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 μg./ml., but once it had developed, it could be subcultured on concentrations as low as 5 μg./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 μg./ml., whether the seedings 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 μg./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 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*).

References

1. Alexander, H. E., and Leidy, G. *J. exp. Med.*, 1947, **85**, 329.
2. Anderson, E. H. *Proc. nat. Acad. Sci., Wash.*, 1944, **30**, 397; Luria, S. E., and Delbrück, M. *Genetics*, 1943, **28**, 491; Luria, S. E. *Genetics*, 1945, **30**, 84.
3. Beadle, G. W. *Physiol. Rev.*, 1945, **25**, 643.
4. Benham, R. S. *Science*, 1947, **105**, 69.
5. Chandler, C. A., and Schoenbach, E. B. *Proc. Soc. exp. Biol. Med.*, 1947, **64**, 208.
6. Miller, C. Phillip, and Bohnhoff, Marjorie. *J. Amer. med. Ass.*, 1946, **130**, 485.
7. Miller, C. Phillip, and Castles, Ruth. *J. inf. Dis.*, 1936, **58**, 263.
8. Tatum, E. L. *Proc. nat. Acad. Sci., Wash.*, 1945, **31**, 215; Gray, C. H., and Tatum, E. L. *Proc. nat. Acad. Sci., Wash.*, 1944, **30**, 404.
9. Welch, H., Price, C. W., and Randall, W. A. *Science*, 1946, **35**, 155.

Irritating Effects of 9,9-Dibromofluorene

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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.

References

1. Cavendish, A. *Brit. J. Dermatol.*, 1940, **52**, 155–163.
2. Sampey, J. R., and Reid, E. E. *J. Amer. chem. Soc.*, 1947, **69**, 234–235.

dl-α-Amino-ε-Hydroxy Caproic Acid in the Rat

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In the course of the synthesis of lysine from dihydropyran, one of the authors (R. Gaudry) prepared dl-α-amino-ε-hydroxy caproic acid by hydrolysis of 5-δ-hydroxy butyl hydantoin. Since this amino acid differs from lysine only because of its hydroxyl group instead of the ε-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 l-lysine; and Group III (Ztl-OH), 1.5 per cent dl-α-amino-ε-hydroxy caproic acid. Eleven days

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later, the rats on the lysine-deficient ration had shown a slight weight loss, while those receiving l-lysine were gaining weight. The largest weight losses were recorded by the rats receiving the synthetic racemic compound (Table 1). One of the rats in this group died on the 11th day, and the experiment was discontinued. The animals were sacrificed and the kidneys were dissected and weighed. Kidneys of rats receiving the α-amino-ε-hydroxy caproic acid were slightly heavier, but histological examination[1] failed to reveal any differences between groups.

TABLE 1

WEIGHT CHANGES (GRAMS) IN RATS RECEIVING α-AMINO-ε-HYDROXY CAPROIC ACID AND IN CONTROL GROUPS

	Zt	Ztl	Ztl-OH
No. of rats per group	4	6	6
Wt. changes during last 10 days of preliminary period (24 days)	−4	−3	−2
Range	−3, −6	−1, −4	−2, −3
Wt. changes during experimental period (11 days)	−2	+5	−8
Range	−1, −3	+2, +9	−6, −10

It is concluded that α-amino-ε-hydroxy caproic acid cannot replace lysine in the diet and that it may have some toxic effect.

Mechanism of Renin Tachyphylaxis—Restoration of Responsiveness by Tetraethyl Ammonium Ion

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The pressor responses to repeated intravenous injections of renin in intact animals diminish progressively. This phenomenon is known as tachyphylaxis. The action of renin is dependent on the presence of plasma renin-substrate, the exhaustion of which might account for tachyphylaxis. But administration of large amounts of α_2 globulin, the fraction of plasma protein containing substrate, does restore the response in tachyphylactic animals (*6*).

Renin through its effector agent, angiotonin, acts directly on peripheral blood vessels. Nevertheless, sectioning of the brain at certain levels causes the pressor response to be reversed to depressor (*3*), a phenomenon which has been further localized by Euler and Sjöstrand (*2*). Widespread direct injury to the central nervous system also temporarily abolishes the pressor response to angiotonin (*4*), as does severe shock produced by a variety of means (*5*). Thus, the nervous system influences vascular responsiveness to angiotonin.

One of the difficulties of accepting the renal-vasopressor system as a cause of renal hypertension has been the phenomenon of renin tachyphylaxis.

Acheson and Pereira have shown that injection of tetraethyl ammonium chloride (*1*) blocks sympathetic ganglia. We have found that repeated (1–4 times) intravenous injections of 100 mg. into normal dogs anesthetized with pentobarbital completely overcame tachyphylaxis induced by repeated injection of renin. The response to angiotonin itself was also augmented. Even more striking was the manyfold increase in the response to adrenalin at a time when the response to nicotine was wholly abolished. The responses to histamine, mecholyl, and barium chloride were usually also augmented. Without anesthesia the same phenomena occur, but to a lesser degree. Adrenalectomy or nephrectomy in brief experiments did not alter these responses.

If the caudal spinal cord was destroyed after section at C_8 and the animal tested the next day after complete recovery from anesthesia, the responses to renin were augmented. Further, 8 injections of renin did not lead to appreciable tachyphylaxis. Administration of tetraethyl ammonium ion now did not further augment the already large response.

[1] Kindly done by C. Auger, of the Pathology Department.

References

1. ACHESON, G. H., and PEREIRA, S. A. *J. Pharm. exp. Therap.*, 1946, **87**, 273.
2. EULER, U. S. v., and SJÖSTRAND, T. *Acta Physiol. Scand.*, 1941, **2** 264.
3. PAGE, I. H. *J. exp. Med.*, 1939, **70**, 521.
4. PAGE, I. H. *J. exp. Med.*, 1943, **78**, 41.
5. PAGE, I. H. *Amer. J. Physiol.*, 1944, **142**, 366.
6. PAGE, I. H. *Spec. Publ. N. Y. Acad. Sci.*, 1946, **3**, 77.

Absence of a Macrocytic Anemia in Dogs Fed Choline or Choline Plus Fat

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Several groups of investigators in this university have been interested in choline since this substance was established as a dietary factor by the work of Best and Huntsman (*1*). While no detailed study of the effect of choline on the blood picture had been made here, a number of hemoglobin and red cell estimations in dogs fed large amounts of choline had failed to reveal any signs of anemia. A series of papers by Davis (*2*), in which it was reported that the administration of choline or choline plus fat produced in dogs a macrocytic anemia, came, therefore, as a surprise. An additional stimulus to the investigation of Davis' claims was provided by the fact that choline or its precursor, methionine, is now being used in many clinics in an effort to determine its role in the prevention of fat deposition or cirrhotic changes in the liver of patients.

A series of four normal male dogs was carefully observed by one of us (M. F. C.), and after the somewhat extended period necessary to obtain satisfactorily constant basal values for the red cell counts and the hemoglobin levels, choline or choline plus fat was added to the stock diet of the animals, which consisted of Canada Packers Frozen Dog Chum, sucrose, and bone meal. Dried yeast and cod-liver oil were provided twice each week and tomato juice once a week. All four dogs were on this diet for at least three months before the feeding of choline or choline plus fat. During this period they all gained weight, and their coats improved in texture.