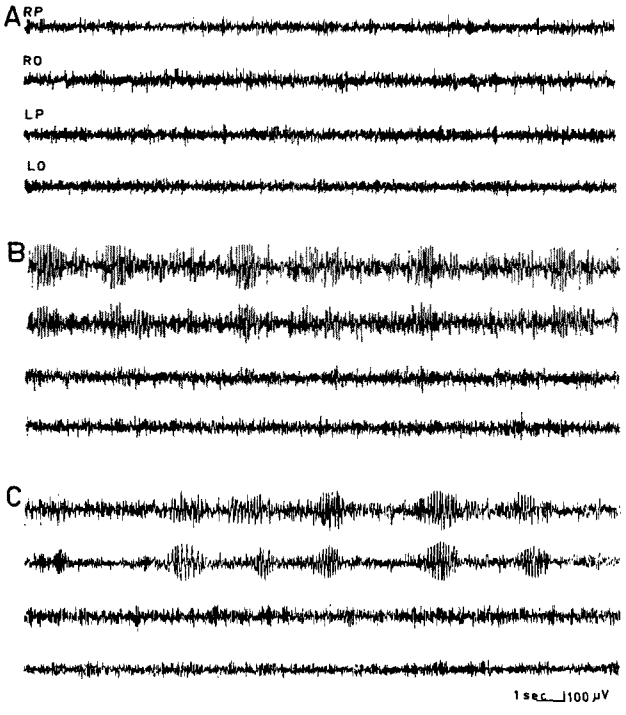

Effects of 5HTP on the EEG of the midpontine-pretrigeminal cat.

(A) EEG pattern symmetrically activated, 2 h after midpontine transection.

(B) Clear EEG asymmetry produced by 1 mg of thiopental injected into the right carotid artery, the controlateral artery being open: only the hemisphere on the side of the injection shows synchronization.

(C) The same EEG pattern produced by 20 mg of 5HTP injected as in (B).

(3) The EEG patterns of the midpontine pretrigeminal cat following mesencephalic hemisection have long periods of asymmetry; in fact the hemisphere overlying the section shows marked synchronization, while the other hemisphere is desynchronized[2]. Olfactory stimulation gives an arousal reaction. In this preparation, 5HT (1–4 μg) injected into the carotid artery – the controlateral artery being closed – frequently produced a clear arousal reaction, which ran independent of changes in the cerebral blood flow, at least when they were recorded at carotid level. In contrast, 5HTP (5–10 mg) not only brought about a symmetric synchronization, but it also frequently blocked the arousal reaction from olfactory or visual stimulation.

Our results are in part at variance with those obtained by other research workers (GANGLOFF and MONNIER[3]; CREPAX and INFANTELLINA[4]; MANTEGAZZINI[5]; ROTHBALLER[6]; MONNIER and TISSOT[7]; COSTA and RINALDI[8]).

A full discussion of our results and a tentative explanation of the discrepancies between our data and those of the above mentioned investigators will be given in the paper *in extenso*. It is evident, however, that differences in animal species, route of administration of the drugs, and experimental methods may be responsible for different results and hence for different interpretation of the physiological action of 5HT and 5HTP on the central nervous system.

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Laboratori Ricerche Farmitalia, Milano (Italia), November 5, 1959.

Riassunto

Il 5HTP, direttamente iniettato nel circolo cerebrale, produce un quadro elettroencefalografico di sonno, indipendente da modificazioni di flusso nel circolo cerebrale e da eventuali metaboliti che si formano al di fuori del cervello. La 5HT, iniettata nelle stesse condizioni, o è senza effetto o dà una reazione elettroencefalografica di risveglio.

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Glutaminase I Activity in Guinea Pig and Rabbit Liver and Kidney Tissues

Phosphate-activated glutaminase (Glutaminase I) has been demonstrated in rat and mouse brain, spleen, liver and kidney and in brain and spleen of rabbit and guinea pig; but in extracts of guinea pig, rabbit liver, kidney tissue, and of mitochondrial preparations, the phosphate activation of glutaminase activity has as yet not been demonstrated[1]. The object of the present report is to demonstrate glutaminase I activity in guinea pig and rabbit liver and kidney tissues.

Methods: Tissue extracts and mitochondrial preparations were made according to the procedures of ERRERA *et al.*[1], and the methods of SCHNEIDER[2] were followed when sucrose was employed for the preparation of mitochondria. The enzyme activity was determined by measuring the ammonia formed in presence of (5×10^{-3} *M*) L-glutamine (0·05 *M* Veronal Buffer pH 7·2 or 0·05 *M* Veronal Acetate Buffer pH 8·4). Incubations in Warburg's vessel at 37°C for 1 h, ammonia estimated by the method of BRAGANCA *et al.*[3]. Enzyme preparations equivalent to 50 mg of tissue (wet weight) for kidney and 100 mg for liver in all cases.

Results and Discussion: Glutaminase activity of tissue extracts and mitochondrial suspensions of guinea pig and rabbit liver and kidney, as shown in Table I, indicates that phosphate activation is most prominent at pH 7·2, especially with the mitochondrial system. The effect of DL-glutamic acid (Table I) on the enzyme activity in absence of any added phosphate showed a slight inhihtion with kidney tissues, while the liver enzyme was not found to be inhibited in accordance with KREBS[4]. However, in the presence of added phosphate, the inhibition of glutamic acid in all the tissue preparations was most significant. Varied concentration of phosphate showed (not shown in the Table) the phosphate-glutaminase activity curve to assume a flattened plateau after reaching the maximum of the optimal phosphate concentration of 0·2 *M* with no further increase in enzymatic activity.

[1] M. ERRERA and J. P. GREENSTEIN, J. biol. Chem. *178*, 495 (1949).
[2] W. C. SCHNEIDER, in *Manometric Techniques* (Ed. by W. W. UMBRIET, R. H. BURRIS, and J. F. STAUFER, Burgess Publishing Co., U.S.A., 1957), p. 188.
[3] B. M. BRAGANCA, J. H. QUASTEL, and R. SCHUCHER, Arch. Biochem. Biophys. *52*, 18 (1954).
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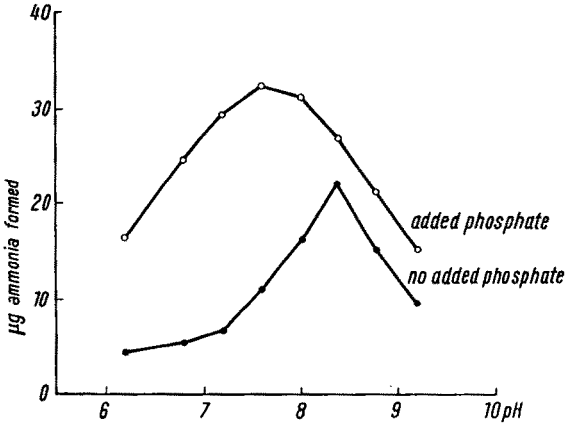
Table I

Glutaminase I activity of guinea pig and rabbit liver and kidney (a) tissue extracts and (b) mitochondrial preparations

Tissue Preparations	Additions	µg Ammonia formed per h at pH 7·2		pH 8·4	
		Preparation (a)	Preparations (b)	Preparation (a)	Preparation (b)
1. Guinea pig Liver	None	12·0	6·2	19·0	22·0
	PO_4	20·0	27·0	19·8	27·0
	DL-glutamate	11·0	6·0	19·0	22·0
	PO_4 + DL-glutamate	16·0	17·0	19·5	23·0
2. Guinea pig Kidney	None	9·0	9·0	18·0	26·5
	PO_4	26·5	28·0	25·0	35·5
	DL-glutamate	6·0	6·0	16·0	26·0
	PO_4 + DL-glutamate	16·0	12·0	15·0	29·0
3. Rabbit Liver	None	7·0	8·0	14·0	17·0
	PO_4	18·0	25·0	20·0	26·5
	DL-glutamate	7·0	8·0	13·0	16·0
	PO_4 + DL-glutamate	11·0	16·0	14·0	20·0
4. Rabbit Kidney	None	6·0	9·0	12·0	13·0
	PO_4	20·0	26·0	22.0	26·0
	DL-glutamate	4·0	7·0	9·0	10·0
	PO_4 + DL glutamate	8·0	18·0	16·0	20·0

0·2 *M* Phosphate and 0·05 *M* DL-glutamate were used. Other conditions are given in the text.

The pH activity curve of glutaminase of guinea pig liver mitochondria (Fig.) indicates that the optimum range for phosphate activation lies between pH 7·2–7·8, and the net effect of phosphate activation decreases as the pH is further raised.



pH-activity curve of Glutaminase I of guinea pig liver mitochondria

It is evident from Table II that the glutaminase of guinea pig liver mitochondria, prepared and suspended in hypertonic sucrose medium, is not activated even at pH 7·2, with the usual phosphate concentration (0·2 *M*). However, a higher concentration of phosphate (0·5 *M*) is required to produce significant activation of glutaminase, which suggests that the effect of phosphate is in some way counteracted by hypertonic sucrose medium.

Table II

Effect of tonicity of medium on Glutaminase I activity of guinea pig liver mitochondria (100 mg wet weight) at pH 7·2

System	µg Ammonia formed per h	
	Without PO_4	With PO_4 (0·2 *M*)
1. Suspension prepared according to ERRERA *et al.*[1]	6·2	27·0
2. 0·3 *M* Sucrose suspension	15·0	27·0
3. 0·88 *M* Sucrose suspension . . .	16·5	17·0
4. 0·88 *M* Sucrose suspension + 0·5 *M* PO_4 (in place of 0·2 *M*) .	16·5	24·5

Although it is well known that phosphate, glutamic acid, higher pH, or hypertonic sucrose produce profound influences on the structural morphology and function of the mitochondrion, it cannot definitely be said at this stage whether the observed effects of these factors on the glutaminase activity and on the mitochondrial morphology are correlated phenomena.

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Indian Institute for Biochemistry and Experimental Medicine, Calcutta (India), October 12, 1959.

Zusammenfassung

Aktive Glutaminase I wurde in Meerschweinchen- und Kaninchenleber, in Nierengewebsextrakten und in Mitochondrienpräparaten der erwähnten Organe bei pH 7,2 nachgewiesen. DL-Glutaminsäure bewirkte eine ausgesprochene Hemmung des Enzyms nach Zusatz von Phosphat. In hypertonischer Sukrosenährlösung lässt sich eine Aktivierung durch Phosphatzusatz gewöhnlich nicht nachweisen, selbst nicht beim pH-Optimum 7,2, es sei denn bei sehr hohen Phosphatkonzentrationen.

PRO EXPERIMENTIS

A Method for Avoidance of Electrostatic Flashing in Preparing Autoradiographs with Stripping Film

In unusually dry climates static electricity complicates the use of stripping film for autoradiography. Peeling the film from its base, however gently, tends to produce bright flashes which raise the background to intolerable levels unless special precautions are taken to humidify the film or the atmosphere to the proper degree. (If it is too damp, the film cannot be separated from the plate.)

Accurately controlled hydration can be achieved by immersion of the film plate in aqueous alcohol. The following procedure has been found effective: autoradiographic plates (Kodak AR 10) are immersed briefly (15–30 s) in 100% ethanol and then transferred to a shallow dish of 95% ethanol. While submerged, the film is scored with a razor blade into strips of requisite size, the pieces are peeled off and floated on water emulsion-side down in the usual way. The film strips flatten rapidly and can be mounted on slides within a few seconds. The water on which the film is floated must be changed at frequent intervals, because alcohol that is carried over alters the