muscle of the rabbits that were not collared than occurred in collared animals. The results of the fractionation of the S^{35} in liver hydrolysates (Table I) indicate that there was a significant amount of S^{35} incorporated into cystine and methionine in the case of the uncollared rabbit, and that a marked reduction in the amount of S^{35} appearing in organic forms in the collared rabbit. There was about 30 times as much S^{35}-labeled cystine and methionine in the liver of the uncollared rabbit, and the S^{35}-labeled sulfate fraction was twice as great as was the case in the liver of the collared rabbit. These results suggest that the increased amounts of labeled cystine and methionine found in the liver of the uncollared rabbit may have been acquired as the result of fecal refection, followed by the digestion and assimilation of organic sulfur compounds synthesized by intestinal microorganisms.

Summary. The metabolic fate of S^{35}-labeled sulfate in young rabbits was studied. Absorption of S^{35} after labeled sulfate was administered by stomach tube was very rapid, with the peak appearing in blood around 6 hours after dosing. Some synthesis of labeled cystine and methionine took place, with S^{35}-labeled cystine and methionine appearing in the liver. When the habitual practice of coprophagy was interfered with by fitting the rabbit with a collar which prevented fecal refection, the amount of labeled cystine and methionine was greatly reduced.

1. Block, R. J., Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 391.

2. Block, R. J., Stekol, J. A., Loosli, J. K., *Arch. Biochem. Biophys.*, 1951, v33, 353.

3. Kulwich, R., Struglia, L., Pearson, P. B., *J. Nutrition*, 1957, v61, 113.

4. Odeblad, E., Bostrom, H., *Acta Pathol. et Microbiol. Scand.*, 1952, v31, 339.

5. Bostrom, H., Odeblad, E., *Anat. Rec.*, 1953, v113, 505.

6. Kulwich, R., Pearson, P. B., Lankenau, A. H., *Arch. Biochem. Biophys.*, 1954, v50, 180.

7. Stein, W. H., Moore, S., *Cold Spring Harbor Symposia Quant. Biol.*, 1950, v14, 179.

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Synaptic Effects of Systemic γ-Amino Butyric Acid in Cortical Regions of Increased Vascular Permeability. (23740)

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Topically applied to the cerebral cortex, aliphatic ω-amino acids exert rapid, reversible synaptic effects dependent on carbon chain length(1-3). Electrophysiological analyses indicate that the acids with less than 5 carbons selectively block excitatory synapses on apical dendrites, whereas longer chained ω-amino acids selectively block inhibitory synaptic electrogenesis. Of the group with less than 5 carbons, γ-amino butyric acid (GABA), the most potent inactivator of dendritic excitatory synapses, occurs in relatively high concentration in normal brain(4,5). Selective blockade of excitatory dendritic synapses following topical application of GABA has been shown to account for the alterations induced in a variety of evoked cortical responses(3). In contrast to the effects caused by topical application, intravenous administration of GABA produces neither central synaptic actions nor detectable increase in brain concentration of the amino acid(6). These results suggest that under

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ordinary circumstances GABA does not effectively penetrate the blood-brain barrier; its behaviour in this respect being similar to that of one of its precursors, glutamic acid (7). Rapid penetration of GABA into the brain following alterations in the permeability characteristics of the barrier, should therefore produce synaptic effects similar to those resulting from topical application. Although little is known about the essential nature of the barrier, a variety of agents, chemical and physical, are capable of affecting it. Two of these, ethyl chloride and chloroform-methanol permit local destruction of the barrier with preservation of physiological activity in most regions of barrier loss. Under these conditions systemic administration of ω-amino acids, exerts synaptic actions in regions of local barrier breakdown comparable to those produced by topical application. The present report primarily concerns the overt effects of systemically administered GABA.

Methods. Twenty succinylcholine-paralyzed cats were prepared under ether anesthesia and with procaine infiltration of the skin and muscles at the operated sites for cortical registration of evoked dendritic postsynaptic potentials in a manner similar to that previously described in detail(8). In some of these, small amounts of pentobarbital sodium (15 mg/K) were administered prior to recording periods. Local breakdown of the blood-brain barrier was produced either by application of a few drops of chloroform-methanol (2:1) solution to the cortical surface, or by restricted freezing with ethyl-chloride utilizing the technique of Morrell and Florenz(9) for producing experimental epileptogenic lesions. Cortical regions of barrier loss were delineated by vital staining with Evans blue dye (W761-1)‡ injected prior to, or immediately after, production of the lesion. Spontaneous or evoked potentials recorded in these regions over long periods of observation exhibited no change attributable to the presence of the dye in the cortex. Although a detailed analysis of the histological and electrophysiological changes occurring in cortex treated with these agents will be presented elsewhere(10), in the experiments described below the criteria for viability were evaluated only in physiological terms. It should be noted, however, that application of ethyl-chloride or chloroform-methanol resulted in localized destruction of cytoarchitecture (Fig. 1), more pronounced following ethyl chloride freezing than application of the chloroform-methanol. In all cases staining of a wedge shaped region of the entire thickness of cortex could be readily observed, extending in many instances deeply into the subcortical white matter (Fig. 1A). Of considerable importance was the fact that blood-brain barrier destruction, as evidenced by the penetration of Evans blue, extended well beyond the regions of maximal histological changes. With the exception of the latter regions, of little or no responsiveness, potentials could be readily evoked by surface stimulation in surrounding areas exhibiting marked blood-brain barrier destruction. *Smaller areas of unresponsiveness* to direct stimulation were generally encountered in chloroform-methanol treated regions than in those sprayed with ethyl-chloride. Despite relatively less cellular damage induced by chloroform-methanol, the severity and extent of blood-brain barrier loss produced by this lipid solvent exceeded that caused by ethyl-chloride. *Significent physiological differences* were observed in regions of ethyl-chloride freezing and chloroform-methanol application, related in part, to the degree of localized cellular destruction. Regions treated with chloroform-methanol never gave rise to spontaneous paroxysmal discharges within the period of observation (6-8 hours), while these were repeatedly observed following ethyl-chloride spraying, depending on the cortical location of the lesion. Their presence in regions of barrier destruction permitted analysis of systemically administered amino acids on the activity of the epileptogenic focus. At the conclusion of all procedures, the animals were anesthetized with 30 mg/K pentobarbital sodium and the brain perfused with 10% formalin.

Results. A. Effects of systemic GABA on evoked dendritic postsynaptic potentials.

‡ Generously supplied by Warner-Chilcott Laboratories.

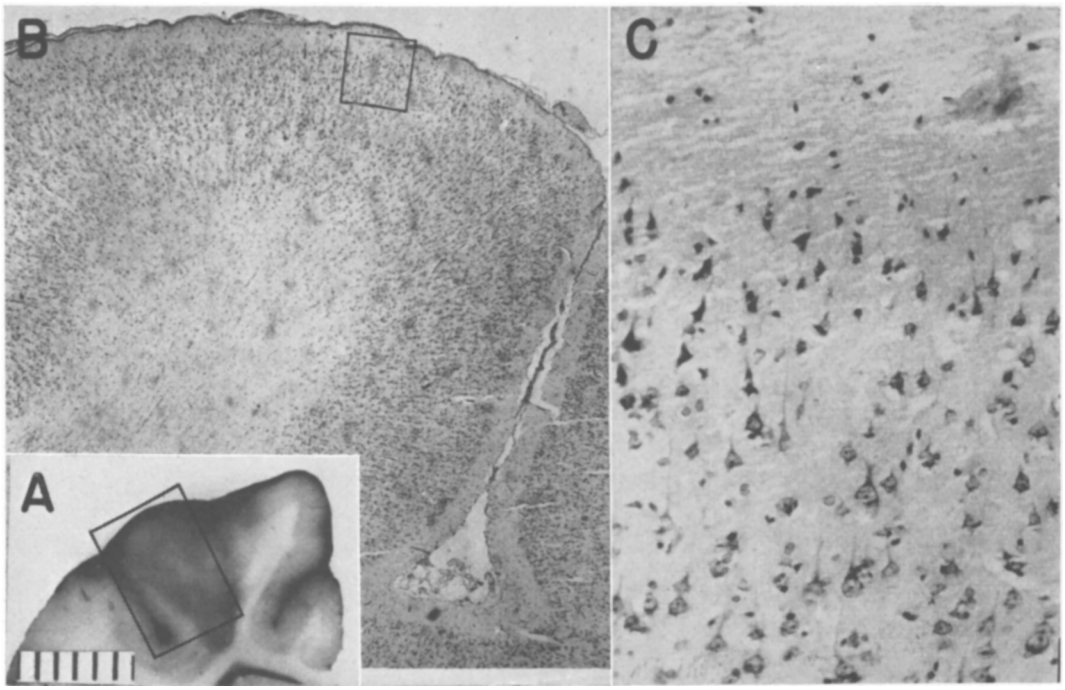

FIG. 1. *A*. Gross appearance of vitally stained fixed brain following application of 3 drops of chloroform-methanol solution to suprasylvian gyrus, 6 hr prior to fixation. Evans blue dye detectable throughout gyrus and deeply into white matter. Evidence of massive blood-brain barrier destruction also observed in adjacent gyri. Scale in mm. *B*. Nissl preparation, of rectangular area demarcated in *A* magnified ten-fold, showing regions of normal and abnormal cytoarchitecture. In this preparation absence of responsiveness to direct stimulation was limited to a small region of maximum destruction. *C*. Ten-fold enlargement of region in *B*, taken through area of maximal cellular destruction.

Topical application of GABA reverses the surface negative evoked potential of the cerebral cortex to postivity by "unmasking" countervailing hyperpolarizing postsynaptic potentials. when the depolarizing responses are blocked(1). Responses evoked from wide areas of blood-brain barrier destruction, were altered in a similar way by systemic amino acids (Fig. 2), whereas no effects were detectable in unstained cortical regions of normal vascular permeability.

In stained cortex following an ethyl chloride lesion, the evoked response (Fig. 2A. upper channel recording) was essentially similar to that recorded from a contralateral, homologous site (Fig. 2A. lower channel). Within 15 seconds after intravenous administration of 50 mg/K GABA. rapid abolition of evoked surface negativity occurred in stained cortex (shown in single channel records, B) without changes in responses from unstained cortex (C). Blockade of excitatory p.s.p. s and unmasking of dendritic inhibitory p.s.p.'s in stained regions (B) persisted for more than 45 minutes, gradually reversing and recovering (D) at the end of 1 hour. Similar results were encountered in minimally narcotized preparations (I-L).

In chloroform-methanol treated cortex blockade of excitatory synapses on apical dendrites also occurred with dramatic onset following intravenous GABA (Fig. 2, E-H). Effects were detectable (lower channel) with as little as 8 mg/K (F), complete blockade being produced by an additional 25 mg/K (G). Recovery was considerably delayed in these preparations, for as long as 90 minutes (H).

The depth of the blood-brain barrier lesions produced in sensorimotor cortex by application of chloroform-methanol (Fig. 1) permitted testing the effects of systemic GABA on axo-somatic synaptic activity generated by collateral excitation of cortico-spinal neurons

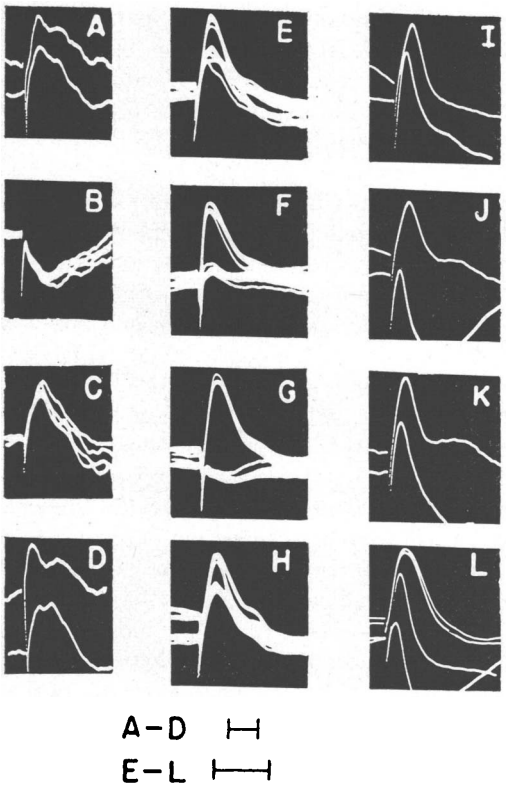

FIG. 2. Differential action of systemically administered γ-amino butyric acid (GABA) in cortical regions of blood-brain barrier loss. In all records dendritic postsynaptic potentials recorded monopolarly following direct cortical stimulation. In this and subsequent records negativity at stigmatic electrode indicated by upward deflection. *A-D:* (unanesthetized-paralyzed cat, ethyl-chloride induced barrier lesion). Upper channel records in *A* and *D* and *B* from stained cortex; lower channel records and *C*, normal cortex. *B* and *C* superimposed responses evoked 40-50 sec. after GABA, 50 mg/kg intrav. *D*, 60 min. later. *E-H:* (pentobarbitalized cat, 15 mg/kg; chloroform-methanol induced barrier lesion). Upper channel records from normal cortex, lower channel records from regions of barrier breakdown. In *E*, *F*, after 8 and 25 mg/kg GABA, respectively. *H*, 90 min. after *G*. *I-K:* (ethyl-chloride induced barrier lesion). *I*, dendritic p.s.p.'s before inj. of 25 mg/kg GABA. Effect of inj. detectable only in area of blood-brain barrier loss (lower channel records) is maximal at 1 min. *(J)* and slowly reversible (45 min., *K*). *L*, superimposed records before and after another similar inj. Time calibrations 20 msec.

or by indirect stimulation of specific thalamocortical afferents. Stimulation of the cortical surface in such regions evoked discharges into the pyramidal tract consisting of directly and indirectly relayed components, the latter presumably being axo-somatically generated (2,8). Comparison with axo-dendritic activity produced by direct surface stimulation in stained cortical regions and with responses evoked by indirect stimulation of thalamocortical fibers from the cortical surface (Fig. 3), indicates that, like the topically applied, the systemically administered GABA (30 mg/K) is without effect on axo-somatic synaptic activity. Abolition of surface negative dendritic components, however, unmasks surface positivity (Fig. 3A2, B2).

B. Effects of γ-amino butyric acid on experimental epileptogenic focus. Cortical regions treated with ethyl-chloride often exhibited spontaneous paroxysmal activity(9). High-voltage discharges of varying frequency

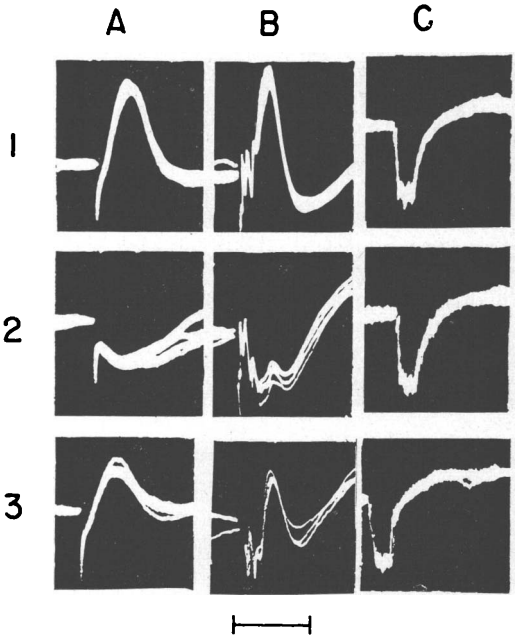

FIG. 3. *A:* Dendritic p.s.p.'s evoked by surface stimulation, post-sigmoid gyrus. *B:* Responses evoked from same site as in *A*, following strong stimulation of pre-cruciate cortex which also evokes discharges in the cortico-spinal tract, recorded at the mid-olivary level in the medulla *(C)*. Entire anterior and post-sigmoid gyri treated with chloroform-methanol solution 2 hr prior to recordings. *1*, controls; *2*, effects induced 1 min. after 30 mg/kg GABA, intrav.; *3*, beginning recovery, 60 min. after *2*. Note unmasking of surface positive dendritic response *(A2, B2)* with no effect on axo-somatically generated activity *(C2)*. Alterations in spike sequence in *B2* presumably due to developing slow surface positivity. Individual spike-like responses signaling axo-somatic discharges are not abolished by the systemically administered amino acid. Time 20 msec.

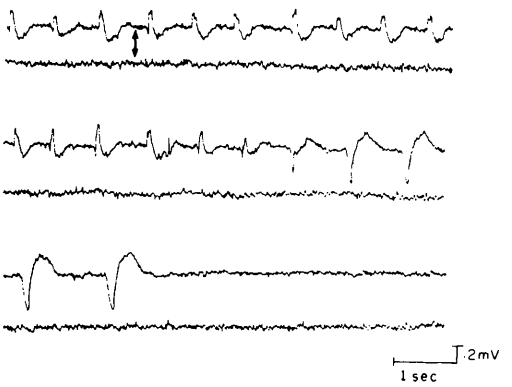

FIG. 4. Effects of systemic GABA on paroxysmal discharges spontaneously developing in region of blood-brain barrier loss induced by ethyl-chloride. Continuous record. *Upper channel*, monopolar recording from center of spike focus, post-sigmoid gyrus. *Lower channel*, bipolar recording from normal cortex, ant. suprasylvian gyrus. Focus discharged regularly for 2 hr prior to start of inj. (signaled by arrow and continued throughout recording). Total inj. was 30 mg/kg, but less than 15 mg/kg was sufficient to induce prompt reduction and reversal of spontaneous paroxysmal discharge prior to its disappearance.

were always localized well within areas of intense cortical staining with Evans blue dye and consequently were available to analysis following intravenous administration of GABA. Regularly discharging foci of relatively low frequency were affected by GABA in low concentration (10-15 mg/K). Monopolarly recorded negative-positive discharges were reduced, *reversed* and abolished for periods depending on the quantity of injected GABA. Injections of 30 mg/K (Fig. 4) abolished paroxysmal discharges for hours, none reappearing for the remainder of the experimental period in many cases. In preparations with rapidly discharging foci (3-5/sec. or more) GABA was less effective. In these, abolition of paroxysmal activity could be effected for short periods (5-10 min.) with 50-75 mg/K GABA. Discharges recurring after this were always at a lower frequency than prior to injection. Abolition of paroxysmal discharges was not accompanied by significant alteration in background electrocortical activity.

Comments. The foregoing results indicate that the powerful blocking action of γ-amino butyric acid on depolarizing synapses of apical dendrites is independent of its mode of administration. However, in contrast to the transient effects produced by topical application, prolonged, slowly reversible blockade of excitatory dendritic synapses occurs in regions of blood-brain barrier loss, following intravenous injection of GABA. As a consequence of this, paroxysmal discharges spontaneously developing in ethyl-chloride treated cortical regions are abolished or markedly reduced in frequency for long periods of time. The synaptic mechanisms responsible for polarity reversal of paroxysmal discharges prior to their abolition are not revealed by the present experiments. Topical application of GABA induces similar reversals in such discharges, but only rarely abolishes them, suggesting that systemic administration delivers more effective concentrations at excitatory axo-dendritic synaptic sites than is achieved by topical application.

Failure to demonstrate significant alterations in spontaneous or evoked activity in normal cortex following intravenous administration of GABA is therefore directly attributable to the presence of the blood-brain barrier. The latter may also be an effective mechanism for preventing diffusion of free GABA from the brain into the blood stream. In local cortical regions of increased vascular permeability the marked rapid electrophysiological alterations shown above are consistent with rapid increases in GABA content. Conversely, outflux of endogenously synthesized GABA from brain in such regions may also result in equally profound functional disturbances. Thus, in the presence of increased blood-brain barrier permeability, relatively small changes in brain concentration of synaptically active free amino acids like GABA may contribute to the production of neurological and psychiatric defects associated with abnormal plasma amino-acid levels such as are commonly observed in various states of hepatic insufficiency(10.)

Finally, the fact that GABA fails to block excitatory axo-somatic synapses when delivered to the latter systemically, supports the hypothesis(1-3) that the amino acid acts selectively on axo-dendritic synapses.

Conclusion. Under ordinary circumstances systemically administered γ-amino butyric

acid does not produce electrophysiological effects. Following destruction of the blood-brain barrier the synaptic actions are similar to those produced by topical application. In regions of increased barrier-permeability, intravenous GABA abolishes or reduces the frequency of spontaneous paroxysmal discharges arising from such areas. Therefore systemically administered GABA produces no synaptic effects because of failure to penetrate the blood-brain barrier. By permitting rapid exchanges of GABA between blood and brain during electrophysiological studies following barrier lesions, information was obtained relating to the role of γ-amino butyric acid in the central nervous system not attainable by the method of topical application. Production of experimental, localized regions of blood-brain barrier loss may have applications for the study of substances that are otherwise denied access to the cells of the central nervous system.

1. Purpura, D. P., Girado, M., Grundfest, H., *Science,* 1957, v125, 1200.

2. ———, PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 791.

3. Purpura, D. P., in, Int. *Symposium on Reticular Formation of the Brain,* in press.

4. Awapara, J., Landua, A. J., Fuerst, R., Seals, B., *J. Biol. Chem.,* 1950, v187, 35.

5. Roberts, E., Frankel, S., *ibid.,* 1950, v187, 55.

6. Roberts, E., Baxter, C., *Proc. Am. Acad. Neurol.,* 1957.

7. Schwerin, P., Bessman, S. P., Waelsch, H., *J. Biol. Chem.,* 1950, v184, 37.

8. Purpura, D. P., Grundfest, H., *J. Neurophysiol.,* 1956, v19, 573.

9. Morrell, F., Florenz, A., *Proc. Am. EEG Soc.,* 1957.

10. Walshe, J. M., *Quart. J. Med.,* 1953, v22, 483.

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Calory and Protein Intake as Related to Growth. (23741)

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Numerous recent papers on calory and protein nutrition of fowls have been concerned with the "Calory-Protein Ratio." This ratio is the nutritional caloric value of the diet divided by the percentage of protein. There has appeared no consistent relation of this ratio to biological results unless either protein or caloric value of the diet has been relatively constant.

Exp. In an experiment on protein and caloric needs, B. B. Bronze hens on range were provided definite intakes of practical formula feeds and grain per day (Table I). Protein intake was calculated from analyses. Caloric intake was expressed as "Productive Energy Values" of Fraps, revised by Titus(1). Most of the reports referred to have also employed such values. These have been expressed as calories per lb. of feedstuff. Correlation of calory-protein ratio to growth results was found to be negligible. It was observed that the *product* of the caloric and protein intakes showed a good correlation with body weights (coefficient 0.83).

Discussion. The ratio remains the same whether calculated from composition of diet or from intakes, and is independent of the magnitude of intakes. Hence such anomalous findings appear as a decrease in growth when the ratio is increased, due to decreased total feed or decreased protein intake or both(2,3). Protein is represented in the numerator of the ratio as protein calories and in the denominator as percent protein. This implies that all protein simultaneously is consumed to produce calories, and also is utilized for growth purposes, which, obviously, is impossible. Since the ratio includes all the protein calculated both as calories and as percentages of protein the ratio may be expressed:

$$\text{Calory-protein ratio} = \frac{\text{Protein calories}}{\text{Percent protein}} + \frac{\text{Non-protein calories}}{\text{Percent protein}}$$