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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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

periods varying from 16 hours, as recommended by Meister and Abendschein (*6*), to 5 hours, as recommended by Kun and Garcia-Hernandez (*7*). The clear supernatants were spotted, along with known standards of glutamic acid, alanine, and α-amino butyric acid, on strips of filter paper; developed in phenol and water in an atmosphere of ammonia; dried; and sprayed with ethanolic Ninhydrin.

α-Ketoglutaric and pyruvic acids were present in all the hydrolyzates examined (*8*). The derivatives of these keto acids appeared in three bands, the lowest one running parallel to known α-ketoglutaric acid hydrazone and the higher two running with the two bands of pyruvic acid derivatives (*3*).

In an attempt to explain the presence of α-ketoglutaric acid in the commercial hydrolyzates, the following experiment was made. Five grams of pyruvic acid and 5 g of glutamic acid were added to 150 ml of 10*N* HCl and refluxed for 14 hours, after which time the HCl was distilled off directly (*9*). The remaining semisolid mass was taken up in 100 ml of distilled water, and the solution was filtered. The acid-carbonyls present in the filtrate were isolated and identified by the methods described previously for the isolation and identification of the α-keto acids in the casein hydrolyzates examined.

α-Ketoglutaric acid was present in the filtrate from the acidified and heated pyruvate-glutamic mixture.

Franck and Knoke (*1*) found that during acid hydrolysis of casein the β-hydroxy α-amino acids serine and threonine gave rise to pyruvic and α-ketobutyric acids, respectively. They found, under the conditions of their experiment (6*N* HCl, 14 hours, 140°C), no other α-keto acids.

The logical precursor of the α-ketoglutaric acid found in the commercial casein hydrolyzates would be glutamic acid. The keto acid could arise under the rigorous conditions of hydrolysis (*9*) by the condensation of pyruvic acid initially coming from the degradation of serine, and the glutamic acid would be freed during hydrolysis. This condensation product, presumably a Schiff base, could be rearranged and split in such a manner as to yield α-ketoglutaric acid as one of the cleavage products.

These findings may be of interest in nutritional studies in which acid-hydrolyzed casein provides the source of amino acids in experimental media. If the cultures for which the media are prepared possess active transaminase systems, the fact that α-ketoglutaric and pyruvic acids are present initially in the media might (i) lead to misinterpretation of differences in the levels of amino acids before and after growth (that is, glutamic acid and alanine levels could be affected by transamination involving the α-keto acids present initially in the media); (ii) account in part for the differences in efficiency of casein hydrolyzate media and completely synthetic media in supporting bacterial growth.

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Activation of Single Lateral Geniculate Cells by Stimulation of Either Optic Nerve

Abstract. The lateral geniculate nucleus is organized in such a way that, initially at least, information from the one eye is almost exclusively segregated from that from the other eye. Single-unit recording, however, confirms the histological evidence that bilateral integration does take place. A small number of cells (< 8.5 percent) receive afferents directly from both optic nerves and are discharged by stimulating either nerve (direct interaction). More common is delayed interaction, where the cells are discharged independently by either optic nerve but only after a relatively long latency. Indirect interaction effects also occur.

The lateral geniculate nucleus is a synaptic center on the direct path between retina and cerebral cortex. In the higher mammals the acquisition of binocular vision is associated with the development of a partial decussation of optic nerve fibers at the chiasma where fibers from both eyes now pass to each lateral geniculate nucleus. While these changes are taking place, distinct cellular laminae develop in the nucleus, but the fibers from each eye terminate in separate cell layers. Many studies have been made, particularly in the cat, regarding the possibility of binocular integration taking place in the lateral geniculate nucleus. Earlier histological (*1*) and electrophysiological (*2*) studies gave negative results (see *3*). Later, Bishop and Davis (*4*) provided clear evidence of some degree of binocular interaction. At that time this interaction was regarded as being due to extracellular flows of current from active cells affecting the excitability of resting cells in adjacent inactive layers. Recent work in this laboratory (*5–7*) indicates that this factor is probably of minor importance and that the existence in the geniculate of bilateral synaptic connections of varying complexity provides a basis for the small degree of binocular interaction that takes place at this level.

By studying the patterns of degenerating nerve terminals following section of one optic nerve in the cat, Hayhow (*5*) confirmed that each cell layer receives fibers from one eye only. He demonstrated, however, that the interlaminar regions which contain large cells (*nucleus interlaminaris centralis* and *nucleus interlaminaris medialis*) receive fibers from both eyes. This suggests that these regions may be concerned with the integration of information from the two eyes.

The technique of recording from single cells provides confirmation of the supposition that there are cells in the lateral geniculate nucleus which may be activated independently from either eye. Thus, Erulkar and Fillenz (*8*) have recorded from single units which responded to light flashes presented to either eye. Using glass micropipette electrodes filled with 3*M* KCl (direct-current resistance, 5 to 10 megohms) under Horsley-Clarke stereotaxic control, we have now recorded, extracellularly, in the region of the lateral geniculate nucleus, from about 270 postsynaptic units that have responded to electrical stimulation of the optic nerves. Of these, only 23 (8.5 percent) responded to stimulation of either optic nerve with a latency in each case of less than 10 msec. Final confirmation that binocular interaction occurs in the lateral geniculate requires, however, a clear demonstration that the recording sites were actually intrageniculate and that the units concerned were not fibers of passage on their way through the nucleus.

As regards the latter point we now have satisfactory criteria (*9*) which enable us to distinguish between the responses from the region of the cell body (Fig. 1, *A*) and those from an axon (Fig. 1, *B*). In various ways the cell response can be fractionated into the separate components concerned in impulse generation (*9*). Twenty-three units responded to stimulation of either optic nerve with latencies of less than 10 msec.