part in the reaction showed a marked increase over that of controls. In an attempt to analyze the significance of the various factors which might play a part, the leucocytes and the streptococci were treated in various ways before setting up the tests for phagocytosis. It was found (1) that when leucocytes were well washed of plasma, (2) that when the leucocytes were treated with varying dilutions of the drug and then well washed, or (3) that when the streptococci were treated with varying concentrations of the drug and then washed, no more phagocytosis occurred than in controls. It appears that serum or a factor in it is necessary to obtain the effect of the drug.

The protocol given is typical of the results obtained when sulfanilamide or Prontosil was used. These drugs

THE EFFECT OF VARYING DILUTIONS OF SULFANILAMIDE ON PHAGOCYTOSIS OF HEMOLYTIC STREPTOCOCCI

Dilution of drug	Number of cocci phagocytosed	Percentage of leucocytes taking part
1/1000	192	20 per cent.
1/10,000	246	32 " "
1/50,000	514	60 " "
1/100,000	296	48 " "
1/250,000	82	12 " "
Control	96	16 " "

clearly enhanced the phagocytosis of hemolytic streptococci *in vitro*. Fresh serum or plasma appeared to be necessary for the completion of the reaction. Not only did these drugs increase the number of bacteria phagocytosed per leucocyte but also the number of leucocytes taking part in the reaction.

While our work was in progress, a paper by Osgood[4] appeared in which are reported results essentially the same as ours; he used bone-marrow cultures as leucocytic suspensions. He concluded that increased phagocytosis is due to a neutralization of the bacterial toxins by sulfanilamide and that the drug has no direct effect on either the leucocytes or bacteria. The increased phagocytosis in the extremely high dilution ranges that we observed compares very favorably with his findings.

Just how sulfanilamide enhances phagocytosis is not clear, but our results briefly reported here suggest that sulfanilamide or a serum-sulfanilamide complex acts as an opsonin.

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INCREASE IN VITAMIN A ACTIVITY OF CORN CAUSED BY DOUBLING THE NUMBER OF CHROMOSOMES[1]

A QUANTITATIVE gene action in corn has been demonstrated by comparing the carotinoid content of pure yellow diploid and tetraploid strains. Since tetraploid corn contains twice as many chromosomes as ordinary diploid corn, it was of interest to see what effect doubling the number of genes for yellow would have on the amount of pigment. The interesting possibility presented itself that the vitamin A activity of corn might be increased by formation of the tetraploid.

The diploid corn was found to contain 0.0267 $\pm$ 0.0004 milligrams and the tetraploid corn 0.0380 $\pm$ 0.0007 milligrams of carotinoid per gram of dry meal. These values are the means of seventeen analyses of the diploid and sixteen of the tetraploid together with their standard errors. Thus the doubling of the number of chromosomes and genes for yellow, resulted in an increase of 43 per cent. in the total carotinoid content per unit weight. There was the same percentage increase in vitamin A activity, since both the active carotinoids, beta carotin and cryptoxanthin, and the inactive zeaxanthin were increased to the same extent. Approximate measurements showed no difference in the density of diploid and tetraploid kernels. Hence the 43 per cent. increase per unit weight is also the increase in carotinoid content per unit volume.

The volume of the endosperm cells in the tetraploid yellow corn used in these analyses was approximately 3.5 times as great as the volume of the endosperm cells of the comparable diploid, as determined by direct measurements of cell dimensions in different regions of the endosperm. Because of this marked increase in cell size which resulted from chromosome doubling, the concentration of the genes per unit volume was actually less in the tetraploid than in the diploid, even though the individual cells of the former contained twice as many genes as did the cells of the latter. However, the amount of carotinoid per cell was five times as great in the tetraploid as in the diploid. In terms of gene concentration within the endosperm tissue, there was in the tetraploid 2.5 times as much carotinoid per gene as there was in the diploid. These proportional differences may be summarized as follows:

	Diploid	Tetraploid
Cell volume	1	3.5
Carotinoid per unit volume	1	1.43
Carotinoid per cell	1	5
Genes per cell	1	2
Genes per unit volume	1.75	1
Carotinoid per gene	1	2.5

The diploid and tetraploid strains of yellow corn selected for this study were produced by crossing the F_1 hybrid between an inbred line of Webbers Dent

[4] E. A. Osgood, *Jour. Amer. Med. Asn.*, 110: 349, 1938.

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Departments of Botany and Dairy Industry, New York State College of Agriculture, aided by a grant from the Committee on Radiations of the National Research Council.

and Illinois A with an inbred line of Luces Favorite. Individual tetraploid plants induced in this three-way hybrid by the heat treatment technique of Randolph[2] were mass pollinated during two subsequent generations and from this tetraploid strain samples of grain from 10 ears selected at random were taken for analysis. Diploid sister plants of the original tetraploids were intercrossed in a similar manner for two generations to provide a comparable diploid strain for comparison with the tetraploid.

For the fractionation and determination of the carotinoids the procedure of Kuhn and Brockmann[3] was adopted with certain modifications. The pigments were extracted directly from the corn meal with anhydrous methyl alcohol, saponified and fractionated with petroleum ether. For determining concentration we used a photoelectric colorimeter equipped with Corning glass filters 428 and 585 and calibrated against standard solutions of crystalline beta carotin.

We attribute the observed differences between diploid and tetraploid yellow corn to quantitative rather than qualitative gene differences, since the comparison was made between strains having a common origin and an essentially identical genetic constitution. There is a possibility that tetraploid corn will be of practical importance due to its increased vitamin A activity.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SIMPLIFIED SCHAEFFER SPORE STAIN

BEGINNING students of bacteriology frequently find it difficult to follow the original Schaeffer[1] technique, which calls for heating a flooded slide over an open flame, without breaking the slide. Consequently, the following technique was first worked out for beginning students in bacteriology at the Agricultural and Mechanical College of Texas, and since then, over a period of time and with a variety of cultures, it has given better results than the technique originally described. In addition, the method of drying and the time of staining have been modified.

A simple, inexpensive steam bath, perhaps a tin can or beaker of proper diameter or a metal tray about three inches deep and two inches wide, on an asbestos centered wire gauze, is used to heat the slides.

(1) Smears, prepared from spore suspensions, are dried for staining by laying the slide on the table top near the base of the burner used for heating the steam bath.

(2) Dried slides are placed across the steam bath until definite droplets of water collect on the bottom of the slide.

(3) The slides are then flooded with 5 per cent. aqueous malachite green and left on the steam bath for one minute.

(4) Stained slides are removed from the steam bath with the thumb and index finger of one hand and dropped into cool water. This is done by spanning the length of the slide. The overhanging ends of the slides are cool enough to do this without danger of burns.

(5) The slides are thoroughly rinsed and, while still wet, are counterstained with 0.5 per cent. aqueous safranin for thirty seconds and again rinsed in cool water.

(6) Rinsed and dried slides are easily examined under the microscope. As in the original Schaeffer staining technique, the spores stain green and the vegetative cells stain red.

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A METHOD FOR FIXING AND STAINING EARTHWORMS

NOTHING furnishes more satisfactory material for classroom work than sections of earthworms properly fixed and stained. In almost every course in general biology or zoology some time is devoted to the histological study of the common earthworm. It is unfortunate that the material used is often poorly fixed and the sections do not show up well. I have found the method given below simple, quick and one which gives excellent results.

Collect several earthworms and rinse off the dirt. Place these in a covered dish and sprinkle a small amount of well-sifted corn meal and powdered agar mixed in equal proportions on the bottom of the dish. Some finely chopped lettuce may also be added. Cover the worms with a moist paper and leave in a cool dark place. Transfer the worms to clean dishes and change the food each day for three days. By this time their alimentary tract should be free of all dirt and grit.

The specimens are fixed by cutting them into pieces about three fourths of an inch long and dropped directly into warm (about 50° C.) Allen's B-15. Fix for twelve hours and then rinse in water and run through 35, 50, 70, 80, 95 and two changes of 100 per cent. alcohol. Leave in each alcohol one hour. Now run

[2] L. F. Randolph, *Proc. Nat. Acad. Sci.*, 18: 222–229, 1932.

[3] R. Kuhn and H. Brockmann, *Zeit. Physiol. Chemie.*, 206: 41, 1932.

[1] Alice B. Schaeffer and McDonald Fulton, SCIENCE, 77: 194, 1933.