of LC_1:LC_2 in C-myosin from control rats amounts to 124:100, a relation of 146:100 results in the case of the C-myosin of rats conditioned by swimming (Figure).

These results are consistent with those of OGANESSYAN et al.[3]. Their results indicated that the alterations in enzymatic activity of the cardiac contractile proteins in dogs following work overload (experimentally-induced aortic stenosis) were associated with concomitant local changes in HMM-region of myosin. In this molecule-region (HMM), light chains are associated with the myosin core[9].

The changes in the specific ATPase activity and pattern of light chains of myosin associated with the alterations of physiological state or with the contractile capability of respective muscle, can be explained on the basis of isoenzymes. It is evident that there are differences between individual myosin species which originate in the different muscle types. Moreover, stoichometry of essential light chains in myosin preparations indicates that in all myosin preparations of fast, slow and cardiac muscle, there may be several different molecule types present, at least with respect to light chains combination of myosin molecules[10].

Moreover, according to studies of SARKAR[11], the relative contribution of essential light chains to ATPase activities of myosin is unequal. In F-myosin, LC_1 contributes significantly more to this activity (60–70%) than LC_3 (30–40%).

Hence it is possible that the changes in light chains pattern and specific ATPase activity of a given myosin preparation, as found by us in foregoing experiments, may be based on variations in the combination of particular isoenzymes in the given myosin preparation.

The variation in the relation of the particular subunits of myosin to each other may originate in the kind of biosynthesis and degradation of myosin molecule. With respect to the formation as well as the degradation, the myosin molecule is a dynamic protein. It is neither synthetized nor degraded as a functional unit. As shown in several studies, its single subunits are produced[12] and degraded[13] independently and at heterogeneous rates[14]. There is consequently no one-to-one coordination of the synthesis and degradation of its individual subunits. The synthesis of myosin is significantly accelerated in response to work overload[15]. As a consequence of severe physical exercise, hemodynamic load of the heart is increased and therefore the production of myosin-protein is significantly accelerated. This accelerated production of myosin along with a different speed of synthesis and degradation of the individual components leads to the changes in the relations of light chains to each other and accordingly to changes in the specific ATPase activity. Hence, the control-mechanism to synthese the individual subunits and consequently the particular isoenzymes could together to be regulative mechanism to produce a myosin of the specific ATPase activity appropriate to the activity pattern of respective muscle.

Zusammenfassung. Im Myokard schwimmtrainierter Ratten fand sich eine signifikante Steigerung der spezifischen ATPase-Aktivität von Aktomyosin und Myosin, die von einer Änderung in der Relation der leichten Ketten des Myosinmoleküls begleitet war.

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Neurochemical Correlates of Alloxan Diabetes: I. Ribonucleic Acid and Protein Levels of Amphibian Brain

Various lines of evidence have implicated insulin in the modification of CNS activity and function[1,2]. Studies in the rat involving administration of alloxan have demonstrated that total phospholipid contents of the brain increase markedly in diabetic condition[3]. FIELD and MASSACHUSETTS[4] reported a depression of the activity of the acetic thiokinase enzyme in diabetic nerves. However, recent studies[5] indicate that the spontaneous electrical activity of the brain, the cerebral glucose consumption and the rate of efflux of K^+ from the brain of rats are not affected by insulin. In view of such a discrepancy in the information available on the CNS of diabetic vertebrates, the present study was proposed. The present investigation presents information about the changes occurring in the levels of RNA and proteins in the different regions of the brain of normal and alloxan-diabetic frogs.

Frogs, *Rana cyanophlictis*, of medium size (22–28 g) were used for the study. They were purchased from local dealers and stocked in the laboratory aquaria at 25 ± 2 °C. These animals were force-fed once in 3 days on the leg muscle of frog.

Diabetes was induced by i.m. injections of freshly prepared aqueous solution of alloxan monohydrate (40 mg/kg body weight)[6]. Animals were analyzed 48 and 96 h after alloxanization. They were killed by decapitation and the brains were quickly removed and washed in ice-cold saline (to remove the adhering blood). The fore, mid and hind brain regions were separated with sterilized fine bent forceps and scalpel, weighed in an electrical balance in amphibian Ringer[7] and immediately extracted with distilled water. Proteins from the aqueous extract were

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precipitated by trichloro-acetic acid (BDH). The precipitated proteins were estimated by the micro-biuret method[8].

RNA was extracted by the method of SCHMIDT-THANHAUSSER-SCHNEIDER[9] and estimated by orcinol color reaction following the colorimetric procedure described by GLICK[10]. The levels of glucose in the blood (collected by cardiac puncture) were determined colorimetrically[11].

Table I. Changes in glucose levels of blood in the frog, *R. cyanophlictis*, as a function of alloxan-diabetes

Time after alloxan administration	Body wt. (g)	Brain wt. (mg)	Blood glucose (mg/100 ml)
Controls (normal frogs)	25.2 ± 2.4	78 ± 12	32.5 ± 2.3
48 h diabetic	21.2 ± 1.1	74 ± 5	50.6 ± 4.2
96 h diabetic	22.1 ± 1.5	71 ± 6	69.2 ± 4.1

Each value represents mean ± SD of 6 observations.

Table II. Protein concentrations in different regions of the brain of normal (controls) and alloxan-diabetic frogs

	Proteins (mg/g wet weight)		
	Controls	Diabetic (48 h)	Diabetic (96 h)
Fore-brain	80 ± 16.3	176.7 ± 10.5	123.3 ± 27.6
Mid-brain	100 ± 6.7	183.7 ± 12.5	120.0 ± 61.7
Hind-brain	106 ± 5.5	195.0 ± 8.6	160.0 ± 8.1
Change (%) and level of Significance			
Fore-brain		+120.7 ± 8.9 ($p > 0.001$)	+54.1 ± 2.4 ($p > 0.01$)
Mid-brain		+83.75 ± 2.2 (NS)	+20.0 ± 3.9 ($p > 0.01$)
Hind-brain		+82.8 ± 1.7 (NS)	+49.91 ± 9.6 ($p > 0.001$)

Signs + and − indicate increase and decrease in protein contents over controls respectively. NS, not significant. Each value represents mean ± SD of 6 observations.

Table III. Changes occurring in the levels of RNA in the fore, mid and hind brain regions of controls (normal) and experimental (alloxan-diabetic) frog, *R. cyanophlictis*

	RNA (µg/mg wet wt.)		
	Controls	Diabetic (48 h)	Diabetic (96 h)
Fore-brain	1.2 ± 0.13	1.3 ± 0.15	1.2 ± 0.11
Mid-brain	2.2 ± 0.40	3.2 ± 0.50	2.8 ± 0.21
Hind-brain	0.9 ± 0.17	1.2 ± 0.14	1.1 ± 0.10
Change (%) and level of significance			
Fore-brain		+12.3 ± 1.06 ($p > 0.06$)	No change
Mid-brain		+23.4 ± 1.8 ($p > 0.05$)	+12.7 ± 1.4 ($p > 0.05$)
Hind-brain		+27.2 ± 2.8 ($p > 0.05$)	+12.2 ± 1.3 ($p > 0.05$)

Each value represents mean ± SD of 6 observations.

The results of the present study are presented in Tables I–III. It is evident that the weight of the animal and brain exhibited considerable decrease as a function of the duration of the disease (Table I). The blood-sugar levels demonstrated 55–60% and 93–100% elevation as a function of disease-duration (Table I).

In general, the protein content increased in the fore, mid and hind brain regions on in vivo administration of alloxan (Table II). Paralleling the increase in proteins, RNA levels also increased considerably on alloxanization (Table III). Further, marked differences in the protein and RNA contents of the fore, mid and hind brain regions were noted. For instance, the protein content of the fore-brain of the control group is less compared to the mid and hind brain (contents) (Table II). On the other hand, mid-brain has more RNA in comparison with the other two regions. A similar trend is exhibited in the experimental animals also. However a remarkable feature was that the fore-brain demonstrated least response for changes in RNA and highest for proteins in the diabetic state. Maximum response for changes in RNA levels was exhibited by the hind-brain on inducing alloxan-diabetes (Tables II and III). Such a differential response of the different regions of the brain may be related to the differences in the functional status of the region concerned.

The general increase in protein levels in the fore, mid and hind brain of diabetic frogs appears to be the direct consequence of insulin deficiency / absence caused by alloxan. A similar effect of insulin deficiency in increasing the incorporation of amino acids into protein by kidney ribosomes was shown earlier[12]. As indicated by these authors, such an increase may be due to a change in protein synthesis. Considerable increase in RNA levels observed in the present investigation also points to augmentation in the activity of the protein synthetic machinery in diabetic animals.

However, it is also possible that alloxan is responsible for the protein changes in the brain rather than the diabetic state. This is supported by the fact that the protein levels were higher 48 h after alloxan administration than after 96 h.

Summary. The levels of RNA and protein were higher in the brain of alloxan-administered frogs. It is possible that alloxan is responsible for the protein changes in the brain as the protein levels were higher 48 h after alloxan injection than after 96 h.

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