Perhaps no definite conclusions are justified by the colchicine feeding experiment, in view of the small number of animals used. It may well be that death in half the cases was from starvation rather than toxicity of the drug. However, the starvation must have been voluntary, since large amounts of food material were left untouched in the cage, and the duration of the experiment was not sufficient to suggest death from malnutrition.

The resistance to colchicine displayed by the hamster strongly resembles the natural resistance of the rabbit to aconite. In this respect, it is of interest to note that colchicine, which is usually extracted from the meadow saffron, *C. autumnale,* has also been isolated from other species of *Colchicum.* Although *C. autumnale* is distributed throughout Europe, the center of distribution for the genus (with 30 species) is in Asia Minor (*5*). This suggests that resistance in the hamster may have developed from close association with the plant in the natural habitat, *Colchicum* probably being used by the hamster as food. How high this resistance may extend, and what mechanism is involved, would be interesting to determine.

References

1. POE, C. F., and JOHNSON, C. C. *Acta Pharmacol. et Toxicol.,* **5**, 110 (1949).
2. SOLLMANN, T. H., and HANZLIK, P. J. *Fundamentals of Experimental Pharmacology,* 2nd ed. San Francisco: Stacy (1939).
3. CUSHNY, A. *Pharmacology and Therapeutics,* 13th ed. Philadelphia: Lea & Febiger (1947).
4. SOLLMANN, T. H. *Manual of Pharmacology and its Applications to Therapeutics and Toxicology,* 7th ed. Philadelphia: Saunders (1948).
5. KRYTHE, J. M., and WELLENSIEK, S. J. *Bibliog. Genetica,* **14**, (1), 1 (1942).

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The Turnover Time of Phosphorus in Normal, Sickle Cell Trait, and Sickle Cell Anemia Blood *in Vitro* as Measured with P^{32} [1,2]

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The close relation between oxidation and phosphorylation in the living cell would lead one to expect that a disease which affects one would have some affect upon the other. Thus in sickle cell anemia, sickle cell trait, or sicklemia (henceforth called trait), and normal blood, a study of rate of uptake of phosphate by the red blood cell under various conditions should give insight into the mechanism of phosphorus transfer and its disturbance in this serious blood dyscrasia.

In order to obtain comparable data with some statistical reliability, we secured blood samples from 10 normal, 9 trait, and 7 sickle cell anemia patients. The sickle cell patients were known cases which had been treated on the Pediatric Service of Freedmen's Hospital for typical crises of the disease. Four slides were made of each patient in the study, using the methods of Singer and Robin (*1*) and Daland and Castle (*2*). All the anemia and trait patients showed sickling. None of the anemia patients had received blood transfusions in the three months preceding this study. The trait cases were siblings or parents of known sickle cell anemia patients. The normal cases were asymptomatic and, of course, showed no sickling. The age distribution of the subjects is given in Table 1.

TABLE 1

	Normal	Trait	Sickle cell anemia
Lowest	9 years	4 years	2 years
Highest	14 "	49 "	10 "
Average	10.5 "	23 "	9 "

Approximately 23 ml of blood was taken from each patient. On 3 ml a complete hemogram was done. Exactly 20 ml was mixed with 0.8 ml of the ammonium potassium oxalate solution (2 g potassium oxalate and 3 g ammonium oxalate in 100 ml solution), following the method of Taylor *et al.* (*3*). This solution caused no swelling of the erythrocytes. The blood samples were incubated in stoppered brown bottles with 13.7 ml air above the sample. To each sample of a group, 0.5 ml of isotonic NaCl containing a tracer amount of P^{32} as PO_4 in weak HCl was added (e.g., 0.54 mc in 0.5 ml solution to each sample of normal blood). The activities were checked against the Tracerlab simulated P^{32} source.

The blood samples were incubated for 4 hr at $38° \pm 0.1°$ C. There was no sickling in the blood samples during incubation. After incubation, total, acid-soluble, and inorganic phosphorus were determined, following the method of Fiske and Subbarow (*4*), on the whole blood, the plasma, and red blood cells. The radioactivity was determined on an aliquot of each sample, evaporated to dryness and counted with a mica end window Geiger-Müller tube, thickness 3 mg/cm^2, feeding into a Nuclear Instrument and Chemical Corporation Model 172 scaler.

Blood samples were centrifuged for 15 min at 3000 rpm to separate the plasma from the red blood cells. The plasma was drawn off as completely as possible. The cells were resuspended in isotonic saline, centrifuged, and the supernatant saline was drawn off and discarded. This washing was repeated. The red cells were then frozen at $-20°$ C to produce hemolysis and to prevent hydrolysis of the organic phosphorus compounds. For analysis the frozen cells were thawed and diluted with distilled water.

The results are expressed in specific activities defined as the counts/sec/mg of phosphorus corrected for radioactive decay (Table 2).

In this preliminary report we shall consider only

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TABLE 2

Total phosphorus	Plasma		Red blood cells	
	mg/ml	SA*	mg/ml	SA
Normal (av of 10)	0.165	3.41	0.60	1.65
Trait (av of 9)	0.270	2.05	0.74	0.88
Sickle cell (av of 7)	0.160	1.60	0.93	0.39

* SA = specific activity as counts/sec/mg of P corrected for radioactive decay.

the total phosphorus. Qualitatively, the behavior of all other fractions was similar to that of the total phosphorus.

In this experiment, the important consideration is the rapidity with which equilibration of P^{32} is being reached between the plasma and the red blood cell (rbc). A measure of this equilibration is the ratio or the specific activity of the red blood cell to that of the plasma. Gourley and Gemmill (*5*) have shown that the uptake of P^{32} as phosphate by the red blood cells, at least during the initial period, follows a first order reaction. If we assume that the first order is an acceptable approximation over the 4-hr period, we have

$$\frac{\text{SA of rbc}}{\text{SA of plasma}} = (1 - e^{-\alpha t}).$$

Using the numerical values, we have α in hours^{-1} in Table 3.

Having the values of the α's, we may calculate the turnover times in the following manner. The factor $e^{-\alpha t}$ is the metabolizing function (*6*) for phosphorus in the red blood cell. Since the exchange of phosphorus (as phosphate) may be looked upon as an instance of dynamic equilibrium, we have for the amount of the normal phosphorus M(O), in the red blood cell (*7*),

$$M(O) = M(O)e^{-\alpha t} + \int_0^t R(\theta)e^{-\alpha(t-\theta)}d\theta.$$

The rate of entry, $R(t)$, determined from this equation is $M(O)\alpha$, where M(O) is the constant amount of phosphorus in mg/ml of red blood cells. With the numerical values from Table 2 for M(O), we have the rates given in the third column of Table 3.

The turnover time is defined as that interval during which the amount entering the cell equals the amount present, or (*7*)

$$M(O) = \int_0^\tau R(\theta)\, d\theta,$$

where τ would be the turnover time. From this equation, $\tau = \frac{1}{\alpha}$. The turnover times are given in Table 3.

TABLE 3

CHARACTERISTICS OF THE BLOOD SAMPLES

	SA of rbc / SA of plasma	α(in hr^{-1})	Rate of uptake in mg/ml of rbc/hr	Turnover time of P (hr)
Normal	0.48	0.160	0.096	6.3
Trait	0.43	0.140	0.103	7.1
Sickle	0.24	0.065	0.060	15.3

The turnover time of P in the sickle cell anemia red blood cell is, therefore, approximately 2½ times greater than that in the normal red blood cell under these experimental conditions. This large difference indicates that, whatever the basic malfunctioning system in sickle cell anemia (*8*), it reveals itself also as a marked reduction in the rate of turnover of total phosphorus in the red blood cell *in vitro*, in the presence of ammonium potassium oxalate. The turnover times for all phosphorus fractions so far examined have this relation: τ normal $< \tau$ trait $< \tau$ sickle cell.

These differences in turnover times cannot be attributed at present exclusively to differences in permeability of the red blood cell membrane or to reactions within the cell. They seem not to depend upon a difference in the surface/volume ratio, since the erythrocytes in the three groups did not differ significantly in size. Experiments are in progress which, it is hoped, will elucidate the specific mechanism of the phosphorus exchange which is altered in the trait and sickle cell cases.

References

1. SINGER, K., and ROBIN, S. *J. Amer. Med. Assoc.*, **136**, 121 (1948).
2. DALAND, G. A., and CASTLE, W. B. *J. Lab. Clin. Med.*, **33**, 1082 (1948).
3. TAYLOR, F. H. L., LEVINSON, S. M., and ADAMS, M. D. *Blood*, **3**, 1472 (1948).
4. FISKE, C. H., and SUBBAROW, Y. *J. Biol. Chem.*, **66**, 375 (1925).
5. GOURLEY, D. R. H., and GEMMILL, C. L. *J. Cell. & Comp. Physiol.*, **35**, 341 (1950).
6. BRANSON, H. *Cold Spring Harbor Symposia Quant. Biol.*, **13**, 35 (1948).
7. BRANSON, H. A.E.C. Technical Report, No. 3-1951. Dept. of Physics, Howard University (1951).
8. PAULING, L., *et al. Science*, **110**, 543 (1949).

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Control of Covered Smut of Wheat by Rapid Seed Treatment with an Antibiotic[1]

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In a previous paper (*1*), it was reported that covered smut of oats and covered smut of wheat, or bunt, were controlled in the field by seed treatment with the antibiotic actidione. The method of treatment used was not considered a practical one as it involved a prolonged soaking of the seed for 4 hr. Moreover, in the case of wheat, the results in bunt control were not too convincing, because of a low percentage of infected plants in the checks. They also were not very promising because of rather serious phytotoxicity expressed in seed injury. It was thought that the treatment might be modified in certain ways and in consequence made more practical. To this end, preliminary greenhouse experiments were made in the spring of 1951 with rapid treatments using actidione[2] in dust

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[2] Kindly supplied by the Upjohn Company of Kalamazoo, Mich., through the courtesy of J. H. Ford.