semi-logarithmic paper. Background counts on the counting fluid alone at the 2 AMP settings used were subtracted from the cell counts. In order to present the counts for the 3 suspensions—fibroblasts, lymphocytes, and mixtures—on the same graph, the midpoints on the axis for the lymphoid cell counts were divided by a factor of 4 to account for the 4-fold difference in the AMP settings. Examination of the size distributions of 3 independent plots (Fig. 2 and 3) shows that with the lower threshold set at 15, all the Le-1 or L cells are counted. When set at 25, the counter selects 80% of the Le-1 cells, and when set at 30 it counts 90% of the L cells while excluding all lymphoid cells even when present in a proportion of 100:1.

Discussion and conclusions. The results presented above show that the selective counting of one or the other of 2 cell types, differing with respect to volume present in the same suspension, is possible with the aid of an electronic counter. This is accomplished by electronically screening out smaller or larger cells from the desired counting interval. From knowledge of the proportion of cells expected to be present within the limits of that counting interval, the total number of cells of a particular type present can be computed. The applicability of this discriminative counting procedure depends in large part on sufficient volume differences among the cell populations present. In the event the cell volumes closely overlap when present as mixtures the counter would be unable to resolve them into distinct populations. In the present studies, a tissue culture line of Lewis rat tumor cells having a size distribution of 12.5-24 μ in diameter could be selectively counted when present in suspensions containing a high (100:1) multiplicity of lymphoid cells having a diameter of 6-15 μ. The same was true of L cells present (diameter = 14.0-24 μ) in mixtures with mouse lymph node cells (diameter = 6-15 μ).

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Hemoglobin, White Cell Count, Packed Cell Volume and Weight Gain in Turkeys Fed Certain Antibiotics.*† (30284)

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The growth promoting effect of orally administered antibiotics in young animals has been well established. Only a few investigations have concerned the effect of continued antibiotic ingestion on hematology. Lichtman *et al*(1) treated Addisonian pernicious anemia in human patients with 2 to 5 g of chlorotetracycline (Aureomycin) orally each day. A definite but submaximal increase in hematocrit and red cells resulted. Toon and Wangensteen(2) reported that macrocytic or normocytic anemia produced in the rat by formation of blind intestinal segments could

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TABLE I. Effect of Continuous Ingestion of Certain Antibiotics on Hemoglobin, Packed Cell Volume and White Cell Count in Turkeys.*

Antibiotics	Mg/kg diet	Hemoglobin (g/100 ml)	P.C.V.†	White cells‡ per mm³
None	—	11.06	38.56	26,800[a]
Nystatin	22	11.15	41.33	22,400[bc]
Chlorotetracycline	110	11.22	39.63	20,050[c]
Zn-bacitracin	110	11.29	40.69	24,100[ab]
Chlorotetracycline	110			
+ nystatin	22	11.55	40.39	12,800[d]
Zn-bacitracin	110			
+ nystatin	22	11.77	41.55	12,700[d]

* Each hemoglobin, P.C.V. and white cell count is the average of 10 individual determinations.

† Packed cell volume.

‡ Values that are *not* followed by the same letter are significantly different from each other ($P < .05$).

be prevented by Aureomycin orally. Scudi *et al*(3) observed no significant changes in blood morphology following prolonged daily administration of crude bacitracin concentrates in dogs and monkeys. Squibb *et al*(4) reported that Aureomycin ingestion had no apparent effect on 8 blood constituents in New Hampshire hens. In this study Aureomycin did not affect red-cell count, hematocrit, hemoglobin, total serum proteins, riboflavin, ascorbic acid and vit. A. Serum carotenoid levels were higher in hens fed Aureomycin. Burgess *et al*(5) obtained similar results with chicks fed penicillin. Glick(6) observed a significant increase in white cell counts in chicks fed a diet containing 110 mg of penicillin per kg.

The data in this communication demonstrate that hemoglobin and packed cell volume were consistently higher in turkeys receiving dietary antibiotic supplements continually from day-old to 12 weeks of age. In addition, the striking effect of certain antibiotic combinations in decreasing white cell numbers is reported.

Methods. One hundred unselected, unsexed, day-old Broad Breasted Bronze poults were assigned at random to each of 6 experimental groups. The composition of the practical type, corn-soybean meal, basal diet was listed in a previous publication by Sullivan *et al* (7). The dietary protein level was 28% until the birds reached 8 weeks of age; after 8 weeks the protein level was decreased to 24% by adjusting the dietary levels of ground yellow corn and soybean oil meal. Dietary treatments consisted of the basal diet plus different feed-grade antibiotic supplements to furnish the levels listed in Tables I and II. The experimental diet and tap water were provided *ad libitum* throughout the 12-week experimental period. Each group of 100 birds was maintained on wood shavings litter in a 9′ × 12′ floor pen. Therefore, during the last 3 or 4 weeks of the experimental period, some stress from over-crowding was probably present.

Individual body weights and the feed efficiency of groups were recorded at 4-week intervals. After 12 weeks blood samples were drawn from the branchial vein of 10 birds (5 males and 5 females) in each experimental group. Hemoglobin and packed cell volume (hematocrit) determinations were run on each of these heparinized samples according to the methods described by Hawk *et al*(8). White cell counts were determined by the method of Natt and Herrick(9). Body weight and hematology data were subjected to analysis of variance. Duncan's(10) multiple range test was employed to compare treatment means when significant differences were detected.

Results. Data pertaining to hemoglobin, packed cell volume and white cell count are presented in Table I. Hemoglobin and packed cell volume were higher for birds in all groups fed antibiotic supplements, but the differences were not statistically significant ($P<0.05$). Hemoglobin levels were the highest in those groups receiving combinations of 22 mg/kg of nystatin with 110 mg/kg of

TABLE II. Effect of Continuous Ingestion of Certain Antibiotics on Body Weight Gain of Turkeys.

Antibiotics	Mg/kg diet	No. of birds	Avg 12-week body wt (g)*
None	—	92	3697[b]
Nystatin	22	58	3442[a]
Chlorotetracycline	110	94	3806[bc]
Zn-bacitracin	110	93	3919[cd]
Chlorotetracycline + nystatin	110 22	91	3887[cd]
Zn-bacitracin + nystatin	110 22	88	4060[d]

* Values that are *not* followed by the same letter are significantly different from each other ($P < .05$).

either chlorotetracycline or zinc-bacitracin. Birds receiving the combination of nystatin and zinc-bacitracin exhibited the greatest packed cell volume. White cell counts of birds in those groups receiving the control diet and diets with a single antibiotic supplement were within the normal range, although significant differences were evident. When combinations of nystatin with chlorotetracycline or zinc-bacitracin were fed, white cell counts were significantly reduced ($P<0.01$).

Body weight data are presented in Table II. Both chlorotetracycline and zinc-bacitracin increased body weight gains in relation to the control (no antibiotic); however, only the increase with zinc-bacitracin was statistically significant ($P<0.05$). Nystatin alone significantly reduced body weight gains as compared to the control. In the group receiving nystatin, only 58 of 100 birds survived the 12-week experimental period. This unusual mortality was due primarily to severe outbreaks of *Histomonas meleagridis* (Blackhead) during the 6th and 9th weeks of the experiment. Since the severe Blackhead outbreaks occurred only in the group receiving nystatin singly, it is possible that nystatin altered the intestinal microflora in favor of the protozoan, *Histomonas meleagridis*.

The effect of dietary antibiotics on hemoglobin level and the packed cell volume appeared to be similar and possibly related to their effect on body weight gain in young animals. Decrease in white cell numbers following ingestion of combinations of nystatin with chlorotetracycline or zinc-bacitracin could have been due to the inhibition of certain microorganisms. Folic acid and other B-vitamins needed for blood cell formation are synthesized by microorganisms in feces and floor litter. Mickelson(11) has reviewed the intestinal synthesis of vitamins in the non-ruminant. Intestinal synthesis of folic acid by *Escherichia coli* in chickens has been demonstrated by Miller and Luckey(12).

Summary. The continuous ingestion of combinations of nystatin with either chlorotetracycline or zinc-bacitracin significantly ($P< 0.01$) reduced white cell counts. Hemoglobin and packed cell volume were consistently higher in all groups receiving antibiotics, but the differences were not statistically significant ($P<0.05$). When the antibiotics were fed singly, white cell counts, hemoglobin levels and the packed cell volume were affected, but to a lesser extent. The combinations of nystatin with chlorotetracycline or zinc-bacitracin, and zinc-bacitracin alone increased body weight gains significantly ($P<0.05$) in relation to the control or nystatin alone.

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