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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on drops of blood obtained by needle puncture of the marginal vessels of the ear. The blood was drawn directly into a leukocyte pipet, the diluting fluid and the counting chamber used being those adopted by Thorn, Forsham, Prunty and Hills(8) in their modification of Dunger's method(9). A saline suspension of cortisone acetate containing 25 mg per ml, kindly supplied by Merck Co., Inc., Rahway, N. J., was used throughout this study. The lyophilized adrenocorticotropic hormone (ACTH) employed in the study, kindly provided by the Armour Laboratories, Chicago, Ill., had a biological potency of 100% of Armour La-I-A standard. The amounts needed each day were weighed out, then dissolved in physiologic saline solution and injected within 30 minutes. Cortisone and ACTH in doses of 30 and 15 mg respectively were injected in volumes of 1.2 ml and this same volume of physiologic saline solution was used in the control series of animals. All of the injections were given intramuscularly.

Tests of the effects of the hormone on anaphylactic shock were carried out on groups of 6 to 15 sensitized animals. In each group approximately equal numbers of animals were given cortisone, ACTH or physiologic salt solution. Those receiving the salt solution alone served as controls. Nine to 204 hours after receiving the cortisone or saline and 9 to 88 hours after receiving ACTH, the animals were given a challenging injection of egg-white solution. Twenty-seven of the guinea pigs received cortisone, 26 ACTH and 23 were given salt solution only. The distribution of males and females was approximately equal in each group. Eosinophil counts were carried out on 21 of the animals in each of the test groups and on 18 of the animals in the control series. Control eosinophil counts were made during the 3 or 4 days just prior to the hormone or saline injections. The majority of the animals had two or more such counts. From one to 9 counts on each animal were then done at various intervals following the injections. For each guinea pig, the number of eosinophils found after the hormone or saline injections was expressed as a per cent of the mean of its control or preinjection counts. An attempt was made to test the susceptibility of the animals to anaphylactic shock during the early, mid and late periods of the eosinopenia which followed the injections of hormone. After death from anaphylactic shock the lungs of all animals were carefully examined.

Results. Effect of cortisone and ACTH on the number of eosinophils in the blood. Control eosinophil counts. The number of eosinophils in the blood of 28 males and 32 females was counted before hormone or saline injections (Table I). The mean of the 56 preinjection counts made on the 28 males was 45 eosinophils per cmm with a standard error of the mean of ±6 cells per cmm (the mean of the first count made on the males was 45 ± 11). The mean number of eosinophils observed in the 64 preinjection counts made on the 32 females was 155 per cmm with a standard error of the mean of ±11 (the mean of the first counts made in the females was 148 ± 17). Because all of these animals had been sensitized and many of the females

TABLE I. Eosinophil Counts in Guinea Pigs.

Group	Mean body wt, g	No. of Counts	No. of Animals	Mean No. eosinophils per mm³ of blood*
Males				
Sensitized	566	28	56	45 ± 6
Nonsensitized	393-844†	3	29	39 ± 6
Females				
Sensitized				
Primigravida	544	32	64	155 ± 11
Nonsensitized				
Virgin	392-758†	10	82	105 ± 7
Nongravid multipara	1,098	10	25	165 ± 19
Gravid multipara	1,368	7	16	84 ± 12

* Values following the ± signs are standard errors of the means.

† Counts were made repeatedly on these animals over a period of 4 months during which the body weights approximately doubled.

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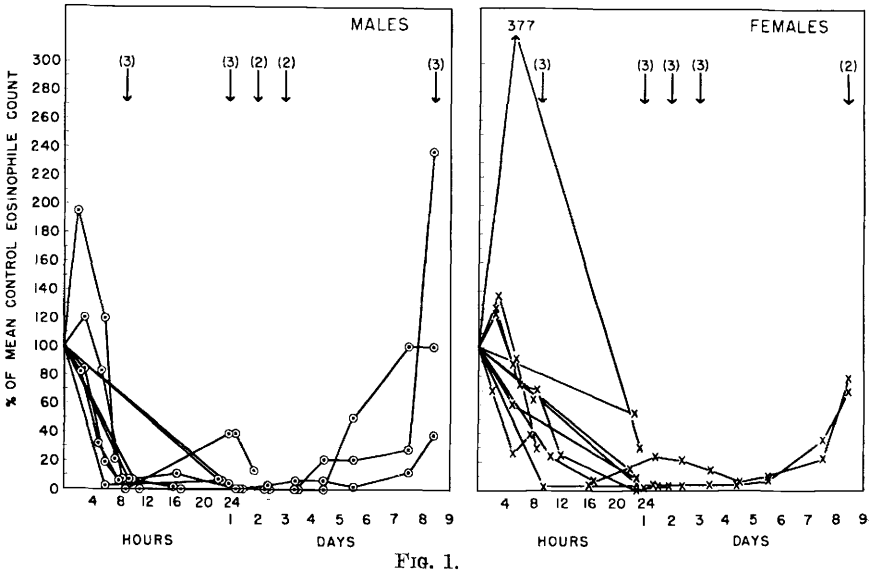

FIG. 1.

Effect of injection of 30 mg of cortisone on the number of eosinophils in the blood of guinea pigs. The arrows indicate the times at which the susceptibility of the animals to anaphylaxis was tested and the numerals in parentheses are the number of animals tested on each occasion.

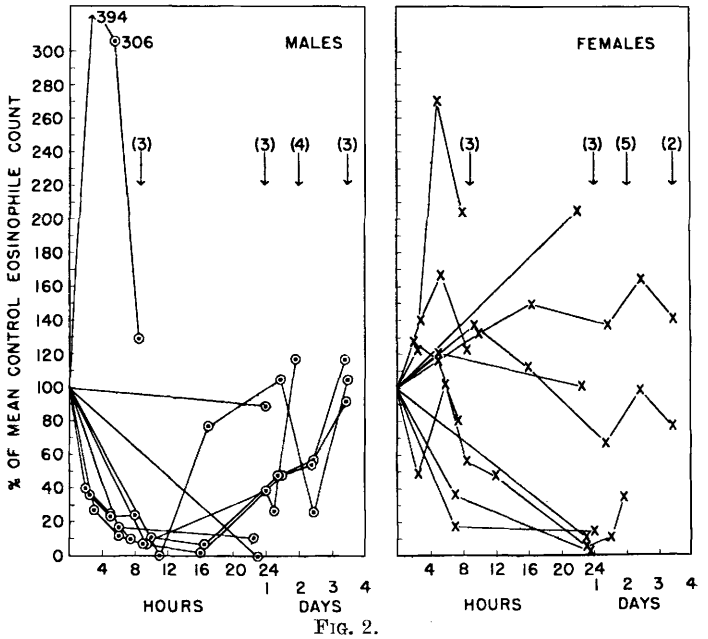

FIG. 2.

The effect of injection of 15 mg of ACTH on the number of eosinophils in the blood of guinea pigs. The arrows indicate the times at which the susceptibility of the animals to anaphylaxis was tested and the numerals in parentheses are the number of animals tested on each occasion.

were found to be pregnant, a series of eosinophil counts were done on 10 nulliparous nonsensitized females and a series of 25 counts were made on 3 unsensitized males. The mean number of eosinophils per cmm of blood was 105 ± 7 in the females and 39 ± 6 in

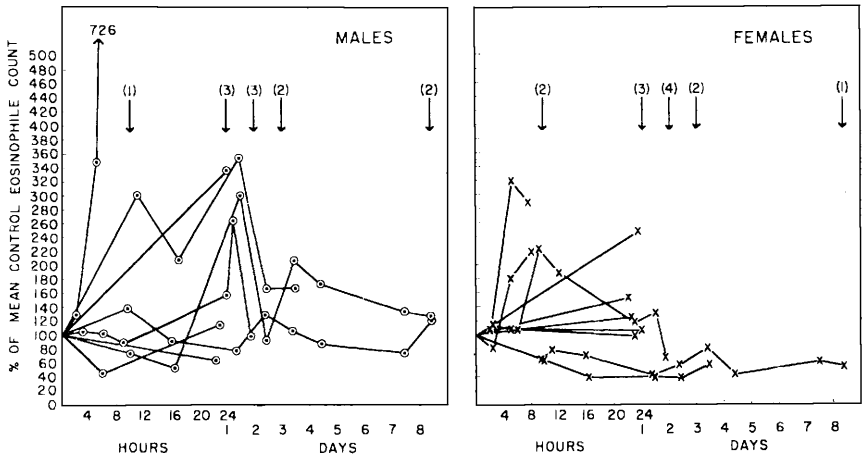

FIG. 3.

The effect of injection of saline solution on the number of eosinophils in the blood of guinea pigs. The arrows indicate the times at which the susceptibility of the animals to anaphylaxis was tested and the numerals in parentheses are the number of animals tested on each occasion.

the males. The mean number of eosinophils in the males was therefore significantly different from that in the females.

Effect of cortisone. The injection of 30 mg of cortisone produced a pronounced reduction in the number of eosinophils in the blood of all the animals tested. Counts made 8 to 12 hours after injection of the cortisone into the male animals were all less than 10% of the preinjection values (Fig. 1). In the females the decline was more gradual, ranging at the 8 to 12 hour period between about 70% and 2% of the control counts although by the end of the first 24-hour period most of the counts in the female animals were below 20% (Fig. 1). In 4 of the 5 animals in which counts were made for 8 days or more, the eosinopenia persisted for 7 days, after which the counts approached normal.

Effect of ACTH. Eosinophil counts were made on 10 male and 11 female animals after the injection of ACTH. In 7 of the 10 males the eosinophils were reduced in number within 12 hours to less than 20% of preinjection values. The eosinopenia may have been missed in 2 of the 3 animals because a count was not made until 23 to 24 hours after the injection (Fig. 2). The duration of the eosinopenia was much shorter after the ACTH than after the cortisone injections, the counts having returned to normal 24 to 72 hours after the injections of ACTH (Fig. 2). ACTH did not have a consistent effect on the number of eosinophils in the blood of the female animals. In 4 animals there was a definite eosinopenia while in the remaining 7 there was an increase or no consistent change in the number of eosinophils (Fig. 2).

Injections of saline solution. The injections of saline solution did not produce a consistent change in the number of eosinophils in the blood of the male or female animals. Approximately as many animals showed a rise in numbers of eosinophils as displayed a fall and none had a reduction in number below 39% of the control values (Fig. 3).

The abdomens of about half the females were examined at necropsy and in every instance a gravid uterus was found. Since the male and female animals were mixed in the cages it is likely that nearly all, if not all, of the females studied were pregnant when the tests were made. Because the females in the foregoing series did not exhibit a consistent eosinopenia after ACTH and because most of them were pregnant the effect of ACTH was tested on an additional series of 7 pregnant and 10 nonpregnant unsensitized females (Table I). All of these had been pregnant previously and were larger than those of the other groups. Their body weights ranged from 0.9 to 1.4 kg and the amount

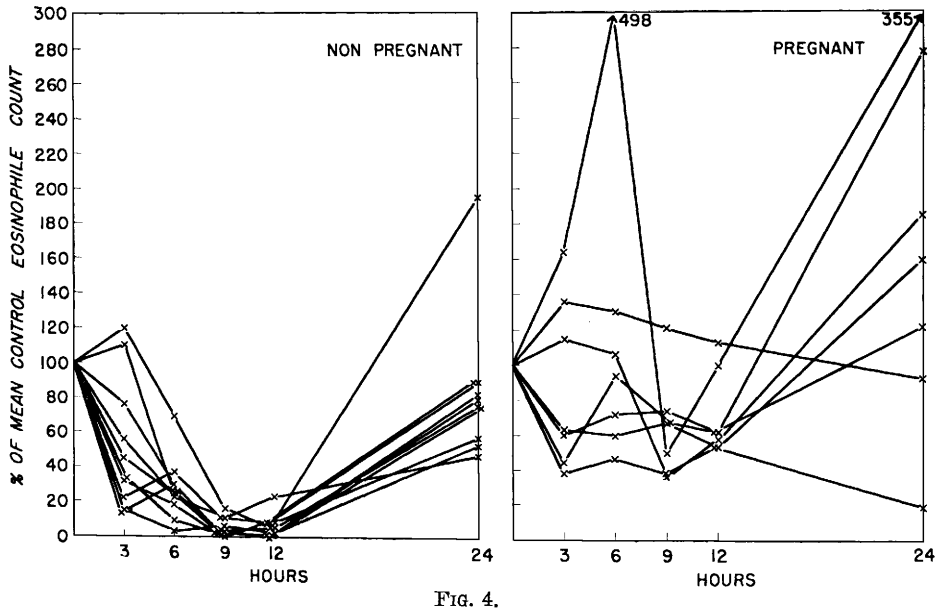

FIG. 4.
The effect of injection of ACTH on the number of eosinophils in the blood of pregnant and nonpregnant female guinea pigs.

of ACTH given was therefore increased proportionally. It was injected after 2 or 3 daily control eosinophil counts had been made. The counts were then repeated 3, 6, 9, 12, and 24 hours after the injection. The mean of 25 control counts on the nonpregnant animals was 165 ± 19 and that of 16 counts on the 7 pregnant animals was 84 ± 12. After the injection of ACTH the nonpregnant animals showed a consistent eosinopenia similar to that obtained in the males. The pregnant females did not show a consistent change in numbers of circulating eosinophils (Fig. 4). It was concluded that pregnancy abolishes the eosinopenic response to ACTH in female guinea pigs.

Effect of cortisone and ACTH on anaphylactic shock. The challenging injections of egg-white solutions were given to the animals 9, 24, 48, 73, 88 and 204 hours after the hormone or saline injections (see arrows in Fig. 1, 2 and 3). This timing was carried out in an attempt to test the sensitivity of the animals to the egg-white solution while the number of eosinophils in the blood was declining, when the eosinopenia was maximal and during the return of the counts to the control values. All the animals experienced severe grades of anaphylactic shock a few moments after receiving the egg white. Seventy-four of the 76 injected died within 5 minutes and the remaining 2 succumbed within 20 minutes. The animals dying promptly all showed the voluminous lungs typical of anaphylaxis. The 2 in which death was delayed showed patchy emphysema. Therefore the administration of cortisone and ACTH in these experiments did not protect the animals against anaphylactic shock.

Comment. The eosinopenia produced by ACTH in the male and nonpregnant female guinea pigs is similar to that observed by Hills, Forsham and Finch(10) in human beings. The eosinopenia produced by cortisone in the guinea pigs was, on the other hand, more pronounced and consistent than the changes produced by this hormone in the number of circulating eosinophils in human beings (11,12). The duration of action of the single

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30 mg injection of cortisone was approximately one week. This prolonged effect was probably due, in part at least, to the insolubility of the compound and its resultant slow absorption, because upon examination the material could often be seen at the site of injection 2 or 3 days and sometimes longer after it had been given.

The failure of ACTH to affect anaphylactic shock in guinea pigs confirms the earlier observations of Leger, Leith and Rose. Employing a larger dose of ACTH in the present study and testing at various intervals after the administration of the hormone did not affect the results. Wolfram and Zwemer(4) noted a brief period from 2½ to 5½ hours after the administration of adrenocortical extract during which sensitized guinea pigs were apparently partially protected against injections of the sensitizing agent. In the present study tests for sensitivity were not made during this early period.

Summary. Guinea pigs were used throughout this study. The number of eosinophils in the blood of males was significantly lower than that in females. Cortisone reduced the number of eosinophils in the blood of males and females. ACTH produced a pronounced eosinopenia in males and nonpregnant females. Pregnancy abolished the eosinopenic effect of ACTH. Neither cortisone nor ACTH had any effect upon the degree of anaphylactic shock produced in guinea pigs by the intravenous injection of the agent to which the animals had been sensitized.

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Effect of Specific Antibody on the Metabolism of *Serratia marcescens.** (18146)

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From time to time studies have been carried out to determine any possible effect which specific antisera might have on the growth and metabolism of various bacteria. We shall not attempt at this time to survey the literature on this subject which has been adequately covered by Oldfelt(1) and Sevag (2). Suffice it to say, with few exceptions, there is agreement that when a bacterium comes in contact with its specific antiserum little or no change can be demonstrated in its metabolism as measured by oxygen consumption, providing lysis of the organism does not occur. Because of some preliminary experiments we have felt that this problem should be re-studied, using simple substrates, purified (ethanol precipitated) antibody and suspensions of resting (washed) organisms.

Experimental. Rabbits were injected intravenously 6 times at 2- or 3-day intervals with emulsions of live *S. marcescens* prepared from cultures grown for 24 hours on pancreatic digest agar. Twelve days after the last injection the animals were bled from the heart. Each serum was inactivated at 60°C for 3 minutes and was then titrated against live organisms. After finding the sera to be active (titer: 1-20,480 to 1-40,960) all were pooled. A portion of this was frozen and used as necessary. The remainder was treated with ethanol to obtain the gamma globulin fraction as described by Cohn *et al.*(3). Normal rabbits were bled; after inactivation

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