This was first described by Dr. F. G. Young, of London, who used normal dogs; he could not make normal cats diabetic. We have likewise failed with the intact cat, but have found that by the removal of part of the pancreas (leaving enough to prevent any signs of diabetes), this species is rendered susceptible to pituitary extract. A number of permanently diabetic cats have thus been prepared. They manifest the same type of diabetes observed in the dog as measured by the blood and urine sugar, but the pancreatic islands (the source of insulin) present a type of abnormal appearance (hydropic degeneration) which is different from that in the dog (atrophy). In the cat anatomical and functional restoration of the islands takes place under insulin treatment, and the recovery of the animal is maintained after the withdrawal of insulin. Recovery does not occur in animals in which the diabetes has lasted for five months, and no recovery has been reported in pituitary diabetic dogs treated with insulin. The relation of various factors to the recovery of damaged islands of the pancreas in the diabetic animal can thus be studied. Species difference, the type of anatomical disorders, the duration and severity of the diabetes and the variations of treatment are under investigation.

The tetramite stage of Orthopteran auxocytes: C. E. McCLUNG. During the development of the male germ cells of the grasshopper a critical stage is reached during which equivalent contributions by the male parent and the female parent are brought into intimate physical contact and there react upon each other so as to bring about new combinations in the character controls of the two. This seems to be the most significant feature of biparental reproduction, and every detail of the phenomena of germ cell production at this time is of great theoretical importance. Because these details concern very minute elements of the cell exact determinations of their behavior are difficult to arrive at. It is, however, now generally agreed that the chromosomes, the cell elements which carry the material genetic controls, are greatly elongated and lie parallel with homologous regions apposed. Just how the exchange of units, representing genetic controls, is accomplished, is not completely understood. One significant stage of the process is of very short duration and has not previously been described. At this time the four strands of each chromosome, two from each parent, lie extended and parallel with each other, without the presence of any of the so-called chiasms which have been credited by some as the means by which these threads are held in contact. Various other assumptions of "attraction" and "repulsion," "precocity," etc., are definitely disproved by the existence of the "tetramite," a chromosome in the form of four parallel threads.

Biographical memoir of Harvey (Williams) Cushing: W. G. MACCALLUM.

Biographical memoir of Calvin Blackman Bridges: T. H. MORGAN.

Biographical memoir of Arthur Edwin Kennelly: VANNEVAR BUSH.

Biographical memoir of Floyd Karker Richtmyer: HERBERT E. IVES.

Biographical memoir of Albert Sauveur: REGINALD A. DALY.

SPECIAL ARTICLES

ATTENUATION OF CELL STIMULATING BACTERIA BY SPECIFIC AMINO ACIDS[1]

FACTORS influencing virulence have been studied as part of a larger program aimed at clarifying the means whereby the crown gall organism, *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.,* incites diseased growth in plants. After a considerable range of toxic substances and unfavorable conditions failed to change pathogenicity, glycine proved effective. Attenuation was accomplished by 20 to 30 transfers in a mannitol nitrate mineral salts medium containing .1 to .3 per cent. glycine adjusted to pH 6.8. The amount of glycine was increased gradually in successive transfers, according to the tolerance of the culture, so that growth was slow. Attenuation was considered complete when puncture inoculations into tomato failed to induce crown gall. Several original strains have been kept in culture for over ten years without loss of virulence. Mechanically picked single cell cultures were isolated at the beginning, at various intermediate stages and at the end to avoid any differential selection in an originally mixed bacterial population.

Some attenuated cultures when placed on yeast infusion mannitol medium regained virulence within a year. Others remained attenuated even after four years. The permanency of attenuation was partially dependent upon the number of transfers in glycine medium after attenuation.

The reaction of the medium affected the rate of attenuation. A medium at pH 8.0 was inhibitory and readily attenuating, while one at pH 5.0 was not.

Various other compounds having structural similarity to glycine have also been employed. Among over 50 compounds most of the amino acids were included. With the exception of dicyandiamid (which gave only partial attenuation) and the dipeptide of glycine, all the attenuating compounds were amino acids. These were: glycine, alanine, serine, alpha amino n-butyric acid, threonine, valine, norvaline, methionine, leucine, norleucine, isoleucine and lysine. Their relative effectiveness in mannitol-nitrate-mineral-salts solution is indicated in Fig. 1. Only racemic mixtures of opti-

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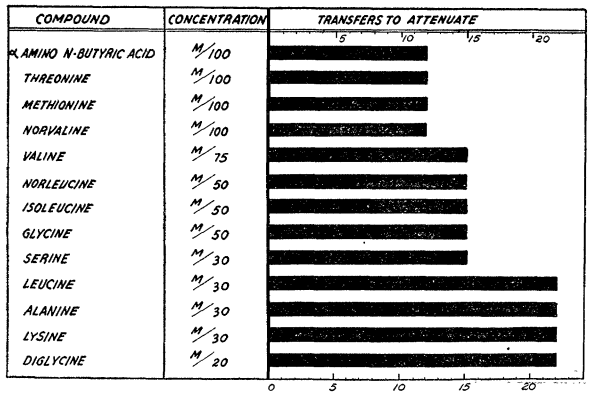

FIG. 1. Attenuating compounds, concentrations used in mannitol-nitrate-mineral-salts medium, and number of successive transfers necessary to attenuate single-celled virulent crown-gall cultures. The concentrations given were the highest used.

cally active amino acids were studied except with leucine. In this case both the l- and dl-compounds were found active. Except for phenylalanine there was direct correlation between inhibition of bacterial growth and attenuation.

Thus all the attenuating amino acids were aliphatic in nature. With the exception of lysine they contained only single amino and carboxyl groups. The other diamino mono-carboxylic acids, the mono-amino di-carboxylic acids and the cyclic amino acids were inactive. Although glycine was active, the ethyl ester of glycine, the anhydride of glycine and sarcosine were inactive. This indicated that the free amino and carboxyl groups might be required for attenuation. Also since diglycine was less effective than glycine and triglycine was inactive the length of peptide chain appears influential.

Glycine and alpha amino n-butyric acid did not specifically inhibit glucose or succinate dehydrogenase, aspartic acid deaminase or aerobic respiration in the Warburg respirometer. The active amino acids likewise did not specifically inhibit the peptidases splitting leucylglycine, leucyldiglycine or alanyldiglycine.

Over 1,000 attenuated cultures have been produced during these studies.

The details of these investigations will be published elsewhere. Meantime, this preliminary statement is given because of its bearing on such basic problems as (1) the attenuation of pathogenic bacteria, (2) the physiological specificity of the amino acids and (3) the mechanism of atypical and pathological cell growth.

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LOCALIZATION OF CHOLINE ESTERASE IN NERVE FIBERS

RECENT investigations have revealed that choline esterase is highly concentrated in all nerve fibers, but rises to strikingly high concentrations at synapses and motor end plates.[1,2] Previous observations on the superior cervical ganglion of cats, after section of preganglionic fibers, and on denervated muscles of guinea-pigs[3] suggested strongly that the enzyme may be concentrated at or near the surface of nerve cells. In these experiments the concentration in preganglionic fibers inside the ganglion appeared to be several times as high as the concentration in the same fibers before they enter the ganglion. This increase was interpreted as possibly being connected with the increase of surface due to the extensive end-arborization of preganglionic fibers in this ganglion.

Direct evidence that choline exterase is highly concentrated at or near the surface of nerve fibers has now been obtained. The enzyme activity has been determined separately in the axoplasm and the sheath of the giant fiber of the squid (*Loligo paealii*). The method used was the Cartesian diver technique as developed by Boell, Needham and Rogers.[4]

Practically the total enzyme activity is found in the sheath, whereas the amount of enzyme present in the axoplasm is negligible. The figures obtained in one experiment are given in Table 1.

TABLE 1

	Material in mg fresh weight per diver	Output of $CO_2 \times 10^3$ cmm in 160 min.	$Q_{CH \cdot E \cdot}$* (average)
Whole axon	0.123 0.123	53 69	0.150
Sheath	0.09 0.09	135 115	0.420
Axoplasm	0.20 0.20 0.20	8 28 18	0.027

* $Q_{CH \cdot E \cdot}$ = mg. ACh split by 100 mg tissue in 60 min. t = 27.5° C.

As at least 80 to 90 per cent. of the sheath is connective tissue,[5] the values obtained for the sheath have to be multiplied by at least 5 to 10 times. The enzyme concentration may be actually much higher than the activity per unit of tissue weight indicates,

[1] D. Nachmansohn, *Bull. Soc. Chim. biol.*, 21: 761, 1939.
[2] D. Nachmansohn, *Yale Jour. Biol. and Med.*, 12: 565, 1940.
[3] R. Couteaux and D. Nachmansohn, *Proc. Soc. Exp. Biol. and Med.*, 43: 177, 1940.
[4] E. J. Boell, J. Needham and V. Rogers, *Proc. Roy. Soc.*, (B) 127: 322, 1939.
[5] Personal communication from F. O. Schmitt. See also figure in Bear, Schmitt and Young, *Proc. Roy. Soc.* (B) 123: 496, 1937.