teleosts[2,8]. The greater fall in the 'insoluble' protein fraction, which consists largely of myofibrillar and connective tissue proteins, in the white muscle reflects the noticeable atrophy of these fibres during starvation[2,8]. In contrast to the white muscle little degeneration of the red muscle myofibrils was observed at the ultrastructural level in carp starving for 16 weeks[8]. The marked loss of low molecular weight proteins and decrease in RNA concentration in the red muscle probably parallels the considerable degeneration of mitochondria in this muscle during starvation[8]. The changes in RNA and DNA levels found in the plaice would seem to be correlated with the observed loss of euchromatin material from the nuclei of both red and white fibres of the carp[8], but whether this alone could account for the reductions is uncertain.

The present study indicates that the white muscle contractile proteins are preferentially utilized by the fish during starvation. There would, therefore, appear to be a differential response by the two principle muscle types of teleosts to inanition. It seems possible that severe changes in the nutritional state of a fish might influence the division of labour between the myotomal muscles. Such temporal changes are already thought to occur with respect to seasonal changes and migrations[9,10].

Zusammenfassung. Die Wirkung von experimentellem Hunger auf rote und weisse Muskeln der Scholle, *Pleuronectes platessa*, wurde untersucht. In roten wie in weissen Muskeln wurde eine starke Verminderung von Protein und Glycogen gefunden, während der RNS- und DNS-Gehalt in beiden Muskeln herabgesetzt war.

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10 January 1974.

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Free Amino Acids of *Hevea brasiliensis* Latex

Very little information is available on the free amino acids of *Hevea* latex. Former investigations[1-4] showed a very limited number of these compounds present in latex. In the more recent works of SOEI[5] and of D'AUZAC and PUJARNISCLE[6,7], this number has been extended to include most of the classic amino acids. However, the evaluation of the respective quantities of each compound was based on paper chromatography and could therefore only be considered as approximative. The only quantitative results were those concerning the total amino acid content of latex[7,8] and the sum of aspartic and glutamic acids[7]. In the present work, the amino acid composition of both cytoplasmic and lutoidic serums were investigated.

Materials and methods. Two batches of trees of clone PR107 were chosen on experimental plots of the I.R.C.A. (Institut de Recherches sur le Caoutchouc en Afrique), in Bimbresso, Ivory Coast. The trees were tapped, on full spiral, twice a week (d/3, d/4). The latex was allowed to run for 1 min in order to eliminate the fraction containing the organelles which had suffered from the traumatic effects of tapping. A 35 ml subsequent fraction was collected in a tube immersed in ice. Half-an-hour later the latex samples were centrifuged for 30 min at 40,000 rpm (Spinco, rotor 50 Ti) at 0 °C. The tubes were pierced, and the clear cytoplasmic serum was withdrawn. The upper part of the tube was cut and discarded with the rubber. The pellet consisting mainly of lutoid particles was recovered and resuspended in water to which ethanol was immediately added. Ethanol was also added to the cytoplasmic serum to a final concentration of 85%. After breaking of the lutoides and precipitation of the proteins of the 2 fractions (lutoidic and cytoplasmic), the ethanol extracts were centrifuged. All operations were carried out at 2–4 °C.

Free amino acids of the 2 fractions were determined quantitatively with a Technicon Autoanalyzer using the one-column technique and an elution system composed of 3 buffers: pH 2.875, 3.8 and 5.0 forming a continuous gradient[9,10]. The temperature of the column was maintained at 60 °C. Under these conditions the 2 amides (glutamine and asparagine) and threonine run together. As shown by paper chromatography, glutamine and threonine are the ever present constituents of both cytoplasmic and lutoidic serums of *Hevea* latex, whereas asparagine is virtually absent. In order to evaluate each of these 2 compounds separately, the aliquots were hydrolyzed in 1 *N* HCl at 40 °C for 24 h and then chromatographed. Threonine was measured directly. Glutamine evaluation was based on the increase of glutamic acid or on the decrease of the 'threonine' peak.

Results and discussion. The determinations of the free amino acids have been carried out on 16 latex samples coming from 12 trees, taken during the period from July to October 1972. The data in Table I represent the average values of these determinations.

The free amino acid content varies, of course, between the samples taken at a given moment from different trees, as well as the samples taken from the same tree at

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Table I. Free amino acids and related ninhydrin-reactive compounds in *Hevea brasiliensis* latex, clone PR 107 (Means of 16 analysis)

Compounds	µmoles per 100 ml latex		Serum C[a]		Serum L[b]		Serum L[c]
	Serum C	Serum L	µmoles per ml	%	µmoles per ml	%	Serum C
Cysteic acid	5.2	1.0	0.10	0.3	0.08	0.3	0.8
Taurine	10.7	1.7	0.20	0.7	0.14	0.4	0.7
X_1	trace	1.9	trace	—	0.16	0.5	—
X_2	1.5	1.8	0.03	0.1	0.15	0.5	5.0
X_3	2.0	1.7	0.04	0.1	0.14	0.4	3.5
Aspartic acid	298.3	22.4	5.74	19.0	1.87	6.0	0.3
Threonine	34.0	6.3	0.65	2.1	0.52	1.7	0.8
Serine	26.5	4.2	0.51	1.7	0.35	1.1	0.7
Glutamine	279.5	33.8	5.38	17.8	2.82	9.0	0.5
Glutamic acid	297.5	21.6	5.72	18.9	1.80	5.8	0.3
Proline	trace	trace	trace	—	trace	—	—
Glycine	32.1	4.1	0.62	2.0	0.34	1.1	0.5
Alanine	404.0	34.6	7.77	25.7	2.88	9.2	0.4
Valine	32.8	10.2	0.63	2.1	0.85	2.7	1.3
Isoleucine	6.8	2.5	0.13	0.4	0.21	0.7	1.6
Leucine	3.1	3.0	0.06	0.2	0.25	0.8	4.2
Tyrosine	28.4	12.5	0.55	1.8	1.04	3.3	1.9
Phenylalanine	5.6	2.0	0.11	0.4	0.17	0.5	1.5
γ-Aminobutyric acid	trace	trace	trace	—	trace	—	—
Ethanolamine	9.3	27.5	0.18	0.6	2.29	7.3	12.7
X_4	1.6	5.9	0.03	0.1	0.49	1.6	16.3
α,γ-Diaminobutyric acid	2.3	9.5	0.04	0.1	0.79	2.5	19.8
Ornithine	20.2	30.6	0.39	1.3	2.55	8.2	6.5
Lysine	31.7	40.8	0.61	2.0	3.40	10.9	5.6
Tryptophane	7.3	15.2	0.14	0.5	1.27	4.1	9.1
Histidine	2.0	1.8	0.04	0.1	0.15	0.5	3.8
X_5	trace	2.3	trace	—	0.19	0.6	—
X_6 (Guanidic derivative)	19.2	53.7	0.37	1.2	4.48	14.3	12.1
X_7	trace	5.6	trace	—	0.47	1.5	—
Arginine	11.3	17.0	0.22	0.7	1.42	4.5	6.5
Total	1572.9	375.2	30.26	100	31.27	100	1.0

[a] Serum C, cytoplasmic serum; [b] Serum L, lutoidic serum; [c] Ratio of the 2 concentrations.

different periods. However, all analyzed samples show the same general features. The major part of the free amino acids of latex – 78 to 83% of the total content which ranges from 16.9 to 23.2 µmoles per ml of fresh latex – is localized in the cytoplasmic serum.

However, when the difference of volume between the lutoidic and cytoplasmic serums is taken into consideration, it appears that the total amino acid concentration in each of the 2 compartments is of the same order: 30.3 µmoles/ml of cytoplasmic serum and 31.3 µmoles/ml of lutoidic serum. The latter value is somewhat too low. In fact, the volume of lutoidic serum has been assimilated to that of the lutoid pellet and, therefore, overestimated. Neither has the interstitial volume been taken into account, nor that of the membranes. The essential differences appear in the distribution of individual amino acids in the two compartments (Table I).

Table II. Distribution of acidic, neutral and basic free amino acids in the cytoplasmic and lutoidic serums

Amino acid group	Free amino acid pool (%)	
	Serum C	Serum L
Acidic	56.9	22.9
Neutral	36.4	21.1
Basic	6.6	56.0

The predominant amino acids of the cytoplasmic serum are glutamic acid and its amide, alanine and aspartic acid. They account for 81% of the total amino acid content. The other amino acids are present in much smaller amounts, sometimes in trace quantities.

The lutoidic serum is distinguished from the cytoplasmic serum by a high proportion of compounds basic in character (Table II). A guanidic derivative, not yet identified (X_6) is the principal constituent of the group. It is followed by lysine and ornithine which also occur in considerable amounts. Their concentrations are, respectively, 12 times (guanidic derivative) and 6 times (ornithine and lysine) superior to those found in the cytoplasmic serum.

An opposite concentration gradient between the 2 compartments exists at the level of the principal constituents of the cytoplasm: alanine, aspartic acid and glutamic acid. Their concentrations in the lutoids is distinctly lower.

The differences systematically noticed in the distribution of the free amino acids between the two compartments, cytoplasmic and lutoidic, become even more important when the inevitable contamination of one fraction by the other is taken into consideration. Thus, it is very likely that certain compounds (for example α–γ-diaminobutyric acid, unknowns: X_4, X_5 and X_7)

found in the cytoplasmic serum in trace quantities, or in very small amounts, come from the degradation of the lutoids during the manipulations. If this is so, the differences between the 2 fractions would not only be quantitative but also qualitative.

The above results set the problem of distinct metabolic pathways in each of 2 compartments, as well as that of the selective permeability of the lutoidic membranes to the amino acids.

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Résumé. Les études des acides aminés libres ont été effectuées sur deux compartiments du latex d'*Hevea brasiliensis*: les serums cytoplasmique et lutoidique. La majeure partie des acides aminés est localisée dans le cytoplasme dans lequel dominent Glu et son amide, Asp et Ala. La fraction particulaire se distingue par une dominante des composés basiques.

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Elektronenmikroskopische Beobachtungen über die Fusion myogener Zellen bei *Antheraea polyphemus* (Lepidoptera)

Von verschiedenen Untersuchungen an Wirbeltieren und Insekten ist bekannt, dass während der Muskelentwicklung Zellfusionen auftreten können. Während bei Vögeln die Verschmelzung von myogenen Zellen (in vitro) elektronenoptisch schon seit längerem gezeigt werden konnte[1,2], fehlte der ultrastrukturelle Beweis bei Insekten bis vor kurzem. PEREZ[3] vermutete als erster eine Inkorporation von Myoblasten in die dedifferenzierten larvalen Muskelfasern (bei *Calliphora*). Verschiedene Autoren haben diese Annahme an anderen Insekten lichtmikroskopisch bestätigt[4–7]. Ultrastrukturelle Untersuchungen bei *Lucilia*[8] zeigten, dass sich Myoblasten während der Metamorphose an die aus der Larve stammenden entdifferenzierten Muskelfasern eng anlagern; in späteren Stadien werden dann mehrkernige Zellen beobachtet, deren mosaikartige Feinstruktur auf eine erfolgte Zellfusion schliessen lässt. Erst kürzlich gelang es CROSSLEY[9], den Verschmelzungsprozess bei *Calliphora* im elektro-

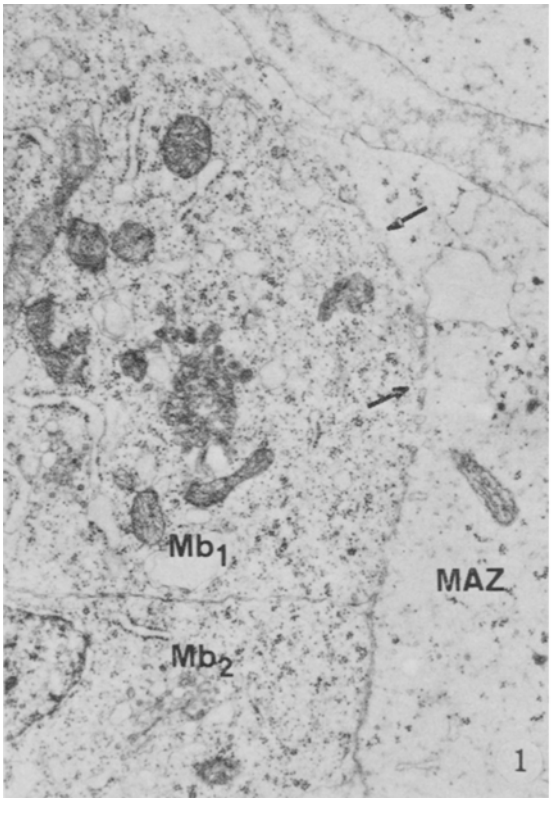

Fig. 1. Fusion eines Myoblasten (Mb_1) mit einer Muskelanlagezelle (MAZ) über verschiedene Zytoplasmabrücken (Pfeile). Sarkolemm und Myoblastenmembran zum 2. Myoblasten (Mb_2) sind noch intakt. ×18000.

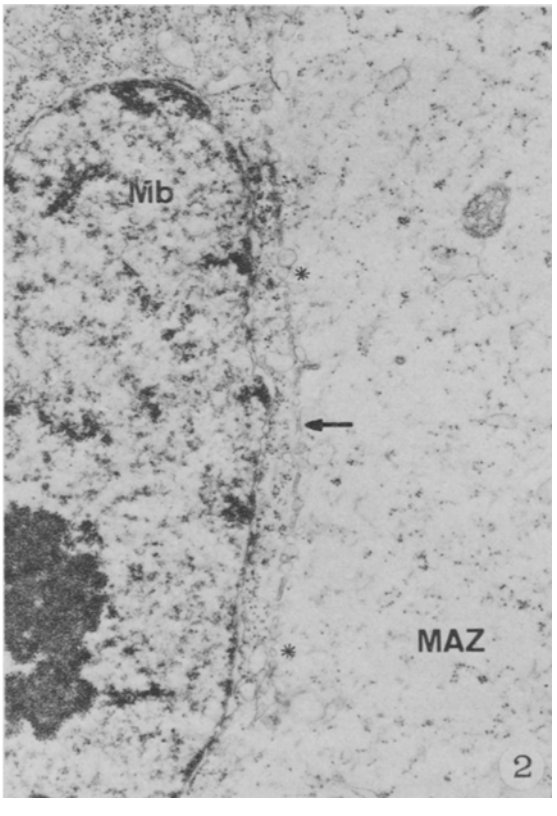

Fig. 2. Myoblast mit Interphasekern in Fusion mit einer Muskelanlagezelle. Man beachte das hantelförmige Anschwellen der Membranreste (Pfeil) und deren Umwandlung in Vesikel (*). ×18000.