A stimulation of the respiration of rat cerebral cortical slices *in vitro* by a medium in which rat sciatic nerve had been previously incubated has been shown by the above experiments. These results extend the earlier observations of Heller and Hesse(1) to show that the oxygen uptake of cerebral cortical slices, as well as of nerve, are stimulated by some factor which diffuses from sciatic nerve. A stimulation was observed if the medium in which the nerve was incubated contained no added substrates, but was absent if the incubation medium was well substrated. The factor increases the respiration of brain slices by a small amount compared with the marked stimulation produced by the presence of added substrates (Tables II and III). Since sheaths of nerves, incubated in the same manner as nerves, are capable of producing a similar stimulation, nerve is not a unique source of the "activating" substance.

The nature of the activating factor is unknown. Diffusible substances, as yet unidentified, have been recently shown to be released by peripheral nervous tissue during an incubation period(5). The nature of and the role played by these diffusible "activating" substances from peripheral nerve require further investigation.

Summary. Stimulation of the *in vitro* oxygen uptake of rat cerebral cortical slices by some factor which diffuses from rat sciatic nerve or the sheath of sciatic nerve during a 2-hour incubation period at room temperature has been shown. The incubation medium used was phosphate buffered, possessing the normal ionic constituents except for calcium and lacking exogenous substrates. The control QO_2 determined in this medium was 3.0 with values of 4.9 ($P<0.01$) and 4.1 ($P<0.015$) resulting from nerve- and sheath-activated media respectively. QO_2 values determined in a well-substrated medium (Krebs III solution lacking calcium) did not show any stimulation. The results of these experiments suggest that both the nerve and its sheath are good sources of an oxygen uptake stimulating material. The nature of the substance is unknown.

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Cerebral Cortical Oxygen Uptake Stimulating Factor from Cerebral Cortical Slices.* (28885)

DAVID A. RINGLE† AND ROBERT J. DELLENBACK (Introduced by Shu Chien)
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

A factor which diffuses from rat sciatic nerve during *in vitro* incubation in a substrate-free medium at room temperature has been reported(1). In the presence of this factor freshly excised nerves(1) and cerebral cortical slices(2,3) show a stimulation of their respiration. This material has been referred to as the "activating" substance (1,2,3). Sciatic nerve and its connective tissue sheath both appear to be a source of this material.

The present experiments were designed to test if an activating substance is also released from rat cerebral cortical slices during room temperature incubation which is subsequently capable of stimulating the respiration of cere-

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† Present address: Midwest Research Inst., Kansas City, Mo.

TABLE I. Results of Non-Activated Control and Brain-Activated* Non-Substrated Phosphate Saline Solutions on Oxygen Uptake of Rat Cerebral Cortical Slices.

Medium	No. of exp	Regression equation†	40 min values‡	QO_2
Control	15	$Y = .055X + .29$ (n=60)	2.4 ± .7	3.6
Brain activated	15	$Y = .087X + .42$ (n=60)	3.9 ± .2 ($P < .01$)	5.6

* 2-hr room temperature (24-26°C) incubation.
† Values in parentheses represent total number of determinations from which regression equations have been calculated.
‡ Values are mean ± S.D.

bral cortical slices. The influence of the temperature of incubation and the presence of substrates have also been investigated.

Methods. The oxygen uptake of cerebral cortical slices from adult male Sherman rats was measured by a standard Warburg manometric technique as previously described(3). Experiments were conducted in 5 ml reaction vessels gassed with pure oxygen and run at a temperature of 37.34 ± 0.02°C. QO_2 values (μl oxygen consumed/mg dry wt. tissue/hour‡) were calculated from regression equations formulated using the 10, 20, 30 and 40 minute readings, disregarding zero time as a point. Statistical significance was evaluated for the 40 minute values by the "Student" t test.

The incubation media used in these experiments were the non-substrated phosphate saline medium of Heller and Hesse(1) and a calcium-free version of medium III of Krebs(4). These media contained the following (in mM/l): for phosphate saline, Na^+ 153.5; K^+ 3.0; Mg^{++} 1.2; Cl^- 125.0; $SO_4^=$ 1.2; Phosphate 17.5; and for the substrated medium III, Na^+ 141.4; K^+ 5.9; Mg^{++} 1.2; Cl^- 122.8; $SO_4^=$ 1.2; HCO_3^- 3.6; Phosphate 3.5; Pyruvate 4.9; Fumarate 5.4; Glutamate 4.9; and Glucose 11.5. The Krebs III medium was calcium-free to afford a closer comparison to the non-substrated phosphate saline solution of Heller and Hesse which lacked calcium. In certain experiments described in the *Results* section, the phosphate saline solution was substrated by addition of glucose (11.5 mM/l).

Prior to their use in manometric analyses, media were subjected to a 2 hour period of incubation with surface cortical tissue slices (0.4-0.5 mm thick) from the brains of 3 animals. Four slices were cut, using a template and razor blade, from each hemisphere. The details of tissue preparation are the same as those previously described(3). Tissues were incubated at room temperature (24-26°C) in Parafilm covered Pyrex beakers with occasional agitation. In the experiments in which the temperature effect was studied, tissues were incubated at 37.34°C during the recording of O_2 uptake. Control media were subjected to identical treatment throughout except for inclusion of cortical slices.

Results. A. *Non-substrated media.* Rat cerebral cortex slices yielded an activating medium following 2 hours of incubation (24-26°C) in the non-substrated phosphate saline. Freshly cut cerebral cortical slices incubated in this activating medium consumed significantly more ($P < 0.01$) oxygen than did slices in control medium (QO_2 values = 5.6 and 3.6 respectively for activating and control media). These results are presented in Table I. The incubation of cerebral cortex slices in non-substrated medium thus alters the medium so that subsequently incubated freshly-cut cortical slices will consume more oxygen.

B. *Substrated media.* 1. *Prior incubation at room temperature.* To determine whether an activating medium is produced by incubation of cortical slices in the presence of known substrates, Krebs medium III(4) was used. Table II shows that incubation of cortex slices in this substrated medium at room temperature produced a QO_2 of 21.7 which is not a significant increase over the control QO_2 of 21.1.

Krebs medium III contains glucose, pyruv-

‡ Dry weights were determined on a series of fresh cortical slices.

TABLE II. Results of Non-Activated Control and Brain-Activated* Krebs III Solution Lacking Calcium on Oxygen Uptake of Rat Cerebral Cortical Slices.

Medium	No. of exp	Regression equation†	40 min values‡	QO_2
Control	15	$Y = .353X + .56$ (n=60)	14.6 ± 1.0	21.7
Brain activated	15	$Y = .342X + .53$ (n=60)	14.1 ± 1.1 (P >.1)	21.1

* † ‡ See legend of Table I.

TABLE III. Results of Non-Activated Control and Brain-Activated* Phosphate Saline Solution to Which a Substrate Has Been Added on Oxygen Uptake of Cerebral Cortical Slices.

Medium	No. of exp	Glucose conc, M/l	Regression equation†	40 min values‡	QO_2
Control	12	1.15×10^{-2}	$Y = .290X + .19$ (n=48)	$11.7 \pm .9$	17.6
Brain activated	12	1.15×10^{-2}	$Y = .297X + .20$ (n=48)	$12.0 \pm .8$	18.0

* † ‡ See legend of Table I.

ate, fumarate, and glutamate, all 4 of which are absent from the non-substrated phosphate saline medium. To determine whether the presence of pyruvate, fumarate and glutamate as opposed to glucose could account for the lack of stimulation seen in the Krebs III medium subjected to prior incubation, an experimental series was run in which glucose (11.5 mM) was added to the phosphate saline medium. Table III shows that a brain-activated, glucose-containing medium failed to produce a further stimulation over the glucose-containing control.

It is seen from the QO_2 values presented in Tables II and III, that tissue slices in substrated media do not significantly alter the effects of these media on oxygen uptake of freshly cut slices.

2. *Prior incubation at 37.34°C.* The temperature of incubation may play a role in the appearance of the "activating" factor. Indeed, the lack of stimulation observed by prior incubation in substrated media may be the result of insufficient amounts of this material appearing in the media due to the low incubation temperature. A series of experiments was thus run in an attempt to investigate this. Table IV summarizes these results. Prior incubation of cortex slices at 37.34°C in a substrated media lacking calcium did not produce a further stimulation over the control. The QO_2 of the cortical slices used in the activation procedure averaged 23.8 and is referred to as activating control in Table IV. The QO_2 of freshly cut tissue subsequently determined in this incubation media averaged 22.0 and is designated as brain activated in Table IV. A comparison of these two suggests that there may actually be a slight (although not significant) reduction in the latter. In Table IV are also listed the control QO_2 values obtained from cortical

TABLE IV. Results of Non-Activated Control and Brain-Activated* Krebs III Solution Containing Calcium (2.5 mM/l) and Lacking Calcium on Oxygen Uptake of Cerebral Cortical Slices.

Medium	No. of exp	Regression equation†	40 min values‡	QO_2
Zero calcium				
Activating control§	6	$Y = .390X + .43$ (n=24)	15.9 ± 1.2	23.8
Control	6	$Y = .377X + .59$ (n=24)	$15.6 \pm .8$	23.2
Brain activated	6	$Y = .356X + .64$ (n=24)	14.8 ± 1.1 (P >.1)	22.0
Normal calcium				
Activating control§	6	$Y = .317X$ (n=24)	$12.6 \pm .9$	19.0
Control	6	$Y = .339X + .36$ (n=24)	$13.9 \pm .6$	20.7
Brain activated	6	$Y = .342X + .50$ (n=24)	$14.1 \pm .8$ (P >.1)	21.0

* 2-hr incubation at 37.34°C.
† ‡ See legend of Table I.
§ Results obtained during 2-hr incubation period.

tissues suspended in the media which had been used as thermobarometer fluid during the activation procedure. The agreement between the activating control QO_2 and this control (23.8 and 23.2 respectively) further indicates that the brain-activated QO_2 may be lower.

Calcium was omitted from the Krebs solution to eliminate the influence of this ion which was not included in phosphate saline medium. The absence of this ion from a substrated saline medium is known to stimulate brain respiration(4). A series of experiments was performed, therefore, to determine whether its presence, by inhibiting brain slice respiration slightly, would unmask the effects of any activator present. The results of this series are presented in Table IV. The control QO_2 of 20.7 obtained in Krebs III solution containing calcium was found not to be significantly different from the brain-activated QO_2 of 21.0. The stimulation of brain slice respiration produced by a reduction in calcium ion can be seen by a comparison of the normal calcium control ($QO_2 = 20.7$) with the zero calcium control ($QO_2 = 23.2$).

Discussion. These results show that brain slices incubated in non-substrated medium can alter this medium so that subsequently incubated cortical tissue will consume more oxygen. The oxygen uptake stimulating effect is similar to that reported for rat sciatic nerve(1,2,3,5) and sheath(2,3) incubated in non-substrated media. The factor or factors released from the incubation of brain slices and responsible for increasing the QO_2 of brain tissue may not be identical to those released from sciatic nerve incubation. There is some evidence to suggest that the 2 "activating" media, *i.e.,* from sciatic nerve and from cerebral cortex slice incubation, are not entirely the same. Heller, Wolfe and Hesse (5) have reported that "activating" media containing glucose (10 mM) resulted in a higher oxygen uptake for sciatic nerve than did control media containing glucose. The studies reported here did not show a similar response either in the presence of 11.5 mM glucose (Table III) or in the presence of glucose plus other substrates (Table II). A lack of stimulation has been reported by Dellenback and Ringle(2,3) when rat sciatic nerve or its sheath is incubated in a substrated Krebs III medium. It is possible that the stimulated oxygen uptake in "activating" medium is due to the presence of some diffusible energy source. Preliminary studies (unpublished observations) suggest, however, that at least part of the increased QO_2 was caused by some factor other than a diffusible carbohydrate energy source. A further stimulation of cortical slice respiration in a substrated Krebs III medium was not observed either when calcium ions were added to the incubation medium or when the temperature of incubation was raised to 37.34°C. The presence of substrates in the incubation media seems to mask the stimulation in all conditions studied. This might be expected if one compares the amount of the stimulation produced by the activating factor with the enormous stimulation resulting simply from substrate addition.

It is not now possible to identify the factor or factors responsible for the increased oxygen uptake by brain slices in "activating" medium. Heller, Wolfe and Hesse(5) have reported that the nerve "activating" substance seems to be thiamine, since not only are its effects mimicked by added thiamine but also its activating effects are blocked by thiamine antagonists. The high levels of thiamine occurring in peripheral nerve(6) and the apparent importance of thiamine in normal nerve function(7) further suggest that thiamine might very well be involved in the "activating" phenomenon displayed by nerve. Thiamine content in brain, moreover, is even higher than in peripheral nerve(6).

Summary. Incubation of rat cortical slices for 2 hours in a non-substrated phosphate saline medium at room temperature, produces an "activating" medium which is capable of stimulating the oxygen uptake of freshly cut cortical slices when subsequently determined in it. The nature of the activating material is unknown. The presence of glucose (11.5 mM/l) or glucose plus additional substrates (Krebs III medium(4)) in the incubation medium masks the stimulation. Neither an increase in the temperature of incubation to

37.34°C, possibly allowing more "activating" material to diffuse into the medium, nor the presence of calcium in the incubation medium which would partially inhibit the respiration of brain tissue was sufficient to allow a stimulation to be observed in the presence of added substrates.

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Blood Non-Protein Sulfhydryl Levels in States of Acidosis.* (28886)

H. M. SMITH† AND U. D. REGISTER (Introduced by L. D. Seager)
Departments of Physiology and Biochemistry, School of Medicine, Loma Linda University, Loma Linda, Calif.

Though extensively studied, the relationship of sulfhydryl (SH) moieties to diabetes mellitus remains obscure. The finding of Illig (1), that blood glutathione (GSH) levels in human diabetes are inversely related to degree of ketosis rather than to glycemia, and of Nath(2), that intravenous administration of acetoacetate in rabbits causes a prompt decline in blood GSH levels implicate ketone bodies and an altered fat metabolism in the SH-diabetes relationship. This paper is concerned with the question: are these blood SH changes due to an altered acid-base state, or are they related to the ketone body level? If the latter is the case, is the reduction in SH level due to chemical combination of SH compounds with ketones? This paper reports studies on rats, rabbits, and human subjects during starvation, or on rats in metabolic acidosis and alkalosis, and by *in vitro* experiments.

Methods and materials. Protein sulfhydryl (PSH) and non-protein sulfhydryl (NPSH) concentrations were determined by the amperometric technique of Benesch and Benesch (3). Ethylenediaminetetraacetate (EDTA) was used to prevent heavy metal reaction with NPSH. Blood proteins were precipitated with 20% sulfosalicylic acid. Acetone, acetoacetic acid, and total acetone bodies in blood and aqueous solution were determined by the method of Thin and Robertson(4). Standard solutions of acetone covering the expected range of values for each experiment were simultaneously determined as a control. pH determinations were made on blood taken by heart puncture under ether anesthesia. The anesthesia apparently produced a respiratory acidosis in all normal rats. This artifact augmented any induced acidosis, and mitigated any induced alkalosis. If the normal control, though indicating an acidosis, was the standard of reference, the abnormal state was clearly definable. Nitrogen was determined by the micro-Kjeldahl, and glucose by the Somogyi-Nelson techniques. The hematocrit was determined by the Wintrobe method. Acidosis was induced in rats by feeding NH_4Cl; 0.5 g/kg/day for 5 days; alkalosis by feeding $NaHCO_3$, 1 g/kg/day for 5 days. The control rats were pair-fed.

Results. In vitro studies. To explore the chemical combination of SH and ketone bodies, GSH was incubated with acetone in

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† Present address: Schering Corporation, Bloomfield, New Jersey.