stems, and leaves of treated plants; the level in the roots and leaves was significantly higher than in the checks. There was also a rise in the inorganic phosphorus content of stems and roots of check plants, but it was less than in treated plants. The leaves of check plants showed no increase as did the leaves of treated plants in this phosphorus fraction, indicating that the most pronounced effect of 2,4-D on phosphorus metabolism occurred in the leaves at this time.

Forty-eight hours after treatment the level of inorganic P in leaves, stems, and particularly roots of treated plants was significantly higher than in check plants. In the roots, stems, and leaves of uprooted plants it was about the same as in the check plants. One week after treatment inorganic P in the roots of treated plants had increased significantly and this coincided with a sharp decline in the leaves, indicating that it may have been translocated from the leaves to the roots. There was practically no change in the inorganic phosphorus fraction in treated stems. Although by this time the level in the leaves and stems of check plants also declined, there was no corresponding increase in the check roots as there was in the treated roots.

Inorganic P in leaves and stems of treated plants fluctuated in most instances like that in the check plants, but this fraction was consistent, and at most sampling dates, except the first, significantly higher in roots, stems, and leaves of treated plants.

This experiment provides a clue to the mode of action of 2,4-D, e.g., it may inhibit or interrupt the phosphate metabolism in the plant. These data and the fact that very small amounts of 2,4-D produce drastic effects suggest that 2,4-D may inhibit or poison the enzyme or system responsible for the hydrolysis or synthesis of the high energy phosphates.

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Colorimetric Method for Determination of Aureomycin, Carbomycin, Erythromycin, and Terramycin in Aqueous Solution

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We have observed that the acid hydrolyzates of Aureomycin (*1*), carbomycin (*2*), erythromycin (*3*), and Terramycin (*4*) react with the arsenomolybdate reagent to produce blue colored complexes. The optical density of the color formed has been found to be a function of the quantity of antibiotic present. Satisfactory results have been obtained with the following procedure.

Aliquots containing from 10 to 40 μg of antibiotic are added to colorimeter tubes and the solution evaporated to dryness using an air jet. Two milliliters of $6N$ H_2SO_4 and 1 ml of arsenomolybdate reagent (Nelson's [*5*] reagent diluted with 2 parts of distilled water) are added. The tubes are plugged with loose fitting corks and placed in a boiling water bath. After a 15-min heating period the tubes are cooled to room temperature and the contents diluted with 5 ml of distilled water. Color intensity is determined using a photoelectric colorimeter equipped with 660 mμ filter. A series of tubes containing known quantities of the antibiotic are prepared and treated simultaneously with the tubes containing unknown quantities of the antibiotic. The values obtained with this series of known solution are used to calculate the constants of Beers' law and to standardize the determinations.

The sensitivity of the method varies somewhat with the particular antibiotic under consideration. The practical working range for Terramycin and Aureomycin is from 2 to 40 μg/tube; for erythromycin it is 5–80 μg/tube; and for carbomycin it is 10–160 μg/tube. Smaller quantities may be determined if only 1 ml of distilled water is added after the heating period, and micro cells are used to determine the optical densities. Apparently the hydrolysis with 6 N acid is necessary to obtain maximum sensitivity of the method, and use of more dilute acid resulted in reduced sensitivity. Only Terramycin will reduce the arsenomolybdate reagent without a preliminary hydrolysis, and in this instance the working range has been found to be from 20 to 160 μg/tube. Some of the data collected in analyzing aqueous solutions containing known quantities of Terramycin are summarized in Table 1.

TABLE 1

ANALYSIS OF SOLUTIONS FOR TERRAMYCIN CONTENT

Solution	Terramycin added* μg/ml	Terramycin found† μg/ml
Distilled water	0	0
	10	9.7; 9.9; 10.2
	30	29.8; 30.7; 30.7
	100	98.6; 99.7; 101.5
2% Glucose	0	0
	10	9.5; 9.7; 10.0
	30	27.7; 30.6; 30.6
2% Starch	0	0
	10	9.1; 9.7; 9.7
	30	28.8; 29.0; 29.6

* Terramycin hydrochloride was used in these studies. All analyses are presented in terms of the free base.

† Antibiotic extracted from aqueous solution with methyl isobutyl ketone.

This method cannot be applied directly to solutions containing carbohydrates and other substances which react when heated with the arsenomolybdate reagent. These four antibiotics may be separated from carbohydrates by extraction from aqueous solution (pH 7.2) into chloroform, amylacetate, n-butanol and methylisobutyl ketone. All the antibiotic has been recovered in the solvent phase when equal volumes of the solvent and aqueous solution have been used.

The arsenomolybdate reaction employed in this study measures the presence of reducing substances present in the samples after acid treatment. Other reagents for determining reducing substances may be substituted for the arsenomolybdate reagent, including Fehling's reagent and the Folin-Malmros reagent. The arsenomolybdate reagent was preferred as smaller quantities of the antibiotics could be determined than when these other reagents were used.

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Effect of Strenuous Physical Activity on Blood Vitamin A and Carotene in Young Men

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In a recent study of the effects of certain proteins in the diet on the utilization of carotene by growing albino rats (*1*), one of our metameters was the vitamin A content of the blood, micrograms vitamin A/100 ml serum. For groups of 11 rats the average values of this metameter, determined at 3-day intervals, changed in definite consistent patterns during the 38-day vitamin A depletion period and the subsequent 6-wk carotene repletion period. Unexpectedly, for each of the individual 44 experimental animals, puzzling irregular large fluctuations of the blood vitamin A values were observed throughout the experiment. The closely controlled conditions of the experiment and the magnitude and irregularity of the fluctuations suggested that a factor (or set of factors), readily available, immediately effective, and more potent than dietary protein in affecting the blood vitamin A level, existed within the body of the rat. A factor possibly capable of meeting these specifications is physical activity.

Since an estimate of the effect of physical activity on blood vitamin A values is more easily made with human beings as the subjects than with rats, a preliminary test of the effect of physical activity was made with the cooperation of Coach A. C. Moreau and twelve members[2] of the Louisiana State University track team. Vitamin A and carotene analyses were made by the method of Bessey *et al.* (*2*) on samples of finger blood collected from each man about 3 min before the start of a 40–50 min period of strenuous physical activity which consisted of a 15-min "warm-up" period, followed by running five or six 220-yd dashes at full speed (26–29 sec/dash, at intervals of 5 min). About 6 min after the completion of the last dash, the 2nd sample of blood was collected. The exercises were performed between 3 and 4 P.M. Samples of blood were collected also at these times from 2 controls who remained seated while the 12 men were running.

In Table 1 are given the observed levels of serum vitamin A before and after the exercise, as well as the per cent change following exercise; similarly, in Table 2 are given the carotene values.

TABLE 1

Subject no.	μg vitamin A/100 ml serum			Per cent change
	Before exercise	After exercise	Change	
1	25.2	43.7	18.5	73
2	30.4	62.7	32.3	106
3	44.9	61.6	16.7	37
4	51.0	74.8	23.8	47
5	46.4	36.0	−10.4	−22
6	48.4	68.6	20.2	42
7	39.3	69.1	29.8	76
8	55.9	67.0	11.1	20
9	34.3	44.0	9.7	28
10	31.2	49.0	17.8	57
11	40.7	56.8	16.1	40
12	29.4	48.9	19.5	66
Controls				
13	95.8	92.0	− 3.8	− 4
14	61.7	73.3	11.6	19

The average blood vitamin A level of the group increased 43% during the work-out with individual changes varying from an increase of 106% to a decrease of 22%; the average carotene level decreased 10%, with individual changes varying from +17 to −50%.

According to the coach, subject 2, whose vitamin A

TABLE 2

Subject no.	μg carotene/100 ml serum			Per cent change
	Before exercise	After exercise	Change	
1	63.1	60.3	− 2.8	− 4
2	79.9	93.4	13.5	17
3	93.4	85.6	− 7.8	− 8
4	101.4	98.6	− 2.8	− 3
5	113.5	56.8	−56.7	−50
6	131.4	103.1	−28.3	−22
7	104.7	113.9	9.2	9
8	154.4	141.0	−13.4	− 9
9	143.9	97.4	−46.5	−32
10	117.7	127.4	9.7	8
11	126.9	123.0	− 3.9	− 3
12	63.1	57.5	− 5.6	− 9
Controls				
13	143.4	133.7	− 9.7	− 7
14	94.6	109.3	14.7	16

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