the height of synaptic blockade by flushing the cortical surface with Ringer's solution (Fig. 1, *D* and *E*). Synaptic blockade again developed in 30 to 60 seconds when rinsing was discontinued, presumably due to a continued influx of the amino acid (Fig. 1*E*). This sequence (blockade; recovery by surface application of Ringer's solution; blockade), once established, was reproducible for 20 to 40 minutes. At the end of this period no further reduction in surface-negative components of evoked responses occurred after a brief rinsing of the cortex. Unequivocal synaptic effects of systemic γ-aminobutyric acid resulting from alterations in blood-brain barrier activity induced by relatively short periods of exposure of the cortex or by preloading with the amino acid were regularly observed in kittens less than 10 days old and were rarely seen 2 to 3 weeks postnatally.

The presence in newborn cats of a blood-brain barrier to a pharmacologically active metabolite is suggested by the observations that single injections of γ-aminobutyric acid do not effectively alter cortically evoked responses until some change has occurred, presumably in the interposed elements between plasma and neurons which constitute this barrier. Then, influx of γ-aminobutyric acid is signaled by a blockade of axodendritic excitatory postsynaptic potentials that is rapidly reversed during and immediately after surface application of Ringer's solution. Rapid reversibility, as illustrated in Fig. 1, *D* and *E*, is seen after topical application of the amino acid (*6*) but has not been observed in mature cats with experimentally induced lesions of the blood-brain barrier (*8*). Apart from any differences in the degree of blood-brain barrier damage which may have been produced by lipid solvents in mature animals and by exposure of the cortex in the newborn kitten, differences in reversibility may be attributable to the presence of a greater number of subsurface axodendritic synapses in the mature cortex which are blocked by systemic γ-aminobutyric acid but are inaccessible to Ringer's solution applied to the surface. The rapid reversibility of systemic γ-aminobutyric acid effects observed in the immature animal emphasizes the superficial location of the axodendritic synapses blocked by the amino acid. This indicates the existence of well-developed synaptic pathways in the superficial neuropil of immature cortex at a time when its spontaneous electrical activity is poorly organized.

Little information has been obtained concerning the nature of the processes which operate to prevent blood-borne γ-aminobutyric acid from gaining access to cortical neuronal surfaces, or why these mechanisms are so labile in the neonatal period. The relative ease with which functional changes in the blood-brain barrier to injected γ-aminobutyric acid are produced in newborn kittens may be a consequence of the incomplete development of perivascular glial elements at birth (*9*). Since it has been shown that in mature animals the blood-brain barrier restricts net uptake but not rapid exchange of some amino acids between plasma and brain amino acid pools (*10*), a process may be envisoned whereby changes in the activity of poorly developed amino acid transport mechanisms in "immature" glial elements could result in the rapid and sustained influx of γ-aminobutyric acid demonstrable in the neonatal animal. Alternative hypotheses based on postulating different permeability properties of capillary-glial membranes at different stages of maturation may also account for the changes in blood-brain barrier activity resulting from short periods of exposure of the cortex. At present, however, it is difficult to interpret the effects of loading with γ-aminobutyric acid or of subsequent test-injections of the amino acid exclusively in terms of changes in membrane permeability (*11*).

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Effects of Veratrine and Cocaine on Cerebral Carbohydrate-Amino Acid Interrelations

Abstract. The rate of aerobic transformation of glucose-U-C^{14} into radioactive amino acids by slices of rat-brain cortex is greatly influenced by the K^+/Ca^{++} ratio in the incubating medium. Protoveratrine has effects on the amino acid pattern resembling that due to an increase in the K^+/Ca^{++} ratio. These effects are antagonized by cocaine and may be correlated with the neurophysiological activities of these drugs.

We have shown (*1–3*) that the conversion of uniformly (C^{14}) labeled glucose into radioactive amino acids (glutamic acid, glutamine, gamma aminobutyric acid, aspartic acid, and alanine) in the presence of slices of rat-brain cortex involves processes whose rates are markedly influenced by the K^+/Ca^{++} ratio in the medium bathing the slices and by the presence of a respiratory inhibitor such as malonate or of small concentrations of a narcotic such as Amytal. It was demonstrated that the changes in relative yields of the radioactive amino acids derived from glucose-U-C^{14} brought about by the presence of an increase in K^+ concentration, and by the presence of malonate, may be satisfactorily explained on the basis of the conclusions that the amino acids are derived from glucose by transamination of the alpha ketonic acids obtained during the operation of the citric acid cycle in the brain cell and that the potassium ion stimulation of brain cortex metabolism is due to an acceleration of a pace-making step, the oxidation of pyruvate by diphosphopyridine nucleotide to acetyl-CoA. It was further demonstrated that the effects of the presence of small concentrations of the narcotic Amytal on the relative yields of radioactive amino acids are adequately explained on the basis of the conclusion that the main effect of the narcotic is to suppress the oxidation of reduced diphosphopyridine nucleotide by cytochrome oxidase and its associated phosphorylations.

It is shown in the results described in this report that the presence of cocaine and of protoveratrine has effects on the relative yields of radioactive amino acids from glucose-U-C^{14} by slices of rat-brain cortex which throw light on the mode of action of these drugs and which may be correlated with their known neurophysiological effects.

The experimental work was carried out by the method we have described (*2*). Slices of rat-brain cortex were allowed to respire in oxygen at 37°C for 60 minutes in 1 ml of Krebs-Ringer phosphate medium, *p*H 7.4, containing

5m*M* glucose-U-C^{14} (10^6 count/min). After incubation the slices were homogenized in a final volume of 8 ml of 80-percent ethanol, the homogenate was allowed to stand at 5°C for 2 hours, and the ethanolic extract was evaporated to dryness at 30°C. The dried extract was dissolved in 0.5 ml of water, and 0.4 ml of the extract was chromatographed two-dimensionally on Whatman No. 1 paper.

Measurements of the radioactivities of the spots on the paper (corresponding to the amino acids) have been corrected for background and are expressed as counts per minute for 100 mg of tissue (wet-weight); (the initial activity of glucose-U-C^{14} was 10^6 count/min).

The results quoted are the means of at least four experiments, standard mean errors being given.

Wollenberger (*4*) has demonstrated that the addition of protoveratrine (well known for its excitation of the neuromuscular and nervous systems) to slices of guinea-pig brain cortex brings about large increases in the respiratory rate and in the rate of aerobic glycolysis. The work of Hill (*5*), of Schmitt and Gasser (*6*), and of Schmitt (*7*) had suggested this possibility after the demonstration that heat production and oxygen consumption of peripheral nerves are increased by veratrum alkaloids. Protoveratrine, like an increase in K^+, accelerates the rate of oxygen consumption of the brain cortex in the presence of glucose, lactate, or pyruvate but not in the presence of succinate. Moreover, like an increase in K^+, protoveratrine is not effective with brain homogenates. Stimulation by protoveratrine of the respiratory rate of rat-brain cortex in the presence of glucose, like stimulation due to an increase in K^+, is highly sensitive to narcotics and to malonate (*3*). There is in fact a striking parallelism, as was pointed out by Wollenberger (*4*), between the actions of protoveratrine, potassium ions, and electric impulses on brain-cortex metabolism.

Results given in Table 1 show that the addition of $5 \times 10^{-6} M$ protoveratrine to slices of rat-brain cortex incubated aerobically in the presence of glucose-U-C^{14} gives rise to a radioactive amino acid pattern which differs from that obtained in the absence of protoveratrine. The difference consists largely of increased yields of labeled glutamic acid, glutamine, and gamma aminobutyric acid, a phenomenon characteristic of the effects of an increase in K^+ (*1, 2*). Effects of an increase in K^+ on the amino acid pattern under similar experimental conditions are shown in Table 2. The changes in the relative yields of labeled amino acids brought about by the addition of the protoveratrine resemble, however, more closely the effects obtained when the slices of brain cortex are incubated in the Ringer medium from which calcium ions have been omitted. Results describing these effects are given in Table 1. The effects of an increase in K^+ in the presence of 3.6 m*M* $CaCl_2$ (Table 2) and of the absence of Ca^{++} in the presence of a normal concentration of K^+ (5 m*M* KCl) (Table 1) are similar but not identical; this indicates that, although the primary change is due to the increase in the ratio K^+/Ca^{++}, secondary effects are due to specific effects of these ions. It has been shown (*1, 2*) that the major change, the increased labeling of the amino acids, is due to acceleration of the conversion of pyruvate to acetyl-CoA by an increase in the ratio K^+/Ca^{++}. The effects of the addition of 5 μM protoveratrine, in a normal Ringer medium, may be satisfactorily explained if the effect of protoveratrine consists of immobilization of, or competition with, Ca^{++}, resulting in a virtual increase in the ratio K^+/Ca^{++}. However, a similar result would be obtained if the protoveratrine affected the relative permeabilities of Na^+ and K^+ in the brain cortex, so as to yield, in fact, an increase in the ratio K^+/Ca^{++} in the extracellular medium. It is well known (*8*) that the veratrine alkaloids bring about marked changes in ion permeability and bioelectric phenomena, and it has been suggested (*9*) that veratrine and Ca^{++} compete for sites on the cell surface. Gershfeld and Shanes (*10*) have indeed demonstrated a model (monolayers of stearic acid) in which there occurs a competition between veratrine and Ca^{++} for carboxyl groups in the fatty acid film.

It is evident from the results given in Table 1 that the action of protoveratrine on metabolic changes in brain cortex in vitro may be satisfactorily explained by the neurophysiological effect of protoveratrine—that is, its antagonism for Ca^{++}.

If this view is correct, it may be

Table 1. Effects of protoveratrine and cocaine on radioactive amino acid formation from glucose-U-C^{14} in the presence of slices of rat-brain cortex. Glucose, 5 m*M*. Radioactivity is given in counts per minute per 100 mg of tissue (wet weight), per 10^6 counts of glucose-U-C^{14} per minute.

KCl, 5 m*M*; $CaCl_2$, 3.6 m*M*	Protoveratrine, 5 μM; KCl, 5 m*M*; $CaCl_2$, 3.6 m*M*	Cocaine, 0.5 m*M*; KCl, 5 m*M*; $CaCl_2$, 3.6 m*M*	Protoveratrine, 5 μM; cocaine, 0.5 m*M*; KCl, 5 m*M*; $CaCl_2$, 3.6 m*M*	KCl, 5 m*M*; $CaCl_2$, nil	Cocaine, 0.5 m*M*; KCl, 5 m*M*; $CaCl_2$, nil
			Glutamic acid		
5565 ± 172	7869 ± 276	5928 ± 569	5845 ± 92	9280 ± 365	6846 ± 569
			Glutamine		
1302 ± 92	1957 ± 105	1365 ± 96	1079 ± 97	1762 ± 97	1233 ± 58
			Gamma aminobutyric acid		
966 ± 57	1832 ± 56	795 ± 20	739 ± 22	1669 ± 94	1301 ± 23
			Aspartic acid		
1353 ± 59	1727 ± 56	1751 ± 65	1541 ± 59	2123 ± 65	2003 ± 173
			Alanine		
721 ± 52	631 ± 65	744 ± 42	826 ± 25	955 ± 34	1086 ± 42
			O_2 uptake [mm³/100 mg of tissue (wet weight)]		
194 ± 5.3	234 ± 6.4	158 ± 8.2	152 ± 7.3	232 ± 2.1	176 ± 6.4

Table 2. Effects of cocaine (4 m*M*) on radioactive amino acid formation from glucose-U-C^{14} in the presence of slices of rat-brain cortex. Glucose, 5 m*M*; $CaCl_2$, 3.6 m*M*. Radioactivity is given in counts per minute per 100 mg of tissue (wet weight), per 10^6 counts of glucose-U-C^{14} per minute.

KCl, 5 m*M*	Cocaine, 4 m*M*, plus KCl, 5 m*M*	Effect due to cocaine (%)	KCl, 105 m*M*	Cocaine, 4 m*M*, plus KCl, 105 m*M*	Effect due to cocaine (%)
			Glutamic acid		
5565 ± 172	7850 ± 45	+ 41	6208 ± 405	9027 ± 481	+ 45
			Glutamine		
1302 ± 92	2823 ± 326	+117	2557 ± 234	5704 ± 474	+113
			Gamma aminobutyric acid		
966 ± 57	1237 ± 88	+ 27	1587 ± 114	2081 ± 344	+ 31
			Aspartic acid		
1353 ± 59	1959 ± 41	+ 45	1418 ± 69	1762 ± 172	+ 24
			Alanine		
721 ± 52	1133 ± 87	+ 57	809 ± 54	1407 ± 95	+ 73
			O_2 uptake [mm³/100 mg of tissue (wet weight)]		
194 ± 5.3	158 ± 7.3	− 18	283 ± 8.9	218 ± 15.3	− 23

expected that the known neurophysiological antagonism between protoveratrine and cocaine (*8, 11*) will be reflected by an antagonism in their metabolic effects.

The results given in Table 1 demonstrate that the addition of cocaine (0.5 m*M*) to slices of rat-brain cortex incubated in a normal Ringer medium brings about a fall in respiratory activity with relatively little change in the pattern of labeled amino acids derived from glucose-U-C^{14}. The addition, however, of cocaine to protoveratrine in the presence of slices of rat-brain cortex not only suppresses the stimulation of the respiratory rate due to protoveratrine but causes a marked change in the amino acid pattern, reducing the labeling of glutamic acid, glutamine, and gamma aminobutyric acid to approximately the same levels found with cocaine alone. This result is to be correlated with the known effect of cocaine in reducing the action of veratrine on ionic exchange and is consistent with the conclusion that cocaine (0.5 m*M*) acts in a manner similar to Ca^{++}, its presence resulting in an effective diminution of the ratio K^+/Ca^{++} at the brain-cell surface.

The latter conclusion may be put to a further test by examining the effects of cocaine (0.5 m*M*) on the metabolic activities of slices of rat-brain cortex in a Ca^{++}-free Ringer medium. If cocaine (0.5 m*M*) can indeed replace Ca^{++}, or give rise to a diminished ratio K^+/Ca^{++}, the amino acid pattern, as well as the respiratory rate, obtained in a Ca^{++}-free medium should change towards the values obtained in a normal Ringer medium.

The results (Table 1) show that the addition of cocaine (0.5 m*M*) to a Ca^{++}-free medium brings about marked falls in the labeling of glutamic acid, glutamine, and gamma aminobutyric acid and in the respiratory rate, in the manner anticipated from the neurophysiological results, but the effects of cocaine (0.5 m*M*) and of Ca^{++} (3.6 m*M*) are not identical. The fall in respiratory rate is greater than that expected if cocaine is simply replacing Ca^{++}, and the labeling of aspartic acid and alanine is not appreciably diminished.

It is concluded, therefore, that cocaine (0.5 m*M*) exerts a twofold effect, essentially replacing Ca^{++} (or diminishing the ratio K^+/Ca^{++} by the effects on ionic permeability) and diminishing the rates of breakdown of pyruvate or oxalacetate so that more of these ketonic acids are available for transamination to alanine and aspartic acid. The latter effect is consistent with the fall in respiratory rate brought about by the addition of cocaine.

In order to explore further the action of cocaine on the metabolic activities of rat-brain cortex, the effects of a higher concentration (4 m*M*) of the drug were investigated.

Results given in Table 2 show that the presence of cocaine (4 m*M*) in a normal Ringer medium (KCl, 5 m*M*) brings about a marked stimulation in the labeling of all amino acids, the largest effect being observed with glutamine. The increase in labeling in the total count of the amino acids amounts to 54 percent. It is important to observe, however, that the presence of cocaine (4 m*M*) in a Ringer medium containing 105 m*M* KCl brings about almost identical increases in the labeling of the amino acids, the effect again being greatest with glutamine. This increase in labeling takes place even though the presence of K^+ of high concentration has, itself, brought about increases in the yields of labeled amino acids. The percentage of stimulation in the labeling of the amino acid is approximately the same whether the concentration of KCl is 5 m*M* or 105 m*M*.

Evidently the increased yields of labeled amino acids derived from glucose-U-C^{14} by the addition of cocaine (4 m*M*) are not due to an increase in the rate of turnover of the citric acid cycle, for the respiratory rate falls by about 20 percent, with normal or high concentrations of K^+. It is more reasonable to conclude that the increase in the yields of labeled amino acids is due to an increase in the availabilities of the corresponding alpha ketonic acids for transamination, through diminution in the velocities of oxidation of the ketonic acids. This is a problem for further investigation (*12*).

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Liquid Scintillation Counting of Aqueous Solutions of Carbon-14 and Tritium

Abstract. A method is reported whereby aqueous solutions containing weak beta emitters are dispersed as stable emulsions in liquid scintillator counting solutions. This permits the routine counting, with about 10-percent efficiency, of large numbers of samples containing tritium. Self-absorption does not present a problem when less than 5 percent aqueous phase is present.

The lyophobic nature of the common solvents (*1*; *2*, p. 185) used in liquid scintillation counting imposes a restriction upon the biological materials whose radioactivity can be determined in homogeneous solution. Various additions can be made to the solvent to increase its ability to dissolve water and certain aqueous solutions, but this usually results in a considerable loss of counting efficiency (*3*). This lowered efficiency is particularly great when the biologically very useful but weak beta-emitter tritium is used.

In addition, methods have been developed for counting solid insoluble materials as a suspension (*4*) or embedded in a gel (*5*). Aqueous solutions have been counted by the use of spiral tubes (*6*), sheets (*7*), or fibers or beads (*8*) of plastic scintillator. The scintillator is relatively expensive, and the counting efficiency is low for weak beta emitters. Raden (*9*) has described attempts to count emulsions, but states that tritium cannot be counted in his system and that a continuous breakdown of the emulsion occurs.

In preliminary experiments, it was hoped that treatment with ultrasound of a mixture of viscous counting solution and aqueous solution of radioactivity would create a stable emulsion which could be counted. A series 600 Narda "SonBlaster," with a maximum output of 60 watts at 40 kcy/sec, was used to prepare the samples in 20-ml low-potassium glass vials obtained from the Wheaton Glass Company. Counting was done in a Packard Tri-Carb liquid scintillation counter. In one experiment, the viscosity of a toluene counting solution was increased by the incorporation of various amounts of Thixcin R (a modified technical hydroxystearin) obtained from the Baker Castor Oil Company. Gels could be obtained readily, but the emulsions produced with ultrasound were not stable. In another experiment, a counting solution was prepared with a heavy mineral oil to replace the toluene. It was found that benzoic acid–C^{14} could be counted with a 56-percent efficiency and toluene-H^3 with a 6-percent efficiency. However, emulsions produced