NOTE

**Relationship between Amylopectin and Viability
of *Eimeria tenella* Sporozoite**

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It has been found that sporozoites of *Eimeria* contain periodic acid-Shiff staining (PAS)-positive polysaccharide granules [1, 2, 6]. Ryley et al. [5] extracted the polysaccharide from *Eimeria tenella* sporozoites and identified it as amylopectin. Hence, the polysaccharide storage of sporozoites of *Eimeria* is thought to be in the form of amylopectin. It was reported that the amylopectin catabolized to lactic acid and small amounts of CO₂ and glycerol when sporozoites were incubated at 41°C [4], and that through the storage of oocysts both the amylopectin content and the infectivity to chickens gradually decreased [6]. Little is known, however, about the metabolism of the amylopectin contained by the free sporozoites, and it has not been clarified what role the amylopectin plays on the activity of the parasites. Therefore, we investigated the relationship between the viability of *E. tenella* sporozoites and the amount of PAS-positive granules by counting the number of viable sporozoites and by measuring the content of the granules during the course of storage of the sporozoites in a phosphate buffer solution at different temperatures.

Sterilized sporozoites prepared from freshly sporulated oocysts by the method

mentioned previously [3] were suspended in 0.1 M phosphate buffer, pH 7.0. In each test tube of 5-ml capacity, 0.5 ml of the sporozoite suspension was placed. The tubes were stoppered tightly and placed in water baths set at 0, 29, 37, and 41°C.

The viability of sporozoites was determined by the trypan blue staining method. The parasites not stained were regarded as viable and those stained as dead when they were stained with 0.5% trypan blue in 0.1 M phosphate buffer, pH 7.0, of the same volume as that of the sporozoite suspension.

Smears of sporozoites were fixed in Bouin's solution and stained with PAS.

Fig. 1. PAS individual index.

The sporozoites stained with PAS were classified into six "PAS individual indices" depending on the amylopectin granule contents (Fig. 1). The mean degree of PAS individual indices obtained with 100 sporozoites per sample was called the "PAS index".

As shown in Fig. 2, the viable sporozoites gradually decreased in number as time went by. At 0°C, the viable count decreased slowly; even on the 5th day of incubation, 85% of sporozoites still survived. At 29°C, most sporozoites survived for 2 days; after the 2nd day, live sporozoites markedly decreased in num-

Fig. 2. Effect of incubation temperature on viability of sporozoite.

Fig. 3. Effect of incubation temperature on PAS-positive granule contents.

ber, resulting in 16% survival on the 5th day of incubation. At 37°C or at 41°C, the viable sporozoites decreased in number to 65% and 8%, respectively, in 16 hr, and no viable sporozoite was detected after 48 hr at 37°C or 24 hr at 41°C. Thus, the higher the temperature, the shorter the period of survival.

In this experiment, only few sporozoites stained with trypan blue or dead sporozoites were found. It may have been due to rapid lysis of the body.

The average amylopectin contents of sporozoites fell with the passage of time (Fig. 3). At 0°C, the content little decreased, and its PAS index was larger than 4 even on the 5th day of incubation. At 29°C, the content decreased comparatively slowly to 0.2 of the index on the 5th day. At higher temperatures, the content decreased rapidly to 0.4 of the index in 24 hr at 37°C and 0.1 in 16 hr at 41°C. So it was concluded that the higher the temperature, the more rapid the decrease in amylopectin contents and that the decrease ran nearly parallel that in number of viable sporozoites.

These phenomena may suggest that the polysaccharide storage of the sporozoite is used as an energy source for maintaining the viability. Therefore, at low temperatures the amylopectin may be utilized slowly because of low metabolic turnover, and the sporozoite may be able to survive for a long time, while at higher temperatures it may be consumed rapidly because of accelerated metabolism, and the sporozoite may die in a short time.

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要 約

Eimeria tenella sporozoite の生存性とアミロペクチン含有量について(短報): 中井 裕・扇元敬司¹⁾(茨城大学農学部家畜衛生学教室,¹⁾東北大学農学部家畜衛生学教室) — *Eimeria tenella* の新鮮 oocyst から人工脱殻によって無菌 sporozoite を得, 無菌的に 0.1M pH7.0 リン酸緩衝液に浮遊して, 0, 29, 37, 41°C における生存数および PAS 染色陽性顆粒含有量の消長を観察した. 高い温度ほど, 虫体の生存数およびアミロペクチン含有量の低下が早い傾向が認められたことから, アミロペクチンは sporozoite が生存するためのエネルギー源であることが推察された.