prototype of a more general phenomenon in the mode of crystallization of proteins. This conjecture can be readily verified (*19*).

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Virulence Transformation of a Trichomonad Protozoan

Abstract. Treatment of an avirulent strain of *Trichomonas gallinae* with a cell-free homogenate of a virulent strain resulted in enhanced virulence as evidenced by the size of lesions produced in mice. Addition of deoxyribonuclease to the homogenate cell mixture blocked the transformation.

Marked differences in virulence in strains of *Trichomonas gallinae*, a flagellate parasite of columbiform and galliform birds, can be demonstrated readily in natural hosts (*1*) and in laboratory mice (*2*). It seemed feasible to determine whether transformation of the virulence of a relatively nonpathogenic strain to enhanced virulence might be accomplished by treating the cells with cell-free material from a highly pathogenic strain, although, as far as can be ascertained, such transformation of protozoans has not been reported previously.

Differences in pathogenicity among strains of *T. gallinae* from axenic cultures can be evaluated quantitatively by comparison of the mean volumes of 5-to-6 and 12-to-14 day lesions produced in mice by subcutaneous flank inoculation of 7.5 to 9.0 $\times$ 10^5 parasites in 0.5 ml of medium (*3*). This assay for virulence was employed in the present investigation.

Two strains of *T. gallinae* were used: the very pathogenic Jones' Barn strain (JB), isolated in axenic culture from a liver abscess of a pigeon in the summer of 1958, and the relatively nonpathogenic Lahore strain (YG), isolated in a similar culture from the mouth of an apparently healthy pigeon in the early fall of 1956. Both experimentally infected birds came from Robert Stabler. It must be noted that in the long series of transfers on fluid thioglycollate (FT) with 1 or 5 percent normal horse serum (FTS), YG strain has become further attenuated in pathogenicity and will be referred to henceforth as the YGA strain.

Strain JB flagellates were transferred from FTS to CPLM medium (cysteine, peptone, liver infusion, maltose) with 5 percent serum but without agar (CPLMNA), on which they were maintained for three transfers. Twenty-four-hour cultures (total of 6 liters) from the fourth transfer were centrifuged at 2000 rev/min for 10 minutes at 5°C, and the centrifugate was washed twice with Earle's balanced salt solution containing penicillin and dihydrostreptomycin. The washed centrifugate was suspended in 5 ml of sterile 0.154*M* sodium chloride–0.01*M* sodium citrate and frozen immediately. (Standard sterility tests for the presence of microorganisms other than the trichomonads gave uniformly negative results.) The suspension was homogenized at 0°C under sterile conditions in a Potter-Elvehjem tissue grinder with a small amount of alumina. A microscopic examination of the homogenate revealed a very few intact nuclei. (A series of media, including FTS, which were inoculated with the homogenate remained sterile during 14 days of incubation.)

The total nitrogen of the homogenate was 3.94 mg/ml as determined by Lang's method (*4*). The homogenate was analyzed for nucleic acid according to the modified Schmidt-Thannhauser procedure (*5*), with the use of salmon sperm deoxyribonucleic acid (DNA) and *d*-xylose as standards. The homogenate contained 76 μg of DNA (as DNA) and 780 μg of ribonucleic acid (as pentose) per milliliter.

All experimental and control inoculations involved 6-to-8-week-old mice of the C57 B1/6 strain. The cultures which were to be tested on mice were grown routinely for 48 hours in tubes containing 10 ml of FTS. In experiments I and II, as well as in control experiment B, 0.5-ml aliquots of trichomonad suspensions were inoculated into each tube.

In experiment I, (i) a 48-hour culture of YGA strain maintained for three transfers on CPLMNA was centrifuged, and the centrifugate was washed twice with Earle's balanced salt solution containing penicillin and dihydrostreptomycin. (ii) The final centrifugate was resuspended in 5 ml of 0.137*M* sodium chloride and, upon addition of 1 ml of the JB homogenate, was incubated at 37°C for 8 hours. (iii) Following incubation, 0.5-ml aliquots of the suspension were inoculated into tubes, each containing 10 ml of CPLMNA. The organisms were carried through two transfers on this medium. (iv) A 48-hour culture from the second transfer was treated as in (ii) and (iii) above, except that 1.5 ml of JB homogenate was added to the suspension of trichomonads. (v) After 8 hours' incubation, the suspension was grown on FTS. Mice were inoculated with trichomonad cultures from this medium.

In experiment II, the contents of seven large lesions from experiment I were suspended in FTS containing penicillin and dihydrostreptomycin. Appropriate aliquots of the suspension were inoculated into FTS, which provided the material for injections into mice.

In control experiment A, mice were inoculated with 48-hour cultures of untreated YGA strain. In control experiment B, YGA cells were treated as in steps (i) to (iii) of experiment I, except that 8 μg of crystalline deoxyribonuclease (Sigma Chemical Co.) were added to the suspension containing the parasites and the JB homogenate. After 8 hours' incubation, 0.5-ml aliquots of the suspension were inoculated into FTS. As in experiments I and II, the mice were injected with 48-hour cultures grown on this medium.

All control and experimental lesions were routinely spot checked for sterility. No foreign microorganisms were found on any media, but invariably excellent trichomonad cultures were obtained on FT and FTS.

All experimental data are summarized

Table 1. Effect of treating avirulent *Trichomonas gallinae* with homogenate of virulent strain as evidenced by lesions produced in mice.

Expt.	No. of mice	Treatment of avirulent (YGA) strain	Mean volume of lesions (mm^3)			
			6 days	s*	14 days	s*
I	30	Treated twice with virulent (JB) homogenate	27.82	16.62		
II	16	Cells from lesions of expt. I.	35.64	26.64	46.27	36.59
Control A	16	Untreated	9.28	8.12	10.97	10.01
Control B	21	Treated with JB homogenate and deoxyribonuclease	13.02	10.87	17.02	16.63

*s = sample standard deviation.

in Table 1. The results obtained were compared statistically with the aid of the nonpaired t-test. In all instances the mean volumes of the lesions produced by cells treated with homogenate alone (experiments I and II) were found to be significantly different, on all levels, from the corresponding (6- and 14-day) controls ($P<0.001$). There were, however, no significant differences between controls A and B on the 6th or 14th day after inoculation ($0.1<P<0.2$). Further, no statistically significant differences could be demonstrated between the mean volumes of the 6-day lesions observed in experiments I and II ($0.1<P<0.2$).

The foregoing results suggest strongly a DNA-dependent transformation of the YGA strain, with respect to virulence. It seems probable that virulence is a genetically controlled character among the strains of *T. gallinae*. It must be noted that the mean volume of lesions resulting from inoculations of the transformed YGA strain is very much smaller than the mean volume of lesions observed after inoculation of mice with JB strain. In experiments performed several weeks earlier with the latter strain, the mean volume of 6-day lesions was 158.53 mm^3 ($s = 97.38$).

Two possibilities may be considered in explaining these results. It could be assumed that the conditions for transformation present in the foregoing experiments were far from optimum, and that thus only a relatively small proportion of organisms became transformed. This hypothesis can and will be tested by modification of the conditions under which YGA organisms are exposed to the JB homogenate. On the other hand, we may be dealing with a stepwise transformation. This latter assumption can be proved unequivocally only by the establishment of the homogeneity of the transformed cultures.

It may be of interest to add that recent studies on the mechanisms of pathogenicity of the JB strain in chick cell cultures have not revealed characteristic inclusion bodies, ascribable to the presence of the flagellates. Consequently, if viruses influence the pathogenicity of this strain, they are evidently restricted to the protoplasm of the parasites (*6*).

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Proton Flux during the Great Aurora of 3–4 September 1959

Abstract. Photoelectric measurements of the Hβ intensity show that it continued to increase after a corona formed, and reached a maximum as the corona broke up into rays, decreasing rapidly to nearly zero within the next 10 minutes. The maximum flux of protons incident on the earth during the aurora, deduced from these measures, was at least 1.4×10^8 auroral protons per square centimeter, per second.

On the night of 3–4 September 1959 an extremely bright aurora was visible throughout Canada and the northern United States. At the Pine Bluff Observatory of the University of Wisconsin, a small telescope designed and ordinarily used for photoelectric measurements of diffuse and planetary nebulae was on this occasion used to measure the Hβ radiation from the aurora, and thereby to determine the flux of protons incident on the earth's upper atmosphere.

The telescope is a 5-inch f/5 achromatic refractor, and the Hβ radiation is isolated by an interference filter, made by Baird-Atomic, Inc., with measured peak wavelength 4864 A and nominal band width 30 A. Light transmitted by this filter passes through a circular diaphragm in the focal plane, which was 3.86 mm in diameter and therefore defined a field of 21.7′ in diameter for all the observations reported here. The light is then measured by a conventional astronomical photoelectric photometer, built around a refrigerated 1P21 photomultiplier. The only strong emission line of either the aurora or a gaseous nebula transmitted by the filter is the Hβ λ4861 line, and the measurements were calibrated into energy units by comparison with measurements made with the same equipment of planetary nebulae having known Hβ fluxes (*1*). This calibration was made indirectly; the aurora was compared with the very bright star α Lyrae on 3–4 September, and the star was in turn compared with the planetaries as part of the regular nebular program on nights before and after the aurora.

On the night of 3–4 September the first auroral measurement was made at 2124 C.S.T.; the aurora then had already been visible for over an hour, growing steadily brighter, and was still in the quiet-arc stage. At this time the sky was also measured at a point 58° south of the zenith, far from the bright part of the aurora, in order to determine the sky correction. The sky light is due to the continuous radiation of all the faint stars included within the area of sky measured, as well as to the faint scattered radiation from the lights of the city of Madison, 15 miles distant, and of the nearer but smaller villages of Mazomanie and Cross Plains. A mean sky correction was subtracted from all the measurements to find the residual intensity due to the aurora alone, but since the true sky brightness undoubtedly varies with position and time, this procedure introduces some error. The maximum measured auroral intensity is over seven times larger than the sky correction, and is therefore quite accurate, but the correction is a larger fraction of the fainter auroral intensities, which are therefore less well determined. The measurements were also corrected slightly for atmospheric extinction according to a coefficient derived from observations on previous nights.