TABLE 2

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

* 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 P³² 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^{-a_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),

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

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)

$$\mathbf{M}(\mathbf{O}) = \int_{\mathbf{o}} \tau \, R(\theta) \, \mathrm{d}\theta,$$

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 $2\frac{1}{2}$ 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).
- J. TAYLOR, F. H. L., LEVINSON, S. M., and ADAMS, M. D.
 Blood, 3, 1472 (1948).
 FISKE, C. H., and SUBBAROW, Y. J. Biol. Chem., 66, 375
- (1925). 5. GOURLEY, D. R. H., and GEMMILL, C. L. J. Cell. & Comp.
- Branson, H. Cold Spring Harbor Symposia Quant. Biol., 13, 35 (1948).
- 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 Antibiotic¹

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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 actidione² in dust

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and liquid form. The main results are briefly reported here.

The seed used in the experiments was a sample of spring wheat naturally contaminated with spores of Tilletia spp., which cause covered smut of wheat. It was further artificially contaminated by the addition of 0.5 g of similar spores to each 100 g of seed. Portions of the infested seed were treated with different amounts of actidione dusts consisting of mixtures of a finely powdered clay and the antibiotic in one type of treatment, and with actidione diluted with a 0.02%aerosol and water mixture in another. Three types of checks were included: one untreated, one to which the clay dust only was applied, and one which was immersed for 1 min in a 0.02% aerosol and water mixture. The seed was treated one day and sown the next. Four replicates (100 seeds/replicate) of each treatment and each check were sown in randomized rows in a bed of black soil in the greenhouse. The most important results are presented in Table 1.

TABLE 1

EFFECT OF RAPID SEED TREATMENTS WITH ACTIDIONE ON THE EMERGENCE OF SPRING WHEAT AND ON ITS INFECTION BY SEED-BORNE COVERED SMUT FUNGI

Treatment	Concentration and form	Amount or time	Av % emergence	Av % smutted 。 heads
Actidione	.5% dust	½ oz ∕bu	85.0	0.0
" "	.5% ''	1 '' ''	85.0	.0
"	1.0% "	1/2 ** **	84.5	.6
	1.0% ''	1	73.0	.0
" "	10 ppm			
	liquid	1 min	84.0	0.0
Clav diluent (ck)	Dust	1 oz	91.3	6.1
Aerosol-water (ck)	Liquid	1 min	91.3	8.3
None (ck)			90.7	22.9
LSD			7.9	

The above results of greenhouse experiments show that rapid seed treatment of wheat with actidione in both dust and liquid form gave complete or almost complete control of covered smut of wheat. Emergence appeared to be reduced slightly, but the reduction was significant from the emergence of the checks only with the highest dosage of the antibiotic in dust form. The entire effect in smut control cannot be attributed to the antibiotic, since it is evident from the check results that the diluents had considerable influence themselves in reducing the severity of the smut infection. This, however, may be considered an advantage of the treatments, especially in view of the fact that the diluents had no injurious effects on the seed. It will be noted that control of covered smut of wheat was obtained with actidione in highly diluted form and that the procedures followed in applying it were similar to those at present used in the treatment of wheat and other grains with standard fungicides.

Moreover, field data which will soon be available tend to confirm in large measure the results of the greenhouse experiments given here. These will be reported in another paper.

Reference

1. HENRY, A. W., et al. Science, 113, 390 (1951).

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An Improved Method for Measuring Coagulation Time

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Numerous methods for measuring the clotting time of blood have been proposed. The most satisfactory technique for the purpose, however, is the so-called Lee and White method (1). This consists in withdrawing blood from a vein, or in smaller animals directly from the heart, introducing 1 or 2 ml of the blood into flat-bottomed glass shells or homeopathic vials, and measuring the time it takes for the blood to clot as indicated by tilting the tubes every minute until the blood no longer flows.

We have recently devised an improvement in the Lee and White technique which we find very useful for careful research investigations. This consists in the employment of an instrument that enables the investigator to measure accurately the exact angle at which the surface film of the whole blood in the homeopathic vial is broken and begins to flow. The apparatus, which is made of plexiglass, consists of a vertical quadrant fixed on a plastic base (Fig. 1). At the center of the quadrant there is attached a radial pointer which carries at its base a small platform on which the vial containing the blood is placed. The quadrant is subdivided into degrees and half-degrees from 0 when the pointer is in a vertical position.

Blood is obtained from rabbits by direct cardiopuncture and immediately transferred to flat-bottomed vials 15 mm in diam and 45 mm in height. As soon as 1 or 2 ml of the blood is introduced into the perfectly clean vial the experimenter notes the posi-

