controls (group 8). The thyroids of these rats were apparently nonfunctional, as indicated by the virtual absence of I^{131} in the glands. In fact, the radioactivity in these thyroids did not differ statistically from that present in the atmosphere (background). Although the administration of 40 μg of vit. B_{12} (group 12) completely overcame the inhibitory effects of the thyroprotein on growth, it did not counteract the effects of the latter on thyroid activity.

Discussion. These experiments indicate that the administration of vit. B_{12} does not alter normal thyroid function in young rats, even when given in amounts sufficient to increase body growth. Apparently, vit. B_{12} does not affect the metabolism or excretion of endogenous thyroid hormone, since such changes would have altered thyroid activity and uptake of I^{131}. It seems reasonable to conclude that vit. B_{12} can increase body growth in rats independently of the thyroid.

On the whole, these data also do not support the view that vit. B_{12} increases the turnover within the body of administered thyroid materials, since the vitamin did not at all overcome the thyroid-inhibiting action of thyroprotein. However, it is possible that the dose of thyroprotein required to completely inhibit thyroid activity was lower than the amount given, and hence its increased metabolism by vit. B_{12} was not reflected by the thyroid.

Summary. Crystalline vit. B_{12} was fed to immature rats of both sexes in order to determine whether the vitamin could alter thyroid function in normal or thyroprotein-treated rats. The growth rate of the rats supplemented with the vitamin was increased above that of the normal or thyroprotein-treated controls, but there was no significant effect on thyroid weight or uptake of I^{131}. It is concluded that vit. B_{12} does not alter normal thyroid activity in rats.

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Studies on the Destruction of Red Blood Cells. VIII. Molecular Orientation in Sickle Cell Hemoglobin Solutions.* (18144)

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Sherman(1) reported "without interpretation" the observation that "under the polarizing microscope characteristic sickle cells exhibit a definite birefringence". Since that time, several writers have used this finding as evidence that the hemoglobin molecules assume an orderly arrangement in sickled erythrocytes(2,3). When hemoglobin was removed from the red cells, Ponder(2) demonstrated that the "ghosts" would not become sickle shaped on exposure to low oxygen tensions. Recent studies by Pauling *et al.* (3,4) have shown that certain physical and chemical properties which differentiate sickle cell hemoglobin from normal hemoglobin result from an alteration in the structure of the globin portion of the molecule. Human deoxygenated hemoglobin is said to be less soluble than the oxygenated form, suggesting that the phenomenon of sickling might be related to incipient crystallization of the

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hemoglobin(5). Rebuck *et al.*(6) have observed that in the early stages of sickling the intracellular hemoglobin forms anisotropoid aggregates with intense peripheral spiculation "suggestive of incipient crystallization". Ponder(2) has proposed that, in the state of oxygen unsaturation, the intracellular hemoglobin molecules assume an orderly or "paracrystalline" arrangement which results in the various shape alterations by means of "expansive" or "turgor-producing" forces arising from a physico-chemical interaction of the paracrystalline hemoglobin with the enveloping ultrastructure. However, aside from the fact that sickling is basically dependent upon the removal of oxygen from the hemoglobin, these few observations constitute the only *direct* evidence concerning the physical basis of the sickling process.

In the present study, Sherman's observation (1) of the birefringence of sickled cells was readily confirmed by examining intact cells under the polarizing microscope after sickling had been induced by any of the methods usually employed(7). Normal intact erythrocytes did not become birefringent when treated in the same manner. It was therefore considered desirable to establish, if possible, further evidence for molecular orientation or alignment of the abnormal hemoglobin as a basis for the sickling phenomenon. Accordingly, certain observations were made employing stroma-free solutions of hemoglobin in distilled water, prepared by a modification of the method described by Drabkin(8).

Viscosity measurements were made at 37.5°C with the Ostwald capillary viscometer, using concentrations of hemoglobin in distilled water ranging from 15 to 25 g/100 ml. When the oxygen saturation of sickle-cell hemoglobin was decreased progressively from 100 to the vicinity of 10%, marked increases in viscosity occurred in the lower range of oxygen saturations. Indeed, the more concentrated hemoglobin solutions assumed a semi-solid gel-like state. All such increases in viscosity could be readily reversed by reoxygenation of the hemoglobin solutions, and the cycle could be repeated as desired. However, no increase in viscosity occurred when the concentration of sickle cell hemoglobin was decreased to 10 g/100 ml or less. Solutions of normal hemoglobin, in concentrations ranging from 15 to 25 g/100 ml, showed no changes in viscosity when treated by the same methods. The increased viscosity of solutions of deoxygenated hemoglobin from sickle cell anemia is evidence for a grouping of the individual hemoglobin molecules such that asymmetric aggregates are formed, resulting in increased internal friction(9) of the solution. The absence of change in viscosity of solutions of less than 10 g/100 ml suggests that molecular interactions or attracting forces are effective only at limited distances. On the average, the distance between two hemoglobin molecules at closest approach in the red cell, as determined by x-ray diffraction, is of the order of magnitude of two water molecules(5). Furthermore, the increases in viscosity take place only at low saturations of oxygen, indicating that molecular interactions are possible only when oxygen is absent from the hemoglobin unit, thus in some way activating or uncovering intermolecular attracting forces.

Microscopic observations of wet preparations at a magnification of 1000x showed no differences between oxygenated or deoxygenated solutions of normal hemoglobin and of oxygenated solutions of sickle cell anemia hemoglobin ranging from 15 to 25 g/100 ml. However, when the solutions of sickle cell hemoglobin were in the deoxygenated and viscous state, spindle-shaped bodies varying in length from 1 to 15 μ were observed in the wet preparations examined under a sealed coverslip (Fig. 1). These bodies proved to be birefringent when examined under the polarizing microscope and thereby showed the requisite characteristics of tactoids(10). They disappeared upon reoxygenation of the hemo-

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TABLE I. Comparison of Normal and Sickle-Cell Hemoglobin in Stroma-Free Solutions and in Intact Erythrocytes.

	Hemoglobin solutions—stroma-free				Intact erythrocytes	
	Viscosity relative to distilled water Oxygen saturation (%)		Birefringent tactoids Oxygen saturation (%)		Birefringence Oxygen saturation (%)	
Sample	100	10	100	10	100	10
Normal hemoglobin						
15.1 g/100 ml	4.34	3.67	Absent	Absent	Absent	Absent
25.0 " "	46.1	47.2	"	"		
Sickle cell anemia hemoglobin						
6.9 g/100 ml	2.06	1.88	"	"		
15.2 " "	7.60	37.1	"	Present	"	Present
23.5 " "	41.1	Semi-solid gel-like state	"	"		

globin solution and were reformed when the oxygen was again removed.

The tactoid form is characteristic of an orderly grouping of long, thin, rod-like particles which are arranged parallel and equidistant to each other(11). Similar bodies have been described in solutions of the asymmetric tobacco mosaic virus and in some colloidal suspensions(11). Since the individual hemoglobin molecule is not markedly asymmetric (57Å x 57Å x 34Å)(5), the formation of tactoids by the oxygen unsaturated hemoglobin of sickle cell anemia is evidence of a specific arrangement or linkage of the individual molecules with the formation of long chains of hemoglobin elements and the subsequent alignment of these elements into an anisotropic grouping. Evidence that this process occurs in the intact sickle cell is found in the observation that the sickled forms of intact cells are birefringent(1,6). The intact cells contain approximately 30 g/100 ml of hemoglobin(15), a concentration ample for tactoid formation of the hemoglobin when in solution(5). It is probable, therefore, that the sickled erythrocyte is in essence a hemoglobin tactoid thinly veiled and somewhat distorted by the cell membrane (Fig. 2).

In conclusion, it would appear that beginning with the genetically(12) abnormal hemoglobin molecule(3) and extending to the clinical manifestations, the following sequence of events would be adequate to explain the major aspects of the pathologic physiology of sickle cell disease. The alignment and parallel aggregation of the molecules of deoxygenated hemoglobin derived from the red cells of patients with sickle cell disease are manifested as increased viscosity of hemoglobin solutions and the formation of hemoglobin tactoids. These effects take place in solutions at concentrations comparable to those of the intracellular hemoglobin. The resemblance of tactoids to sickled erythrocytes is so striking that the sickled red cell in all probability is essentially a membrane-covered hemoglobin tactoid. Due entirely to the sickled form of the erythrocytes, the viscosity(13) of the whole blood and the mechanical fragility(14) of sickled cells are significantly increased at low oxygen tensions. The increase in viscosity appears to explain the multiple venous thromboses, and the increase in mechanical fragility may largely explain the hemolytic anemia—phenomena which are characteristic of the active disease. Thus, in sickle cell anemia (genetically homozygous)(12), the erythrocytes contain 100% abnormal hemoglobin(3). Sickling and increases in viscosity and in mechanical fragility of the red cells occur within the range of oxygen saturations of venous blood

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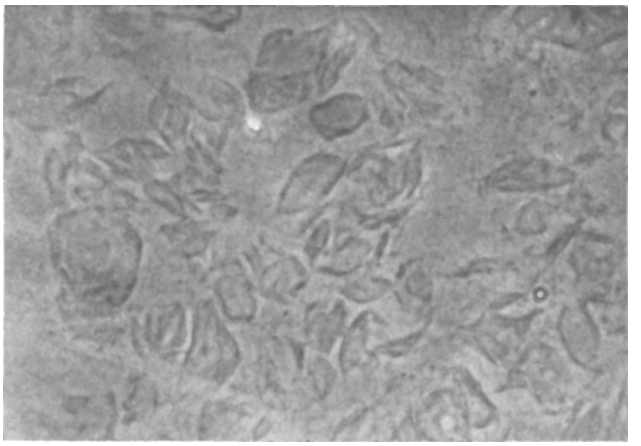

FIG. 1.
Hemoglobin tactoids formed in stroma-free solutions of deoxygenated sickle cell anemia hemoglobin. (Phase microphotography ×375.)

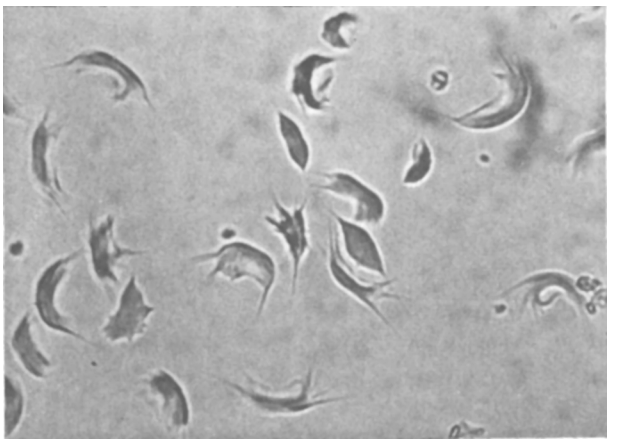

FIG. 2.
Sickled erythrocytes in oxygen unsaturated whole blood from a patient with sickle cell anemia demonstrating the similarities in shape to that of tactoids formed in stroma-free solutions of their deoxygenated hemoglobin. (Phase microphotography ×375.)

(15). When transfused into normal recipients, sickle cell anemia erythrocytes do not long survive(16). In sickle cell trait (genetically heterozygous)(12), only 25 to 44% of the hemoglobin is abnormal(4). The condition is without clinical manifestations and sickling and increases in viscosity and in mechanical fragility of the red cells can be produced only by oxygen saturations well below those usual for venous blood(15). When transfused into normal recipients, sickle cell trait erythrocytes survive normally(16). Thus, the clinical and pathologic manifestations of sickle cell disease apparently derive from a single basic abnormality: the orderly molecular orientation of the peculiar hemoglobin that occurs in concentrated solutions at the reduced oxygen saturation of normal venous blood.

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Summary. 1. In the oxygen unsaturated

state the abnormal sickle cell hemoglobin molecules undergo orderly orientation, forming—by specific linkage of the individual molecules—long chains of hemoglobin elements. Subsequent parallel alignment of these elements results in birefringent tactoids.

2. The birefringent sickled erythrocyte is in all probability a membrane-covered hemoglobin tactoid.

3. The clinical and pathologic manifestations of sickle cell disease apparently follow as a consequence of the effects of the abnormal hemoglobin molecules upon the physical behavior of the erythrocytes.

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Effect of Cortisone and ACTH on Eosinophils and Anaphylactic Shock in Guinea Pigs. (18145)

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Adrenalectomy has been reported to increase the susceptibility of guinea pigs(1) and rats(2,3) to anaphylactic shock. On the other hand, administration of adrenocortical extracts has been found to confer some protection to guinea pigs and dogs against anaphylactic shock(4,5) and some protection to rats against peptone shock(6). Leger, Leith and Rose(7) have found, however, that the administration of 3.5 to 4 mg of adrenocorticotropic hormone (ACTH) to sensitized guinea pigs 6 to 8 hours prior to giving the challenging dose of the antigen did not influence the course of the ensuing anaphylactic reaction. Since the effect of cortisone on anaphylactic shock in guinea pigs had not been studied and since some protective action of ACTH might conceivably have been missed by Leger, Leith and Rose owing to the dosage and time schedule they followed, this investigation was undertaken to extend their study and at the same time to determine whether administration of cortisone would affect anaphylactic shock in guinea pigs and the number of eosinophils in their blood.

Methods. Thirty-seven male and 39 female guinea pigs each weighing between 250 and 300 g were sensitized by 4 consecutive daily subcutaneous injections of 0.5 ml of egg-white solution. The egg-white solution was prepared by diluting the white of fresh eggs with physiologic saline solution in the proportion of 1:5 and thoroughly shaking the mixture until the gelatinous character of the egg white had disappeared. The mixture was then strained through gauze and a clear solution, subsequently referred to as egg-white solution, was obtained. Eight to 12 weeks after the last sensitizing injection a challenging injection that varied from 1.0 ml to less than 0.1 ml of egg-white solution was administered intravenously. The body weights of the animals had by then approximately doubled. Eosinophil counts were performed

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