posterior part and move successively along the follicle. The sections revealed that the nuclei with the heterochromatin labeled are the more advanced ones. Thus, the heterochromatin synthesizes DNA later than does the euchromatin (*3*).

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3. The animals used in this experiment were raised from eggs obtained from Dr. T. Tahmisian of the Argonne National Laboratory, Lemont, Ill. A detailed report of these experiments is in preparation.
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25 March 1959

Ballistics of Dwarf Mistletoe Seeds

Abstract. The explosive fruit of *Arceuthobium* expels the seed for several feet, but the ballistics of seed flight has not been previously investigated. The data reported here for *A. vaginatum* f. *cryptopodum* indicate that the seeds have an initial velocity of about 1370 cm/sec and an initial acceleration of nearly 5000*g*.

The explosive fruit of the dwarf mistletoes (*Arceuthobium* spp.) is one of the most efficient mechanical seed dispersal mechanisms in any of the higher plants (*1*). As far as I know, no calculations have been made of the initial velocity or other ballistic factors of the dwarf mistletoes or any other higher plants with explosive fruits. However, Buller (*2*) studied the ballistics of the glebal masses projected by the fungus *Sphaerobolus stellatus* and found that they were thrown to a height of 14.5 ft; this indicates an initial velocity (when air resistance is disregarded) of at least 30 ft/sec (*3*).

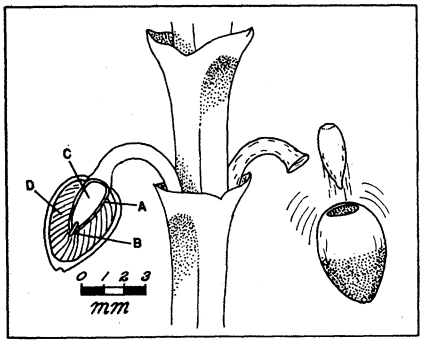

Fig. 1. Semidiagrammatic drawing of a portion of a dwarf-mistletoe shoot bearing mature fruits. Left, a longitudinal section through a fruit showing a seed (*A*), embryo (*B*), endosperm (*C*), and viscin cells (*D*). Right, a fruit immediately after the expulsion of the seed.

Each fruit of *Arceuthobium* contains a single semifusiform seed (Fig. 1). When the fruit is ripe, the pedicel is elongated and recurved so the perianth end points downward. An abscission zone develops between the tip of the pedicel and the base of the fruit. A layer of viscin cells between the seed and the exocarp of the fruit creates a considerable internal pressure, and finally the fruit is sheared from the pedicel and the exocarp contracts rapidly and hurls the seed upward (*4*). The forward end of the seed is rounded and the other end is pointed; thus, their shape approaches the ideal for the most efficient projectile.

The dwarf mistletoe used in this work (*5*) was *Arceuthobium vaginatum* f. *cryptopodum*, which is a widespread and important pathogen of ponderosa pine (*Pinus ponderosa* Laws.) in the southwestern United States. The seeds of this species average 1.1 mm in diameter and 2.9 mm in length. They are expelled for an average horizontal distance of 530 ± 30 cm, with a maximum of about 1280 cm.

The following are the experimental data obtained: Average vertical height of seeds expelled directly upward, 460 cm; terminal velocity of seeds, 750 cm/sec (*6*); average seed weight, 2.4 mg; and seed specific gravity, approximately 1.0. If it is assumed that the forces acting on the seed in flight are the force of gravity and a frictional force proportional to its velocity, then a formula may be derived relating the maximum height to which a seed goes and its initial velocity (*7*). When the data shown above are used in this formula, an average initial velocity of 1370 cm/sec or about 45 ft/sec is indicated. The kinetic energy of the seed as it leaves the fruit is thus $\frac{1}{2}\ mv^2 = 2.3 \times 10^3$ ergs.

From the initial velocity and dimensions of the seed, the time taken for the seed to leave the fruit was calculated as 4.4×10^{-4} second. The computed initial acceleration of the seed was 4.7×10^6 cm/sec^2, or nearly 5000*g*.

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3. This calculation was based on the formula $V_0 = (2gH)^{\frac{1}{2}}$, where V_0 is the initial velocity, g is the acceleration due to gravity, and H is the height of projectile expelled directly upward.
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5. I wish to thank R. B. Setlow and W. R. Henson of Yale University for advising me in this work and for reviewing the manuscript, and Dr. Setlow for providing the formula given in reference (*7*).
6. The terminal velocity was determined by passing air upward through a vertical tube containing a dwarf mistletoe seed. The rate of flow necessary to suspend the seed was recorded, and the average value for 24 seeds was taken as the approximate terminal velocity.
7. $H = \frac{m^2}{c^2} g \ln \left(\frac{g + \frac{c}{m} V_0}{g} \right) - \frac{m}{c} V_0$,
when H is the maximum height of seed expelled directly upward, m is the mass of seeds, c is the ratio of frictional force (mg) to the terminal velocity (V_t), g is the acceleration due to gravity, and ln is the natural logarithm of V_0, the initial velocity.

26 March 1959

Acetylcholine Effects of γ-Carbomethoxypropyltrimethyl-Ammonium Bromide

Abstract. γ-Butyrobetaine, in comparison with its methyl ester, γ-carbomethoxypropyltrimethyl-ammonium bromide, is biologically inert. When injected into mice and insects or assayed on the frog's rectus abdominis muscle, γ-carbomethoxypropyltrimethyl-ammonium bromide has pharmacological properties resembling those of acetylcholine. Although reported to be present in rat brain during the convulsions induced by dieldrin poisoning, γ-butyrobetaine has not been found in the nervous tissue of the roach after treatment with dieldrin.

Burgen and Hobbiger (*1*) reported a similarity in the pharmacological properties of acetylcholine and the methyl ester of γ-crotonic betaine (γ-carboxyallyltrimethyl-ammonium chloride). More recently Hosein (*2*) stated that γ-butyrobetaine (GBB) was found in the brain of rats during convulsions after administration of a large dose of dieldrin. Hosein (*3*) showed that some pharmacological effects of GBB resembled those of acetylcholine. This finding is of importance, since in insects treated with chlorinated hydrocarbons, no explanation has yet been found for the manifestation of convulsions which occur in the central nervous system (*4*). Colhoun (*5, 6*) showed that after treatment of cockroaches with DDT and dieldrin a high titer of acetylcholine was found in the nerve cord at a late stage of prostration. The finding of Hosein (*3*) therefore necessitated a reevaluation of these results.

γ-Butyrobetaine was synthesized and tested for biological activity by intraperitoneal injection into mice. It was inert at the concentrations used by Hosein (*1*) and Linneweh (*7*). Further tests showed that the methyl ester of GBB, γ-carbomethoxypropyltrimethyl-ammonium bromide, had a toxicity for mice comparable to the reported toxicity of GBB injected by Hosein (*1*). Significantly, the ester was the first intermediate product in the synthesis of GBB. γ-Carbomethoxypropyltrimethyl-ammonium bromide was prepared by the reaction of anhydrous trimethylamine with methyl γ-bromobutyrate. On purification, the resulting material melted at 147° to 149°C. The actual bromide con-

Table 1. Comparison of some effects of acetylcholine and γ-carbomethoxypropyltrimethylammonium bromide.

Experiment	Acetylcholine	γ-Carbomethoxypropyltrimethyl-ammonium bromide
Intraperitoneal injection, mouse	6.7 mg/kg: slight muscular symptoms only	6.7 mg/kg: salivation, bloody tears, some convulsions, LD_{30}
Abdominal injection, roach	5 mg/g: some tremors, not toxic	5 mg/g: toxic, tremors, prostration
Electrical conduction in 6th abdominal ganglion of roach	$10^{-2}M$: no effect	$10^{-2}M$ to $10^{-3}M$: excitation, prolonged repetitive volleys
Assay of rectus abdominis muscle of frog		
Rates of activation on eserinized muscle	1 μg	30 μg
Atropine	Blocked	Blocked
Alkaline hydrolysis	No contraction	No contraction
Cholinesterase hydrolysis	No contraction	Contraction
Both substances combined	No antagonism	No antagonism
Activity on uneserinized muscle	Slight initial contraction	Contraction greater than on eserinized muscle
Cholinesterase hydrolysis (Warburg vessels)	pS optimum $10^{-2}M$ to $10^{-3}M$	No hydrolysis
Cholinesterase hydrolysis with both compounds combined	No inhibition of hydrolysis	

tent agreed with the theoretical, and the infrared absorption at 1730 and 1197 cm^{-1} is characteristic for esters of this type. The R_f was 0.20 when the compound was chromatographed on No. 4 Whatman paper with wet *n*-butanol as the developing solvent. Removal of the bromide with moist silver oxide, followed by warming and concentrating, yielded γ-butyrobetaine with an R_f of 0.03. The infrared absorption characteristics of the ester had disappeared, while a new peak appeared at 1575 cm^{-1}, corresponding to that for a carboxyl. The compounds tested for toxicity to mice were the same as those used for chromatograph, as described above.

Some of the pharmacological properties of γ-carbomethoxypropyltrimethylammonium bromide are given in Table 1. It is at once evident that, although the pharmacological effects of the compound resemble those of acetylcholine, there are distinct differences. The most striking are the effect on the uneserinized rectus abdominis muscle of the frog and the complete lack of hydrolysis of the compound by cholinesterase or in tissue breis of nerve cords of cockroaches. It is more toxic than acetylcholine when injected into cockroaches and excites electrical activity in the sixth abdominal ganglion of the ventral nerve cord of the cockroach at $10^{-2}M$ to $10^{-3}M$, whereas acetylcholine is inert at this concentration (*8*). The compound has pharmacological properties very similar to those of γ-carbomethoxyallyltrimethyl-ammonium chloride (*1*).

After treatment of cockroaches with DDT and dieldrin, a substance was found in large amounts in the nerve cord (*5*) which was correctly termed acetylcholine, for it is readily hydrolyzed by cholinesterase in vitro and in homogenates of nerve cords of roaches in which no anticholinesterase was included. However, it is thought (*5*) that the rise in acetylcholine in DDT- and dieldrin-prostrated roaches is secondary and not responsible for the convulsions that occur during the early phase of poisoning. Unless it were present in large amounts it would be difficult to detect by bioassay the occurrence of γ-carbomethoxypropyltrimethyl-ammonium bromide in the nerve cords of roaches, for this is 30 times less effective than acetylcholine. This problem is being investigated by chemical means to determine a possible primary neurological lesion in chlorinated hydrocarbon poisoning.

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2 March 1959

Alpha-Keto Acids in Vitamin-Free Casein Hydrolyzates (Acid)

Abstract. α-Ketoglutaric and pyruvic acids were isolated as their 2-4-dinitrophenylhydrazones from five different commercial samples of vitamin-free acid hydrolyzates of casein. In addition, one sample yielded traces of α-ketobutyric acid. The hydrazones were converted by hydrogenation to glutamic acid, alanine, and α-aminobutyric acid and identified by paper chromatography.

Franck and Knoke (*1*) in 1957 reported the presence of pyruvic and α-ketobutyric acids in acid hydrolyzates of egg albumin, zein, gelatin, and casein, while Neuman and McCoy (*2*) in 1958 reported that pyruvate, oxalacetate, and α-ketoglutarate possessed growth-promoting properties with respect to isolated Walker carcinosarcoma 256 cells. These facts prompted an investigation of the α-keto acids present in commercially available casein hydrolyzates (acid) which are not uncommon components of semidefined bacteriological media.

The analysis of the α-ketoacids present involved the following procedures: (i) conversion to the 2-4-dinitrophenylhydrazone derivatives; (ii) chromatographic separation of the acidic carbonyl derivatives; (iii) isolation of each component by paper chromatography; (iv) catalytic reduction of the isolated derivatives; and (v) identification of the resulting amino acids by paper chromatography.

The carbonyl derivatives were formed by the method described by Cavallini and Frontali (*3*). The hydrazones were extracted with diethyl ether or ethyl acetate, and the acid carbonyl derivatives were extracted from the solvent with 1*N* Na_2CO_3. The alkaline extracts were washed with chloroform containing 20 percent ethanol, then acidified in the cold with 6*N* HCl. The hydrazones were then reextracted into diethyl ether or ethyl acetate and evaporated to dryness at room temperature.

The derivatives were taken up in a small quantity of methanol and applied in a band about 2 in. from the bottom of a large sheet of Whatman No. 1 filter paper. The papers were developed with butanol, ethanol, and ammonia (7:1:2) (*4*).

The separated bands were cut out and eluted with 1*N* Na_2CO_3, and the eluates were acidified in the cold with 6*N* HCl (*5*). The hydrazones were extracted with diethyl ether or ethyl acetate, and the extracts were evaporated at room temperature. The derivatives were taken up in 1 ml of distilled water and added to 15-ml centrifuge tubes containing about 2 mg of platinum oxide catalyst. Hydrogenation was carried out in a Parr hydrogenation apparatus for