sostanze, si avvicina al sangue mentre differisce notevolmente dal liquido cerebrospinale.

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Summary

The following aminoacids have been detected in the perilymph from horses by filter paper chromatography: aspartic acid, glutamic acid, phosphocolamine, glycine, serine, taurine, threonine, alanine, glutamine, lysine, arginine, γ-aminobutyric acid, proline, valine, leucine, tyrosine, phenylalanine, cystine.

A comparative study of chromatograms obtained from blood, perilymph and liquor showed that the perilymph is much more similar to the blood than to the cerebrospinal fluid either for qualitative and for quantitative content in aminoacids.

The Formation of Flavin-Adenine Dinucleotide in Liver Cells Prepared from Normal and Alloxan Diabetic Rats

In a previous paper[1] we have shown that less FAD[2] is found in the liver of alloxan diabetic rats than in normal animals and that FMN or Rib + ATP administration can restore FAD to the normal level.

In the present paper, experiments have been carried out with the object of reproducing *in vitro* the above-mentioned results.

Attempts have previously been made to find the optimum tissue preparation which could convert FMN to FAD.

In experiments with liver homogenates under different conditions of incubation, no formation of FAD has been observed.

Further experiments with preparations of liver cells led us to reproduce *in vitro* the synthesis of FAD from FMN, thus confirming the above-mentioned observation of TRUFANOV[3], by which the maintenance of cell structure is essential for this reaction. The cells from the liver of both normal and diabetic rats have been prepared in the following manner[4]: immediately after removal from the animals, the liver was perfused with an isotonic solution of NaCl in order to free it completely from blood. The liver was then cut into small pieces and quickly homogenized in 0·16 *M* phosphate buffer at pH 6·9 in a Potter-Elvehjem homogenizer, employing a pestle loosely fitting into a cylinder. The suspension so obtained was passed through a nylon filter to remove the stroma elements which are retained by it.

The suspension passed through the filter was centrifuged at 400 × g for 10 min; the sediment was resuspended in the same buffer and centrifuged at 1500 × g for 15 min. The same operation was then twice repeated.

The sediment so obtained, when observed under a microscope, contained mainly liver cells, very few blood corpuscles and Kupfer cells.

All the operations described were carried out at 5°C.

The sediment was suspended in phosphate buffer 0·16 *M* at pH 7·5 and this suspension was used for the incubation. In each experiment the suspension of cells was divided into 5 fractions. One was used for the determination of the dry weight of the cells maintained at 90°C until constant weight was obtained.

The remaining fractions were incubated under identical conditions: one without any addition, the others with the addition of FMN, FMN + ATP, FMN + ATP + insulin respectively.

After incubation the suspension was homogenized in a very close-fitting homogenizer in order to disrupt the cells, and FAD was determined according to the method of BESSEY *et al.*[1].

The incubation and all the manipulations were performed in a dark room.

FAD content of incubated liver cells

Addition	FAD μg/g dry cells from normal rats	FAD μg/g dry cells from diabetic rats
No	49·5 ± 4·7	35·2 ± 3·1
FMN	60·1 ± 5·8	34·1 ± 3·5
FMN + ATP	69·5 ± 6·1	42·3 ± 4·0
FMN + ATP + insulin	70·3 ± 6·4	44·5 ± 4·1

The values represent the mean ± standard error of the mean obtained from 20 normal and 20 diabetic rats.

The incubation mixture contained 30 mg of liver cells (dry weight); 30 μM $MgCl_2$ and 56 μM of phosphate buffer at pH 7·5 in a volume of 4 ml. The substances were added to the mixture in the following amounts: 8 μM ATP · 4 Na; 0·1 μM FMN, 4 U.I. insulin powder (Lilly and Co.). Incubation for 3 h at 38° C.

The results shown in the Table indicated that the FAD content of liver cells of alloxan diabetic rats is lower than that of liver cells in normal rats.

The FAD of cells of diabetic rats is not affected by addition to the incubation mixture of FMN. When FMN is added together with ATP, a significant increase is obtained. The addition of FMN together with ATP and insulin is even more effective in increasing the formation of FAD by the cells of diabetic rats. In cells of normal animals, FAD is, on the other hand, increased by the addition of FMN, and the addition of FMN + ATP is even more effective to increase the yield of FAD. Insulin does not increase the effect of FMN + ATP on the normal cells.

These results are in agreement with those previously obtained *in vivo*, except for the effect of FMN on FAD formation by diabetic liver. While *in vivo* FMN enhances the formation of FAD, *in vitro* it fails to produce this effect unless ATP is added at the same time. In any case, both content and formation of FAD in alloxan diabetic rats is lower than in normal animals.

The maintenance of cell structure seems to be essential in promoting *in vitro* the formation of FAD from FMN.

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Institute of Biological Chemistry, University of Rome, Italy, May 23, 1955.

[1] F. NAVAZIO and N. SILIPRANDI, Exper. *11*, 280 (1955).

[2] ADP = Adenosindiphosphate; ATP = Adenosintriphosphate; PP = Pyrophosphate; FMN = Flavinmononucleotide or Riboflavinphosphate; Rib = Riboflavin; FAD = Flavin-adenindinucleotide.

[3] A. V. TRUFANOV, Biokhimiya *6*, 301 (1941); *7*, 188 (1942); Chem. Abstr. *35*, 7499 (1941); *38*, 131 (1944).

[4] We are greatly indebted to Prof. M. BRACCO of the Biochemistry Department of "Villaggio Sanatoriale di Sondalo" for having suggested this procedure.

[1] O. A. BESSEY, O. H. LOWRY, and R. H. LOVE, J. biol. Chem. *180*, 755 (1949).

Riassunto

Viene studiata la formazione del flavin-adenin dinucleotide (FAD) da parte di cellule di fegato di animali normali e diabetici per allossana a partire da flavin mononucleotide (FMN).

Le cellule dell'animale normale possono sintetizzare il FAD direttamente dal FMN, mentre le cellule dell'animale diabetico possono effettuare questa sintesi dal FMN, solo se viene aggiunto anche ATP.

Tale sintesi è possibile solo se viene conservata l'integrità cellulare. Viene descritto un procedimento per la preparazione delle cellule isolate di fegato.

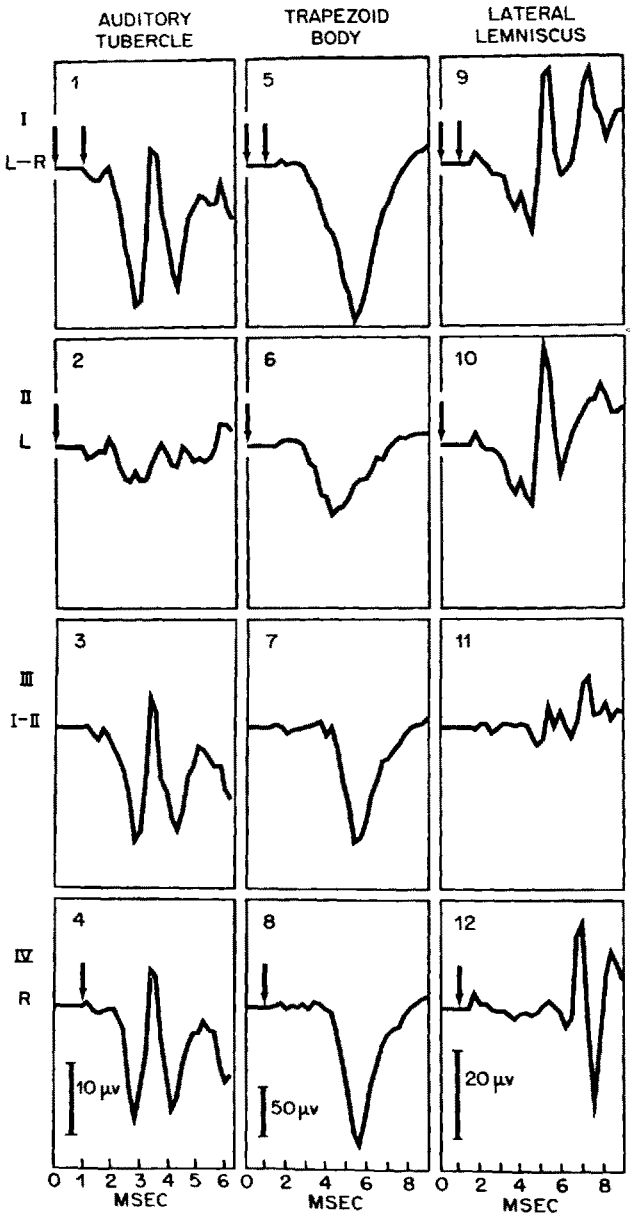
Binaural Interaction in the Medulla of the Cat[1]

The ability to locate the source of a sound in space is considered to depend upon the interaction of neural impulses originating at the two ears. Binaural interaction has been shown in the responses of the auditory cortex[2], but it is not yet known at what level of the auditory system interaction first occurs. JEFFRESS[3] suggested that the inferior colliculi are probably the site of the first binaural interaction, and interaction has recently been demonstrated at this level[4]. There does not seem to be any clear evidence in the literature of binaural interaction below the colliculi. KEMP and ROBINSON[5] interpreted their experiments at the lateral lemniscus as showing no binaural interaction at that level. A preliminary report indicated that binaural interaction might occur in the cochlea itself, impulses being transmitted over a cochleo-cochlear tract[6], but later work has not confirmed this[7].

We have sought to determine whether binaural interaction occurs at the auditory tubercle, the trapezoid body, the superior olivary complex, and the lateral lemniscus. We have not found evidence of interaction at the tubercle, but we have found significant evidence of interaction at the 3 other stations.

Methods. If the two ears are represented at a particular region by independent populations of neural units, then the response to stimulation of both ears should equal the sum of the responses to monaural stimulation of the separate ears. If a significant deviation from equality is found, then we conclude that interaction between the responses of the two ears has occurred and that the ears are not represented by independent populations.

In our experiments, the ears were stimulated independently of each other with brief click stimuli. The intensity of the clicks was usually set between 30 and 40 db above threshold, so that crosshearing was avoided. The amplified electrophysiological responses were recorded photographically from the face of a cathode-ray oscilloscope. To record from the auditory tubercle, a gross electrode was placed on the dorsal surface of the tubercle. To record from the other stations, a needle, insulated except at the tip, was inserted into the medulla. The brains were preserved for histological determination of the exact sites of recording; the histology has not yet been done, and the positions of the needle tip have been determined provisionally from external measurements.



Electrophysiological responses to click stimuli recorded at the auditory tubercle, the trapezoid body and the lateral lemniscus. Each graph is the average of 8 to 10 successive responses. Each of these electrode locations was taken from a different animal. The tubercle and lemniscus locations were on the right side; the trapezoid location was in the midline.

The subjects were ten adult cats, anesthetized with Nembutal or with Dial in urethane. Seven animals were used in experiments at the tubercle; five, including two of the former group, were used in experiments at the other stations.

[1] This research was aided by a grant from the National Science Foundation.

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