Au-Ge composition, crystallizing also in the hexagonal close packed structure, apparently does not, at least above 0.32°K. As shown in Table 1, $AgTe_3$ and Au_3Te_5 were also found to be superconducting, at 2.6° and 1.6°K, respectively.

We conclude, from our results, that metastability has very little bearing on the question of whether or not a compound will become superconducting. Superconducting metastable compounds of Bi with copper (*5*) and various other metals (*6*) had been found previously by Alekseevskii and co-workers. We have found superconductivity in at least one, and probably two new crystal structures. These are the primitive (one atom per unit cell) cubic (*7*) structures, $AgTe_3$ and Au_3Te_5 (the noble metal and tellurium atoms are randomly distributed over the cubic sites), and the complex undetermined phase of the Au-Ge system.

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7. Several superconducting compounds ($AuZn_3$, $Cu_{18}As_4Sb_3$, $LaCd_{11}$, $Pd_{17}Se_{15}$) have been indexed as primitive cubic (see *2*, p. 21) but with large lattice parameters, indicating more than one atom per unit cell.
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11. This work has developed from the researches of Prof. P. Duwez and B. T. Matthias, and we are indebted to them and to W. Zachariasen for advice and encouragement. We also thank A. R. Sweedler for experimental assistance. The research was supported in part by the Air Force Office of Scientific Research and the Atomic Energy Commission.

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Differential Estimation of Gamma-butyrolactone and Gamma-hydroxybutyric Acid in Rat Blood and Brain

Abstract. *A sensitive and specific gas chromatographic technique for estimating concentrations of the anesthetic adjuvants, γ-butyrolactone and γ-hydroxybutyrate, in tissues has been developed. These substances do not appear to occur endogenously in either the blood or the brain of common laboratory animals. The onset and duration of anesthesia caused by the administration of either compound is correlated with the concentration of γ-hydroxybutyrate in the brain, rather than with that of the corresponding lactone.*

Recent reports have focused attention upon the neuropharmacology and biochemistry of γ-butyrolactone (GBL) and the product of its hydrolytic cleavage, γ-hydroxybutyrate (GHB). After initial observations in this laboratory of the ability of γ-butyrolactone to depress the central nervous system (*1*) explorations of its potential use in man have been undertaken recently (*2*).

Fishbein and Bessman have described an isozyme of lactic dehydrogenase in mammalian brain that catalyzes the reduction of succinic semialdehyde to γ-hydroxybutyrate (*3*). Utilizing several modifications of a method that is based upon the formation of hydroxamates of esters, these investigators, contrary to the findings reported here, have claimed that both GHB and GBL are present as normal brain metabolites in a combined concentration of $10^{-3}M$ (*4*). Furthermore, Bessman and Skolnik stated recently that, regardless of whether the lactone or the acid is administered to rats, the onset and duration of anesthesia are correlated with the presence of the lactone in brain and not with that of the anion (*5*). Our data do not support this conclusion.

A method has been developed in this laboratory for simple, rapid, direct, and specific estimation of γ-hydroxybutyrate and γ-butyrolactone in tissues, based upon the use of gas chromatography (*6*). By the new method one can readily detect $10^{-6}M$ GHB or GBL in tissues. At a concentration of $10^{-4}M$, the recovery from blood of added GBL is 70 percent, while that from brain is 80 percent. It is clear from Fig. 1 that the retention time for GBL is comparable to that for material extracted from the brain of rats anesthetized with GBL.

In view of the report of the natural occurrence of γ-hydroxybutyrate and γ-butyrolactone in mammalian brain in a concentration of $10^{-3}M$ (*4*), we have extracted and analyzed concentrates of such extracts of rat brain and blood, rabbit brain, cat brain, and dog blood for total GBL and GHB contents. None of the tissues was found to contain these substances. While it is conceded that the lactate dehydrogenase of brain can reduce succinic semialdehyde to GHB, our results suggest

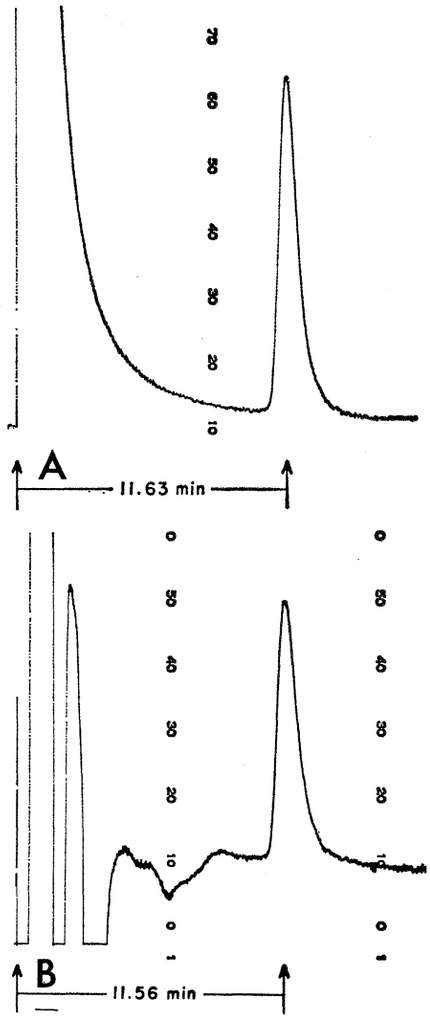

Fig. 1. Gas chromatograms of (*A*) authentic GBL and (*B*) of an extract of brain of an animal anesthetized with GBL prepared as described (*6*). The irregularity at the beginning of record *B* is caused mainly by the trichloroacetic acid in the extract. This can be removed by adsorption with Dowex-2 chloride; it has been found, however, that the presence of trichloroacetic acid does not alter the retention-time of GBL on the column.

strongly that this pathway does not lead to an appreciable endogenous accumulation of either GHB or GBL.

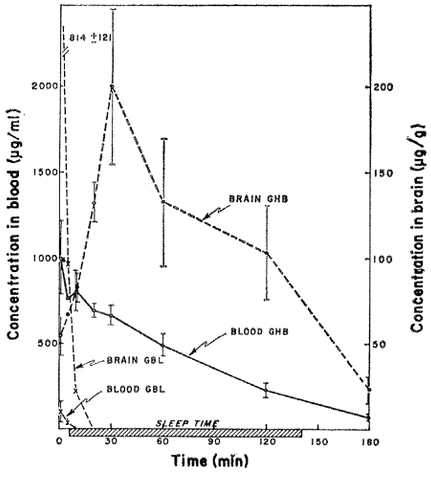

Fig. 2. Differential brain and blood concentrations of GBL and GHB after the intravenous administration of GBL, 500 mg/kg. Each point is the mean value for at least four animals. Brain values are corrected for the volume of residual blood in the cerebral vasculature after decapitation (0.02 ml/g) based upon a calculation derived from studies of the amount of I^{131}-serum albumin in the circulation 5 to 10 minutes after administration.

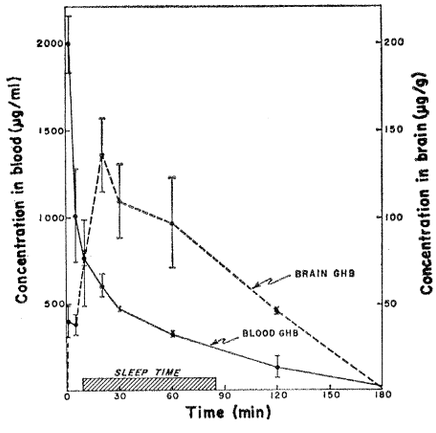

Fig. 3. Concentrations of gamma-hydroxybutyrate in the brain and blood after the intravenous administration of the sodium salt of γ-hydroxybutyric acid (732 mg/kg). Each point is the value obtained from analyses derived from each of at least four animals; the total number of animals studied was 38. The vertical bars indicate the standard deviation of the mean. The amount of γ-butyrolactone was below the limits of detection in both tissues during the entire time-course depicted. Brain values are corrected for the volume of residual blood in the cerebral vasculature after decapitation (0.02 ml/g), based upon a calculation derived from studies of the amount of I^{131}-serum albumin in the circulation at 5 to 10 minutes after administration.

The distribution of GBL and GHB in brain and blood of rats was investigated after the production of anesthesia with each of these compounds (*7*). Figure 2 shows the results obtained after the animals were given 500 mg of GBL per kilogram intravenously. The entry of GBL into brain obviously is very rapid, and very high concentrations (about $10^{-2}M$) are achieved within 1 minute. Once in the brain, this large amount of GBL disappears rapidly, presumably by redistribution out of the brain and by quick conversion to GHB. When the amount of GHB in the brain reaches what appears to be a critical concentration, about 70 μg/g ($8 \times 10^{-4}M$), the animal loses the righting reflex and a state of anesthesia ensues. It is clear, therefore, that the onset of anesthesia correlates with the amount of anion in the brain, and not with the amount of the lactone, as has been suggested by Bessman and Skolnik (*5*). This conclusion is supported strikingly 1 minute after GBL is given (when the brain content of GBL is 800 μg/g), at which time the animal is awake and appears normal. It is also apparent from Fig. 2 that, as the amount of the anion (GHB) falls below the critical concentration of 70 μg/g, the animal awakens and regains the righting reflex.

The contention that the amount of γ-hydroxybutyrate is correlated closely with the onset and duration of anesthesia is also supported by our findings (presented in Fig. 3) when animals were anethetized with an intravenous injection of an equivalent amount of GHB (that is, 732 mg of the sodium salt per kilogram). The GHB does not appear to be converted to detectable quantities of GBL in blood or brain, and the anion, in contrast to the lactone, passes more slowly into brain and reaches an anesthetic level somewhat later (thus, the onset of anesthesia occurs about 8 to 12 minutes after the anion has been injected as contrasted to the 5 to 7 minutes required with the lactone). Reorientation of the animals again follows the fall of the amount of GHB in the brain below the anesthetic level (about 70 μg/g). The time they remain asleep is shorter after the administration of GHB (80 minutes) than it is after GBL (135 minutes), because the concentrations of GHB in the brain do not rise as high after the administration of GHB (about 140 μg/g) as they do after GBL (about 200 μg/g), and, therefore, fall more quickly below the anesthetic level.

From the use of the specific gas chromatographic technique we can conclude that neither γ-hydroxybutyrate nor γ-butyrolactone exist endogenously in the brain or blood of common laboratory mammals in amounts within the limits of our assay, and that, when either of these substances is injected into the rat, the onset and duration of anesthesia correlates with the concentrations in the brain of the anion, γ-hydroxybutyrate, not that of the corresponding lactone.

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6. Method of assay. All tissue extractions are carried out in an ice bath. Blood is deproteinized at approximately 0°C with an equal volume of cold 20 percent trichloroacetic acid (TCA), while with brain and other tissues, 3 ml of TCA are used per gram of tissue. The suspension is centrifuged at 33,000*g* for 7 minutes in a Sorvall refrigerated centrifuge. The supernatant fraction is extracted twice with an equal volume of benzene to remove GBL. The benzene phase is removed and concentrated to about 2 ml by means of a stream of nitrogen directed over the sample placed in a water bath at 60°C (if necessary, further concentration at this step can provide up to 100-fold greater sensitivity). With the aid of a Hamilton microliter syringe (701 NW/G) a small volume (0.4 to 0.8 μl) of the concentrated benzene extract is placed into a model 10 Barber-Colman gas chromatographic column (1.8 m × 3 mm, glass) containing 12 percent ethylene glycol succinate coated on Anakrom solid support, 70–80 mesh. The column temperature was 125°C. The Argon ionization detector temperature was 200°C, and the flash heater was maintained at 240°C. Argon flow rate was about 80 ml/min. This procedure permits the determination of the amount of GBL present in a tissue.

   To determine the amount of γ-hydroxybutyrate present, the TCA-supernatant fraction is heated to 100°C in a water bath for 15 minutes to convert GHB to GBL, which is then extracted with benzene and estimated in the manner described above. We gratefully acknowledge the invaluable advice and assistance of S. R. Lipsky of the Department of Internal Medicine, Yale Medical School, in the development of this method.
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