6. Anaerobic glycolysis was also unaffected by the anticonvulsants, until its level was altered by currents of 2000 cyc./sec. It then became susceptible; at 50 cyc./sec. no action of the drugs was seen.

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Action of Protoveratrine on the Metabolism of Cerebral Cortex

1. UNSTIMULATED CEREBRAL-CORTEX TISSUE*

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Protoveratrine is one of the most powerful members of an important group of pharmacological agents, the polycyclic ester alkaloids of veratrum. These compounds, especially the mixture of alkaloids known as veratrine, have been the subject of innumerable researches in the physiological and pharmacological laboratory and have proven to be of particular value in the analysis of certain aspects of the physiology of muscle and nerve (Kramer & Acheson, 1946). During the last few years, protoveratrine has become prominent as an agent in the treatment of hypertension.

The central nervous system has been suspected as primarily involved in some effects of veratrum

alkaloids on the circulatory and respiratory systems as well as in the elicitation of convulsive activity which usually precedes and accompanies death due to poisoning by these compounds; but a direct central nervous action has not yet been demonstrated unequivocally. The work of Hill (1933), Schmitt & Gasser (1933) and Schmitt (1936), who found that heat production and oxygen consumption of peripheral nerve are increased by veratrum alkaloids, suggested the possibility that, if protoveratrine has a direct effect on the central nervous system, a similar change in metabolism might occur and be observable in that tissue. As shown in the present and succeeding communications, this is indeed the case. The present communication will deal chiefly with the action of protoveratrine on 'resting', i.e. electrically non-stimulated pieces of cerebral cortex. It will be seen that minute amounts of the alkaloid are

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capable of bringing about not only marked quantitative, but also profound qualitative changes in the metabolism of this tissue. A preliminary account of some of the present work has appeared (Wollenberger, 1950).

METHODS

Young adult male and non-pregnant female guinea pigs were decapitated with a pair of heavy shears. The brain was removed and cooled in the incubation media described below but lacking bicarbonate or phosphate, and at about 15°. Slices of cerebral cortex approximately 0.4 mm. thick were cut free-hand with a razor blade held in artery forceps. In some experiments the cerebral hemispheres were placed immediately into ice-cold 3.3 mM potassium phosphate of pH 7.0 and homogenized in this buffer, using a Potter-Elvehjem glass apparatus. The cerebral slices were pooled in 4–5 ml. of unbuffered incubation medium, and batches of the sliced tissue, weighing about 50 mg. each, were transferred to the main compartments of Warburg incubation vessels containing 2.95 ml. of buffered incubation fluid. This volume included in most experiments 0.1–0.3 ml. of medium, placed into the side arms and containing protoveratrine. The incubation fluid was either the bicarbonate-saline of Krebs & Henseleit (1932) or a modified Krebs-phosphate solution (Wollenberger, 1947). Unless stated otherwise, it contained 11 mM D-glucose. Other substrates used were: sodium DL-lactate, prepared by neutralization (with NaOH in the cold) of diluted and boiled commercial lactic acid; sodium pyruvate, prepared by neutralization of freshly distilled pyruvic acid with NaHCO₃ followed by precipitation in ethanol; sodium oxaloacetate, prepared from diethyl oxaloacetate according to the method of Krampitz & Werkman (1941); and the commercial C.P. sodium salts of L-glutamic acid and succinic acid. The vessels were gassed with oxygen or with 95% N₂–5% CO₂. Oxygen consumption and anaerobic acid production, the latter presumed to be due to glycolysis, were measured manometrically at 38° by the direct method of Warburg, readings being taken every 7½ or 15 min. After the rate of metabolism in the absence of drug had been ascertained, the contents of the side arms were tipped into the main compartments. In this way each vessel served as its own control. In addition, one or two vessels containing drug-free medium in the side arm were employed as over-all controls. The O₂ uptake of the homogenates was measured in an appropriately fortified medium (see under results) equilibrated with air.

For the estimation of lactic acid and of potassium in the medium, 2 ml. were pipetted out at the desired time, treated with 1 ml. of 20% trichloroacetic acid and centrifuged, and portions of the supernatant taken for analysis. Lactic acid was determined colorimetrically by the method of Barker & Summerson (1941). Potassium was estimated by internal-standard flame photometry, using the Beckman instrument (Hofmann, Knobel & Greep, 1954). In the homogenate experiments analyses for inorganic phosphate were made in samples deproteinized with trichloroacetic acid by the method of Lowry & Lopez (1946). The rates of metabolism are given in Q units (μl. gas/mg. dry tissue/hr.). Q_{lact} denotes the rate of lactic acid production as determined chemically and Q_{CO₂} denotes the rate of acid formation determined manometrically. The dry weight of the tissue was determined after termination of the experiments and includes the weight of debris and of trichloroacetic acid precipitate in the medium.

The investigation was carried out before the resolution of protoveratrine into protoveratrine A and B had been achieved (Nash & Brooker, 1953). The crystalline sample of alkaloid used was a mixture of 60% protoveratrine A and 40% protoveratrine B. The concentrations given, when expressed on a molar basis, refer to the sum of the molar concentrations of the two constituent compounds. The molecular weights of the protoveratrines A and B are 794 and 810 respectively.

RESULTS

Metabolic changes in cerebral cortex taken from guinea pigs poisoned with protoveratrine. To ascertain whether energy metabolism in brain slices is affected by administration *in vivo* of the small quantities of protoveratrine sufficient to produce neuromuscular and other disturbances, the following experiment was carried out. Two guinea pigs were given an intraperitoneal injection of 0.15 mg. protoveratrine/kg. A few minutes after the injection increasingly violent retching movements were noticed, which were followed by vomiting. (So far as we are aware, this is the first time that vomiting has been described in rodents.) The hind legs became immobilized in flaccid paralysis while vomiting continued. When breathing became laboured the animals were decapitated and the brain was removed for the preparation of cerebral-cortex slices.

Table 1. *Respiration and glycolysis of cerebral-cortex slices from guinea pigs poisoned with protoveratrine*

The experimental periods are given in minutes after decapitation. Aerobic quotients were determined in phosphate media and anaerobic in bicarbonate media.

Treatment of guinea pig	Period between injection and decapitation (min.)	Q _{O₂}		Q _{CO₂} Q _{lact}	Q _{CO₂} ^{N₂}			
		30–60 (min.)	60–90 (min.)		30–60 (min.)	60–90 (min.)	135–165 (min.)	165–195 (min.)
Control	—	10.7	10.7	1.1	19.8	18.2	17.3	17.3
Control	—	11.2	10.8	0.7	20.6	18.3	17.7	17.5
0.15 mg. (0.187 μmole) protoveratrine/kg.	39	17.3	17.0	9.6	16.3	11.6	7.7	7.5
	48	17.2	17.3	9.7	15.4	10.8	7.0	6.2

The respiratory and glycolytic activity of these slices is compared in Table 1 with that of sliced cerebral cortex from two normal animals. The rate of oxygen uptake of the tissue of the animals given protoveratrine was increased about 60 %. Aerobic glycolysis increased to half the normal anaerobic level. Anaerobic glycolysis, on the other hand, was inhibited. It declined during the course of incubation, becoming roughly constant after 2 hr. at about 40 % of the control rate. Since the aerobic and anaerobic experiments shown in Table 1 were performed in different media, no exact measure of the Pasteur effect can be presented. However, since the control Q_{lact} values were of the same order of magnitude in the two media, both in the presence and the absence of oxygen respectively, it is clear that the Pasteur effect was inhibited.

Effect of protoveratrine added in vitro on respiration and glycolysis of cerebral cortex. The changes in cerebral metabolism just described could have been mediated through the local vascular system or result from an extracerebral alteration whose effect

persisted in the separated tissue. To show that protoveratrine acts directly on cerebral cortex, it was added to the medium, the final concentration being $9.4 \times 10^{-7} \text{ M}$ ($0.75 \mu\text{g./ml.}$). The results of this experiment (Table 2) reveal the same pattern of response as after administration of protoveratrine *in vivo*, namely stimulation of aerobic and depression of anaerobic metabolism. The changes in aerobic and anaerobic glycolysis, this time measured in the same medium, were even more pronounced. The aerobic glycolysis rose to the normal anaerobic level, whereas the anaerobic glycolysis was completely suppressed. The Pasteur effect was thus abolished. The fact that these changes were of the same kind as those observed in brain cortex of protoveratrine-treated animals may be taken as supporting evidence for a direct action of the alkaloid on the brain *in vivo*.

The sensitivity and the time-course of the respiratory response to protoveratrine are illustrated in Fig. 1. $4.7 \times 10^{-7} \text{ M}$ protoveratrine is the threshold concentration for stimulating respiration

Table 2. *Effect of protoveratrine added in vitro, on respiration and glycolysis of cerebral cortex*

Slices of the guinea-pig tissue were suspended in phosphate-buffered media. Experimental periods are given in minutes after the addition of protoveratrine.

Expt. no.	Treatment	Q_{O_2}	$Q_{\text{lact}}^{O_2}$	$Q_{\text{lact}}^{N_2}$	
		50-110 (min.)	50-110 (min.)	50-110 (min.)	110-170 (min.)
1	Control	10.8	0.3	21.6	—
	$9.4 \times 10^{-7} \text{ M}$ protoveratrine	14.1	21.4	6.5	0.5
2	Control	12.7	-0.3	19.2	18.5
	$9.4 \times 10^{-7} \text{ M}$ protoveratrine	17.5	17.7	3.2	0

Fig. 1. Protoveratrine at various concentrations (μM) on the rate of respiration of cerebral-cortex slices from the guinea pig.

in guinea pig cerebral-cortex slices. It increases metabolism by about 20 %. The rise in respiration produced by $9.4 \times 10^{-7} \text{ M}$ protoveratrine is of the order of 40 %. At a concentration of approximately $4 \times 10^{-6} \text{ M}$ a sharp initial rise in respiratory activity is followed by a gradual decline towards, and possibly to below, the control level.

Effect on cerebral oxygen uptake in various substrates. The response of cerebral-cortex slices to protoveratrine in the presence of several non-carbohydrate substrates known to be oxidized by this tissue is shown in Table 3. The substrates were: lactate, pyruvate, oxaloacetate, succinate, and L-glutamate. Stimulation by protoveratrine of respiration in pyruvate was comparable to that seen in glucose, but stimulation was less in media containing lactate and oxaloacetate. In lactate the increase was followed by a fall. No increase in oxygen consumption occurred with succinate or glutamate or in the absence of added substrate. The finding that slices respiring in pyruvate respond as intensely to protoveratrine as in the presence of

glucose and that some increase in respiration is obtained in oxaloacetate suggests that protoveratrine accelerates reactions in the tricarboxylic acid cycle. This cycle may not be fully operating when succinate is the sole substrate added.

Tissue specificity. Among the various guinea-pig tissues listed in Table 4, heart muscle alone resembles cerebral cortex with respect to the respiratory response to protoveratrine. A concentration of 4.7×10^{-6} M produced a rise in the rate of oxygen uptake of about 50 %. Aerobic glycolysis in heart muscle, however, was not stimulated by protoveratrine. Among the unresponsive tissues were voluntary muscle (diaphragm) and smooth

muscle (intestine). The lack of response in diaphragm is surprising in view of the report of Stozharov (1936) that the resting heat production of veratrinized skeletal muscle is high. In the succeeding paper (Wollenberger, 1955) it is shown that protoveratrine stimulates respiration also in the white matter of brain. Bernheim & Bernheim (1933) have reported that oxygen consumption of skeletal muscle and liver tissues is inhibited by veratrum alkaloids at and above 10^{-3} M.

Most Q_{O_2} values in Table 4 are lower than corresponding values obtained in the same incubation medium in an earlier study (Wollenberger, 1947). The earlier quotients were based on the dry

Table 3. *Effect of protoveratrine on the oxygen consumption of cerebral-cortex slices in the presence of various substrates*

Added substrate (10 mm)	Expt. no.	Concn. of protoveratrine (μ M)	Q_{O_2} Time after addition of protoveratrine		
			Before	5-35 (min.)	95-125 (min.)
Sodium DL-lactate	4	0	9.3	8.9	7.4
		1.87	9.6	12.6	5.4
Sodium pyruvate	4	0	11.2	11.3	10.4
		1.87	10.8	15.5	11.3
Sodium oxaloacetate	3	0	10.7	10.7	9.7
		1.87	10.9	13.2	12.1
Sodium succinate	3	0	8.9	7.8	—
		1.87	8.2	6.6	—
Sodium L-glutamate	3	0	10.9	9.7	6.2
		1.87	10.4	10.4	4.7
None	2	0	4.8	3.1	—
		1.87	5.0	3.2	—

Table 4. *Effect of protoveratrine on the rate of respiration of various isolated guinea-pig tissues*

Phosphate-buffered medium. Protoveratrine was added after 30-60 min. of preliminary incubation. The Q_{O_2} values refer to this preliminary period.

Tissue and state	Q_{O_2}	Protoveratrine (μ M)	Percentage change in Q_{O_2} 45-90 min. after addition of protoveratrine
Diaphragm (intact)	4.2	0	-5
		9.4	-2
Heart ventricle (sliced)	2.5*	0	-7
		4.7	+47†
Muscularis of small intestine (intact)	4.9	0	-20
		9.4	-20
Testis (teased)	7.0	0	+1
		9.4	+3
Liver (sliced)	3.5	0	-5
		9.4	-2
Kidney cortex (sliced)	12.8	0	-13
		9.4	-10
Lung (cut)	5.7	0	-6
		9.4	0
Spleen (sliced)	7.1	0	0
		9.4	0

* After decrease during the first 30 min.

† 75-150 min. after addition of protoveratrine.

weight of slices or other larger tissue pieces only, while present final dry weights include the weight of small tissue debris and of trichloroacetic acid precipitate in the medium.

Reversibility. Cerebral-cortex slices which had been incubated in $1.87 \times 10^{-6}M$ protoveratrine sufficiently long to observe a 50% increase in the rate of O_2 uptake were washed twice for 5 min. at room temperature in 25 ml. of aerated medium free of protoveratrine and transferred to vessels containing fresh, protoveratrine-free medium. This procedure abolished the stimulation of respiration (Table 5). The rate of oxygen consumption at the beginning of the second incubation period was about 20% lower than the initial rate during the first incubation, both in the control and in the experimental vessels. During the second incubation the protoveratrine-treated slices exhibited an additional slight decline in respiratory activity.

These findings need to be reconciled with the observation (Table 2) that cerebral cortex slices after *in vivo* poisoning with protoveratrine respire at an elevated rate when placed in protoveratrine-free medium. One reason for this may be that in

fluoride did not entirely suppress phosphatase activity in the unfractionated homogenate. However, there were marked differences in the ratios between control and protoveratrine-treated samples on the one hand and samples lacking substrate or treated with dinitrophenol on the other hand. While omission of substrate lowered oxygen uptake by about one-half, phosphate uptake fell to one-fifth of the control value. 2:4-Dinitrophenol, known to dissociate oxidation and phosphorylation (Loomis & Lipmann, 1948), caused at $8 \times 10^{-5}M$ a slight increase in O_2 consumption and complete inhibition of phosphate uptake.

Protection by nicotinamide against the inhibition of anaerobic glycolysis. Experiments were conducted to ascertain whether the depression of anaerobic glycolysis by protoveratrine could be prevented by substances likely to exert a stabilizing action. Bicarbonate medium was used in which the anaerobic action of protoveratrine is not as severe as in phosphate-buffered medium (cf. Table 2). Among a number of substances tested only one afforded some protection against the alkaloid, namely nicotinamide. The protective concentration of this

Table 5. *Effect of washing on the respiratory activity of cerebral-cortex slices treated with protoveratrine*

Phosphate-buffered medium was used throughout. Results are expressed in % of the rate of O_2 uptake before addition of protoveratrine.

Time after addition of protoveratrine (min.)	Expt. 1		Expt. 2	
	Control	$1.87 \times 10^{-6}M$ protoveratrine	Control	$1.87 \times 10^{-6}M$ protoveratrine
(Before)	100	100	100	100
5-35	101	154	100	151
36-48	Washing and transfer to protoveratrine-free medium			
62-82	82	81	81	77
152-182	82	74	75	67

the experiment with the animals poisoned *in vivo* the slices were not washed before incubation. For quantitative comparison of the changes after *in vivo* and *in vitro* administration, the concentrations of drug in the tissue are required but have not yet been determined.

Effect on oxidation and phosphorylation in homogenized cerebrum. To determine whether protoveratrine affects oxidative phosphorylation in cerebral tissue, uptake of oxygen and disappearance of inorganic phosphate from the medium were measured in homogenates of cerebral hemispheres supplied with the necessary cofactors, with lactate as the main source of energy and creatine as phosphate acceptor. Protoveratrine was present in concentrations 1.87×10^{-6} and $4.7 \times 10^{-5}M$. As Table 6 shows, neither the low nor the high concentration had a significant effect on oxidation or phosphorylation in the cell-free system. The P/O ratios were rather low, presumably because

Table 6. *Comparison of the effects of protoveratrine and dinitrophenol on oxygen and phosphate uptake by guinea-pig brain homogenate*

Experiments used a 1.7% homogenate of cerebral hemispheres (50 mg. wet tissue/vessel). Concentrations of added constituents: 3.3 mM potassium phosphate buffer of pH 7; 4 mM sodium DL-lactate; 1 mM potassium fumarate; 40 mM creatine; 8 mM-MgCl₂; 1 mM adenosine triphosphate; 0.05% diphosphopyridine nucleotide preparation (Schwartz); 40 mM nicotinamide; 0.023 mM cytochrome c; 20 mM-KF. Final volume: 3.0 ml. Air; 38°; 15 min. incubation.

Treatment	Oxygen uptake* (μ moles)	Phosphate uptake (μ moles)
Control	5.3	4.7
Protoveratrine, $1.87 \times 10^{-6}M$	5.3	4.5
Protoveratrine, $4.7 \times 10^{-5}M$	5.6	4.8
2:4-Dinitrophenol, $8 \times 10^{-5}M$	6.4	-0.4
Lactate and fumarate omitted	2.5	0.9

* Corrected to include equilibration period.

compound was of the same order as that required to inhibit the activity of the nucleosidase which splits diphosphopyridine nucleotide (DPN) (Mann & Quastel, 1941; Handler & Klein, 1942). The co-enzyme itself was ineffective. Addition of adenosine triphosphate and of adenylic acid proved likewise unsuccessful. Neither did these compounds add to the protective action of nicotinamide. A fivefold increase in the external magnesium concentration and addition of pyruvate ($0.0017M$) had moderate protective effects in some experiments, but no effect in others. In one run complete protection against protoveratrine was provided by nicotinamide plus magnesium salts.

Three experiments illustrating the effect of $0.04M$ nicotinamide are summarized in Table 7. It is seen that the loss in glycolytic activity caused by protoveratrine was reduced by about one-half in the presence of the vitamin. The compound was effective only when it was added to the medium before or simultaneously with protoveratrine. Once the inhibition of glycolysis had become established, it was not reversed.

Effect of omission of external potassium salts. According to one theory (Bacq, 1939) veratrum alkaloids act by sensitization to potassium ions. To determine whether the metabolic response of

ions than in media containing sodium, calcium and potassium ions. Gore & McIlwain (1952), however, noted no marked changes in cerebral respiration and glycolysis due to absence of potassium.

While persistence of the protoveratrine effect in potassium-free medium would have been difficult to reconcile with an hypothesis that the alkaloid acts on cerebral cortex via extracellular potassium, the observation to the contrary does not prove the hypothesis. Dickens & Greville (1935) observed that, after incubation in potassium-free media, cerebral-cortex slices fail to respond to high concentrations of potassium ions with an increase in respiration. It is possible that prolonged exposure to K^+ -free medium caused irreversible damage to the tissue and reduced its respiratory capacity.

Effect on accumulation of potassium by washed cerebral-cortex slices. Freshly prepared slices of cerebral cortex were washed for 5 min. in a large volume of potassium-free medium. On the basis of measurements made by Krebs, Eggleston & Terner (1951) it seems safe to assume that at the end of the washing the tissue had lost at least half its normal complement of potassium ions, which amounts to about $100 \mu\text{equiv./g.}$ During subsequent aerobic incubation in glucose media without protoveratrine, potassium ions were gradually

Table 7. *Effect of nicotinamide on the inhibition of anaerobic glycolysis in cerebral-cortex slices by protoveratrine*

Bicarbonate medium. Gas phase: 95% N_2 -5% CO_2 . Results are means of 3 experiments and are expressed in % of the rate of CO_2 evolution before exposure to the substances added.

Time after addition of substances listed (min.)	Substances added			
	None	Nicotinamide, $4 \times 10^{-2}M$	Protoveratrine, $1.87 \times 10^{-6}M$	Protoveratrine, $1.87 \times 10^{-6}M$, and nicotinamide, $4 \times 10^{-2}M$
(Before)	100	100	100	100
5-35	98	96	74	92
80-110	94	93	16	46
110-140	94	92	16	48

cerebral-cortex slices to protoveratrine depends on the presence of potassium, incubation fluid was used in which potassium chloride was replaced by an equivalent amount of sodium chloride. The results of the experiment (Table 8) show that the rate of oxygen consumption and of lactic acid production were not significantly changed by the drug. The rate of oxygen consumption was lower than in the presence of potassium, and interim readings of pressure indicated that it was declining during incubation. On the other hand, the rate of glycolysis was high. This finding agrees with the observation of Dickens & Greville (1935) that aerobic glycolysis of cerebral-cortex slices tends to be higher in media containing sodium and calcium

Table 8. *Effect of protoveratrine on respiration and glycolysis of washed cerebral-cortex slices in potassium-free medium*

The composition of the medium was: $0.098M$ -NaCl, $0.0013M$ - $CaCl_2$, $0.0012M$ - $MgSO_4$, $0.033M$ sodium phosphate of pH 7.4, $0.011M$ glucose. Before being placed in the respirometer vessels the slices were washed for 5 min. in 100 ml. of this medium. Protoveratrine was added 25 min. after start of incubation. 60 min. incubation in protoveratrine.

Concn. of protoveratrine (M)	Q_{O_2}	$Q_{lact.}^{O_2}$
0	6.7	15.3
1.87×10^{-6}	6.6	16.5

regained, as indicated by their disappearance from the surrounding medium (Table 9). This reaccumulation was a slow but steady process, as indicated by the fact that twice as much potassium disappeared during 2 hr. of incubation as in 1 hr. The rate of uptake was 17–18 $\mu\text{equiv. K}^+/\text{g. wet wt./hr.}$ A comparable value (1 $\mu\text{l. K}^+$ (gas)/mg. dry wt./hr.) was obtained by Dixon (1949) in sliced rabbit cerebral cortex subjected to similar experimental conditions.

Table 9 shows that $1.87 \times 10^{-6}\text{M}$ protoveratrine, which markedly accelerates oxygen consumption, prevented the disappearance of potassium from the medium. Since in these and other experiments the loss in dry weight of slices during incubation was unaffected by protoveratrine, the effect of the alkaloid on the content of K^+ in the medium can hardly have been due to increased contamination with potassium present in tissue debris or bound to soluble proteins. This effect may reasonably be attributed to inhibition of the net uptake of the ion by the tissue.

with the present study of protoveratrine because they have been subjected to the same kind of experimental analysis. The similarity of the pattern of responses to the three agencies is striking. A résumé of observed effects is given in Table 10. The parallelism between the action of protoveratrine, K^+ , and electrical pulses extends to the nature of the substrate required for elicitation of the respiratory response, to reversibility of the action, to sensitivity to malonate of the stimulated as contrasted with the unstimulated respiration, to specificity with respect to the tissue affected, and probably also to the role of cell structure. Aebi (1953) finds, in contrast to Dickens & Greville (1935), that the respiration of slices of kidney cortex (but not of liver) is increased by high external K^+ . Apart from this instance, however, stimulation of metabolism by potassium, electric currents and protoveratrine has so far been seen only in excitable tissues. All three agents raise the aerobic glycolysis of cerebral-cortex slices to or close to the normal anaerobic level. The anaerobic glycolysis of the tissue is

Table 9. *Effect of protoveratrine on potassium uptake by washed cerebral-cortex slices*

The slices were washed for 5 min. in 100 ml. of K^+ -free medium (see Table 8) and incubated in 2.7 ml. phosphate-buffered medium containing K^+ in the concentrations indicated. Protoveratrine was initially present in the main compartment of the incubation vessels.

Duration of expt. (min.)	Concn. of protoveratrine (moles/l.)	Initial wet wt. of tissue (mg.)	Q_{O_2}	Concn. of K^+ in medium ($\mu\text{equiv./ml.}$)		Uptake of K^+ from medium ($\mu\text{equiv./g. tissue}$)
				Initial	Final	
60	0	82	12.2	4.80	4.26	17.8
	1.87×10^{-6}	73	17.5	4.80	4.80	0.0
120	0	74	11.3	3.42	2.49	33.9
	1.87×10^{-6}	87	15.6	3.42	3.33	2.8

DISCUSSION

Comparison of the effects of protoveratrine, potassium and electrical pulses. Stimulation of respiration and aerobic glycolysis in brain can be brought about by a variety of agents and circumstances. These include normal functional activity of the brain (Schmidt, Kety & Pennes, 1945; Vladimirov, 1953), induced convulsive activity (Davis, McCulloch & Roseman, 1944; Schmidt *et al.* 1945; Stone, Webster & Gurdjian, 1945; Klein & Olsen, 1947; Vladimirov, 1953), electrical stimulation of isolated cerebral tissue (McIlwain, 1951*a*), high extracellular concentrations of potassium salts (Ashford & Dixon, 1935; Dickens & Greville, 1935) and dinitrophenol (McIlwain & Gore, 1951). Among these agencies high concentrations of potassium ions and electrical pulses, both applied to isolated cerebral tissue, may be singled out for closer comparison

inhibited by both protoveratrine and potassium salts; according to McIlwain (private communication) anaerobic glycolysis falls also on application of electrical pulses.

Possible mechanism underlying the protoveratrine effect. The question arises whether the effects of protoveratrine, potassium ions and electrical impulses on cerebral metabolism may have a common mechanism. McIlwain (1952) has considered this question so far as the application of potassium and electrical stimuli *in vitro* is concerned, and he concluded that in both cases the stimulation of metabolism is probably secondary to intensification of processes involved in functional activity. This is more or less self-evident in the case of electrical stimulation, particularly since the chemical changes caused by application of electrical pulses to brain-cortex slices parallel those seen during heightened electrical activity of the central

nervous system *in vivo*. The relation of the metabolic effects of protoveratrine and electrical stimuli will be dealt with in the succeeding paper.

The increase in metabolic activity caused by raising the external concentration of potassium ions is believed by McIlwain (1952) to occur in support of increased osmotic work performed to re-establish normal ionic concentration differences across the cell membrane. A similar mechanism may underlie the metabolic action of protoveratrine. Shanes (1952) has shown that, in resting nerve, veratrum alkaloids (veratrine) cause net release of K^+ and uptake of an equivalent amount of Na^+ . The effect of protoveratrine on potassium balance in cerebral-cortex slices is in the same direction; i.e. under conditions where the tissue can be presumed to be depleted of potassium, the

ment of calcium ions from the cell surface (Gordon & Welsh, 1948). Since calcium stabilizes biological membranes and may be bound to and immobilize molecules that may act as carriers for Na^+ and K^+ , and since the cell membrane in excitable tissues is highly sensitive to alterations in the external Ca^{2+}/K^+ ratio, such displacement of calcium ions could account for increases in permeability and also for sensitization to potassium (see Bacq, 1939). It may be the primary cause for the increase in metabolic activity. Removal of external calcium ions, which will cause Ca^{2+} bound at the cell surface, in equilibrium with external Ca^{2+} , to leave the cell membrane, has been found to accelerate respiration and glycolysis in cerebral cortex (Dickens & Greville, 1935; Canzanelli, Rogers & Rapport, 1942).

Table 10. *Parallelism of the effects of protoveratrine, potassium salts and electrical stimulation on respiration and glycolysis of cerebral-cortex slices and other tissues*

+ = increase, - = decrease, 0 = no change.

Metabolic event	Agent		
	Protoveratrine approx. $10^{-6}M$	K^+ approx. $10^{-1}M$	Electrical pulses
O_2 uptake in glucose, in lactate and in pyruvate	+ ^a	+ ^{b-c}	+ ^{f-h}
O_2 uptake in succinate and in L-glutamate	0 ^a	0 ^{a, i}	0 ^{a, j}
O_2 uptake in glucose plus 10^{-3} to $10^{-2}M$ -malonate	0 ^k	0, - ⁱ	0 ^m
Aerobic glycolysis	+ ^a	+ ^{b, c}	+ ⁿ
Anaerobic glycolysis	- ^a	- ^{b, c}	- ^o
O_2 uptake on removal of agent	- ^a	- ^b	- ^f
O_2 uptake of minced or homogenized brain	0 ^a	0 ^p	{ + ^q 0 ^r
O_2 uptake of non-excitable tissues	0 ^{sa}	{ 0 ^{a, s, t} + ^t }	0 ^u

* Concentrations of protoveratrine ranging up to $10^{-4}M$.

^a Present paper.	ⁱ Kimura & Niwa (1953).
^b Ashford & Dixon (1935).	^m Heald (1953).
^c Dickens & Greville (1935).	ⁿ McIlwain, Anguiano & Cheshire (1951).
^d Canzanelli <i>et al.</i> (1942).	^o McIlwain (private communication).
^e Lipsett & Crescitelli (1950).	^p Dixon & Meyer (1936).
^f McIlwain (1951a).	^q Aboud, Gerard & Ochs (1952).
^g Kratzing (1953).	^r Narayanaswami & McIlwain (1954).
^h McIlwain (1953).	^s Needham, Nowinski, Dixon & Cook (1937).
ⁱ Dixon (1937).	^t Aebi (1953).
^j McIlwain (1951b).	^u Kratzing (1951).
^k Wollenberger (unpublished).	

drug prevents reaccumulation of the ion. The rate of turnover of potassium may actually be increased by protoveratrine. Such change would fit in with and could explain the rise in respiration and glycolysis. As Shanes (1951) has shown in nerve, veratrine increases the net rates of both loss of potassium during stimulation and of its reabsorption during recovery. According to the same author (Shanes, 1952; Shanes, Grundfest & Freygang, 1953) veratrum alkaloids increase membrane permeability to K^+ or other ions (Na^+ , Cl^-) to whose movements that of K^+ is linked. There is some evidence that the basic effect of veratrum alkaloids is a displace-

Protoveratrine and inhibition of the Pasteur effect. Seits & Engelhardt (1949) and Judah & Williams-Ashman (1951) found that a variety of compounds which inhibit the Pasteur effect in cells and tissue slices also uncouple oxidation and phosphorylation. McIlwain (1950), working with brain tissue, discovered that the inhibitory action of some of these compounds on the Pasteur effect was correlated with inhibition of the enzyme which hydrolyses DPN at the nicotinamide-ribose linkage. These relationships are probably of considerable significance, but they do not constitute a general rule. Exceptions have been noted by McIlwain (1950)

and Lehninger (1951). Protoveratrine, which does not interfere with oxidative phosphorylation in homogenized brain, is probably a different type again of Pasteur effect inhibitor. Its action on the nucleosidase attacking DPN was not tested. However, the ability of nicotinamide, in a concentration which inhibits splitting of DPN (Mann & Quastel, 1941; Handler & Klein, 1942) to give protection against the depression of anaerobic glycolysis by protoveratrine, suggests that the alkaloid, far from being an inhibitor of the DPN-splitting enzyme, may actually, under anaerobic conditions, bring about its activation.

Breakdown of DPN in brain slices is normally restricted, but is rapid when the cells in the tissue are damaged (Gore, Ibbott & McIlwain, 1950). It is conceivable that protoveratrine and anoxia combined cause disorganization of cellular structures, resulting in an 'unmasking' of the DPN-splitting nucleosidase and possibly also of other, normally restrained, hydrolytic enzymes. This need not occur through direct physical action on the part of the alkaloid. If protoveratrine stimulates processes involved in the functional activity of neurons, the increased energy demands of the tissue might lead, anaerobically, to exhaustion of the stores of energy, required not only for specialized function, but for chemical syntheses (including synthesis of DPN) and for maintenance of labile cellular structures. Facilitation by anoxia of the entry of protoveratrine into the cell (see Hill, 1933; Fromherz, 1933) may be an additional factor responsible for the slowing of anaerobic metabolism, by permitting the tissue concentration of the alkaloid to rise to levels which might also be inhibitory aerobically (cf. Fig. 1). Aerobic incubation in media containing low, but anaerobically inhibitory concentrations of protoveratrine did not result in deficiency of DPN, as shown by high rates of aerobic lactic acid production. It is possible, however, that also under aerobic conditions protoveratrine might cause a drain on energy-rich phosphate. Lowered levels of phosphocreatine have been observed in veratrinized muscle (Nachmansohn, 1929) and nerve (Gerard, 1934), as well as in brain in physiological activity, in convulsions, during exposure to high concentrations of K^+ , and in other conditions characterized by high rates of respiration and glycolysis in which energy utilization is believed to be accelerated (see, for example, McIlwain, 1952).

In connexion with the problem of the Pasteur effect it is of interest that protoveratrine, in contrast to its action on cerebral-cortex slices, was found to stimulate respiration in heart-muscle slices without increase in glycolysis. The difference in the behaviour of the two protoveratrine-sensitive tissues may be attributed to differences in enzymic

make-up. Heart muscle is rich in enzymes and coenzymes of the tricarboxylic acid cycle and of the electron-transfer system, but its glycolytic power is low. The glycolytic capacity of brain cortex, on the other hand, is high relative to its oxidative capacity. In brain cortex the extra pyruvate formed by acceleration of glucose breakdown can compete successfully with oxygen for reduced DPN and may for the most part be reduced to lactate. This situation is unlikely to arise in heart muscle. In point of fact, substances which increase aerobic glycolysis in brain cortex have been reported to decrease glucose utilization in tissues characterized by predominance of oxidative over glycolytic capacity, such as lactating mammary gland (Turner, 1952) and heart muscle (Wollenberger, 1953).

SUMMARY

1. Cerebral-cortex slices from guinea pigs poisoned with protoveratrine exhibited accelerated respiration, high aerobic glycolysis and depressed anaerobic glycolysis.
2. The same changes were seen when cerebral cortex of normal animals was exposed *in vitro* to protoveratrine concentrations of the order of $10^{-6}M$.
3. Protoveratrine accelerated oxygen uptake in media containing glucose, lactate, pyruvate or oxaloacetate, but not in media containing succinate, L-glutamate or no added substrate.
4. Acceleration of respiration was restricted to nervous and cardiac tissues.
5. Protoveratrine had no effect on respiration and aerobic phosphorylation in homogenized cerebrum.
6. The accelerating effect of the alkaloid on the respiration of cerebral slices could be reversed by washing. The inhibition of anaerobic glycolysis could partly be prevented, but not reversed, by nicotinamide.
7. External K^+ was necessary for the acceleration of aerobic metabolism.
8. Net uptake of K^+ by washed cerebral-cortex slices was inhibited by protoveratrine in concentrations which stimulated respiration and aerobic glycolysis.
9. These effects of protoveratrine have been compared with the response of cerebral tissue to an increase in the concentration of external K^+ and to electrical impulses and have been discussed in relation to the action of other inhibitors of the Pasteur effect.

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Action of Protoveratrine on the Metabolism of Cerebral Cortex

2. ELECTRICALLY STIMULATED CEREBRAL-CORTEX TISSUE

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In the preceding communication (Wollenberger, 1955) it was reported that addition of small amounts of protoveratrine to freshly cut sections of guinea pig cerebral cortex causes, amongst other metabolic changes, an acceleration of oxygen con-

sumption. The metabolic conditions which were required for this acceleration to take place were similar to those which had previously been found in this laboratory to be essential for acceleration of the metabolism of cerebral-cortex slices by means of electrical pulses. The present experiments were started in order to see whether these pulses and

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