varying "aggregate" concentrations (Fig. 2). The degree of inhibition bears an inverse relationship to the concentration of "aggregate" or probably more properly DNA.

The actinomycin D effect can be reversed by the addition of calf thymus or HeLa DNA to the enzymatic reaction (Fig. 3). In this experiment 2.4 mg protein of HeLa "aggregate" and 4 μg/ml of actinomycin D were used. Heat-denaturation was accomplished by heating a solution of calf thymus DNA (2 mg/ml) in 0.01*M* Tris buffer, *p*H 7.4 and 0.01*M* NaCl at 100°C for 3 minutes and then rapidly cooling it in ice. Heat-denatured DNA appeared to be more effective than native DNA. Single-stranded DNA, which complexes less readily with the antibiotic (*3*), may act as a substitute for the inactivated natural DNA template. Yeast RNA at about 100 times the concentration of DNA used in the experiment described above, relieved the inhibition only in part.

The inhibition of the RNA polymerase system in HeLa cells by minute amounts of actinomycin D and its reversal by addition of DNA support the mode of action proposed by Reich *et al.* (*5*) for this antibiotic in the intact cell (*8*).

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Magnesium Binding as an Explanation of the Mode of Action of Novobiocin

Abstract. Novobiocin affects a wide variety of biochemical and biological processes in bacteria, whereas most other antibiotics seem to affect one specific process. All of the processes known to be affected by novobiocin require magnesium ions, and in a number of cases magnesium deficiency mimics the action of the antibiotic. Evidence is presented that novobiocin complexes with magnesium ions.

Earlier work has shown that the action of novobiocin is reversed by magnesium ions (*1*) and that the antibiotic affects the permeability of *Escherichia coli* ML 35 (*2*). Recently a number of other effects of novobiocin have been noticed. A search for a common element in these effects revealed that all of the processes inhibited require magnesium ions. In addition, novobiocin induces some effects that are also brought about by magnesium deficiency.

Since novobiocin is an inhibitor of growth, it is essential that any effects noted should be attainable at concentrations of the antibiotic which inhibit growth. However, when the antibiotic is added to growing cells of a sensitive organism (*Streptococcus faecium*), growth continues for a period of time. The degree of residual growth is less at higher concentrations of novobiocin. However, it is only at a concentration of $10^{-3}M$ that growth stops immediately after the antibiotic is added, although a concentration of $10^{-6}M$ will eventually bring about complete inhibition. In short-term biochemical experiments with nongrowing cells, inhibitions achieved at $10^{-3}M$ are therefore considered significant in explaining growth inhibition.

A summary of the magnesium-related processes affected by novobiocin is given in Tables 1 and 2.

Novobiocin is not a general cytotoxic substance, since cells can be incubated for several hours in the presence of $10^{-3}M$ concentrations with no significant killing. Further, the accumulation of C^{14}-uracil in the cell pool is not inhibited but is in fact three times higher than in the control. The incorporation of C^{14}-lysine into the cell wall is only slightly affected by concentrations that completely prevent lysine incorporation into soluble RNA.

Webb (*3*) has shown that zinc ions potentiate magnesium deficiency. Zinc ions also potentiate novobiocin action against *Escherichia coli*. Sodium azide inhibits most of the same processes inhibited by novobiocin and, when used at subinhibitory concentrations, also potentiates novobiocin action against *E. coli*.

Novobiocin is able to form insoluble salts with a variety of metal ions (magnesium, calcium, manganese, and zinc). In addition, novobiocin is able to form weak complexes of another type with magnesium ions, as shown by the following observations:

1) An aqueous mixture of 0.01*M* novobiocin monosodium salt and 0.01*M* $MgCl_2$ at *p*H 7.5 or 10.0 fluoresces under ultraviolet light when frozen or when dried on filter paper but does not fluoresce in the liquid state. A fluorescent complex is not formed with sodium or calcium ions.

2) Mixtures of 0.01*M* novobiocin and 0.01*M* $MgCl_2$ are more yellow than solutions of the antibiotic alone. Difference spectra reveal a peak for the mixture at 360 mμ at *p*H 7 or 10,

Table 1. Processes requiring magnesium that are inhibited by novobiocin.

Magnesium-requiring process	Novobiocin inhibits completely at
Growth	$10^{-3}M$
Nitrate reductase in *Escherichia coli* (*2*)	$10^{-3}M$
Succinic dehydrogenase in *Micrococcus lysodeikticus**	$10^{-3}M$
Ethanol dehydrogenase in *M. lysodeikticus**	$10^{-3}M$
Adenosine triphosphatase in *E. coli*† and *Streptococcus faecium**	$10^{-2}M$
Oxidative phosphorylation in rat liver (*5*)	$7.8 \times 10^{-4}M$
Amino acid incorporation into soluble RNA in *S. faecium**	$10^{-4}M$
RNA synthesis in *S. faecium**	$10^{-4}M$
Amino acid transport in *S. faecium**	$10^{-3}M$

* My observations. † D. Kessler and H. V. Rickenberg, unpublished observations.

Table 2. Effects induced by both magnesium deficiency and novobiocin.

Effect induced by magnesium deficiency	Same effect induced by novobiocin at
Increased permeability to ortho-nitrophenyl-β-D-galactoside in *Escherichia coli* ML 35*	$10^{-3}M$
Loss of 260 mμ absorbing material in *E. coli* ML 35*	$10^{-3}M$
Filamentation in *E. coli* (*1*)	$10^{-3}M$–$10^{-5}M$
Chaining in *Streptococcus faecium**	$10^{-6}M$
Slow death of cells in *E. coli* (*2*) and *S. faecium**	$10^{-3}M$
Slow autolysis of cells in *E. coli* ML 35*	$10^{-3}M$

* My observations.

whereas novobiocin itself absorbs at 311 mμ at *p*H 10 and 308 mμ at *p*H 7. No peak is obtained when $CaCl_2$ is substituted for $MgCl_2$.

3) Potentiometric titrations of 0.01*M* solutions of novobiocin in the presence and absence of 0.02*M* $MgCl_2$ revealed a small but definite and reproducible alteration in the titration curve brought about by magnesium ions.

Binding studies with tritium-labeled novobiocin revealed that the antibiotic is bound quickly to bacterial cells, even at 0°C. If it is assumed that novobiocin is distributed equally throughout the cells, calculations reveal that concentrations of $10^{-2}M$ are obtained when the external concentration is $10^{-3}M$. If the antibiotic is bound preferentially to surface components of the cell, the local concentration may be considerably higher than $10^{-2}M$. Since complexes of magnesium ions and novobiocin form with $10^{-2}M$ novobiocin in solution, it seems reasonable to assume that such complexes may also occur within the cell.

There seems to be no specificity in the binding of novobiocin, since sensitive and resistant bacteria as well as serum albumin and other proteins bind it equally well. The selectivity of novobiocin action must therefore have some other basis. Novobiocin is more effective against Gram-positive than Gram-negative bacteria, and Webb (*3*) has shown that Gram-positive bacteria have much larger magnesium requirements than Gram-negative bacteria. Thus the selectivity of the antibiotic may be due to the varying requirements of organisms for magnesium. Residual growth occurs in the presence of low concentrations of novobiocin. This lag in the effectiveness of the antibiotic is not due to slow uptake of the antibiotic, for novobiocin is bound very rapidly. Similar residual growth occurs when cells are transferred to a magnesium-deficient medium. Therefore, the residual growth observed in novobiocin-treated cells probably occurs at the expense of uncomplexed magnesium ions, and growth ceases when these are diluted out.

Since novobiocin forms insoluble salts with many metals, it may be that other essential metals—such as iron, copper, and zinc—are also bound by the antibiotic in the bacterial cell. However, magnesium is quantitatively the most important metal in the cell, and it is reasonable that the effects of novobiocin would first resemble a magnesium deficiency. Recently there has been much interest in intracellular magnesium levels, because of the stabilization of ribosomes by this metal. Novobiocin may induce ribosome degradation and thus bring about a number of indirect effects on protein synthesis.

I recognize the difficulty of proving with complete certainty that the primary action of novobiocin is to induce an intracellular magnesium deficiency. Reversal studies with magnesium may be misleading, and all of the other evidence is indirect. However, because of the large number of points of correspondence between the effects of novobiocin action and the effects of magnesium deficiency, the present conclusions seem justified (*4*).

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Chromatic Response Mechanisms in the Human Fovea as Measured by Threshold Spectral Sensitivity

Abstract. Threshold sensitivity was measured with 40-degree surrounds of 5.3 and 800 trolands of white, and 509, 580, and 690 mμ spectral light. With the brighter white surround there appeared peaks at 550, and 570 and a shoulder at 600 mμ. These were selectively eliminated by the spectral surrounds.

The so-called humps and notches in the luminosity curve have received increasing attention in the past 10 years. Although their existence is no longer questioned, there has been little more than speculation to relate them to visual functioning. They are presumed related to cone vision, since they do not appear in the fully dark-adapted "rod" sensitivity curve (*1, 2*). Thomson (*3*) established by statistical tests that they are not merely random fluctuations of sensitivity. Stiles and Crawford (*4*), in an early study, found three submaxima at 440, 540, and 600 mμ, which they could selectively eliminate by adapting to bright, broad-band colors in the blue, green, and red parts of the spectrum.

Although our data show humps in the blue region of the spectrum, the emphasis of our work here is on the occurrence of similar but much larger dips and humps in the long wavelength side of the visible spectrum. Sloan (*5*) first showed a notch in the spectral sensitivity function at 580 mμ. This notch appears from time to time in subsequent determinations (*2–7*). Sperling and Lewis (*6*) and Sperling (*8*), by using a stimulus of 45 minutes in diameter in a dark surround, have shown that the notch at 580 mμ goes from a deep cleft at absolute threshold to an almost smooth curve at high brightness. They discuss their results in terms of variable summation between underlying red and green receptor mechanisms.

In the present experiments we have introduced a light surround field which is variable in luminance and spectral color. The apparatus was in all details similar to that of Sperling and Lewis (*6*). A D246 Hilger and Watts double monochromator fitted with glass prisms was used. A 6-volt, 18-ampere ribbon filament source provided sufficiently intense spectral light over the wavelength range from 410 to 670 mμ to allow threshold determinations under all conditions of surround with bandwidths no wider than 4 mμ. A maxwellian view and an artificial pupil 2 mm in diameter were used. The relative energy content of the stimuli was determined by direct nonselective radiometry using a 0.75 mm^2 platinum black thermocouple in place of the artificial pupil. The surround field was provided by an integrating sphere 1 foot in diameter and an intense projector system which was adapted to accommodate interference filters. The stimulus filled a 20-minute diameter circle cut out of the 40° surround field and viewed at 62 cm. The eye was held behind the artificial pupil by fixing the head by means of a dental impression mounted on a three-way micromanipulator. The stimulus was of 40 msec duration as produced by a sector disc rotating in an image of the exit slit. A calibrated neutral density wedge and filters were used to reduce the stimulus to threshold. Fixation of the observer's eye was achieved by instructing him to look at the center of the small circular stimulus. Between trials he was instructed to fixate a point on the sur-