Typical curves of upper channel activity versus date are shown in Fig. 1 for Bismarck, N.D., and for New Orleans, La. The constancy of the potassium-40 assay is indicated by the reproducibility of the results for the first 6 weeks.

The peak concentrations given in Table 1 can be compared with the International Commission on Radiological Protection's maximum permissible concentration for barium-140/lanthanum-140 in drinking water of 300 mμc/lit (*3*). The latter value is for continuous exposure for an indefinite period of time, while the exposure resulting from weapons testing is of short duration. Unlike strontium-90, barium-140 cannot present a cumulative hazard because of its very short half-life. Barium-140 has not been observed in any human subjects, although a search has been made for it.

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28 October 1957

Action of Blood-Borne Gamma-Aminobutyric Acid on Central Synapses

When substances are identified in the brain, it is natural at the same time to inquire into their function. Thus they become candidates for various roles, including that of potential neurohumoral transmitters. Such, indeed, has been the case with serotonin (*1*), and such is now the case with gamma-aminobutyric acid (GABA). Bazemore, Elliott, and Florey (*2*) have identified the latter as an active principle of factor I, which Florey and McLennan (*3*) had extracted from mammalian brain and had shown to have inhibitory actions.

One of the readiest methods of acquiring preliminary information of this sort is to paint a solution of the material upon the exposed cerebral cortex. The high doses thus applied and the unusually high concentration gradients that result serve to uncover any possible actions. Effects achieved in this highly abnormal way are undeniable but difficult to interpret in terms of physiological function, even when specificity can be asssured. Although the usefulness of this method, as in the topical application of strychnine (*4*) to fire brain areas, in order to map them, has gained it considerable respectability, this should not be extended to other uses. Thus, Kato (*5*), in studying conduction in nerve, found it convenient to make use of mechanical stimulation by a miniature mallet, but there was no suggestion that this was a normal way to activate or that this mechanical stimulus played a part in propagation of the nerve impulse. Nevertheless, the actions of GABA have been studied almost exclusively by topical application.

We have, therefore, wished to study the effects of blood-borne GABA and have resorted to the method we have previously used to help establish the roles of acetylcholine, adrenaline and nor-adrenaline (*6*), and serotonin (*1*) as neurohumoral transmitters in mammalian brain. This has been the relatively close arterial injection in the common carotid artery serving effectively to bring across the blood-brain barrier relatively small doses which, therefore, on dilution in the systemic blood stream become subthreshold for peripheral actions and consequently exhibit the cerebral actions in isolation or in relatively pure form—that is, not complicated by the peripheral actions and the resulting barrage of afferent impulses which bombard the brain. In this manner, by activating cortical synapses through the transcallosal pathway and recording the response as evoked cortical potentials in the lightly anesthetized cat, we have demonstrated that GABA, when delivered through the natural route (that is, when it is blood-borne), can, like adrenaline, nor-adrenaline, and serotonin, inhibit synaptic transmission. It does this in doses of 50 to 500 μg/kg (Fig. 1); thus it has a potency of about 1/50 that of serotonin, intermediate between that of nor-adrenaline and adrenaline, the series being nor-adrenaline, 1; GABA, 7×; adrenaline, 15×; serotonin, 300×. Unlike the effects of topical application reported by Purpura and Grundfest (*7*) the surface negative evoked response is usually reduced without affecting the positive wave or inverting the negative wave into a positive one.

Comparison with serotonin brings out further significant differences. The time course of the GABA action is faster in all respects. As the continuous plot of the surface negative evoked responses shows, the time of onset and the duration of action are remarkably short. The latter suggests an enzymatic destruction of GABA as was supposed by Florey and McLennan (*3*) or a binding into an inactive state by adsorption as believed by Elliott (*8*). Successful interference with this enzymatic or this binding process would result in abnormal accumulation of GABA, which would be evi-

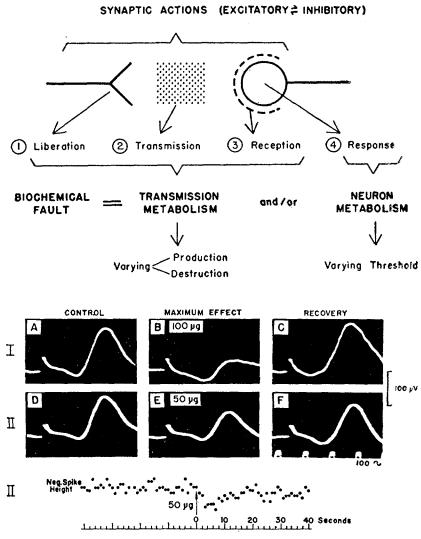

Fig. 1. Cerebral synaptic action of gamma-aminobutyric acid in a two-neurone intercortical (transcallosal) system. (Top) Potential factors in disturbed synaptic equilibrium. (Bottom) Potentials evoked in the cerebral cortex of the cat by electrical stimulation of the contralateral cortex every second. Gamma-aminobutyric acid was injected into the ipsilateral common carotid artery.

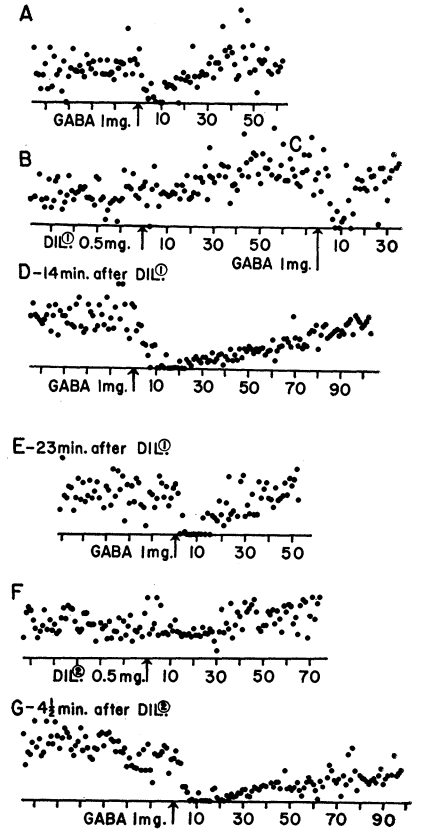

Fig. 2. Augmentation of GABA cerebral synaptic inhibition by dilantin. Negative cortical spike heights from transcallosal system potentials evoked by contralateral cortical stimulation (one per second). Injections were made into the ipsilateral common carotid artery.

denced by a prolongation of the duration of action. We would hope, in this way, also to accumulate naturally present GABA to threshold levels, which would then be recordable as synaptic inhibition.

On the basis that any alteration of GABA might be enough to terminate its inhibitory action, we attempted to interfere with its transamination with alpha-ketoglutaric acid. We have utilized *p*-benzoquinone, which is cited by Braunstein (*9*) as being a highly potent transaminase inhibitor against glutamic aminopherase, but we have been unable to alter the course of GABA effects in this way.

Meantime, because of the long-entertained possibility that events in the glutamic acid–gamma-aminobutyric acid equilibrium might play a role in altered excitability states of the brain and abnormalities—for example, in epilepsy—Woodbury (*10*) had examined the influence of diphenylhydantoin (dilantin) on GABA metabolism and had found in preliminary work that it increases the GABA content of cat brain. We therefore sought to prolong the GABA effects with dilantin. Figure 2 illustrates that we have succeeded in doing just this with intracarotid doses of dilantin having, per se, no effect on synaptic transmission. In fact, the prolongation starts only after a latent period, so that the effect of GABA introduced immediately after dilantin is not prolonged, but a test dose several minutes later does show a prolongation that persists in repeated trials over many minutes.

Perversion of GABA formation or destruction hypothetically constitutes one of the possible biochemical faults diagrammatically indicated at the top of Fig. 1, pointing out the potentially disturbed factors which might be the cause of synaptic dysequilibrium. If GABA is to be considered as a transmitter, then we may say further that it does not act at the same inhibitory receptors as serotonin, since we can effectively prevent or block the synaptic inhibitory action of serotonin with chlorpromazine but cannot similarly block that of GABA. Though not as potent as serotonin, GABA is of great interest because it is even more plentiful in the brain than serotonin and because its site of action is evidently not identical to that of serotonin (*11*).

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22 October 1957

Use of Charcoal to Separate Mixtures of Inorganic, Ester, and Nucleotide Phosphates

Several years ago, the use of charcoal for the separation of inorganic and nucleotide phosphorus fractions, with particular application to experiments involving radioactive phosphorus, was described (*1*). Briefly, the procedure called for the addition of charcoal to a trichloroacetic acid extract containing inorganic and nucleotide phosphorus, the removal by centrifugation of the charcoal with its adsorbed nucleotide, and heating of the charcoal suspended in acid solution to release the nucleotide phosphorus as inorganic phosphate. This procedure has been accepted and used routinely in a number of laboratories in spite of the limitation that the nucleotide is not recovered in substance.

In this laboratory, charcoal has been used principally for the removal of nucleotide from mixtures in which phosphate esters have been prepared by means of the hexokinase reaction (*2*). In the course of these experiments, it was found that the use of hydrochloric acid in place of trichloroacetic acid to terminate the reaction resulted in the loss of ester by its adsorption to the charcoal (*3*). Recent experiments on the adsorption of nucleotide by charcoal have led to the finding that desorption can be accomplished by suspension of the charcoal in trichloroacetic acid (TCA) to which has been added the ammonium salt of trichloroacetic acid. These observations are substantiated by the data of Table 1.

The experiments were carried out as follows: Five milliliters of a solution containing 1 μmole per milliliter each of inorganic phosphorus and of one of the phosphate esters shown in Table 1 were placed in a centrifuge tube. To this was added 0.05 ml of 5*N* hydrochloric acid and 0.3 g of acid-washed (*3*) Norit-A charcoal. The contents of the tube were mixed by inversion, 0.01 ml of 10 percent Triton X-100 (*4*) was layered on top to reduce the amount of charcoal which floats, and the tube was centrifuged. The supernatant solution was decanted and assayed for inorganic and total phosphorus by the method of Fiske and Subbarow (*5*). The charcoal was extracted twice with 5 ml portions of 0.05*N* hydrochloric acid by suspension and centrifugation and once with 4 ml of 10 percent trichloroacetic acid. The trichloroacetic acid extract was assayed for total phosphorus. The results of this experiment are given in the first two columns of Table 1.

A similar experiment was carried out with adenosine triphosphate and inosine triphosphate without the addition of inorganic phosphate. The charcoal, after the washing with 10 percent trichloroacetic acid, was suspended in 4 ml of 25 percent trichloroacetic acid, and a solution of ammonium trichloroacetate (prepared by the addition of 8.3 ml of 28 percent NH_3 to 20 ml of 100 percent trichloroacetic acid) was added to a final volume of 10 ml. The charcoal was mixed in by inversion and shaking and removed by filtration. The nucleotide in the various fractions was assayed by phosphorus analysis (*5*) of aliquots subjected to heating at 100°C in 1*N* sulfuric acid for 11 minutes (*6*). The results of this experiment are shown in Table 1.

These experiments show that it is possible, by the use of Norit-A charcoal, to

Table 1. The adsorption of inorganic, ester, and nucleotide phosphates by acid-washed Norit-A charcoal. TCA is an abbreviation for trichloroacetic acid. The concentrations of these solutions are given in the text. The percentage desorbed is calculated on the basis of the amount adsorbed, not on the amount in the original solution.

Compound	Adsorbed from 0.05*N* HCl (%)	Desorbed in TCA (%)	Desorbed in TCA–NH_4TCA (%)
Inorganic ortho-P	8.4		
Adenosine tri-P	100	< 1	91
Inosine tri-P	100	31	60
Glucose-6-P	91	97	
Galactose-1-P	96	100	
Mannose-6-P	93	88	
Galactose-6-P	91	92	
Fructose-6-P	92	100	
1,5-Sorbitan-6-P	96	100	
Fructose-1,6-diP	97	95	
Glucose-1-P	97	81	
Ribose-5-P	100	75	
L-Sorbose-1-P	97	72	
2-Deoxyglucose-6-P	100	53	
Glucoheptulose-7-P	77	83	