It was observed that the MEC of all the drugs apparently restrained inocula up to 25,000 cells per cc, but that visible growth invariably occurred with larger inocula, even when much greater amounts of the drugs were added to the cultures. In view of these results it was considered possible that the bacteria might be capable of undergoing a *definite, limited number of cell divisions in the presence of any effective drug concentration, regardless of the inoculum employed.* This hypothesis was examined by inoculating decreasing numbers of *E. coli* into media containing a bacteriostatic concentration of sulfathiazole (5×10^{-4} M), counting the number of viable organisms which developed from each inoculum at 6, 12 and 24 hours, and computing the number of cell divisions from the following formula:

$$S = A \frac{(r^n - 1)}{r - 1}$$

where S = count on developing culture
A = number of cells in inoculum
R = factor of increase (2)
N = number of cell divisions.

The results are recorded in Table II, and show that all the inocula underwent almost exactly the same

TABLE II

THE UNIFORM RESTRICTION OF CELL DIVISION BY SULFATHIAZOLE WITH VARIOUS INOCULA OF E. COLI

Inoculum per cc	Count at 6 hours	Number of divisions	Count at 12 hours	Number of divisions	Count at 24 hours
11,800,000	152,000,000*	3.61	170,000,000	3.80	116,000,000
1,180,000	58,000,000*	5.60	64,000,000	5.70	15,000,000
118,000	13,500,000*	6.85	14,000,000	6.86	5,180,000
11,800	1,190,000	6.65	1,600,000	7.08	267,000
1,180	112,000	6.56	110,000	6.54	5,800
118	11,000	6.54	11,600	6.61	210
11.8	1,180	6.64	1,250	6.72	Sterile

* Visible growth.

number of cell divisions within the first 6 hours, with the exception of the largest inoculum, which actually divided fewer times. At 12 hours no further significant change had occurred; at 24 hours the number of organisms had diminished.

The conclusion seems warranted that, in the presence of a bacteriostatic concentration of a sulfonamide, bacteria possess the ability to undergo only a certain limited number of cell divisions, regardless of the size of the inoculum. This readily explains why no apparent bacteriostasis may be observed when large inocula are employed, since even a small number of cell divisions will bring such cultures into the range of visible turbidity (*circa* 10,000,000 cells per cc). For example, Table II shows that cultures inoculated with more than 50,000 *E. coli* per cc attained visible growth, whereas those which received smaller inocula remained clear. The number of cell divisions in each culture, however, was approximately the same. Furthermore, the data also offer a reasonable explanation for the well-known but previously obscure finding that organisms subjected to the action of the sulfonamides grow as rapidly as do controls for a few hours before bacteriostasis becomes manifest.

The reason for this phenomenon is as yet unknown, but it seems possible that the bacterial cell contains a substance necessary for reproduction which is synthesized under normal conditions of growth. In the presence of bacteriostatic concentrations of the sulfonamides the synthesis of this substance is prevented, and the organism is forced to distribute its original supply in diminishing amounts to its progeny. After a certain number of cell divisions the quantity of the substance in the individual organisms becomes insufficient to permit further multiplication. PAB may be concerned with this substance in some way. The observed facts indicate that the antagonism between PAB and the sulfonamides is independent of the number of bacteria, but instead is related principally to critical concentrations of these compounds.

HARRY M. ROSE
CHARLES L. FOX, JR.

DEPARTMENTS OF MEDICINE
AND OF BACTERIOLOGY,
COLLEGE OF PHYSICIANS AND SURGEONS,
COLUMBIA UNIVERSITY

THE RELATIVE EFFICIENCY OF STRAINS OF RHIZOBIUM TRIFOLII AS INFLUENCED BY SOIL FERTILITY[1]

Arachis hypogaea (peanut) was recently tested in the greenhouse to determine how the response to phosphatic and potassic fertilizers is conditioned by the strains of Rhizobia with which the plants are inoculated. The results suggested that certain cultures were better adapted than others to fix nitrogen in poorly nourished plants, although the cultures differed only slightly in efficiency in well-nourished plants. Since the design of this preliminary experiment did not permit reliability to be placed on the small differences observed, a second, more comprehensive experiment was set up, using *Trifolium pratense* (mammoth red clover) and proper cultures of Rhizobia.

In the second experiment five cultures of *Rhizobium trifolii* were compared on clover grown in Plainfield sand receiving four fertilizer treatments. Cultures 209, 238 and 239 were obtained from the University of Wisconsin, and cultures H and D were isolations from old commercial cultures which had been in the laboratory for two years. The four fertilizer treatments were (1) no fertilizer, (2) P, 100 p.p.m., (3)

[1] Journal Paper No. 9 of the Purdue University Agricultural Experiment Station.

K, 50 p.p.m., and (4) P, 100 p.p.m.-K, 50 p.p.m. Treatments were based on parts per million of elemental phosphorus supplied as $Ca(H_2PO_4)_2 \cdot H_2O$ and elemental potassium supplied as KCl. The soil, when gathered from the field, was deficient in nitrogen, phosphorus and potassium. Commercial seed was surface sterilized, inoculated as desired, and planted, ten seeds to each pot, in steam-sterilized, limed (to pH 6.5) soil. Five-inch pots were used as containers. The experiment was set up in seven randomized blocks. The pots were seeded on April 19, 1941, and harvested on July 15, 1941. Total dry weight and total nitrogen content of the plants were measured.

TABLE I

Culture	Gms. dry wt. produced* in soil fertilized as indicated				Mgs nitrogen produced* in soil fertilized as indicated			
	None	P, 100 p.p.m.	K, 50 p.p.m.	P, 100 p.p.m.-K, 50 p.p.m.	None	P, 100 p.p.m.	K, 50 p.p.m.	P, 100 p.p.m.-K, 50 p.p.m.
209 ...	21.5	19.3	21.5	24.9	515	463	478	604
238 ...	19.3	17.9	22.5	25.5	455	427	500	584
239 ...	18.8	17.5	24.6	27.3	448	414	557	648
H	18.3	16.7	24.0	26.3	461	416	562	636
D	15.5	15.1	19.8	19.4	293	323	419	432
No culture	16.3	15.0	17.1	18.1	307	298	330	324

* Total from the seven replications.

The results of this second experiment (Table I) confirmed the suppositions made from the data of the first experiment. The dry weight and total nitrogen content of plants inoculated with cultures 209 and 239, with and without the addition of potassium, may be taken as an example. When inoculated with culture 209, plants produced a somewhat greater total dry weight and total nitrogen content in unfertilized soil than plants inoculated with culture 239. However, when inoculated with culture 209 dry weight and total nitrogen content of plants do not increase as a result of fertilization with potassium, whereas the increase in dry weight and total nitrogen content of plants inoculated with 239 is quite appreciable. From this it appears highly probable that culture 209 is better adapted to fix nitrogen in potassium deficient clover than culture 239, whereas 239 is better adapted to fix nitrogen in well-nourished plants than is 209. This single example suffices for the purpose of this report.

An analysis of variance of the data on dry matter produced, and subsequent calculation of the mean squares for individual degrees of freedom for interaction between culture and fertilizer treatments, indicates that in the example used above differences are highly significant. In the data on total nitrogen content of the plants, differences in the example cited are highly significant if the data relative to plants inoculated with cultures 209 and 239, with and without potassium, are isolated from the remaining data and analyzed. This procedure is believed justified since there are heterogeneous factors contributing to experimental error in the nitrogen data. For example, several plants receiving no inoculum became contaminated, thus raising the per cent. nitrogen content unduly high. Contamination was less likely in the inoculated groups of plants.

There is no evidence that fertilization with phosphorus changed the relative order of efficiency of the cultures studied.

The occurrence of strains of Rhizobia particularly well adapted to potassium deficient plants is not unexpected in view of what is known of the behavior of Rhizobia. Wilson, Burton and Bond,[2] as well as others, have previously shown that strains of Rhizobia differ in their adaptation to physical and chemical conditions prevailing in given varieties of leguminous plants. For example, two strains of Rhizobia may be of comparable efficiency on one variety of a leguminous plant but differ greatly in their efficiency on another variety of the same species of leguminous plant. However, it seems to us that the demonstration of cultures especially suited to potassium deficient plants has added significance because it suggests the possible development of commercial inoculants especially suited for use with legumes to be grown on particular soil types.

JAMES L. ROBERTS
FRANK R. OLSON
DEPARTMENTS OF BOTANY AND AGRONOMY,
PURDUE UNIVERSITY AGRICULTURAL
EXPERIMENT STATION

THE EFFECT OF ARTIFICIAL CHANGES IN THE BRAIN OF MAZE-LEARNING IN THE WHITE RAT

ONE of the authors has published a number of papers[1, 2, 3, 4] on the effect of injections of the pituitary growth hormone (Antuitrin G and Phyone) in tadpoles and in pregnant white rats on the proliferation of brain cells in the young. In general the mammals at birth showed an increase of about 36 per cent. in weight of the cerebral hemispheres, but only about 19 per cent. in body weight. This differential is further emphasized by the fact that the number of cells per volume of cortex increased 86.5 per cent. over that of the controls. Moreover, the increase in volume was

[2] P. W. Wilson, J. C. Burton and V. S. Bond, *Jour. Agr. Research*, 55: 619, 1937.

[1] S. Zamenhof, "Possibilities of Increasing the Higher Functions of the Cortex," pp. 1–28. Lancaster: Science Press, 1940.

[2] *Idem, Growth*, 5: 123–139, 1941.

[3] *Idem, Nature*, 148: 3744, 143, 1941.

[4] *Idem, Physiol. Zool.*, 1942. (In press).