Estimation of hemoglobin and determination of the volume of packed red cells were also made. Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were determined.

CONCLUSIONS

Ninety-five per cent of the above cases showed a red cell count below four millions per cu.mm.; in 51 per cent only was the hemoglobin below 10.2 gms. (70%). In no case normal levels for hemoglobin and red cells were observed. The degree of anemia for the entire group was considered marked, the mean red cell count being 2,510,000 $\pm$ 0.064 millions per cu.mm., and the mean hemoglobin, 9.77 $\pm$ 0.23 gms. (67.5%).

A macrocytic type of anemia was found in ninety per cent of the cases, normocytic in 7 per cent, simple microcytic in 1 per cent, and hypochromic in 2 per cent. Examination of stained smears revealed anisocytosis, poikilocytosis, macrocytes, oval macrocytes, microcytes, hypochromia, polychromatophilia, basophilic stippling, Howell-Jolly bodies and Cabot's ring bodies. In ninety-nine individuals the mean leukocyte count was 4,960 ± 0.144 cells. Leukopenia (below 5,000) was present in 56.5 per cent of the cases. Differential leukocyte counts (82 cases) gave the following mean values: Juvenile neutrophiles (non-segmented), 10.12 per cent; segmented neutrophiles, 44.22 per cent; eosinophiles, 6.34 per cent (not considered significant because of presence of intestinal parasitism in some cases); no basophiles were encountered in 81 per cent of the cases; lymphocytes, 36.40 per cent, and monocytes, 3.01 per cent. Considering the series of cases as a unit, the Schilling hemogram did not reveal a regenerative shift in the granular leukocytes. A relative lymphocytosis was found in only 34 per cent and absolute lymphocytosis in 2.45 per cent of the cases. Morphological abnormalities in the granular leukocytes, such as vacuolated neutrophilic metamyelocytes with cytoplasmic basophilia, giant staff cells with broad, tortuous, vacuolated, pycnotic nuclei, and "pernicious anemia neutrophiles," or macropolycytes, were observed in some instances. Unusually large lymphocytes with pycnotic or perforated nuclei and scant cytoplasm were also occasionally encountered. The mean reticulocyte count was 1.8 per cent. A reduction in the number of platelets was observed, the mean count being 142,000 per cu.mm.

When peripheral blood values are referred to, it is suggested that the anemia of sprue should not be called aplastic or unregenerative, as these terms are confusing and misleading. In the author's opinion, the peripheral blood picture in sprue and Addisonian anemia are indistinguishable. The blood picture in sprue reflects not alone a disturbance of the hemoglobiniferous cells of the marrow, but also a disturbance of the white cells and platelets as well. The existence of an involvement of the entire marrow or panmyelopathy in this condition is assumed.

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REFERENCES

1. Ashford, B. K.: Bol. Asoc. Méd. de P. R., 141:1-26, March 1923.
2. Serra, A.: Am. J. Trop. Med., 9:49. 1929.

3. Suárez, J.: P. R. Jour. Pub. Health & Trop. Med., 7:145. 1931.
4. Castle, W. B., Rhoads, C. P., Lawson, H. A. and Payne, G. C.: Arch. Int. Med., 56:627-699. October 1935.
5. Suárez, R.: Bol. Asoc. Méd. de P. R., No. 10:239-249. October 1935.
6. Reed, Alfred C.: Am. Jour. Trop. Med., 16(5):499-527. September 1936.
7. Wintrobe, M. M. and Landsberg, W.: Am. Jour. Med. Sciences, 189:102-115. 1935.
8. Wintrobe, M. M.: Arch. Int. W. ed., 54:256-280. 1934.
9. Davenport, C. B. and Ekas, M. P.: Statistical Methods. John Wiley & Sons, Inc., Chapman and Hall Limited, London. 1936.
10. Manson-Bahr, P. H.: Manson's Tropical Diseases. William Wood & Co., Baltimore, Md. 1936.
11. Mackie, T. T.: Nelson's Loose-Leaf Living Medicine, Vol. F:465-470. 1937.
12. Ashford, B. K.: Am. Jour. Trop. Med., 12:199. 1932.
13. Rhoads, C. P. and Castle, W. B.: Am. Jour. Path., 9:813-825. 1933.
14. Fairley, N. H., Mackie, F. P. and Billimoria, H. S.: Ind. Jour. Med. Research, 16:831. January 1927.
15. Manson-Bahr, P. H.: The Practice of Medicine in the Tropics. Byam and Archibald, Oxford Medical Publication, page 2257. 1923.
16. Smith, L. W.: Phil. Jour. Sci., 24 (4):447-465. April 1924.
17. Cooke, W. E.: J. Lab. & Clin. Med., 19:453. February 1934.
18. Tempka, T. and Brown, B.: Folia Haemat., 48:355. 1932.
19. Jones, O. P.: Arch. Int. Med., 60:1002-1015. December 1937.
20. Dameshek, W. and Valentine, E. H.: Arch. Path., 23:159. February 1937.
21. Davidson, L. S. P. and Gulland, B. L.: Pernicious Anemia. London, Henry Kimpton. 1930.