cellular constituents show most of it. The behavior of reducing activity in normal and leukemic blood cells is paralleled by the findings as obtained by the Warburg technic. The immature cells have a purely oxidative metabolism without any aerobic lactic acid formation. In contrast, the more mature granulocytes show strong lactic acid formation under aerobic conditions(4). No significant changes are present between granulocytes from normal and leukemic blood(5).

Summary. Reducing activity in blood and bone marrow cells can be demonstrated with the aid of pp' diphenylene bis 2-(3,5 diphenyl tetrazolium chloride). Under the microscope, sites of reduction are indicated by small purplish granules due to the formation of insoluble formazan. A positive reaction is seen in the myeloid elements with the exception of the myeloblasts. Lymphocytes and monocytes show a varying degree of activity. Nucleated red cells and megakaryocytes are occasionally positive. In leukemic blood, myeloblasts are consistently negative while the more mature elements show reducing activity.

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Significance of the Intestinal Flora in Nutrition of the Guinea Pig.* (17665)

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Considerable work has been done to develop a purified ration which will give growth in guinea pigs equivalent to that obtained with commercial rations. In recent reports (1,2) from this laboratory it was shown that the growth was remarkably improved when gum arabic was added to the purified basal ration. Almost normal growth was obtained when in addition to gum arabic potassium acetate and magnesium oxide were used. The reason for the growth response obtained with gum arabic, potassium acetate, and magnesium oxide could not be explained with certainty. Since it seemed possible that this response was not based on a direct effect on the animal but on a beneficial effect on the intestinal flora, we have investigated the intestinal flora of the guinea pig. The chief purpose was to obtain some indication of the numbers of certain representative organisms and to determine whether there are great differences in the absolute and relative numbers when different diets are fed to the animals. Attention was also paid to the excretion of vitamins from animals on different rations.

Experimental. The procedure for growing the animals was similar to that used by Booth *et al.*(1). Two different rations were used. The *first* one, the synthetic basal ration, consisted of: sucrose 60.9%, casein (vitamin-free) 30%, salts IV[3] 4%, fortified soybean oil 4%, sucrose mixture containing B vitamins 0.8%, and choline 0.3%. The fortified soybean oil supplied 1.2 mg of β-carotene, 12 mg of α-tocopherol, 8 μg of calciferol and 0.2 mg of menadione per 100 grams of ration. The vitamin B mixture supplied 1 mg of thiamine hydrochloride, 1.4 mg riboflavin, 1 mg pyridoxine, 3 mg calcium pantothenate, 200 mg inositol, 10 mg niacin, 10 mg p-aminobenzoic acid, 0.04 mg biotin and 0.3 mg folic

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TABLE I.
Effect of Different Rations on the Number of Cecal Bacteria in Guinea Pigs.

	Ration	
	Basal	Basal + gum + K-acet. + MgO
No. of animals	16	18
Dry wt. of cecum, g	2.6	3.7
pH of cecal content	7.3	6.8
Aerobic bacteria per g dry cecal content	$6.9 \times 10^6 \pm 2.4 \times 10^6$	$9.9 \times 10^6 \pm 2.5 \times 10^6$
Anerobic bacteria per g dry cecal content	$6.8 \times 10^6 \pm 2.4 \times 10^6$	$28.9 \times 10^6 \pm 9.0 \times 10^6$
Coliform bacteria per g dry cecal content	$7.5 \times 10^4 \pm 4.2 \times 10^4$	$33.6 \times 10^4 \pm 8.4 \times 10^4$
Lactic acid bacteria per g dry cecal content	$4.7 \times 10^9 \pm 1.5 \times 10^9$	$12.6 \times 10^9 \pm 4.7 \times 10^9$

acid per 100 g of ration. Vitamin C dissolved in sucrose solution was fed with individual pipettes at a level of about 25 mg every other day. The *second* ration had the same composition, except that 15% gum arabic, 2.5% potassium acetate, and 0.5% magnesium oxide were added in place of an equivalent amount of sucrose.

As the cecum evidently is the main site of the intestinal flora, all the bacterial work was done on cecal contents. The technic was the same as that used by Nath *et al.*(4). The animals which had been on the experimental rations for 2 to 6 weeks were sacrificed, the ceca removed and the contents squeezed aseptically into sterile Petri dishes. Representative 0.5 g samples were transferred to sterile 50 ml water blanks containing glass beads and shaken thoroughly. From these initial 1:100 dilutions serial decimal dilutions were made up to 10^{-8}. Quantitative inoculation was then made of the respective dilutions into various culture media.

An estimate of the total viable population of aerobic and anaerobic bacteria was made by using the BBL thioglycollate medium. For culturing anaerobes the oat jar method was used and a period of two days at 37°C was allowed for incubation of the plates. Eosin methylene blue agar (Difco) was used for culturing coliform organisms and carrot-liver agar (Foster *et al.*(5)) for the lactic acid bacteria. In preliminary experiments enterococci and yeasts were cultured on special media. Since their numbers were very low and no correlation between them and the rations could be found, these groups of organisms were not counted in the main experiments. The results are summarized in Table I.

It may be seen that the dry weight of the cecal content is somewhat higher in the animals receiving the more complete diet. There is also a difference in the average pH of the cecal content, the pH being slightly alkaline in the animals of the basal group and slightly acidic in the other group. This appears at first somewhat strange, but evidently the final pH is not determined by the pH of the food but by the kind of microflora which has developed in the cecum.

In general the counts of aerobic, anaerobic, and coliform organisms per gram of cecal dry matter are very low, much lower than the values of Nath *et al.*(4) for rats on sucrose diets. Only the numbers of lactic acid bacteria are high, even higher than those of the rats; lactic acid bacteria seem to be predominant organisms in the cecum of guinea pigs.

The individual variations in the bacterial counts of different animals were very high. To be able to draw any conclusions regarding the differences in the two groups of animals statistical treatment of the results was used. The standard errors of the means are given in the table. In addition a "t-test" was made according to Fisher(6) to determine whether

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the differences found between the groups of animals were statistically significant. The P values were found to be between .3 and .4 in the group of aerobes, between .02 and .05 in anaerobes, between .01 and .02 in coliforms and between .1 and .2 "lactics." These values show that there are significant differences in the groups of anaerobic and coliform bacteria. In the groups of aerobic and lactic acid bacteria, on the other hand, the differences are not significant.

A more detailed picture concerning relationships between the ration and the intestinal flora of the animals can be obtained only by new extensive studies with large numbers of animals. According to the results obtained, it is evident that there are significant differences in the composition of the intestinal flora between the animals on the basal diet and those on the gum arabic, potassium acetate, and magnesium oxide containing diet, *i.e.*, between slower and faster growing animals. These differences can manifest themselves, *e.g.*, in an increased intestinal synthesis of vitamins or, on the other hand, in an increased destruction of vitamins, or possibly even in an increased synthesis or destruction of toxic substances.

An experiment was made to determine whether there are differences in the vitamin excretion from guinea pigs on the different diets. The vitamins investigated were riboflavin and folic acid. Six animals were put on the basal diet and six on the basal + gum arabic + potassium acetate + magnesium oxide diet. After 3 weeks on such a dietary regimen the animals were placed in metabolism cages for 2 days and the urine was collected. During this collection period the diets did not contain any riboflavin or folic acid. The amounts of riboflavin and folic acid in the urine were determined microbiologically. The results are given in Table II.

As can be seen there are differences in the excretion of both of the vitamins studied. Particularly the difference in folic acid excretion is very distinct. If the amounts excreted are compared with the levels ordinarily fed to the animals, it is evident that they are rather low. In spite of this they point to important differences in the vitamin economy of the animals on different diets. These differences might be in the synthesis, in the extent of retention, or in the rate of destruction of vitamins.

TABLE II.
Effect of Different Rations on Urinary Excretion of Riboflavin and Folic Acid.

	Ration	
	Basal	Basal + gum + K-acet. + MgO
No. of animals	6	6
Riboflavin excreted, μg/day	11.9	19.8
Folic acid excreted, μg/day	0.13	0.76

Summary. An investigation was made on the cecal flora of guinea pigs receiving a purified basal diet and the basal diet supplied with gum arabic, potassium acetate and magnesium oxide. Significantly more coliform and anaerobic bacteria were found in the ceca of animals on the more complete diet. The urinary excretion of riboflavin and folic acid was also greater in this group of animals. It seems probable that the beneficial effect of gum arabic as well as that of potassium acetate and magnesium oxide on the growth rate of guinea pigs is based on the formation and maintenance of a proper intestinal flora. Evidently there is a well balanced symbiosis between the host animal and the different bacteria, and the bacterial flora has important functions in the physiology of the animal. Artificial, purified diets can easily disturb this balance and thus produce nutritional disturbances in the animal. On a basal purified diet growth is always very poor. The gum arabic induces conditions in which a proper flora can develop. For a normal flora and normal growth other supplements like potassium and magnesium salts are usually needed.

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