to construct the *Bufo* phylogeny[7]. All the species here analyzed have only 1 secondary constriction in 1 small chromosomic pair (specially pair 7). The karyotypes of the primitive leptodactylid frogs (Telmatobinae)[14-16] have a primitive formula (2n=26)[17] and also show a secondary constriction on pair 7. If the bufonid toads are leptodactylid derivatives, as many authors have considered[18,19], the karyotypes of *Bufo* species may have some similar characters to the leptodactylid frogs. Among them the secondary constriction could be included. The ceratophrynid toads (Ceratophrydae), other leptodactylid derivatives[18], also have 26 chromosomes and also have a secondary constriction on pair 7[20]. The wide distribution of 1 secondary constriction (specially on pair 7) among the karyotypes of the primitive leptodactylid frogs and the leptodactylid derivatives (Bufonidae and Ceratophrydae) suggests the possibility that this character could be considered to be primitive.

Other South American *Bufo* species with this primitive character are: *B. marinus, B. atacamensis, B. paracnemis, B. granulosus, B. poeppi* and *B. crucifer.* The latter species may be the most similar of all living *Bufo* to the ancestral form to the genus[21]. According to Bogart[7], *B. marinus, B. atacamensis, B. paracnemis* and *B. variegatus* could have arisen from a primitive ancestor possessing only K secondary constriction. If the same idea is applied to the species karyologically analyzed *(B. rubropunctatus, B. chilensis* and *B. spinolosus)* then they could also have derived from the same ancestor as *B. marinus, B. atacamensis, B. paracnemis* and *B. variegatus.*

1 Supported by Proyecto N° S-77-47, Dirección de Investigación, Universidad Austral de Chile.
2 I would like to thank Dr J. M. Cei for collecting the Argentinian toads. Mr N. Diaz collected the specimens from Santiago, Chile.
3 The technical assistence of Sonia Lacrampe is acknowledged with appreciation.
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A view on intramembraneous particles

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Summary. Intramembraneous particles of the outer membrane of *Escherichia coli* show complementary pits on the opposite fracture face. This complementarity characteristic has been discussed in relation to the nature of the particle and the mode of fracturing.

Freeze-fracturing splits membranes into 2 halves, thus allowing an examination of the membrane's interior. The 50-100 Å particles visible on both monolayers are widely assumed to be proteinaceous in nature[2,3].

Most membranes do not reveal complementary impressions (or pits) opposite to particles. Even when it is considered that shadowing, contamination or fracturing itself might obscure complementary pits[4-6], there is no satisfactory explanation why under similar physical circumstances matching halves of other membranes can be visualized. Convincing examples for non-complementarity are the particles of the erythrocyte membrane[4], the purple membrane of Halobacteria[7] and of rhodopsin containing membranes[8]. For the erythrocyte membrane, it is now well established from labelling experiments[9,10] and recombination studies[11,12] that proteins i.e. band III protein and glycophorin represent the particles. These proteins are spanning the membrane[9,13,14]. The number of α-helices penetrating is at least 6 in the native particle. Similarily this has been demonstrated elegantly for the purple membrane[15], which also gives non-complementary fracture faces. In this membrane, about 64-82 α-helices should be involved per particle[16]. A common feature of these membranes is that etching (sublimation of ice) provokes craters in the outer fracture face[8,17]. In these types of membranes, the particle reflects protein; however, shape and mol.wt (tertiary structure) are difficult to assess.

Recently we found that the outer membrane of *Escherichia coli* K12 reveals particles on the outer fracture face (OM i.e. EF) and pits on the inner fracture face (OM i.e. PF)[18,19]. This morphological characteristic has been analyzed in more detail.

Materials and methods. E. coli K12 strain CE 1054 was grown in yeast broth medium as described elsewhere[20]. This strain was used because the fracture plane runs exclusively through the outer membrane. Glycerol was added to prevent freeze damage. The cells were quenched from 23 °C and freeze fractured at −115 °C in a Denton freeze etch apparatus.

Results and discussion. Particles (5000-6000/μm^2) are present on the OM (EF) and pits (4000-5000/μm^2) are observed on the OM (PF) in this membrane. The size of the particles varies between 40-80 Å in diameter. The pits are between 40-60 Å in diameter. It is reasonable to assume that the pits are prints of the particles on the opposite fracture face which means that in this membrane most intramembraneous particles show complementarity upon fracturing.

What might be the biochemical basis of complementarity in these membrane types? As a principle, complementarity

can be expected when only lipid is involved and the fracture plane runs between the acyl chains of the membranes[21]. Strong support for this statement is found in studies on several kinds of lipid phases like the lamellar L*α* and hexagonal II phase. The observations were consistent with the notion that fractures occur along non-polar regions[22]. Also a study on a peculiar lipid phase, the p*β′*, showed that at a resolution level of about 100 Å the fracture halves are complementary[23]. Of special interest in this respect is the complementarity which was detected in erythrocytes treated with sphingomyelinase[6,24]. Micrographs of this material unambiguously revealed small (50–60 Å) particles on the outer fracture face and pits on the inner fracture face. We concluded that in this case globules (particles) consisted of ceramides aggregated into inverted micelles. Next to these complementary structures, the amply described population of intramembrane particles fracture plane runs between the acyl chains of the membranes[21]. Strong support for this statement is found in *E. coli* membrane, we started with the hypothesis that particles represent proteins. So we focussed our study on mutants lacking one or more of the outer membrane proteins and mutants deficient in their lipopolysaccharide. Moreover, we have investigated the effect of EDTA on the morphology, as it was known that this treatment could remove lipopolysaccharide from the *E. coli* outer membrane[25].

The outer membrane of *Escherichia coli* K12 strain 1054 *A* The outer fracture face (OM) without etching, *B* The inner fracture face (OM) without etching ↗ shadowing direction. Magnification ×200,000.

The results from these studies led us to the alternative hypothesis that lipopolysaccharide instead of the intrinsic membrane proteins determines the membrane particle. In this hypothesis, the complementarity aspect is understood in a way that the fracture plane is determined by the lipopolysaccharide micel-like complex. Strong evidence for this hypothesis has recently been obtained from the following experiment. Incubation with Ca^{++} of the mutant that is deficient in all important intrinsic proteins, and shows a particle density of about 25% of that of the wild type, results in outer membrane fracture faces densely occupied with particles. This could be explained from the assumption that Ca^{++} causes aggregation of lipopolysaccharide monomers into micel-like complexes, which upon fracturing exhibit themselves as particles opposite to pits[26].

Another, perhaps the best known, example of a complementary fracture is found in the gap-junction[27]. Particles are present on the PF and complementary impressions on the EF, except in some invertebrates[28] where the distribution is reversed. In addition, as is also the case for other complementary membranes, the junctional membrane is not affected by etching. This may indicate that unlike, for instance, the erythrocyte membrane, junctional proteins do not determine in a direct way the structure of the particle.

What evidence, except for the strange similarity in freeze fracture etch morphology, does strengthen this idea? Both negative staining and freeze etching of the outer membrane of *E. coli*[29,30] showed the presence of a subunit pattern which is also found in gap-junctions[31]. Moreover, the outer membrane of gram-negative bacteria[32,33] and the gap-junction[34-36] have in common that ions and small molecules can freely permeate. It is conceivable that the complementary particles are committed to this transport. As to the composition and frame of complementary membrane particles of junctions and other cells than *E. coli*, it may be worthwhile to envisage the possibility that in the particle site lipidic acyl chains offer an interface along which the fracture plane runs so as to give a matching twin of monolayers. For a proper assessment of complementarity, which in our opinion offers a clue for resolving the architecture of particles, it seems worthwhile to improve and control freeze-etch conditions as is recently reported in a study on yeast cell membranes[37].

1 Acknowledgment. We thank Dr Lugtenberg for his active support and critical comments and Mrs J. Leunissen-Bijvelt for her technical assistance.
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Seasonal variations in liver metabolism of the green frog *Rana esculenta* (L.)

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Summary. Rana esculenta (L.) kept under natural conditions show almost constant b.wt in the annual cycle. Liver weight, however, has a distinct peak in October/November which is also evident in the liver index. The liver storage materials: glycogen, triglyceride and protein, show 2 distinct maxima (spring, autumn).

There are only a few experimental data concerning the seasonal variations of metabolism in Amphibia[1-8]. Less attention was given to natural environmental conditions in some works concerning several aspects of these problems in frogs. Also experiments mostly were not carried out in a uniform population. Therefore, in the present paper, experiments are described which were performed during an annual cycle with a population of *Rana esculenta* (L.) living under natural conditions as far as possible.

In Amphibia fat body and liver represent 2 important organs which are able to store depot substances. Therefore the liver is the preferable organ for experiments concerning anabolism, catabolism and conversion of storage material.

Experimental animals and methods. Rana esculenta (L.) obtained from a commercial dealer served as experimental animals. They were kept outdoor 2 months before experiments started. The open-air grounds (3 × 8 m) consisted of a land area with natural vegetation, stones and sticks and a water compartment with continuous flow. The frogs were fed additionally to naturally offered food with mealworms and flyblows.

Every month a random sample of 10 animals was taken from this population. After decapitation, the liver was removed and homogenized. It was stored at −20 °C until further elaboration. Determination of liver protein was carried out according to[9] with a modified Biuret-method. Liver glycogen values were obtained according to[10] with a modified o-Toluidin-method. Determination of liver triglycerides were made with the test kit Ingotest 567651 (Boehringer)[11]. The data were compared by the t-test according to student.

Results. The b. wt of the experimental animals is relatively constant 60–70 g (figure, A) whereas the liver weight shows a seasonal rhythm: it increases significantly from September on and is not positively correlated to the b. wt (Aug./Sept., Aug./Oct., $p < 0.001$). Because of slight variations of the b. wt in spring, seasonal variations of the liver weight are less distinctive (figure, A).

The liver index established with both parameters is a 1st aspect of the metabolic situation of the liver: it shows a distinct increase in September with a maximum in November (Aug./Sept., Aug./Nov., $p < 0.001$). From this time on a continuous decrease until August is observed, which is interrupted only by a less distinct spring maximum in April (figure, B) (March/April, April/May, $p < 0.001$). The liver protein values of the experimental frogs show 2 maxima in the annual cycle: a spring peak in March and a late summer maximum in September (figure, C) (Feb./Mar., March/April, $p < 0.001$; July/Sept., Sept./Nov., $p < 0.001$).

The content of glycogen in the liver shows a clear maximum in December and a less distinct one in April (Mar./Apr., April/May, $p < 0.001$; Aug., Nov./Dec., Dec./Jan., $p < 0.001$). The lowest glycogen values are determined in the summer months June, July, August. From this time on, the rapid increase towards the December maximum begins, which then decreases to the March minimum. This is followed by the spring maximum (figure, C). The liver triglyceride values also show a distinct seasonal rhythm: an autumn maximum (October, November) is accompanied by a spring maximum in March (Aug./Oct., Nov./Dec., $p < 0.001$; Feb./Mar., Mar./Apr., $p < 0.001$).