TABLE I.
Penicillin Sensitivity of Group A Beta-hemolytic Streptococci Isolated During Penicillin Therapy.

Penicillin sensitivity*	Route of penicillin† administration	Day of treatment and No. of strains of strep.									Total isolations
		2	3	4	5	6	7	8	9	10	
.0156	IM	3	1	1	1	3	1	2	2	1	15
	PO	1	0	0	0	0	0	0	0	0	1
.0078	IM	3	1	2	0	0	0	1	1	2	10
	PO	2	1	2	0	1	2	1	1	0	10
.0039	IM	1	0	0	0	0	0	0	0	0	1
	PO	0	0	0	0	0	0	0	0	0	0
Total		10	3	5	1	4	3	4	4	3	37

* Quantity which inhibited growth.
† IM—intramuscular; P.O.—oral.

known. The circumstances of the appearance of sulfonamide resistant group A beta-hemolytic streptococci in the Armed Forces has been reviewed elsewhere.[10] The possibility that a similar situation might arise as a result of the extensive use of penicillin has been anticipated but not observed clinically.

Despite the failure of group A beta-hemolytic streptococci to develop proved penicillin resistance *in vivo* during treatment, there is good evidence that this phenomenon can be produced with ease *in vitro*. Weinstein and Tsao[11] made a number of strains of this organism 4 to 32 times more resistant to penicillin by frequent subculture in media containing increasing amounts of the drug; this resistance was only temporary, however, and was made to disappear by frequent subculture in antibiotic-free media. Gezon,[12] using a slightly different technic, reported similar results.

Summary. (1) Studies of the penicillin sensitivity of group A hemolytic streptococci isolated from scarlet fever patients before, during, and following penicillin therapy indicate that, with the usual "short" courses of treatment, drug-resistant strains of the organism do not appear.

(2) The likelihood of the development of penicillin resistance in cases of streptococcal infection treated with this agent appears to be very remote.

(3) No penicillin-resistant strains of *Strep. pyogenes* (group A) were recovered in any instance before antibiotic therapy was started.

10 Hartman, T. L., and Weinstein, L., *New Eng. J. Med.*, 1948, **238**, 560.

11 Weinstein, L., and Tsao, C. C. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 598.

12 Gezon, H. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 208.

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Oxygen Saturation of Sternal Marrow Blood with Special Reference to Pathogenesis of Polycythemia Vera.*

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There are two main theories of the pathogenesis of polycythemia vera. The older theory considers that the disease is neoplastic in nature. The other theory supposes that polycythemia vera, like most forms of experimental and clinical polycythemia, is due in

* The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

† Deceased.

some way to anoxia of the erythropoietic cells of the bone marrow. Because in contrast to secondary polycythemias the percentage oxygen saturation of the arterial blood is normal,[1] the application of this theory to polycythemia vera requires that anoxia be produced by circumstances local to the bone marrow. Reznikoff, Foot and Bethea[2] have described a possible cause of local bone marrow anoxia in certain cases of polycythemia vera in marked narrowing and fibrosis of the arteries and arterioles of that organ. Brilliant support for this concept derives from the recent experiments of Schafer[3] with dogs rendered hypertensive and polycythemic by proprioceptor depressor neurotomy.

In other cases of polycythemia vera the white blood cell count in the peripheral blood may be elevated with evidence of myeloid immaturity. Likewise, in the bone marrow myeloid hyperactivity and immaturity may be found.[4] Splenomegaly is prominent and the disease process may evolve sooner of later into anemia associated with the features of myelogenous leukemia, non-leukemic myelosis, or even of osteosclerotic anemia.[5,6] Castle[7] has suggested that in this splenomegalic type of polycythemia vera the hyperactivity of the myeloid cells of the bone marrow causes a reduction in the oxygen available to the erythropoietic cells, either because of competitive utilization of the available oxygen by the myeloid cells or because of their mechanical interference with the vascular supply of the erythropoietic cells.

This paper reports an attempt to confirm the validity of this theory by a direct measure of the oxygen saturation and tension of samples of blood removed from the sternal marrow cavity.

Methods. Two sets of investigations were carried out. In the first study conducted in 1941 5 cc of blood were withdrawn by gentle suction from the sternal marrow cavity. Thereafter, using conventional methods for handling the sample under oil in an ice bath and employing Wintrobe's dry oxalate mixture as anticoagulant, the carbon dioxide and oxygen contents of the sample as well as its oxygen capacity were determined in duplicate by the Van Slyke manometric combined method[8] using the 0.5 cc technic. In several instances the pH of the marrow blood was determined anaerobically by the glass electrode method using the Beckman instrument.

In the second study begun in 1947 blood samples were obtained by sternal puncture without suction. A sterile 16 gauge needle was washed with sterile heparin solution, the stylet was placed in position and sternal puncture was performed as usual. The patient was then turned on his side so that the needle sloped downward away from its point. The stylet was withdrawn and in many instances blood began slowly to fill the hub of the needle. Whenever no blood appeared, gentle probing with a long stylet usually started a flow. The tip of a 22 gauge needle attached to a tuberculin syringe was then introduced into the hub of the sternal puncture needle and the blood carefully sucked up as it welled into the hub of the needle. The dead space in the needle and the syringe was completely filled with heparin solution, 1 mg per cc.

The blood samples so obtained varied from 0.6 to 1.0 cc in volume. They were handled as described by Roughton and Scholander[9] except that a metal bead replaced the drop of mercury for mixing the blood and heparin solution. Peripheral arterial and venous blood samples were taken immediately after the sternal puncture, using the technic of collection and storage employed with the

1 Hitzenberger, K., *Z. f. klin. Med.*, 1934, **126**, 495.

2 Reznikoff, P., Foot, N. C., and Bethea, J. M., *Am. J. Med. Sci.*, 1935, **189**, 753.

3 Schafer, P. W., *Ann. Surg.*, 1945, **122**, 1098.

4 Zadek, I., *Ergebn. d. ges. Med.*, 1927, **10**, 355.

5 Rosenthal, N., and Bassen, F. A., *Arch. Int. Med.*, 1938, **62**, 903.

6 Vaughan, J. M., and Harrison, C. V., *J. Path. and Bact.*, 1939, **48**, 339.

7 Castle, W. B., Chapter on Blood, in *Pathologic Physiology and Mechanisms of Disease*, W. A. Sodeman, editor, in press.

8 Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

9 Roughton, F. J. W., and Scholander, P. F., *J. Biol. Chem.*, 1943, **148**, 541.

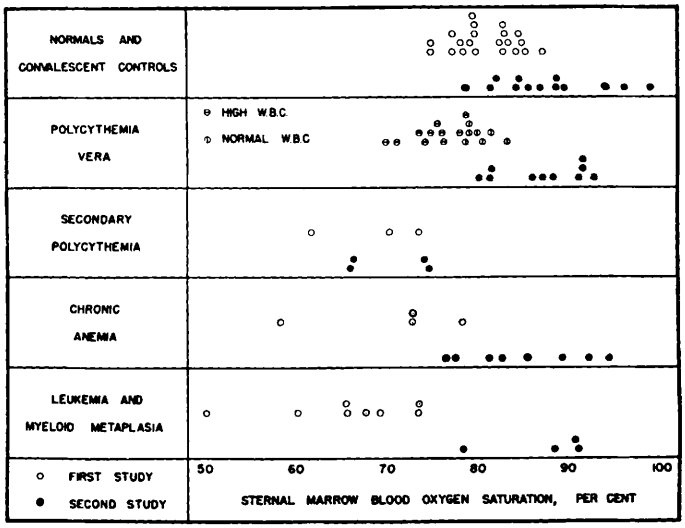

FIG. 1.

Percentage oxygen saturation of samples of blood removed by needle puncture from the sternal marrow cavity of normal subjects, convalescent control patients and patients with polycythemia vera, secondary polycythemia, chronic anemia, and leukemia or myeloid metaplasia. Each symbol represents the result of a single determination. The open dots indicate results from the first study; the solid dots those from the second. The results of the first study on patients with polycythemia vera are divided into two groups, according to whether the white blood cell count of the peripheral blood was normal, 5,000 to 12,900 per cu mm (open dots divided vertically) or high, 17,300 to 40,000 per cu mm (open dots divided horizontally).

marrow blood. Duplicate determinations were made by each of two operators of the oxygen content of the blood samples by the Roughton-Scholander micro method[9] employing 0.04 cc of blood for each analysis. Appropriate allowance was made for the dilution produced by the known volume of heparin solution in the dead space in the needle and syringe. Oxygen capacity was determined by measuring the hemoglobin content of a 0.1 cc sample of blood in an Evelyn photoelectric colorimeter using a factor of 2.58.[10] Control observations were made on the same sample of blood by the Roughton-Scholander, the Van Slyke and photoelectric methods respectively. The results in three such experiments on blood samples with oxygen capacities of 17.3, 17.8 and 18.9 displayed close agreement, the greatest differences being 0.2, 0.3 and 0.4 volumes % respectively. Other control observations on a dummy set-up using partially reduced blood showed no significant exposure of the blood in the hub of the sternal puncture needle to the air before it was sucked into the collecting tuberculin syringe. The accuracy of the Roughton-Scholander technic in our hands when tested on atmospheric air was only slightly beyond the limits claimed for the method by its authors.

Results. The results of the observations are plotted in Fig. 1.

Controls. In the first studies the oxygen saturation of 21 marrow bloods derived either from normal subjects or from convalescent hospital patients ranged from 75.0 to 87.4 with a mean of 81.1 ± 3.49%. The oxygen capacity of the sternal blood samples from these subjects ranged from 17.3 to 23.6 with an average of 19.8 ± 1.75 volumes %. The pH of the marrow blood in the 8 samples studied ranged from 7.45 to 7.54.

In the second study the oxygen saturation of 13 marrow bloods derived from convalescent hospital patients who were in 2 instances

[10] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

moderately anemic, ranged from 79.0 to 99.2 with a mean of 88.6 ± 5.86%. The oxygen capacity of the arterial blood of these subjects ranged from 15.0 to 21.6 with an average of 18.8 ± 1.99 volumes %.

Polycythemia Vera. In the first study, 17 determinations of the oxygen saturation of the sternal marrow blood of 13 patients with polycythemia vera gave readings between 70.1 and 83.4 with a mean of 76.8 ± 3.29%. Of these, 7 determinations were made on 6 patients with normal white blood cell counts in the peripheral blood (5,000 to 12,900 per cu mm. The results showed values between 78.8 and 83.4 with a mean of 80.4 ± 1.64%. Ten determinations made upon 7 patients with elevated white blood cell counts in the peripheral blood (17,300 to 40,000 per cu mm) showed values of 70.1 to 78.9 with a mean of 75.0 ± 2.69%.

In the second study the values obtained for the oxygen saturation of the marrow blood of 10 patients with polycythemia vera ranged from 80.3 to 93.0 with a mean of 87.3 ± 4.49%. In the 3 patients with normal white blood cell counts, the corresponding values were 91.7, 88.5 and 81.7% respectively. The percentage oxygen saturation of the arterial blood samples was within normal limits in all the patients included in this study as well as in 8 other patients with polycythemia vera that exhibited elevated arterial oxygen capacities ranging from 22.8 to 33.0 volumes %.

Secondary Polycythemia. Including the observations from both studies 7 determinations of the percentage oxygen saturation of the marrow blood were made in 6 patients with polycythemia secondary either to cardiac or pulmonary disease. In 5 of these patients the oxygen capacity of the arterial blood was elevated, ranging from 22.7 to 30.7 volumes %. The other patient showed a normal oxygen capacity of the arterial blood because of previous venesections. However, in all the arterial blood was definitely unsaturated and carried only from 66.3 to 87.8% of its oxygen capacity. The oxygen saturation of the sternal marrow blood in all instances likewise fell below normal limits with extremes of 61.8 and 74.8%.

Anemia. Including observations made in both studies the percentage oxygen saturation of the blood in the sternal marrow of 12 patients with chronic anemia due to various causes was determined. The oxygen capacity of the marrow blood of these patients ranged from 6.0 to 14.2 volumes %. Excluding one patient, with the extremely low value of 58.4%, the oxygen saturation of the marrow bloods ranged from 72.9 to 94.5 and averaged 81.3 ± 6.34. In the 8 patients of the second study in which the determination was made the oxygen saturation of the arterial blood samples was likewise normal.

Leukemias and Myeloid Metaplasia. In the first study the oxygen saturation of the marrow blood of 7 cases of leukemia and of myeloid metaplasia was determined, with results ranging from 50.0 to 73.7%. The average value was 65.8 ± 7.8%. However, in the second study the oxygen saturation of the marrow blood of 4 patients ranged between 78.5 and 91.1%, indicating no significant departure from normal values.

Discussion. The results obtained and plotted in Fig. 1 do not in general demonstrate a lowered percentage oxygen saturation of sternal marrow blood in polycythemia vera. Also in the few instances in which, in the first study, oxygen tensions were calculated from the oxygen saturation and the pH of the sternal marrow blood according to the methods of Bock and his associates[11] no significant differences between the controls and the patients with polycythemia vera were demonstrated. In the patients with polycythemia secondary to pulmonary or cardiac disease, the percentage oxygen saturation of the marrow blood was distinctly reduced below that of the normals and convalescent controls. However, this was presumably due to the demonstrated unsaturation of the arterial blood.

It is important to note that even in the cases of anemia, where it is generally acknowledged that an anoxic stimulus to blood forma-

[11] Bock, A. V., Dill, D. B., Hurxthal, L. M., Lawrence, J. S., Coolidge, T. C., Dailey, M. E., and Henderson, L. J., *J. Biol. Chem.*, 1927, **73**, 749.

tion exists, lowered percentage oxygen saturation of the marrow blood was not demonstrable in the present investigation. This result suggests that the failure to find decreased oxygen saturation in the marrow blood of the patients with anemia, and conceivably of those with polycythemia vera, may have been due to failure to obtain samples of blood that truly reflect the environmental conditions of the erythropoietic cells of the bone marrow. Penetration by the needle of the rigid bony cortex surrounding the marrow quite possibly creates a fundamental disturbance of the local pressure relations which, together with the inevitable injury to marrow vessels, may cause a totally abnormal situation with respect to local blood flow.

The values for the percentage oxygen saturation of the marrow blood in all patients tested, with a single exception, lay between those of the peripheral arterial and venous blood. Therefore, in the observations reported here as in those of Grant and Root[12] using a somewhat similar technic in dogs, it would appear that samples containing variable proportions of arterial and venous blood were obtained upon marrow puncture. The fact that, in general, in the first study the oxygen saturations of the marrow bloods were lower than in the second suggests that the suction employed in the first study caused a greater proportional backflow of blood from the venous side of the marrow circulation than occurred in the second study in which suction was not used. Because the venous blood leaving the marrow presumably reflects its total metabolic activity, the samples obtained by suction may have more nearly represented its rate of oxygen consumption than did those obtained without suction in the second study.

In the light of this possibility it is interesting to note that there is a suggestion in the first study that the patients with high peripheral white blood cell counts, that is, the cases with myeloid overactivity, tended to show lower percentage oxygen saturations of the marrow blood than did patients with normal or low white blood cell counts. Another feature of interest in the first study is the consistently low values for percentage oxygen saturation obtained in the cases of leukemia and of myeloid metaplasia. Because increased oxygen consumption by the leukocytes of the blood after withdrawal of the sample from the bone marrow could not be demonstrated, these findings suggest that increased oxygen utilization relative to the blood supply was actually occurring in the bone marrow in these patients.

Summary. An attempt was made to measure the stimulus to erythropoiesis in the human bone marrow by estimating the percentage oxygen saturation of blood removed from the sternal marrow cavity through a needle inserted through the cortex as in the performance of a sternal needle biopsy. By this means, blood samples were collected without effective contact with air and their oxygen content, capacity and in some instances pH were determined. In general no significant differences were demonstrated between normals, convalescent controls, anemic patients, and patients with polycythemia vera. In patients with secondary polycythemia, the percentage oxygen saturation of the blood removed from the bone marrow was relatively reduced, probably entirely as a result of the manifest unsaturation of the arterial blood. In some patients with leukemia and with myeloid metaplasia and in some patients with polycythemia vera with evidence of excessive myeloid activity, the data suggest an increased local oxygen utilization relative to the blood flow in the bone marrow. However, it was concluded that the technics used were not adequate to demonstrate an anoxic stimulus to increased erythropoiesis even in the marrow of patients with chronic anemia, possibly because of the difficulty of obtaining blood samples satisfactorily representative of the undisturbed environment of the erythropoietic cells.

We desire to express our appreciation to Dr. Benjamin Alexander for permission to carry out the determinations on several of the patients in the second study.

12 Grant, W. G., and Root, W. S., *Am. J. Physiol.*, 1937, **150**, 618.