

could be recovered regularly from their hearts' blood in cultures made on streptomycin-containing media but not in duplicate cultures made on streptomycin-free media.

The results of these mouse inoculations indicate that (1) the type B variants are nonvirulent for (untreated) mice; (2) they require streptomycin for their reproduction *in vivo* as well as *in vitro*; and (3) their dependence on streptomycin for growth persists after multiplication within the body of an infected animal host.

The origin of the type B variant is difficult to explain except as current mutation. It never appeared on media containing less than 40  $\mu\text{g./ml.}$ , but once it had developed, it could be subcultured on concentrations as low as 5  $\mu\text{g./ml.}$  It could not be grown on less even after repeated transfer on media containing that concentration. The rare occurrence of a single colony on streptomycin-free media represents an exception to the rule and seems most likely to be the result of mutation back to normal.

The numbers of colonies developing from equivalent inocula are always greatest on concentrations between 100 and 400  $\mu\text{g./ml.}$ , whether the seedlings are made from a parent (stock) strain, as illustrated in Fig. 1, or from a subculture of type B variant taken from a high or a low concentration of streptomycin. This fact seems to indicate that all the B variants are alike genetically and that the higher reproductive rate at those concentrations reflects a physiological response to the drug rather than a differential induction of the variants at different concentrations.

The variation in color and size of their colonies in relation to the concentration of the drug is additional evidence that streptomycin directly affects the physiology of the bacterial cells. On 60–100  $\mu\text{g./ml.}$  they are small and pearl gray. On higher concentrations, they develop greater size and acquire a distinctly yellowish tinge, resembling the large variants described as type A. Small, gray colonies taken from low concentrations and transferred onto higher concentrations grow as medium-sized, pigmented colonies. Conversely, pigmented colonies taken from high and subcultured onto low concentrations develop as small gray colonies.

Benham (4) has noted the stimulating action of streptomycin on the  $\text{O}_2$  uptake of typhoid bacilli. Welch, Price, and Randall (9) report that small doses of streptomycin increased the mortality rate of mice infected with typhoid bacilli.

Studies on the growth requirements of mutants isolated from cultures of *Bacillus coli* after treatment with bacteriophage (2) or X-ray (8) have demonstrated a variety of deficiencies in their metabolic processes. Similar observations have been made on mutants induced in *Neurospora* by X-ray (3).

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## Irritating Effects of 9,9-Dibromofluorene

JOHN R. SAMPEY, ANNE B. KING,  
THOMAS A. ROE, JR., and S. J. CHILDRESS<sup>1</sup>

*Furman University, Greenville, South Carolina*

Alfred Cavendish (1) has reported the irritating effects of 9-bromofluorene. In the further study of the photochemical bromination of fluorene (2), one of us (A. B. K.) worked several weeks with 9-bromofluorene without any irritation, but on the first exposure to 9,9-dibromofluorene, a severe skin eruption developed.

The irritation set in shortly after crystallization of a sample of 9,9-dibromofluorene from hot glacial acetic acid. Red blotches appeared first on the back of the left hand and the inner left wrist, and after a few hours red streaks developed on the face and ear. The rash spread gradually, and after three weeks it covered both forearms and face completely, and one eye was swollen shut.

Administration of benadryl relieved the severe itching at once, and within a few days the face peeled, and the blotches on the hands and arms began to dry up. Six weeks from the time of exposure, small red blotches remain only at the points of initial contact.

A second member of the group suffered some inflammation and itching of the hands following one exposure to 9,9-dibromofluorene, but the condition cleared up within a week, without medical attention.

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## dl- $\alpha$ -Amino- $\epsilon$ -Hydroxy Caproic Acid in the Rat

R. GINGRAS, EDOUARD PAGÉ, and ROGER GAUDRY

*Department of Biochemistry, Medical School,  
Laval University, Quebec, Canada*

In the course of the synthesis of lysine from dihydropyran, one of the authors (R. Gaudry) prepared dl- $\alpha$ -amino- $\epsilon$ -hydroxy caproic acid by hydrolysis of 5- $\delta$ -hydroxy butyl hydantoin. Since this amino acid differs from lysine only because of its hydroxyl group instead of the  $\epsilon$ -amino group, it was thought of interest to investigate its biological properties.

Young albino rats averaging 66 grams in weight were first placed on a diet of the following percentage composition: zein, 10; dl-tryptophane, 0.2; cellu-flour, 2; soybean oil, 4; salt mixture, 4; sucrose, 79.65; choline chloride, 0.15. Each 100 grams of ration contained: thiamine-HCl, 0.4; riboflavin, 0.4; pyridoxine-HCl, 0.5; calcium pantothenate, 3.0; nicotinic acid, 3.0; inositol, 10.0; and 2-methyl-1,4-naphthoquinone, 0.1 mg.

After 24 days on this diet, weight changes were very small (Table 1), and the following additions were made to the ration at the expense of the sucrose: Group I (Zt), 0.6 per cent zein; Group II (Ztl), 0.6 per cent 1-lysine; and Group III (Ztl-OH), 1.5 per cent dl- $\alpha$ -amino- $\epsilon$ -hydroxy caproic acid. Eleven days

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