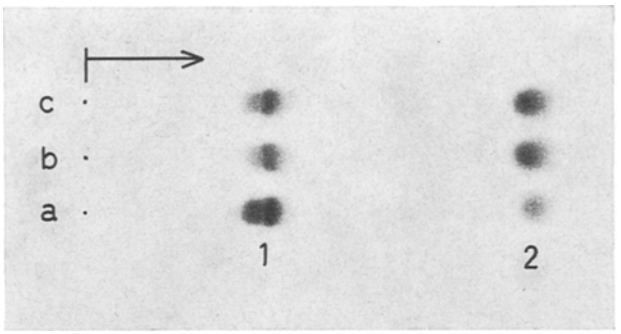
Gamma-Aminobutyric Acid (GABA) in Fish Erythrocytes

Considerable attention has been paid to GABA in nervous tissues[1], which is the only vertebrate tissue so far where this substance has been found[2]. It has been suggested that GABA plays an important physiological role as an inhibitory transmitter in the vertebrate central nervous system, and at the crustacean neuromuscular junction[1]. Other physiological functions have also been suggested[3].

In the present investigation it has been found that GABA, together with taurin, are the major constituents of the intracellular extractable amino acid pool in erythrocytes from flounder (*Pleuronectes flesus*), plaice (*P. platessa*), and dab (*P. limanda*) (Figure).

Protein-free alcohol extracts (80% ethyl alcohol) were prepared from hemolyzed cells which were collected from heparinized blood, and washed in isotonic saline. Thin-layer chromatography was carried out[4,5] using either isopropanol/formic acid/water (40/2/10, v/v), or n-propanol/ammonia 0.2*N* (3/1, v/v) as solvent for the development in one-dimensional chromatography. For two-dimensional chromatography, the first solvent mentioned was used for the first dimension and phenol/water (75/25, w/w, gas-phase equilibrated with 3% NH_4OH), or n-butanol/acetone/diethylamine/water (70/70/14/35, v/v) for the second dimension.

In every chromatogram tested, one and the same ninhydrin-positive spot behaved in an identical manner to a standard containing GABA. The other dominating ninhydrin-positive substance was taurin (Figure) which, together with GABA, makes up most of the intracellular pool of extractable amino acid-like substances (about 45 m*M*/l cells, measured as taurin equivalents[6]). However, traces of β-alanine, alanine, glutamic acid, aspartic acid, glycine and 3–4 unidentified spots could also be detected.



Thin-layer chromatogram (solvent for the development: iso-propanol/formic acid/water, 40/2/10) of alcohol extracts from flounder (a), plaice (b), and dab (c) erythrocytes. The ninhydrin-positive spots 1 represents taurin, the spots 2 represents γ-aminobutyric acid.

Ion-exchange chromatography (Bio-Cal Instruments, sulphosalicylic acid protein-free extracts) showed that the intracellular GABA pool was only half the size of the taurin pool in flounder erythrocytes.

The total pool of extractable amino acid-like substances in erythrocytes from flounder[7], plaice and dab[8] has been shown to vary in concentration concomitant with a variation in plasma osmolality, initiated by a change in the salinity of the surrounding water. In vitro experiments on isolated flounder blood samples have shown that when the erythrocytes were exposed to a diluted plasma, an osmotic swelling of the cells occurred; but this stage was followed by cell volume adjustment back to the original volume. During this process the cellular content of protein-free ninhydrin-positive substances decreased concomitant with an increase in plasma content of similar substances. The phenomenon could not be explained as a temporary lysis of the cells[6].

It has now been shown that both GABA and taurin leave the flounder erythrocytes under similar experimental conditions, and the same substances are in vivo reduced in concentration in flounder, plaice and dab erythrocytes under conditions which lead to a decrease in plasma osmolality. From the above findings it seems that GABA as well as taurin in the 3 fish species erythrocytes play a role as osmotically active solutes in the cell volume regulation under conditions which lead to an alteration in plasma osmolality.

Zusammenfassung. Nachweis, dass γ-Aminobuttersäure und Taurin bei den 3 verwandten Plattfischarten Flunder, Scholle und Kliesche die wichtigsten intraerythrozytären freien Aminosäuren sind.

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[9] This work was supported by the Nansen Foundation (Norway) and the Norwegian Research Council for Science and the Humanities (NAVF).

Cardiac Muscle and Liver Ribosomes of the Rat: the Influence of Laparotomy and Laparotomy Combined with Adrenalectomy

Surgical trauma (laparotomy) induces in rats, in the immediate post-operative period, an increase in the incorporation ability of ribosomal preparations from both cardiac muscle and liver in an in vitro system (Kölbel[1], Kölbel et al.[2]). The increase is due to a temporary increase in the formation of polysomes. As the glucocorticoids are known to be one of the possible regulatory factors in the metabolism of RNA (Garren et al.[3]), we became interested in the reaction of cardiac muscle and liver ribosomes to surgical trauma in adrenalectomized animals.

Material and methods. In 15 male rats (Wistar strain, body weight 200–210 g), bilateral adrenalectomy was performed per laparotomiam under ether anaesthesia. The animals were decapitated on the 5th post-operative day.

Yields of ribosomes from cardiac muscle and from liver of control animals and of animals on the 5th day following bilateral adrenalectomy

	(1) Ribosomal yield (*mg of ribosomes per 1 g wet tissue wt.*)	(2) Amount of phenylalanine-^{14}C (cpm) incorporated (a) Without poly U	(b) With poly U	(3) Incorporation capacity for phenylalanine-^{14}C (cpm per 1 g tissue)
Cardiac muscle ribosomes (control)	0.313	280	1400	408
Cardiac muscle ribosomes (5th day following adrenalectomy)	0.180	430	1710	387
Liver ribosomes (control)	2.70	513	2520	6926
Liver ribosomes (5th day following adrenalectomy)	0.52	875	3150	2272

Amounts of phenylalanine-^{14}C incorporated by cardiac muscle and liver ribosomes in an in vitro system without the addition of external mRNA (2a and 3) and after the addition of 200 µg of poly U (2b). Conditions of the incubation and composition of the incubation media as described under material and methods.

Hearts were immediately removed, weighed on a torsion balance and chilled in ice-cold medium A (0.01 *M* $MgCl_2$, 0.1 *M* KCl, 0.005 *M* β-mercaptoethanol, 0.05 *M* *Tris*-buffer, pH 7.6). For the isolation of ribosomes, the hearts of all animals were pooled. Similarly were also pooled corresponding parts of the left upper liver lobes. After pooling, the hearts were homogenized in a Warringblendor and in a Potter-Elvehjelm, teflon pestle, motor-driven homogenizers. The ribosomes were prepared from the tissue homogenate treated with deoxycholate by means of differential centrifugation as described by RAMPERSAD et al.[4]. The ribosomal pellets obtained from the post-mitochondrial supernatant by centrifugation at 105,000 × *g* at 5 °C for 75 min were carefully washed and stored in a deep-freezer at − 70 °C. The liver ribosomes, as well as the ribosomes from tissues of control animals were prepared identically as described above.

The concentration of ribosomes was determined after careful rehomogenization of the pellets from the absorbance at 260 nm using the absorbance coefficient $E^{1\%}_{1cm} = 125$ (RAMPERSAD et al.[4]). The ratios of absorbances at 260 and 235 nm for cardiac muscle and liver ribosomes were 1.22 and 1.48, respectively.

The ability of the ribosomal preparations to incorporate phenylalanine-^{14}C was measured in an in vitro system containing in a final volume of 1.0 ml 50 µ*M* *Tris*-HCl, pH 7.8, 10 µ*M* β-mercaptoethanole, 80 µ*M* KCl, 15 µ*M* $MgCl_2$, 1 µ*M* ATP, 0.4 µ*M* GTP, 10 µ*M* phosphoenol-pyruvic acid, 0.1 mg pyruvic kinase, 1 µC L-phenylalanine-^{14}C (specific activity 25–30 mC/m*M*), 0.2 mg of ribosomes, 2.0 mg of protein of the pH_5 fraction from normal rat liver. Samples were incubated at 37 °C for 30 min, incubation was terminated by chilling samples on ice. 3 aliquots of 0.1 ml each from each sample were transferred to 3 discs of Whatmann 3-MM filter paper and processed as described by MANS and NOVELLI[5]. The air-dried paper discs were transferred to counting vials, 10 ml of scintillation mixture SLT-31 (Spolana Neratovice, Czechoslovakia) were added to each sample and the radioactivity was measured on the Mark I Nuclear Chicago Spectrometer. As no quenching was observed, the results were corrected for zero time only. The incorporation capacity of amounts of cardiac muscle and liver ribosomes equivalent to 1 g of corresponding tissue was calculated from the ribosomal yield and from amounts of phenylalanine-^{14}C incorporated by 200 µg of the ribosomal suspension. The pH_5 fraction necessary for the incorporation of the labelled amino acid was prepared according to MOLDAVE[6].

Results and discussion. Bilateral adrenalectomy performed per laparotomiam decreased, on the 5th post-operative day, the yields of ribosomes from both the heart muscle and the liver (Table, 1). In an in vitro system containing no external mRNA, the cardiac muscle ribosomes prepared from hearts of adrenalectomized animals were able to incorporate 57% more of the phenylalanine-^{14}C, and the liver ribosomes 70% more phenylalanine-^{14}C than ribosomes from corresponding tissues of control animals (Table, 2a). The decreased reactibility of ribosomal preparations from tissues of adrenalectomized animals after the addition of polyuridylic acid testifies to higher saturation of the preparations with endogenous mRNA (Table, 2b). Owing to the low ribosomal yields, the incorporation capacities of amounts of ribosomes equivalent to 1 g of cardiac muscle and the liver, were lower than the control values (Table, 3).

The decrease in ribosomal yields from both liver and heart muscle is the most striking change in tissues of adrenalectomized animals. Among the reasons for this change could be both the lower rate of formation of ribosomes and their subunits and/or faster degradation of these particles. None of these possibilities was directly tested by our experiments. With respect to the stimulatory effect of glucocorticoids on RNA-polymerase (ELSON[7]), the first of the 2 possibilities seems to be a more probable explanation of our observations.

Zusammenfassung. Nach doppelseitiger Adrenalektomie nimmt der Ribosomengehalt in Leber und Herzmuskel ab. Die Fähigkeit dieser Ribosomen zur Inkorporation von Phenylalanin wird nachgewiesen.

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