of the propositus are mixed in a complete broth tube, and incubated for 6–24 hr. The mixed culture is then harvested, and the washed cells are plated on a minimal agar medium containing 100–1,000 μg/ml streptomycin. The minimal agar selects prototroph cells; the streptomycin selects S^r. The minimal streptomycin agar thus permits the growth only of $T + L + B_1 + S^r$ colonies and suppresses the two parents. This assortment of characters can arise either by recombination, or by mutation of the propositus from S^s to S^r. Fortunately, this mutation occurs at an extremely low rate, about once per 10^{10} cell divisions (*6*), and therefore confusion between recombinants and mutants is minimized. On the other hand, the improbable coincidence of three reverse mutations needed to produce a prototroph from W-1177 has never been observed in extensive controls (*1, 2*).

The principal function of the screening procedure is the rational selection of cultures appropriate for more detailed analysis by the development of auxotroph mutants. Even in this preliminary test, however, recombination of unselected markers (V_1, *Lac, Mal,* etc.) among the S^r prototroph selections usually verified the occurrence of genetic interchange.

Two groups of cultures have been screened so far for cross-fertility with W-1177 (i.e., K-12). About 40 cultures from chicken cecal flora (supplied by courtesy of S. Shapiro) yielded one isolate that crosses, but very poorly. About 100 isolations from human urine cultures (secured through courtesy of the Wisconsin State Laboratory of Hygiene) have given 8 that cross with about the same facility as K-12, and an equal number that appear to be less fertile (if fertile at all), so that the evidence for recombination in the latter is still inconclusive. The possibility that some ecotypic differentiation is revealed by the breeding test deserves further study when it is recalled that K-12 is also of human origin.

Nutritional mutants are being prepared in the new isolates. The three cultures so far tested cross freely with each other, as well as with K-12 and within each strain.

The new strains differ in a number of characteristics, including fermentation patterns (3 are sucrose-positive; 6, sucrose negative; one is a lactose-negative "paracolon" type), colony morphology (*R, S,* and intermediates by the acriflavine test), and patterns of resistance to and production of colicins (*7*) and phages. Preliminary serological studies are under way, in addition to experiments to uncover cryptic genetic differentiation. There is a strong suggestion that colicin and lysogenicity interactions may act as genetic isolation mechanisms.

Unfortunately, the survey method does not reveal other intrafertile, intersterile breeding groups, nor, owing to the dominance of S^s (*8*), can it reveal unreduced diploid hybrids between the different strains. Despite these shortcomings, however, the streptomycin-prototrophy selection method has succeeded in displacing strain K-12 from its position as the only "sexual" bacterium.

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The Mode of Action of Growth Substances and Growth Inhibitors

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Previous experiments have proved (*1*) that alcoholic extracts of different plants show various effects in regard to elongation of cells. Interpreting these results, it is reasonable to suppose there are growth-promoting and growth-inhibiting substances in the plants. Amounts of the two types of growth regulators vary in different kinds of plants. Extracts of *Brassica* sp. show a strong growth-promoting effect, whereas extracts of *Syringa* produce a strong growth-inhibiting effect.

It was therefore of interest to study the effect of a mixture of growth substances and inhibitors by means of the *Avena* test. Larsen (*2*) used the Went test, taking the angle of curvature for a criterion of the effect, but his results are not very clear and are difficult to survey. Hence the question was studied again by means of Linser's (*3*) paste method, permitting measurement of the coleoptyl elongation (*Z*) and the angle of curvature (α°).

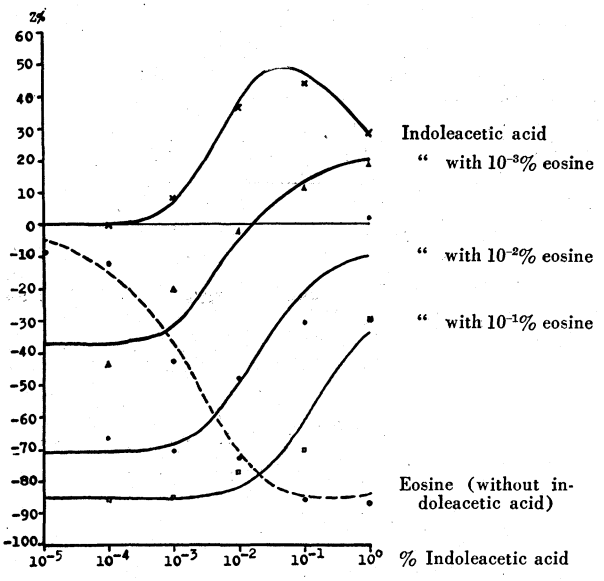

FIG. 1. Mixtures of indoleacetic acid and eosine in different concentrations. The solid lines show the curves calculated for the different mixtures of indoleacetic acid and eosine. On the left, the curve of action is calculated for eosine. The corresponding experimental values are marked as single points.

The growth substances tested in these experiments were indole-3-acetic acid (I-3-E) and an extract of Brussels sprouts; the inhibitors were eosine and a *Syringa* extract. Values for Z in the mixture I-3-E/eosine in different concentrations are shown in Fig. 1. The curves of the other possible mixtures of the above-mentioned substances were analogous but differed from those of Fig. 1 because of varying efficacy of the original substances.

The following ideas about the mode of action of the growth substances and growth inhibitors provide a possible explanation of the results.

The growth-inhibiting or growth-promoting substances must be converted into a part of the living substance, being adsorbed by molecules of "living structure." It is reasonable to postulate that in the molecular system of the living substance there are spaces that can be occupied by the molecules of the growth regulator. This means that the molecule of the growth regulator has a certain affinity for a certain "space." In this connection the ideas of R. Kuhn (*4*) about the mode of action of the sulfonamides may be recalled.

Our hypothesis postulates two kinds of spaces: The first filled in by growth substances effects a growth promotion; the second type also shows a certain affinity, but in this case growth inhibition results.

It is immaterial whether there is a molecular process of self-reproduction, as postulated by Dehlinger (*5*), Neugebauer (*6*), Jordan (*7*), or Friedrich-Freska (*8*), with the growth substance as a "brick" of the molecule of the living substance, or whether the active substance is effective by adsorption in a certain place with a specific metabolic function influencing the growth process.

Any organic molecules brought from outside into the plant will not ordinarily be able to fill the "spaces" of the growth substances with the necessary active compound for the growth effect. The molecules accumulate somewhere on the living structures, where they disturb their functions and exert an inhibiting action. The growth substances, besides promoting growth, have also an inhibiting effect, although the latter is less probable. Thus every growth substance consists of a promoting and an inhibiting compound.

From these theories, the following equation, which should apply to the experimental results, and which shows the relation between the increase of elongation (Z) and concentration of growth substances (c), can be derived:

$$Z = A(1 - e^{-k_1 c_1}) - B(1 - e^{-k'_1 c_1}) .$$

For growth inhibitors the formula is:

$$Z = -C(1 - e^{-k_2 \sqrt{c_2}}).$$

Finally, for mixtures of growth substances and growth inhibitors, the formula is:

$$Z = A(1 - e^{-k_1 c_1 e^{-l_2 \sqrt{c_2}}}) - B(1 - e^{-k'_1 c_1 e^{-l'_2 \sqrt{c_2}}}) - C(1 - e^{-k_2 \sqrt{c_2} \cdot e^{-l_1 c_1}}).$$

A, B, and C denote figures, which are proportional to the number of places of adsorption ("spaces") mentioned above; k and l denote probabilities of hits per unit of concentration. If the constants are chosen correctly, the theoretical curves can be brought into agreement with the curves established by the experiments. The curves for the mixtures I-3-eosine (Fig. 1) could be derived mathematically from the values for the different substances with good conformity.

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Desoxyribonucleic Acid Content of Rat Liver Nuclei Influenced by Diet

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That the desoxyribonucleic acid (DNA) content of all diploid cells of an animal, and the species, is constant has been suggested by a number of investigators (*1–4*). This would exclude the possibility that diet influences the DNA content of nuclei. Kosterlitz *et al.* (*5, 6*) and Davidson (*7*) suggested that the DNA content of liver nuclei of rats was not influenced by fasting or by protein-free diets.

Nuclei in sections of livers of rats on a protein-free diet were observed to stain more intensely by the Feulgen nuclear reaction than those of rats on the laboratory stock diet, and the results of the present investigation obtained by both chemical and cytochemical methods indicate that the diet does influence DNA content of nuclei of rat livers.

Male rats from the Albino Farms, Red Bank, N. J., 130–160 g, were maintained on one of three diets: Fox Food Blox (Allied Mills), which contained a minimum of 26% protein; a semisynthetic diet containing 12% washed casein (Eimer and Amend); and a protein-free diet (Table 1).

Nuclei were prepared from the livers with 5% cold

TABLE 1

COMPOSITION OF DIETS

	12% casein diet (%)	Protein-free diet (%)
Casein	12.0	0.0
Corn oil	5.0	5.0
Dextrose	77.0	89.0
Salt mixture	4.0	4.0
Rice bran extract (Vitab)	2.0	2.0
Riboflavin	0.002	0.002