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ON THE EFFECT OF SOME FIXATIVES UPON MYXOSPORIDIAN SPORES[1]

By
R. Kudo

Although it has generally been recognized that when myxosporidian spores are fixed, stained and mounted as either smears or as section preparations, they appear smaller than in the fresh state, it was Cépède who first called attention to the matter. He recognized differences between fresh and stained spores of the species he studied, and concluded as follows (Cépède, 1906:63): "en présence de telles différences de taille et de l'importance donnée actuellement aux dimensions des spores des Myxosporidies dans la systématique comme caractère distinctif des espèces, je crois utile de faire remarquer qu'il serait bon d'indiquer si les mensurations des spores ont été faites in vivo ou sur des préparations fixées et colorées, et montées au baume."

My observations upon the species which I have studied up to date agree with Cépède's results and have suggested that the dimensions of spores of a species should be accompanied by the statement of conditions under which the measurements were made (Kudo, 1920:49).

Most authors agree that at the present state of our knowledge regarding this group of Protozoa, a satisfactory classification of genera and species of Myxosporidia, must have as its basis the study of the spore (Kudo, 1920:52-59). The size, dimensions and structure of spores show a certain amount of variation even in one species, yet they are far more typical of the species than are the vegetative forms. In every case, the identification of a species has been successfully done only when the spores were present.

Since the characters of the spore vary to a more or less recognizable extent, according to the difference of conditions under which the spores are observed, it naturally follows that the characters of spores of two different species can only be correctly compared when they are observed

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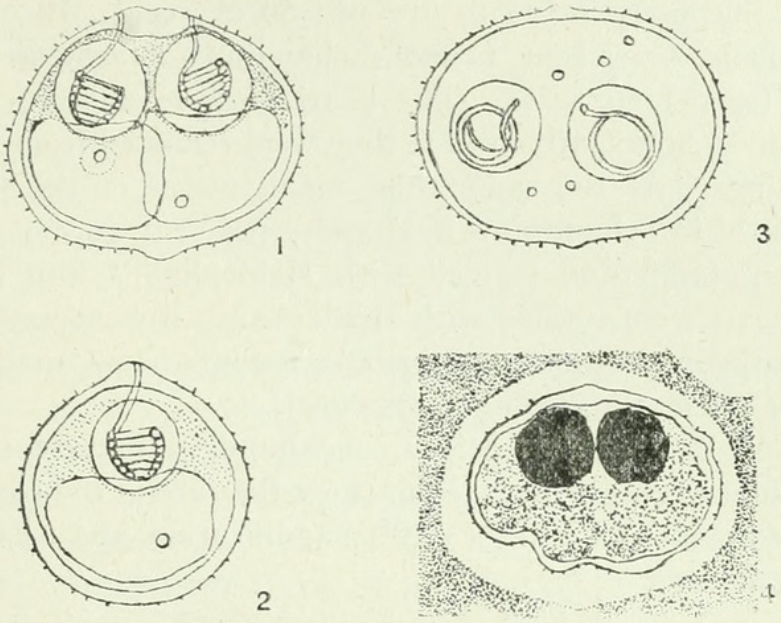
under exactly the same conditions. In other words, if the spores were originally observed and measured in the fresh state, those of the species to be compared with the former, should also be studied in the same condition. This kind of comparison, however, has happened only in a few cases, and can not always be carried out even in future. Many species of Myxosporidia have accidentally been found and described from stained smears or section preparations only by several authors, who were engaged with studies on other topics. Furthermore, even if one deals exclusively with Myxosporidia, one is frequently compelled to omit the study of fresh spores and to confine oneself to that of stained preparations under various circumstances.

The characters of spores observed only in section preparations can, of course, not only be compared properly with those obtained from fresh spores, but also should not be used for the data of establishing a new species. Special precaution must be exercised in cases where two forms are similar in habitat and locality and whose vegetative stages are not known.

Recently Schuurmans Stekhoven (1920) described three new species of Myxosporidia, *Sphaerospora gasterostei*, *Myxidium rhomboideum* and *Henneguya renicola*, found in the uriniferous tubules of the kidney of *Gasterosteus pungitius* (misprinted as *pungiticus*) from Holland. The author studied section preparations of the host kidneys which were fixed with 60 per cent. alcohol and stained with Delafield's hematoxylin, and compared the characters of the spores observed therein with those of already known three species, *Sphaerospora elegans*, *Henneguya media* and *Henneguya brevis* (Kudo, 1920:30). Thélohan (1895) who described these latter forms, seems to have studied them in both fresh and fixed conditions, although his description is unfortunately brief. When the forms are so similar in every respect except the size of the spores, one finds it extremely difficult to decide whether the newly observed species studied only in sections, are identical with the former or not. If, however, we can calculate the dimensions of spores in the fresh state from those obtained from the stained ones, we can undertake a more satisfactory comparison between the species observed under diverse conditions.

In order to see exactly how the fixation, staining and mounting would affect the shape, dimensions and structure of Myxosporidia, a few experiments were performed on the spores of *Leptotheca ohlmacheri*. This Myxosporidian was first found by Ohlmacher and Whinery (1893) in the kidney of *Bufo lentiginosus*. I have recently observed it in the kidneys of *Rana clamitans* and *Rana pipiens*. Although I formerly placed it provisionally in the genus Wardia (Kudo, 1920:83-84), my recent study on its morphology and life history which will be published elsewhere, has shown that it should be placed in the genus Leptotheca as Labbé suggested.

The fresh spore of *Leptotheca ohlmacheri* (Figs. 1 to 3) is oblong with its longest diameter at right angles to the sutural plane. The anterior end is slightly attenuated due to the thickening of the spore membrane at that point, while the posterior end is rounded. In profile, it is nearly circular with the slightly attenuated anterior extremity. In the anterior end view, it is regularly oblong. The shell is moderately thick. The sutural



Spores of *Leptotheca ohlmarcheri* (Gurley) Labbé. Figs. 1, 2 and 3. Three optical sections in front, side and anterior end views respectively of fresh spores which were kept in a hanging drop preparation with physiological solution and which are typical of the species in form, structure and dimensions. Fig. 4. A spore from a smear fixed with absolute alcohol, stained with Giemsa stain and mounted in cedar oil, showing the shrinkage of the entire body. All about X 2100.

ridge is well marked and protrudes conspicuously at the ends. The spore membrane is somewhat irregularly striated. Three to seven striæ run parallel to the sutural line on each valve, the remaining ones make somewhat similar angles with the former. The striæ in lateral view are mostly placed horizontally. The number of striæ on each valve varies from 25 to 35. Two spherical polar capsules usually equal in size in one spore, occupy the anterior portion of the spore. The polar filament, coiled 4 to 6 times, is distinctly visible. Two independent sporoplasms occupy the extracapsular cavity of the spore. They are extremely homogeneous and each to be the karyosome of the nucleus). The size of the spore varies to some extent. There are some larger and some smaller than the average spores as is the case with every species found, doubtlessly due to the malformation. The average dimensions are as follows:

Sutural diameter	9.5 to 12μ; average	10.8μ
Breadth	13 to 14.5μ; average	13.75μ
Thickness	9.5 to 12μ; average	10.8μ
Diameter of polar capsule	3.5 to 4.5 μ; average	4.0μ
Length of polar filament (KOH)	42 to 62μ; average	52.0μ

A drop of the emulsion of fresh spores in physiological salt solution was smeared on a slide. The amount of the emulsion and the area over which it was smeared were made approximately the same in every smear so as to obtain the similarity in the number of spores and the conditions under which the spores existed until they were fixed. Soon after the smear was made, it was fixed in one of the following fixatives before it dried up one smear being purposely made to dry up: 50 per cent., 70 per cent. and absolute alcohols, 4 per cent. formol, Schaudinn's (warmed), Bouin's and Flemming's (weak) mixtures. The fixatives were allowed to act upon the smears for 16 hours, after which they were removed from the latter by proper washings. At the same time, small pieces of the infected host organ were fixed in Schaudinn's fluid and 4 per cent. formol respectively, sectioned in paraffin and stained with Heidenhain's iron hematoxylin. The fixed smears were stained with Heidenhain's iron hematoxylin, Delafield's hematoxylin or Giemsa stain, and mounted in Canada balsam or cedar oil (for Giemsa stained smears only).

From each of the preparations, one hundred mature spores which could be distinguished distinctly from those that are in the course of development, were drawn at a scale of 2,100 magnification, and measured. The results are as follows:

		Sutural Diameter			Breadth			Diameter of Polar Capsules		
		Range in μ	Average in μ	Loss in μ	Range in μ	Average in μ	Loss in μ	Range in μ	Average in μ	Loss in μ
SMEARS	Fresh spores (control)	9.5–12.0	10.8	0	13–14.5	13.75	0	3.5–4.5	4.0	0
	Air-dried, unstained, alcohols and balsam	7.6– 9.5	8.6	2.2	10.5–11.4	10.7	3.05	2.6–3.2	2.9	1.1
	50% alcohol; Giemsa	9.2–10.0	9.6	1.2	10.5–10.9	11.0	2.75	3.0–4.0	3.5	0.5
	70% alcohol; Delafield	9.0– 9.8	9.4	1.4	9.8–10.9	10.4	3.35	2.8–3.9	3.4	0.6
	Absolute alcohol; Giemsa	9.0– 9.5	9.25	1.55	9.5–10.9	10.2	3.55	2.8–4.0	3.4	0.6
	4% formol; Giemsa	8.9– 9.3	9.1	1.7	9.5–11.4	10.5	3.25	2.9–3.8	3.4	0.6
	Schaudinn; Giemsa or Heidenhain	9.2– 9.8	9.6	1.2	10.9–11.9	11.4	2.35	3.0–3.8	3.4	0.6
	Bouin; Heidenhain	8.5– 9.5	9.0	1.8	9.5–10.9	10.2	3.55	2.6–4.0	3.3	0.7
	Flemming; Heidenhain	8.8– 9.6	9.2	1.6	10.0–11.2	10.6	3.15	2.4–3.2	3.3	0.7
	Average	.....	9.2	1.5	.....	10.6	3.09	.....	3.4	0.6
	Per cent. of loss, calculated from the dimensions of spores from smears	.....	.....	16.0%	.....	.....	29.0%	.....	.....	17.0%
SECTIONS	Schaudinn; Heidenhain	8.6–10.0	9.3	1.5	10.6–11.8	11.1	2.65	3.0–3.6	3.3	0.7
	4% formol; Heidenhain	8.4– 9.9	9.15	1.65	9.5–11.3	10.4	3.35	2.8–3.8	3.3	0.7
	Average	.....	9.23	1.58	.....	10.75	3.00	.....	3.3	0.7
	Per cent. of loss, calculated from the dimensions of spores in sections	.....	.....	16.0%	.....	.....	28.0%	.....	.....	21.0%

From the above table, the following may be noted:

1) The amount of loss in sutural diameter of the spore of *Leptotheca ohlmacheri* is greatest when the spore is airdried and smallest when it is fixed with either Schaudinn's fluid or 50 per cent. alcohol. The average loss amounts to about 14 per cent. of the sutural diameter of the fresh spores.

2) The amount of loss in breadth of the spore is greatest when the spore is fixed with Bouin's fluid or absolute alcohol and smallest when it is fixed with Schaudinn's fluid. The average loss amounts to about 22 per cent. of the breadth of the fresh spores.

3) The amount of loss in the diameter of polar capsule is greatest when the spore is air-dried and smallest when it is fixed with 50 per cent. alcohol. The average loss amounts to about 15 per cent. of the diameter of polar capsules in fresh spores.

4) The losses in smear and section preparations are almost similar.

In the case of *Myxobolus cycloides*, Cépède gave the following dimensions: Fresh spores: sutural diameter, 13.5-16μ, breadth 11-13μ. Schaudinn-Heidenhain's spores: sutural diameter 10.5-12μ, breadth 7.5-8μ. Thus in this case, the fixation caused loss of 3.5μ and 4.25μ respectively in sutural diameter and breadth of the spore. These losses amount to 25 and 35 per cent. compared with the fresh spores.

The loss in the latter species is greater than the former species. It, however, shows distinctly from these two different types of Myxosporidia, that the sutural diameter undergoes a smaller amount of shrinkage than the breadth.

Concerning the change in the dimensions of spores by fixation, Cépède states that the reason why the spores appear smaller in fixed and stained conditions than in fresh condition, is simply because the refractive power of Canada balsam makes the unstained spore membrane invisible, and not because the shrinkage of the body caused by fixation takes place.

In the case of *Leptotheca ohlmacheri*, this is not the case. Very frequently spores such as shown in Fig. 4, are seen in the smears. The spore undoubtedly occupied the entire area which appear as blank zone before it was fixed. When fixed, the contents underwent a strong shrinkage, thus leaving a clear unstained zone between it and the smear. In such a spore, one can distinctly see the spore membrane in an irregular outline. I have noticed similar change of spores in smears of many species from various genera suggesting shrinkage as the main cause for the loss in dimensions. I, therefore, consider the decrease in the dimensions of myxosporidian spores, in general, is caused by the shrinkage of the entire spore body under the influence of the fixative and subsequent treatments.

The amount of shrinkage caused by fixation will apparently be different in different genera and species. Unless a large number of measurements

on different species be made, we do not know exactly the data on which the dimensions of fresh spores of the species observed by Schuurmans Stekhoven may correctly be calculated. Assuming that the spores of *Sphaerospora gasterostei* and *Henneguya renicola* underwent shrinkage similar to that of *Leptotheca ohlmacheri*, we obtain the following comparison:

	Sphaerospora gasterostei		*Sphaerospora elegans*
	Schuurmans Stekhoven	Calculated	Thélohan
Length	6.7 μ	7.8 μ	10 μ
Breadth	7.0 μ	9.0 μ	11 μ
Length of polar caps.	3.5 μ	4.1 μ	——

If the calculation is correct, it seems probable that *Sphaerospora gasterostei* is independent from *S. elegans*.

	Henneguya renicola		*Henneguya media*	*Henneguya brevis*
	Schuurmans Stekhoven	Calculated	Thélohan	Thélohan
Length of spore	8 μ	9.28 μ	20–24 μ (?)	10 μ
Breadth	3.5 μ	4.5 μ	5–6 μ	5–6 μ
Polar capsule (length)	4.5 μ	5.4 μ	4–5 μ	4–5 μ
Tail	15 μ	17.4 μ	——	14–15 μ

The calculated value of *Henneguya renicola* resembles closely the dimensions given by Thélohan for *Henneguya brevis*. The form of spore becomes so highly modified in section preparations that it is hard to make out the form in the fresh state. Therefore, it is highly doubtful whether Schuurmans Stekhoven saw a new species or not.

The form of spores changes to a variable extent according to the difference of the fixatives used. The more shrinkage the spore undergoes, the more irregular outlines it assumes. Careful fixation in Schaudinn's fluid often preserves the form of spores very nicely.

When a spore is fixed with any one of the fixatives, the coiled polar filament becomes entirely invisible. This is probably caused by the coagulation of the wall of polar capsule which becomes opaque by the

fixation. The distinction between the two sporoplasms is harder to determine in fixed and stained spores than in fresh spores. The sporoplasms become coarsely reticulated, losing the homogeneous condition seen in the fresh state.

In conclusion, I may again suggest that a new species should be described after studying the spores in fresh as well as fixed and stained conditions and if possible the fixed vegetative forms.

Summary

1) The ordinary fixation causes about 14 and 22 per cent. decrease respectively of the sutural diameter and breadth of fresh mature spores of *Leptotheca ohlmacheri*.

2) The possibility of calculating the dimensions of fresh spores from those of fixed and stained spores is discussed.

3) The decrease in the dimensions of spores is due to the shrinkage of the entire spore body.

4) Fixation makes the coiled polar filament invisible.

Works Cited

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