a ring-form nucleus also incorporate thymidine. Whether these cells are young or mature cannot be determined decisively. Some of these cells have a pale cytoplasm and a nucleus containing coarsely divided chromatin (mature forms); others have a basophilic cytoplasm and a nucleus containing finely divided chromatin (young forms). If we assume that mature forms are not able to divide, thymidine incorporation into these cells would indicate ploidy, or possibly turnover of thymidine, rather than potential to divide. It is not likely that the labeled ring forms arose from labeled precursors since the number of labeled rings did not increase as incubation time increased, nor was there a reduction in the number of grains over the ring forms.

Summary. Using hemoglobin markers to identify donor- and host-type erythrocytes, we have demonstrated that cells in the leukocyte fraction of leukemoid blood are capable of transplanting and giving rise to mature erythrocytes of donor type in lethally irradiated mice. Autoradiograms showed that several cell types in leukemoid blood incorporate H^3-thymidine *in vitro*.

We wish to thank Mr. W. D. Gude of the Biology Division for making the autoradiographs.

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Augmentation of Chlortetracycline Activity Against Two Strains of *Staphylococcus* by Kinetin.* (25471)

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Kinetin (6-furfurylaminopurine) was shown by Miller *et al.*(1,2) to be a cell division factor for tobacco pith tissue in tissue culture. This compound, originally isolated from aged or heated deoxyribonucleic acid (DNA) by these workers, was shown by Hall and de Ropp(3) to be essentially an artefact with respect to DNA. The latter workers established that the presence of kinetin in DNA was due to fortuitous interaction of adenine with 2-deoxyribose. Since the discovery of kinetin, a number of reports have described the influence of this compound and related compounds in several diverse biological systems. In the bacterial field, Lansford *et al.* (4) have shown that kinetin and certain other 6-(substituted amino) purines will augment inhibition of *Lactobacillus arabinosus* by 2,4-diamino-6,7-diphenylpteridine. Braun(5) has demonstrated that kinetin will shift the population of a culture of mixed rough and smooth *Brucella suis* in favor of the smooth cells. This shift is the reverse of that in the absence of kinetin. The increasing incidence of infections caused by antibiotic-resistant staphylococci has prompted us to examine the effects of kinetin and related compounds on these microorganisms. In this paper we wish to report that kinetin (and some analogs) will enhance the action of chlortetracycline (CTC) against an antibiotic-resistant strain of staphylococcus *in vitro*.

Materials. Kinetin, 6-(3-pyridylmethyl-

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TABLE I. Effect of Kinetin and Its Derivatives on Bacteriostatic Action of Chlortetracycline for *S. albus* 69R.

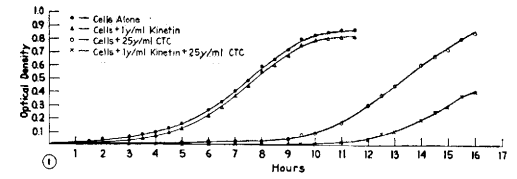
Compound	Conc., γ/ml, of compound	No. of colonies ($\times 10^{-8}$)/ml — Saline control	Compound *per se*	CTC *per se*, 50 γ/ml	Compound + 50 γ/ml of CTC
Kinetin	.1	2.47	2.12	2.09	.00
	1.0	2.47	2.42	2.09	.00
	4.0	2.35	2.22	2.09	.70
Kinetin riboside	4.0	2.43	2.40	2.50	.85
6-(3-pyridylmethylamino)-purine	4.0	2.43	2.43	2.50	1.69
6-(3-pyridylmethylamino)-9-D-ribofuranosylpurine	4.0	2.43	2.44	2.20	1.69
6-(Dimethylamino)-9-D-(3-deoxy-3-amino)-ribofuranosylpurine	4.0	2.43	2.40	2.51	.50
6-(Dimethylamino)-9-D-ribofuranosylpurine	5.0	2.26	2.46	2.14	1.35
Adenosine	5.0	2.35	2.37	2.66	2.37

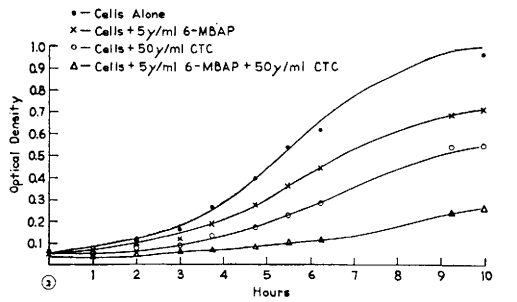
amino) purine, 6-(2-pyridylmethylamino) purine, 6-benzylaminopurine, 6-(N-methylbenzylamino) purine, and 6-furfurylthiopurine were obtained from Dr. M. W. Bullock (6). Kinetin riboside(7), 6-(3-pyridylmethylamino)-9-D-ribofuranosyl purine(7), 6-dimethylamino-9-D-ribofuranosyl purine(8) and 6-dimethylamino-9-(D-3-deoxy-3-amino) ribofuranosyl purine(9) were obtained through Dr. H. Kissman. The 2 indole analogs, 6-[(β-indoyl-3-ethyl)-amino] purine, 6-{[β-(5-methyl-3-indoyl) ethyl] amino} purine, were synthesized in this laboratory by Mr. R. Haselkorn using a general method(6) whereby the corresponding indoylethylamine‡ was condensed with 6-chloropurine. The microorganism used throughout the course of the experiments was *Staphylococcus albus* 69R (*Micrococcus pyogenes* var. *albus* 69R). It was a hospital isolate obtained from Mr. A. C. Dornbush of these laboratories.

Methods. Tubes of trypticase-soy (TS) broth containing the adducts (total vol. 6 ml) were warmed to 37° then inoculated with 0.1 ml of a 1 x 10^{-2} dilution of a 6 hour (late exponential phase) TS broth culture of *S. albus* 69R. After 24 hours incubation at 37° the cultures were serially diluted and plated on to TS agar. The plates were incubated for 24 hours at 37°. Four plates were made per tube and tubes were run in duplicate or triplicate. Because of the tendency of staphylococci to clump, the plate counts reported in Table I are essentially viable units/ml. The growth curves were run with TS broth cultures started as above except 18 hour cultures were used for the inoculum. Optical density readings were made at 610 mμ. *In vivo infection.* Infections were produced in CFI white mice, females 17-20 g (Carworth Farms) by intraperitoneal injection of 0.5 ml of a 1 x 10^{-2} dilution of 5 hour TS blood broth cultures of *Micrococcus pyogenes* var. *aureus*, strain Smith. Kinetin and the antibiotics were suspended in 0.2% aqueous agar. Eleven groups of 10 mice each were pretreated with kinetin by subcutaneous injection with 0.5 ml doses of 64 mg/kg/dose at 24, 6 and 0.5 hours prior to infection. Within 0.5 hour after injection the mice were treated subcutaneously with CTC or tetracycline in graded doses. A parallel series of non-kinetin treated animals was run simultaneously plus the following controls. One group treated with kinetin but not infected, 2 groups not treated with kinetin but infected, and one group not treated with kinetin and not infected. Heart blood cultures were made routinely from mice dying 6 days postinfection. All such mice showed the infecting organism.

Results. Staphylococcus albus 69R will grow in broth culture in presence of at least 125 γ/ml of CTC. The data in Table I show growth of this microorganism as reflected by plate counts in the presence of CTC or kinetin, and in the presence of a combination of

‡ We are indebted to Dr. S. Davis for supplying indole precursors used in these syntheses.





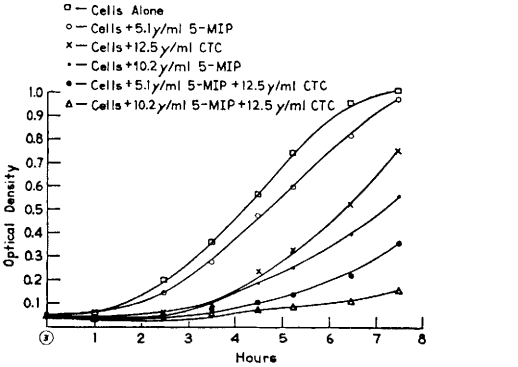


FIG. 1. Effect of kinetin on the response of *S. albus* 69R to CTC.

FIG. 2. Effect of 6-(methylbenzyl)aminopurine (6-MBAP) on the growth of *S. albus* 69R in the presence and absence of CTC.

FIG. 3. Effect of 6-{[2-(5-methyl-3-indolyl)ethyl] amino}-purine [5-MIP] on the growth of *S. albus* 69R in the presence and absence of CTC.

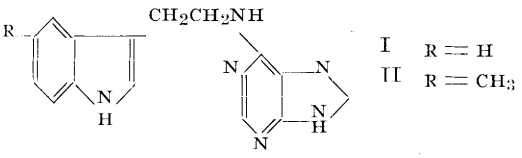
the two. Neither kinetin nor CTC by themselves influenced plate counts after 20 hours of growth, but the combination reduced cell counts by at least 100-fold. Other kinetin analogs listed in Table I gave the same result to a lesser degree. There was no advantage in using kinetin riboside and neither did the riboside of 6-(3-pyridylmethylamino)-purine have an advantage over the free base. Adenosine was inactive at the level tested.

A more accurate picture of the potentiation of CTC by kinetin was obtained by studying turbidimetric growth curves of *S. albus* 69R in presence of CTC and the kinetin compounds. Fig. 1 shows that 25 γ/ml of CTC alone extends the lag phase of growth, although exponential growth rate and total growth were essentially the same as the control. This effect *per se* of sublethal amounts of antibiotics on growth of resistance cells has been studied in detail(10). For our purposes here the relative lag periods are of interest. Kinetin by itself at a level of 1 γ/ml had no effect on the course of growth of *S. albus* 69R, but in combination with CTC the length of the lag phase was considerably extended over that of CTC alone. These data show that plate count differences reported in Table I are not necessarily differences between total growth but differences between control cultures at maximum growth and experimental cultures still in late exponential phase.

This phenomenon was also studied with the kinetin analog, 6-(N-methylbenzylamino) purine (6-MBAP) (Fig. 2). This compound itself at a level of 5 γ/ml was slightly toxic to *S. albus* 69R as evidenced by a slight increase in the lag phase of growth. In combination with CTC it was synergistic. Thus apart from the inherent toxicity of 6-MBAP there seemed to be a potentiation of CTC activity analogous to that of kinetin.

Similar studies were made with 6-benzylaminopurine, 6-furfurylthiopurine, 6-(2-pyridylmethylamino) purine and 6-methylaminopurine. In each case no toxicity was noted at 1-10 γ/ml level and there was no potentiation of CTC action.

A different class of kinetin analogs was studied next, namely, indoylethylaminopurines. The parent compound, 6-[(β-indoyl-3-ethyl)-amino] purine I was first prepared by Lettré(11) who reported that it was toxic to fibroblasts in tissue culture. This particular compound at a level of 10 γ/ml in the cul-



ture had no influence on the shape of the *S. albus* 69R growth curve nor did it potentiate CTC. But the 5-methyl analog 6-{[β-(5-methyl-3-indoyl)ethyl] amino} purine II was slightly inhibitory to *S. albus* 69R

TABLE II. Effect of Pre-infection Treatment with Kinetin upon Activity of Subcutaneously Administered Chlortetracycline and Tetracycline against Staphylococcus Smith Infection in Mice. (Pooled results of 4 separate tests.)

Antibiotic dose, mg/kg	Survival on 14th day post-infection: Without kinetin pretreatment Alive/Total	% effect	With kinetin pretreatment Alive/Total	% effect
	Chlortetracycline treated mice			
2.0	38/40	95.0	40/40	100.0
1.0	29/40	72.5	37/40	92.5
.5	16/40	40.0	26/40	65.0
.25	4/40	10.0	8/40	20.0
.125	2/20	10.0	1/20	5.0
	Tetracycline treated mice			
4.0	37/40	92.5	40/40	100.0
2.0	26/40	65.0	35/40	87.5
1.0	20/40	50.0	27/40	67.5
.5	5/40	12.5	13/40	32.5
.25	0/20	.0	1/20	5.0

Kinetin controls: 95% (38/40) kinetin pretreated-infection control mice were dead within 2 days post-infection.

Infected controls: 100% (80/80) untreated-infected control mice were dead within 2 days post-infection.

Age-conditioned controls: 97.5% (39/40) untreated-uninfected control mice were *alive* on the 14th day when test terminated.

at levels of 5-10 γ/ml (Fig. 3). A combination of this compound with CTC was synergistic as shown by the increased lag phase of growth.

Kinetin and most of the analogs tested were relatively non-toxic to *S. albus* 69R, yet in some way they potentiated the inhibitory action of CTC. We have no evidence as to why this occurs; however, the effect would seem to be more than an anti-metabolic one. The question arises whether the effect is related to stimulation of cell division by kinetin in some plant systems(1,2,3). Guttman(12,13), in a cytological investigation of meristematic onion root cells grown in solutions of kinetin found that kinetin seemed to influence metabolism of nuclear ribonucleic acid. Perhaps there is an analogous effect in staphylococci that makes the cell more vulnerable to the action of CTC. The potentiation of CTC by kinetin was not only observed *in vitro* but was also demonstrated in an *in vivo* system. In cooperation with Mr. G. Redin and Miss E. McCoy, studies were made with an infection of *Micrococcus pyogenes* var. *aureus*, strain Smith, in mice. This is a CTC-sensitive organism. Its use was necessitated by the unavailability of a mouse-virulent CTC-resistant staphylococcus. Data in Table II show that the activity of both CTC and tetracycline was augmented by treating the mice with kinetin prior to infection. Application of the statistical method of Litchfield and Wilcoxon (14) for evaluation of dose-effect experiments showed that the combination of CTC and kinetin was 1.5 times as active as CTC alone (95% confidence limits 1.1-2.0) and the tetracycline-kinetin combination was 1.8 times as active as tetracycline alone (95% confidence limits 1.3-2.5). These data demonstrate first, that kinetin will enhance the activity of the tetracyclines *in vivo* and second, that enhancement of antibiotic activity by kinetin also occurs with an antibiotic-sensitive staphylococcus.

Summary. 1. Kinetin, although non-toxic to an antibiotic-resistant strain of *Staphylococcus albus*, augmented the action of chlortetracycline against this microorganism *in vitro*. 2. Some other 6-(substituted amino) purines had this property. 3. Kinetin caused a slight but statistically significant increase in activity of chlortetracycline and tetracycline against an antibiotic-sensitive strain of *Staphylococcus aureus* in mice.

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Serum Lipoproteins in Atherosclerosis-Susceptible and Resistant Pigeons.*† (25472)

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White Carneau pigeons (both male and female) develop spontaneous atherosclerosis involving aorta and coronary arteries, while Show Racer pigeons appear resistant to atherosclerosis(1,2). Despite striking differences in incidence of the disease, the 2 breeds do not differ significantly in serum levels of total lipides, cholesterol (free or ester), or phospholipides(3). Studies on man(4,5,6) and experimental animals(7,8,9) indicated that atherosclerosis may be associated with changes in levels of serum lipoproteins, especially increases in beta-lipoproteins. Accordingly, we thought it of interest to compare atherosclerosis-susceptible and resistant pigeons for possible differences in levels of beta-lipoproteins, as determined by agar precipitation method of Boyle and Moore(10). It soon became apparent that enormous fluctuations occurred in the K-agar Δ‡ values obtained for female pigeons. These results, and those of a more detailed study of serum lipides in pigeons are reported here, together with observations on reliability of the K-agar precipitation technic.

Materials and methods. White Carneau and Show Racer pigeons ranging in age from 6 weeks to 7 years from Palmetto Pigeon Plant, Sumter, S.C., were maintained on commercial pigeon rations (mixtures of corn, peas, wheat, kaffir, and minerals). Blood samples were obtained from the alar vein. On one sample of serum (within one to 5 hours after drawing), the K-agar Δ values were determined(10). Another sample of whole blood was mixed with ACD§ solution and centrifuged, and the supernatant subjected to low-temperature alcohol fractionation, as described by Lever, *et al.*(11). This procedure yielded 2 fractions, one containing alpha-lipoproteins, and the other beta-lipoproteins. Lipides were extracted from each fraction with boiling alcohol and alcohol-ether, and purified with chloroform(12). "Total lipides" of each extract were determined by weight and an aliquot was used for determination of total cholesterol by the method of Zlatkis, *et al.* (13), as modified by Rosenthal, *et al.*(14). On another aliquot, lipide phosphorus was determined according to Stewart and Hendry (15).

Results. Values for K-agar Δ and total cholesterol in serum of sexually mature pigeons of the 2 breeds are shown in Table I. These birds were actively reproducing. No significant difference in levels of beta-lipoprotein exists among males of the 2 breeds. The extremely wide range of K-agar Δ values determined on female birds, however, suggested that these values were influenced by sequence of events in the ovulatory cycle, thus making difficult any comparison we could make between females of the 2 breeds. This suggestion is supported by results of K-agar Δ de-

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† The authors are indebted to Dr. J. S. King for electrophoretic analyses, and to Katherine King for valuable technical assistance.

‡ K-agar Δ is defined as optical density of sample of serum mixed with buffer and a solution of K-agar, minus optical density of a blank consisting of serum plus buffer.

§ ACD Solution: Citric acid monohydrate 0.8%, sodium citrate ($2H_2O$) 2.2%, and dextrose 2.2%.