## Study Title

The Administration of Gamma-Aminobutyric Acid to Man:
Systemic Effects and Anticonvulsant Action

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# THE ADMINISTRATION OF GAMMA-AMINOBUTYRIC ACID TO MAN: SYSTEMIC EFFECTS AND ANTICONVULSANT ACTION

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## INTRODUCTION

OBSERVATIONS on the effects of γ-aminobutyric acid (GABA) administered to human subjects have been made during investigations of the efficacy of GABA in the clinical control of seizures. These investigations represent extensions of studies carried out on epileptogenic brain samples *in vitro* in order to evaluate the significance of the latter findings more fully. Certain preliminary observations had suggested that glutamic acid metabolism might be deranged in seizure states[29, 41]. Detailed studies on a variety of seizure preparations have been carried out using cerebral cortex slices incubated *in vitro* under standardized conditions, so that the metabolic capabilities of epileptogenic and normal samples could be directly compared. In all seizure states, including those of man, which have been studied in this way there is a significant derangement of glutamic acid metabolism[38, 39]. With the appearance of data on the metabolic role of GABA in brain[20, 22] and on the relationship of GABA to seizures, especially in association with vitamin $B_6$ deficiencies[1, 11, 12, 31, 35], the derangements of glutamic acid metabolism in epileptogenic brain samples assumed added significance.

Some of the pertinent data from our studies are summarized in Table 1. All the epileptogenic samples listed exhibit a profound depression of glutamic acid levels under the conditions noted. Glutamine metabolism, with one exception discussed elsewhere[17, 37], seems to be essentially unaffected, but an apparently comparable derangement of GABA metabolism is present in these samples. The addition of GABA to these samples during *in vitro* incubation partially or, in most cases, completely corrected the abnormality of glutamic acid metabolism[33, 38, 39], as indicated in Table 1. This is in distinct contrast to the lack of efficacy of pyridoxal phosphate added *in vitro* under the same conditions. The latter was effective only for samples from animals with seizures induced by thiosemicarbazide, in conformity with previous observations by a number of investigators[11, 12, 16, 31]. These findings





suggested that glutamic acid and GABA metabolism may be impaired in most seizure states and that such impairment may not necessarily result from interference with pyridoxal phosphate function *per se*. The significance of these findings in terms of seizure mechanisms has been discussed elsewhere[14, 15].

TABLE 1. GLUTAMATE METABOLISM IN INCUBATED SLICES OF NORMAL AND EPILEPTOGENIC CEREBRAL CORTEX

| | (Per cent of normal control levels) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Glutamic acid | | | GABA | | Glutamine | | |
| | Sample | +GABA | +P-P | Sample | +P-P | Sample | +GABA | +P-P |
| Cat | | | | | | | | |
| Normal | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Megimide | 10 | 61 | — | 50 | — | 100 | 100 | — |
| Methionine Sulfoximine* | 68 | 100 | 68 | 55 | — | 10 | 10 | 10 |
| Thiosemicarbazide | 45 | 90 | 85 | 52 | 81 | 80 | 85 | 86 |
| Human | | | | | | | | |
| Normal | 100 | 100 | — | 100 | — | 100 | 100 | — |
| Focal Epileptogenic | 51 | 94 | 51 | 53 | — | 100 | 100 | 100 |

*Conditions:* Slices of cerebral cortex incubated for 1 hr in bicarbonate-saline with 10 mM glucose and 27 mM potassium. Gas phase: 95 per cent oxygen–5 per cent carbon dioxide. Additions to medium were: GABA 10 mM; pyridoxal phosphate (P-P) 1 mM.

Seizures induced in cats by Megimide (3-methyl-3-ethylglutarimide) 25 mg/kg i.p.; methionine sulfoximine 15 mg/kg i.p.; thiosemicarbazide 200 mg/kg i.p. and brain sampled by decapitation after onset of seizures. Human material obtained at operation for excision of cortical epileptogenic foci (local anesthesia only).

* Addition of L-methionine 10 mM resulted in glutamic acid 100 per cent; GABA 89 per cent; and glutamine 10 per cent of normal controls. Addition to normal slices or to slices from other epileptogenic preparations resulted in no detectable differences from unsupplemented samples.

For original data from which table is derived see refs. 17, 33, 36–40.

During the course of the foregoing studies, the observations of Hawkins and Sarett became available to us prior to their actual publication[7]. They were the first to demonstrate anticonvulsant efficacy of GABA and its lactam, 2-pyrrolidinone,* against experimentally induced seizures in mice. Their

* Evidence that cerebral cortex *in vitro* can hydrolyze 2-pyrrolidinone to GABA has been reported[33]. This has been confirmed by incubating cat cortex slices with 1 μC of 2-pyrrolidinone-2-$C^{14}$ (specific activity 1.1 μC/μM). Approximately 15 per cent (0.6 μM/g of tissue) was metabolized during 1 hr incubation. Of the total metabolized, 50 per cent of the $C^{14}$-activity was recovered in GABA, 45 per cent as $C^{14}O_2$, and the remainder as aspartic acid and glutamic acid[40]. This relatively slow rate of conversion, compared to the metabolic rate calculated for GABA under these same *in vitro* conditions[15], may account for the lack of clinical efficacy of 2-pyrrolidinone when administered orally to seizure patients, since its rate of conversion is apparently much slower than its biological half-life in man[40].

data are reproduced in Table 2. More recently other investigators have reported the anticonvulsant potency of GABA in various other seizures states[2, 5, 9, 18].

In view of the two types of evidence illustrated in Tables 1 and 2, it seemed that trials of GABA on a limited, experimental basis in human seizure patients might assist in the evaluation of the significance of the experimental data. Before initiating such trials it was necessary to be satisfied that no toxicity would result from the relatively massive doses contemplated. Dr. Lewis Sarett of the Merck Sharp and Dohme Research Laboratories kindly made available to us the results of their tests for acute and chronic toxicity of the compound. These studies included prolonged chronic administration of large doses (up to 10 mM/kg per day orally) to rats and dogs with complete autopsies at termination of the test periods, as well as intravenous administration of similar doses repeatedly to unanesthetized dogs. No signs of toxicity or untoward effects were observed in any of their animals.

TABLE 2. PROTECTION OF MICE FROM LETHAL SEIZURES

| | | (Per cent protected) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3-Methyl-3-ethyl-glutarimide [25 mg/kg s.c.] | | | | Pentylenetetrazol [100 mg/kg s.c.] | | | |
| Time of premedication (hr)→ | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Compound | Oral dose (mM/kg) | | | | | | | | |
| GABA | 3.9 | 40 | 60 | 30 | 20 | 40 | 60 | 50 | 30 |
| 2-Pyrrolidinone | 4.7 | 70 | 80 | 60 | 50 | 40 | 70 | 30 | 10 |

Derived from Hawkins and Sarett[7].

We have repeated some of these studies on a limited scale with the material actually intended for human administration. Unanesthetized dogs withstood intravenous administration of 10 mM/kg of GABA without incident. On one occasion the serum level 10 min after termination of the infusion was found to be 1 mM of GABA per 100 ml. The effects of this intravenous dose upon blood pressure, respiration, electrocardiogram and electroencephalogram of the anesthetized dog were also studied in collaboration with Dr. Richard Irwin and Mr. Jay Wells of the Section of Clinical Neuropharmacology of our Institute. Representative records are reproduced in Fig. 1. A slight, brief (10 sec) drop in blood pressure and minimal transient increase in respiratory and decrease in cardiac rate occurred during the 30 sec infusion of 10 mM/kg of GABA. Most changes had virtually disappeared before termination of the infusion. These findings conform to those recently published by Elliott and Hobbiger[6] and Takahashi *et al.*[27] At the time it seemed reasonable to ascribe these momentary alterations to the effects of



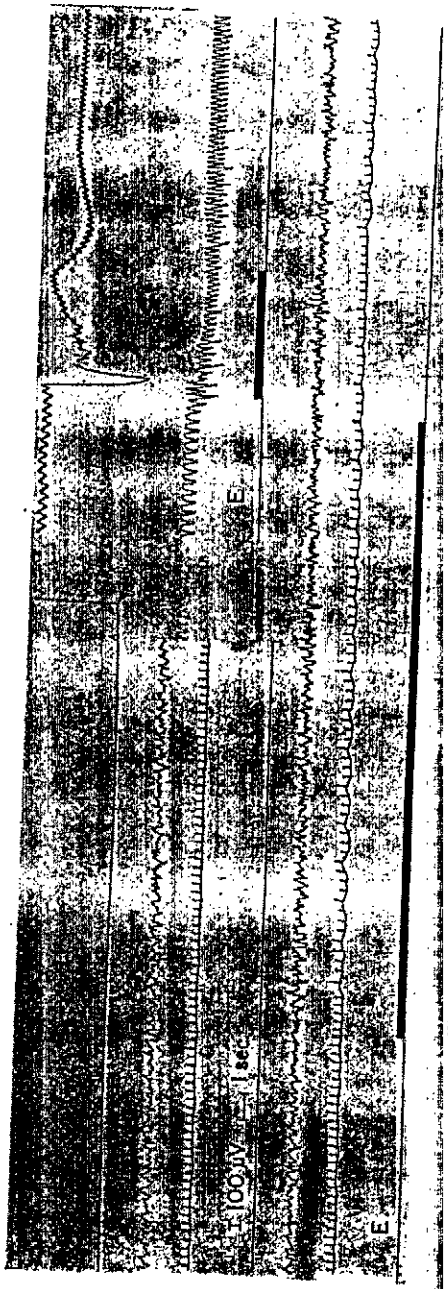


FIG. 1. Effects of 10 mM/kg of GABA administered intravenously (femoral vein) to a dog lightly anesthetized with thiopental. Injection volume 25 ml. Injection time approximately 30 sec (denoted by horizontal black bars beneath appropriate records). Recordings (in order from top) are: carotid blood pressure (recorded from a Hg manometer connected to a siliconed carotid cannula); respiration (recorded by a pneumographic body cuff); electroencephalogram (recorded by needle skull electrodes placed bilaterally over the central region); and electrocardiogram (recorded by needle electrodes led from the two forearms). Time interval markers below BP and Resp. record denote 1 min intervals. Calibration for EEG record given at top left of the record. Injections of equal volumes of saline only (not shown) produced no change in any of the records.

sudden administration of additional fluid volume containing an unbuffered acidic substance. Because of the lack of toxicity of GABA during chronic administration reported to us by the Merck group and in view of the minor effects of relatively massive doses illustrated in Fig. 1, it was deemed safe to initiate oral administration to human patients.

The choice of dose was of necessity somewhat empirical. On the basis of previous experience with administration of L-glutamine and L-asparagine to seizure patients[30, 37–39] an oral dose of 2 mM/kg body weight four times daily (total daily dose 8 mM/kg or about 0.8 g/kg) was chosen. Twice recrystallized reagent grade GABA has been employed.* A total of 14 humans have ingested the compound, 3 in single doses only and 11 on the regular daily schedule outlined above for periods of at least 3 months and in some cases up to 2 years.

### *Systemic Effects of Oral and Intravenous GABA in Man*

In view of the apparent lack of toxicity or side effects in experimental animals given doses 5 times those used clinically, we were surprised to encounter from the start a number of definite side-effects following oral ingestion of GABA. Our experiences are summarized in Table 3 together with representative findings from other reports in the literature dating back nearly 80 years. With only one exception, no significant effects of oral or parenteral administration of GABA to experimental animals have been recorded, and one report in 1933 by Thomas *et al.*[28] of oral administration of 3.3 mM/kg per day for 4 days to a muscular dystrophy patient made no mention of any ill effects. However, Takahashi *et al.*[27], observed a pronounced and prolonged drop in blood pressure and some hyperventilation in rabbits under urethane anesthesia given 0.08–0.12 mM/kg intravenously. These workers also observed a slight transient drop in blood pressure with similar doses administered to unanesthetized rabbits. They also noted paradoxically that with higher doses the effects were minimal. These latter findings are apparently comparable to those for the dog depicted in Fig. 1.

Almost without exception our patients have reported that *within minutes* after ingestion of 2 mM/kg of GABA they experience a sensation of warmth, which coincides with observed flushing of face and extremities. This reaction is often rapidly followed by a general malaise or uneasy feeling, which may be accompanied by nausea and rarely emesis. Two patients have experienced recurrent diarrhea. In addition, the majority of patients have experienced paresthesias of the extremities more or less coincident with the foregoing signs and symptoms. Three normal adults on our clinical staff also experienced these reactions after ingestion of a dose of 1 mM/kg. The duration of these various side effects has generally been 10 and 90 min, and tolerance to the compound as far as flushing, malaise and paresthesias are concerned generally develops within a few weeks. In 3 cases the gastrointestinal symptoms have

* Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. The material met the following specification: m.p. 186–187°C; Nitrogen content 13.6 per cent; moisture content not exceeding 0.1 per cent and ash less than 0.5 per cent.





TABLE 3. SYSTEMIC EFFECTS FOLLOWING ADMINISTRATION OF GABA

| | Dose and route | Effects | References |
|---|---|---|---|
| I. Experimental animals | | | |
| A. Unanesthetized | | | |
| 1. Rat | 5 mM/kg p.o. or i.p. | [None reported] | Van Gelder and Elliott, 1958 |
| 2. Rabbit | 0.3 mM s.c. | None | Schotten, 1883 |
| | 0.08–0.12 mM/kg i.v. | Slight BP ↓ | Takahashi *et al.*, 1955 |
| | 2 mM/kg i.v. | [None reported] | Van Gelder and Elliott 1958 |
| 3. Dog | 5 mM/kg s.c.* | None | Corley, 1926 |
| | 30 mM/kg s.c. | [None reported] | Keil, 1932 |
| | 10 mM/kg i.v. | None | Tower (unpubl.) Sarett (unpubl.) |
| 4. Monkey | 2 mM/kg i.v. | [None reported] | Van Gelder and Elliott, 1958 |
| B. Anesthetized | | | |
| 1. Rabbit | 0.08–0.12 mM/kg i.v. | BP ↓ 50–60% 2 hr; Hyperventilation | Takahashi *et al.*, 1955; 1958; Elliott and Hobbiger, 1959 |
| 2. Cat | 0.1–1.0 mM/kg i.v. | Slight, variable on BP | Elliott and Hobbiger, 1959 |
| 3. Dog | 10 mM/kg i.v. | Slight BP ↓ Slight resp. ↑ Slight pulse ↓ | [See Fig. 1]; also Elliott and Hobbiger, 1959 |
| II. Man | | | |
| A. Intravenous | 0.05 mM/kg | BP (diast) ↓ 40% Hyperventilation Flushing Acute discomfort Duration: 5 min | McKhann and Tower (unpubl.); also Elliott and Hobbiger, 1959 |
| B. Oral | 3.3 mM/kg (4 days) | [None reported] | Thomas *et al.*, 1933 |
| | 1–2 mM/kg† | Flushing Paresthesias Malaise Nausea, vomiting Diarrhea Duration: 10–90 min | Tower, 1959 |

* Phlorhizinized animals.

† Three acute observations; 11 on chronic therapy of dose 4 times daily (total 4–8 mM/kg/day).

persisted for a number of months, but in no case have been severe enough to necessitate abandoning the therapeutic trial. Since many amino acid compounds administered orally produce similar gastrointestinal irritation, these particular effects may not be directly related to the flushing and paresthesias. The rapidity with which the latter occur, at times after ingestion when blood levels are very low (see Fig. 5 below), suggests that these are peripherally rather than centrally mediated and that they may well be autonomic, perhaps vasomotor, phenomena. Dr. Harry Williams at Emory has encountered an analogous situation following administration of β-alanine to human subjects. Shortly after ingestion these subjects experienced extreme pruritus. These reactions are reminiscent of the effects of various amines, but the explanation for these reactions is not yet apparent.

The side effects accompanying oral administration of GABA have not been severe or serious. The fact that some of our patients have been on daily doses of 8 mM/kg for a year or more with no indication of chronic or cumulative toxicity is evidence in this regard. In distinct contrast the effects of GABA administered intravenously to man are striking and severe. Elliott[6] was the first to make this observation and we have subsequently confirmed his observations, as indicated in Table 3. Our patient, a 7-year-old girl, was given 0.05 mM/kg slowly on two occasions, interspersed with injections of the saline vehicle alone. There was no reaction to the latter at all, but when GABA was infused there was an almost immediate drop in diastolic blood pressure (averaging 40 per cent), immediate hyperventilation, flushing and acute discomfort which the patient was unable to describe. No marked change in pulse rate occurred. These changes lasted about 5 min. The intravenous solution used in this case had been carefully checked for sterility and for pyrogens by tests specified for intravenous fluids for human administration. In addition an aliquot was administered in a dose of 10 mM/kg intravenously to an unanesthetized dog without detectable effects. At the present time, on the basis of these observations, we feel that the intravenous administration of GABA to man is contraindicated, despite the fact that this hampers certain aspects of evaluation of this compound as an anticonvulsant and the investigation of mechanisms in cases of pyridoxine dependency[26].

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### *Anticonvulsant Effects of GABA in Man*

The efficacy of oral GABA in controlling clinical seizures is illustrated by results summarized in Figs. 2 and 3. These are the monthly seizure record and representative electroencephalograms of a 14-year-old girl with *petit mal*-type seizures. During 7 months of observation while on presumably adequate doses of trimethadione and diphenylhydantoin, this girl had some 300–500 seizures per month, or a mean of 402 (±68) seizures per month for the 7 month period. When she was switched to only oral GABA 2 mM/kg four times daily, there was a prompt and dramatic reduction of seizure frequency essentially to zero within 2 months. Control was sustained for the ensuing 4 months, after which GABA was stopped. Seizure frequency rose after a short lag towards previous levels. Due to circumstances beyond our control, the records during this period are somewhat incomplete, so that we cannot be



sure of the peak frequencies reached. There is no question that during the period denoted by the dotted lines in Fig. 2, she was having at least 5–10 seizures daily—substantiated for the months actually recorded. Three months ago she was again started on daily oral GABA in a dose of 0.5 mM/kg four times daily. Some decrease in seizure frequency occurred, but it is difficult to assess its significance in view of the variability noted during the control period. However, when the dose was again raised to 2 mM/kg, a very prompt and undoubted decrease in frequency again took place.

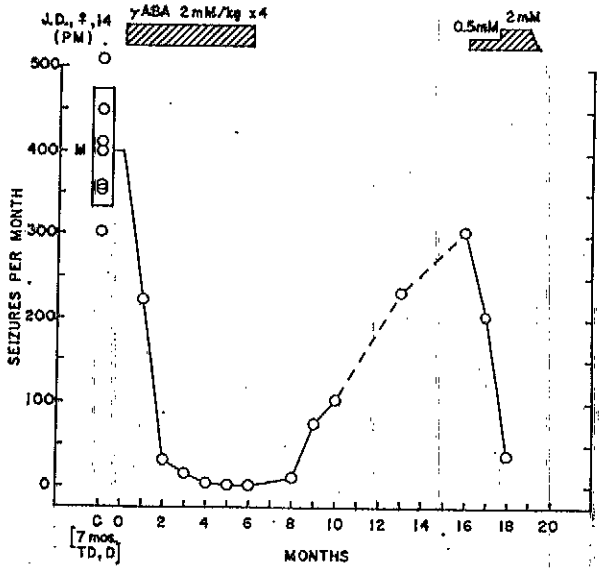


FIG. 2. Monthly seizure record of Patient J. D. discussed in text. *Petit mal* (PM) seizure frequency for the 7-month control period (C) while on trimethadione (TD) and diphenylhydantoin (D) had a mean value (M) of 402 (± 68). Standard deviation of mean value indicated by length of the box.

The electroencephalographic records shown in Fig. 3 complement the clinical data. Record A, taken prior to the second trial on GABA, illustrates the virtually continuous paroxysmal, high voltage epileptiform activity induced after about 2 min of hyperventilation and persisting to the end of the full 5 min test period. Many frank seizures were observed during this period. Record B, taken coincident with the last clinical point (month 18) recorded in Fig. 2, provides a distinct contrast. With the same recording conditions and at the same point during hyperventilation no paroxysmal high voltage activity was observed, and only three brief (2–4 sec) bursts appeared during the whole 5 min hyperventilation test period. It is always difficult to be sure that such comparisons are valid, since uncontrollable factors may enter this method of activation of epileptiform activity. There is no question that the second test period was well performed. The time of recording was in each case about 3 hr after eating, so that differences due to

levels of glycemia and the like would appear to be minimized. Thus within the inherent limitations of such clinical experiments the differences between the two records would appear to be valid and significant and to be in accord with the clinical status at each time.

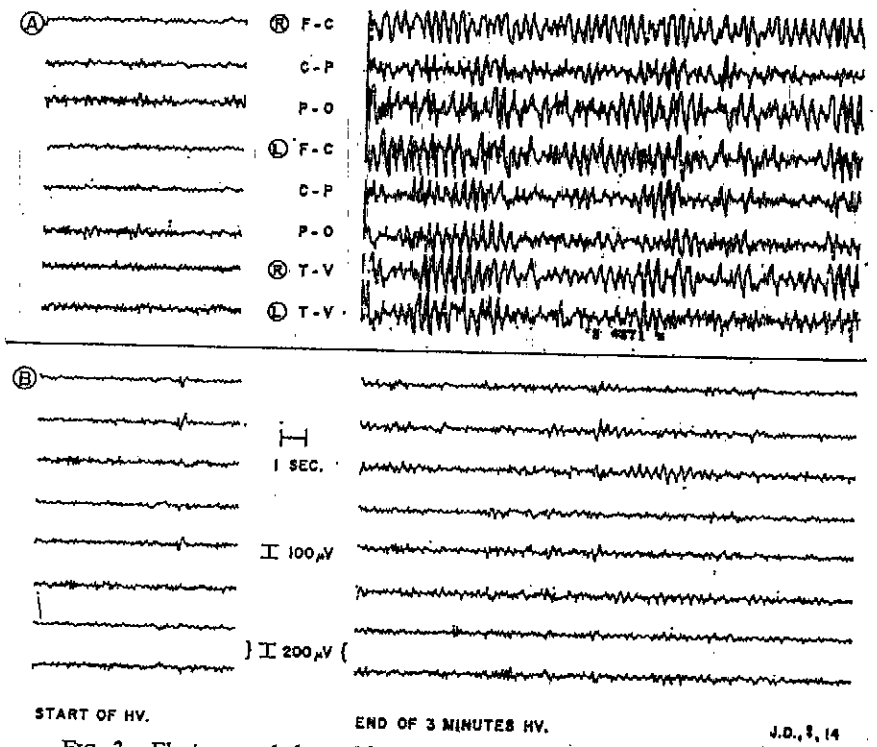


FIG. 3. Electroencephalographic records of patient J. D. (Fig. 2) at the start of hyperventilation (samples on left) and at the end of 3 min of hyperventilation (samples on right). Record A taken before second trial on GABA (see Fig. 2) when frequency of seizures was 200–300 per month. Record B taken at the end of month 18 (see Fig. 2) while on oral GABA 2 mM/kg. 4 times daily. Seizure frequency at this time was less than 40/month. Electrode placements, instrument calibrations and other conditions were the same for both recordings. Time and calibration scales given between lower records. Electrode leads given between upper records (abbreviations: R = right; L = left; F = frontal, C = central, P = parietal, O = occipital—all in the midlateral plane—T = temporal (ear) and V = mid-vertex).

Data on three other patients, illustrated in Fig. 4, indicate that the foregoing case is not an isolated instance. The 13-year-old boy with *petit mal*-type seizures has been followed for nearly 2 years now on oral GABA alone with an obviously significant decrease in seizure frequency from previous levels on other medications. We do not know whether complete control could be achieved at a higher dose level, since the parents have been unwilling to increase the dose. The 14-year-old boy with nocturnal generalized (or



major) seizures has also experienced a significant decrease in seizure frequency since GABA was added to his previous medications. The figure fails to indicate that the seizures which still occur are much less severe than previously, some now being scarcely perceptible. Previously the seizures were always accompanied by micturition, but now none of them are. The third case, a 14-year-old girl, has both minor *petit mal*-type and major or generalized seizures. Since they are less frequent than in the other three cases, the data have been plotted as *total number* of seizures for the entire trial periods indicated instead of as monthly frequency. The totals for 10 months on GABA alone are less for both types of seizures than the totals in the preceding 5

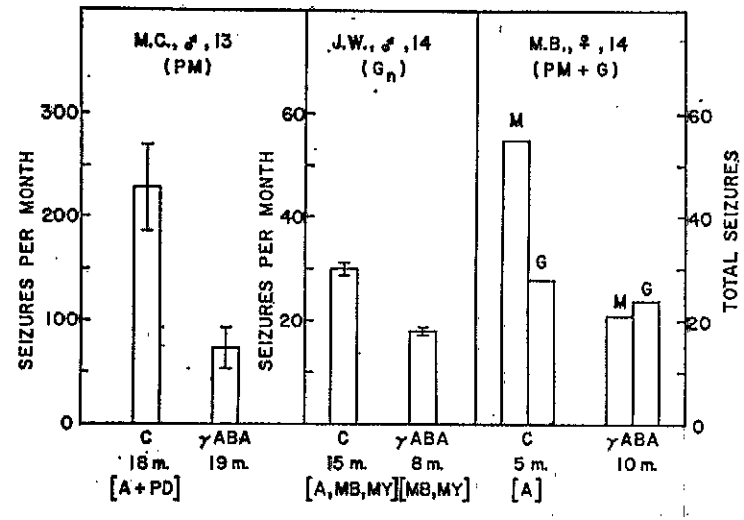


FIG. 4. Seizure frequencies for three patients during control (C) periods and periods on oral GABA 2 mM/kg 4 times daily. Observation periods in months (m) indicated below each bar. For two patients on the left seizures are plotted as mean frequency per month for each period with the standard deviations indicated by the central line atop each bar. For patient M. B. on the right seizures are plotted as *total number* for the entire respective observation periods. (Abbreviations: Seizure types: PM = *petit mal*; $G_n$ = nocturnal generalized or major; G = generalized or major; M = minor or *petit mal*; medications (during control periods): A = L-asparagine; PD = paramethadione; MB = mephobarbital (Mebaral); MY = Mysoline). See text for detailed discussion.

month period. It is of interest that this patient exhibits a definite relationship between occurrence of seizures and menstrual periods, so that the better control on GABA may be of additional significance in this respect.

These four patients demonstrate that daily oral administration of GABA can be an effective anticonvulsant, and it can apparently prove effective against various different types of seizures. The seemingly readier response of the minor or *petit mal* types cannot properly be evaluated on such a limited number of patients. These results must be tempered by others where no response to GABA has been obtained. A 35-year-old man with *petit mal*-

type seizures has been on GABA for nearly 2 years now with no change in seizure frequency from previous "control" period. A 16-year-old girl with *petit mal*-type seizures associated primarily with menstrual periods experienced little, if any, benefit from oral GABA over a 6 month trial period. Other patients during briefer trial periods did not appear to benefit either. Regardless of factors perhaps peculiar to GABA, these differences in clinical response are not too surprising, since there is no known anticonvulsant which has proved effective in more than about two-thirds of a given epileptic population.[32].

The foregoing data must be considered purely as clinical experiments. We are not engaged in promoting new anticonvulsant therapies, nor do we advocate on the basis of these limited results GABA as a new anticonvulsant therapy. Our interest is primarily in the use of such clinical experiments to augment the experimental animal and *in vitro* studies discussed earlier and to test hypotheses suggested by such studies. From this specific point of view the clinical trials may be considered successful. They do not necessarily prove such hypotheses nor do they necessarily constitute confirmatory evidence. However, the fact that significant control of clinical seizures in man by GABA alone can be achieved may be suggestive evidence in favor of the view, derived from *in vitro* data in Table 1 and discussed elsewhere[14], that derangements of glutamic acid and GABA metabolism in epileptogenic brain samples could be important factors in the mechanisms responsible for seizure activity.



## *The Metabolism of GABA Administered to Man*

Some preliminary studies have been carried out on a few of the patients to obtain information about the metabolism of GABA by man. Van Gelder and Elliott[43] have recently reported studies on a variety of experimental animals in some detail. In both cases methods for quantitative determination of GABA have been employed which are not necessarily specific. Van Gelder and Elliott assayed their samples with the crayfish stretch-receptor preparation, which, like all biological assay methods, may respond to unsuspected constituents of blood and particularly "raw" urine samples. We have used a macro modification of the fluorimetric method of Lowe *et al.*[13], which also may be subject to nonspecific interference. In our own studies we can only state that numerous control samples of human blood serum and cerebrospinal fluid and of the amino acid fraction of human urine (isolated by column chromatographic procedures) gave minimal blank values and the recoveries of known amounts of added GABA were eminently satisfactory from these three fluids.

Our data on blood serum and cerebrospinal fluid levels at various times after oral doses of 2 mM/kg of GABA to human subjects are summarized in Fig. 5. With one exception discussed below, the serum level–time curves are similar to each other in terms of peak times after ingestion, peak levels attained, and time of return to "fasting" values. The curves in Fig. 5 do not resemble those for rats ingesting 5 mM/kg, published by Van Gelder and



Elliott[43], which show a perfectly flat level of 50 $\mu$M/100 ml from 10 to 90 min after oral administration. Our serum curves are, however, quite comparable with those obtained with L-asparagine and L-glutamine administered orally to man in equivalent doses[37, 39, 42].

Urine collected up to 24 hr after single or multiple doses administered to these patients did not exhibit any increase in "apparent" GABA content over comparable control samples. This contrasts with the animal studies of Van Gelder and Elliott[42] where "very high" concentrations of GABA were found in urine "shortly after injection". Their findings are surprising

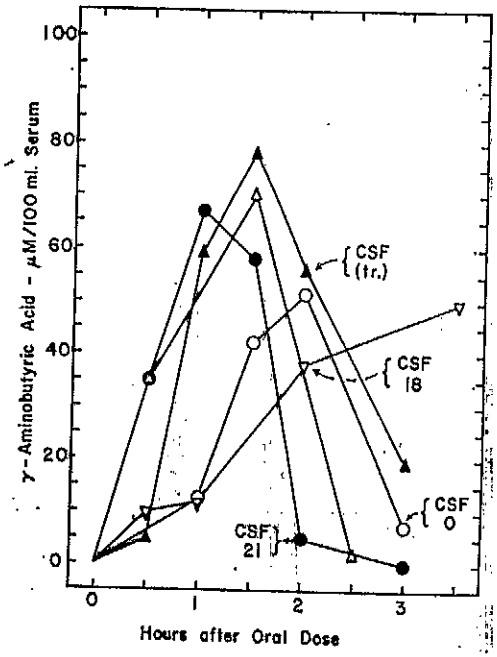


FIG. 5. Blood serum and cerebrospinal fluid levels of GABA at various times after oral administration of 2 mM/kg. All studies carried out in the early morning under fasting conditions at least 12 hr after the last previous dose of GABA and the last meal. Each set of symbols denotes a different patient. See text for discussion.

In view of the apparent capacity of liver and kidney to metabolize GABA rapidly[3, 21]. Metabolic disposal of asparagine and glutamine by liver and kidney occurs promptly, and, since the serum curves for GABA and these two amides are so similar in man, it is likely that the metabolic disposal is also similar. This tentative conclusion receives support from one patient included in Fig. 5 (denoted by the inverted open triangles). This patient had Wilson's disease with frank hepatic cirrhosis. The course of his serum level curve after oral GABA was distinctly different from all the others, being characterized by progressive elevation with time. Since these patients usually

exhibit pronounced amino aciduria and if direct renal excretion were the principal mode of GABA disposal, such a serum curve would seem unlikely. On the other hand this type of serum curve would be expected if hepatic metabolism were a principal mode of GABA disposal, and it is noteworthy that a somewhat comparable observation has been reported by Himwich *et al.*[8] for a patient with hepatic disease to whom glutamic acid was administered orally.

In a few cases, indicated in Fig. 5, cerebrospinal fluid samples were obtained simultaneously with serum samples. In three cases, GABA was definitely present at times closely related to periods of high serum levels. In one case none was detectable an hour after the serum peak. Although the cerebrospinal fluid levels are of the same order as the few Van Gelder and Elliott[43] were able to obtain, there is a distinct disparity between their data and ours in terms of serum to cerebrospinal fluid ratios. For example, they cite for anesthetized cats serum levels of 700–750 $\mu$M/100 ml with simultaneous cerebrospinal fluid levels of 10–20 $\mu$M/100 ml or ratios of about 50 : 1, whereas for two of the patients shown in Fig. 5 the ratios were 4.5 : 1 and 1 : 4 respectively. If these ratios were expressed in the more comparable ratios of blood to cerebrospinal fluid, the differences would be even more striking.

These observations raise several important points. In the first place, from the limited data available there seems to be little apparent relationship between therapeutic response of our seizure patients and their blood or, in two instances, cerebrospinal fluid levels. The patient with the highest cerebrospinal fluid level (solid circles in Fig. 5) is J. W. in Fig. 4. On the other hand, the patient with the highest blood levels (solid triangles in Fig. 5) and a somewhat similar serum level–time course to patient J. W., as well as a detectable amount in cerebrospinal fluid, is the 34-year-old man who has not benefited at all from GABA therapy. For unavoidable reasons it has not been possible to study the two patients with the best response to GABA from this standpoint.

A direct relationship between body fluid levels and therapeutic response should not necessarily be expected for a compound like GABA, which, if it does penetrate from blood into the central nervous system, might pass directly to the tissues rather than into cerebrospinal fluid. In a single experiment, a mouse was injected with 0.5 $\mu$C of GABA-1-$C^{14}$ (specific activity 1.1 $\mu$C/$\mu$M) in 0.25 ml heparinized saline in 30 sec and was sacrificed 8 min later. The blood contained 4220 counts/min per ml, of which 600 counts/min per ml were present as $C^{14}O_2$, and the brain contained 605 counts/min per g with no $C^{14}O_2$, or a presumed blood to brain activity of 6 : 1. Although this finding is not very impressive, it does indicate a limited degree of brain penetration (or exchange) under these conditions and a considerably better blood to brain ratio than might be anticipated from the data of Van Gelder and Elliott.[43]

Another factor, especially pertinent to studies on rodents, has not received much attention in the past. The situation is outlined by the data in Table 4. The availability of compounds in the circulation to the central nervous system



will be greatly affected by two factors: the biological half-life of the compound —which is the composite of the factors that determine the circulating level at various times after administration—and the relative cerebral circulation. The latter is emphasized by the fact that the human brain, which in terms of percentage of total body mass is proportionately less than 3 times the rat brain, receives 10 times the percentage of the total circulation per minute that the rat brain receives. Furthermore, if liver and kidneys are principal sites of disposal of the compound, rat brain is at a 1 : 20 disadvantage compared to the estimated 1 : 2 ratio of cerebral to combined liver and kidneys circulation percentage in man. The practical significance of these differences between rat and human brain is illustrated by the example of hexobarbital in Table 4, where biological half-life, plasma levels on awakening

TABLE 4. FACTORS IN CENTRAL NERVOUS SYSTEM THERAPY: BIOLOGICAL HALF-LIFE AND RELATIVE CEREBRAL CIRCULATION

| | Approximate percentage of total body mass | | | Approximate percentage of total circulation per min | | | Biological half-life (min) | Plasma level on awakening ($\mu$M/100 ml) | Liver enzyme activity ($\mu$M/g hr) |
|---|---|---|---|---|---|---|---|---|---|
| | Brain | Liver | Kidneys | Brain | Liver* | Kidneys | | | |
| Rat | 0.8 | 5.5 | 1.0 | 1.6 | 20 | 15 | 140† | 25.0 | 0.5† |
| Dog | 1.0 | 4.0 | 0.5 | 5.0 | 30 | 11 | 260 | 7.5 | 0.15 |
| Man | 2.2 | 2.5 | 0.5 | 15–20 | 15 | ? | 360 | 7.5 | — |

* Ratio of hepatic to portal flow about 1 : 2.
† Values for female rats. Male rats exhibited biological half-life of about 35 min and liver enzyme activity of 2.6 $\mu$M/g hr. Plasma level on awakening same for both sexes.
Data derived from Quinn *et al.*[19], Sapirstein[23, 24] and Tower[34].

and rates of liver metabolism are indicated. Other factors are obviously involved, some of which are indicated in Table 4 and are more fully discussed by Quinn *et al.*[19] The pertinence of these factors to GABA stems from the fact that most attempts to demonstrate the penetration of systemically administered GABA into brain have been carried out on rodents[1, 22]. In view of the considerations just discussed, it is not necessarily valid to conclude from the failure of these attempts that the same situation would obtain in man.

There is, however, a final factor which is clearly important in dealing with seizure subjects. Purpura *et al.*[18] have shown that when the blood–brain barrier is locally damaged, cerebral penetration of systemically administered GABA definitely occurs. It is not certain whether seizure activity in the brain is or can be associated with alterations in blood–brain barrier permeability locally or generally. The findings of Hawkins and Sarett[7] (Table 2) might be interpreted in favor of this possibility, since their peak protection period coincided with a potent convulsant challenge given at a time when blood

levels of GABA in their animals could be expected to be relatively maximal. In our patients the efficacy or lack of efficacy of GABA as an anticonvulsant may depend upon the extent to which this factor (increased blood–brain barrier permeability) is operative. If this were so, the apparent lack of correlation between anticonvulsant efficacy and blood levels would be readily explicable, and the lower ratios of blood to spinal fluid levels observed in the few cases shown in Fig. 5, compared to those obtained on normal animals, would also be reasonably accounted for.

## SUMMARY

The effects of administration of GABA to 14 human subjects have been reviewed. Eleven have been on oral doses of 2 mM/kg body weight four times daily (a total of 8 mM or 0.8 g/kg daily) for periods from 3 months to nearly 2 years. The effects of minimal intravenous doses were studied in one case. The findings have been discussed in three categories.

(1) *Side effects.*—Immediately following oral administration and persisting 10–90 min subjective and objective flushing, paresthesias of the extremities, and generalized malaise were encountered in most human subjects, and some also experienced nausea, emesis or diarrhea. Tolerance to these side effects usually developed with a few weeks on continued daily administration. No comparable side-effects have been observed or reported for experimental animals. One patient given 0.05 mM/kg of GABA intravenously reacted immediately with flushing, 40 per cent drop in diastolic blood pressure, hyperventilation, and acute discomfort lasting about 5 min. The same solution administered in a dose of 10 mM/kg intravenously to dogs was without visible effects.

(2) *Anticonvulsant potency.*—Four patients with various types of seizures experienced significant improvement and in one case complete control of seizures after being started on oral GABA. The latter case, averaging 402 (±68) *petit mal*-type seizures per month on trimethadione and diphenylhydantoin, was completely controlled within two months by 2 mM/kg of GABA 4 times daily. Striking improvement in the electroencephalographic records of this patient also occurred. A number of other seizure patients experienced little or no benefit from therapeutic trials of GABA.

(3) *Metabolism.*—Limited studies of blood serum and cerebrospinal fluid levels and urinary excretion of GABA after oral administration were also carried out. Peak serum levels of about 60–80 μM/100 ml were observed between 1 and 2 hr after ingestion in all cases studied. Cerebrospinal fluid levels at 2 hr after ingestion ranged in three cases from a definite trace to 21 μM/100 ml. No evidence for significant urinary excretion was obtained up to 24 hr after single or multiple oral doses. Persistent elevation of serum levels in one case of hepatic disease suggested that hepatic metabolism is an important mode of disposal of exogenously administered GABA in man.

The significance and implications of these various observations have been briefly discussed in relation to the problems of seizure mechanisms and the functional role of GABA.

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*Addendum:* Recently G. K. Bunyatyan (Acad. of Sci., Armenian SSR) has reported that small intravenous doses of GABA to dogs are followed by accelerated uptake of glucose and synthesis of glycogen by skeletal muscle coincident with hypoglycemia. These effects are transient and are not seen when larger doses of GABA are infused. These observations may explain part or all of the systemic side effects reported in the foregoing paper for GABA administered to man.