

March 15, 1984

Dr. Kwang W. Jeon
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Zoology
Knoxville, TN 37996

Dear Dr. Jeon:

On talking with a number of cooperating physicians I find that they need **guidance in how** to prepare the knee **effusion** samples. One physician, for example, has refrigerated his two samples for two weeks. Another has used antiamoebics **before submission**.

Enclosed please note characteristics taken from Carter's paper **on Naegleria fowleri**. If our search encompasses some of the physical characteristics I've outlined, then some doctors will be destroying the sample prior to your receipt.

Would you please send me a sample preparation and transportation protocol to mail to doctors?

Meanwhile Tony has collected a large **number** of papers from 19th onward related to medical research with Hartmanella, Vahlkamfia, Naegleria, et. al. Would you like me to copy these and forward on to you?

Cordially,

Perry A. Chapdelaine, Sr. C: Pybus,

Blount, Simoons

EFFECT OF PHYSICAL AND CHEMICAL AGENTS

Refrigeration. Trophic amoebae in fresh mouse-brain water suspensions remain active and culturally viable for up to 4 wks when kept at 21°C. Refrigeration at 0°-4°C overnight (16 hr) usually renders the amoebae non-viable. Direct microscopy during this period shows progressive rounding and loss of activity, with shrinkage and disintegration occurring after about 12 hr. Usually only a few unidentifiable structures remain at 16 hr, although occasionally a few tiny motile amoebae survive and produce slight and easily overlooked growth on agar culture.

Sodium chloride. Trophic forms are immediately immobilised by a sodium chloride concentration of 3 per cent; membrane-sterilised seawater (sodium chloride 4 per cent.) has a similar effect. The organisms shrink to rounded, highly retractile forms that persist without alteration for at least 2 wks. In physiological saline trophozoites appear normal for 24 hr, but then progressively lose activity and are all rounded and refractile after 1 wk. Trophozoites are not viable after 24 hr in seawater, but cysts survive. A sodium chloride content of 0.5 per cent. in the agar medium completely inhibits growth and accelerates disappearance of inoculated amoebae from the culture.

Alcohol, formalin and Savlon. Cultural viability of trophozoites and cysts alike is lost after immersion for 24 hr in either 70 per cent. alcohol, 4 per cent. formaldehyde saline or 1 in 100 aqueous dilution of Savlon Hospital Concentrate (I.C.I.). *pH.* Between pH 4.6 and 9.5 (the highest level tested) trophozoites continue to behave normally for at least 48 hr. At pH 4.3 or lower they are all immobilised and many disintegrate within 24 hr.

Bile salt. Trophozoites are completely lysed within 15 min. by a concentration of sodium deoxycholate of 1 in 320 or greater. Lower concentrations produce no effect during the test period of 48 hr. Cysts are not affected by exposure for 1 hr to a concentration of 1 in 20.

Serum. Exposure to fresh human serum at 21°C produces a progressively damaging effect on trophozoites over a period of 24 hr, all being immobilised and about one-half disintegrated at this time by dilutions up to 1 in 8. When serum is heat-treated by a method used to destroy complement its amoebicidal effect is lost, although with serum diluted 1 in 2 the amoebae tend to become rounded and lose adherence to coverslips. That the latter effect is non-specific and related merely to the high molarity of serum is shown by the similar behaviour of the amoebae in PPG, or in solutions of pure human albumin at concentrations similar to those in serum. The mouse serum tested, whether fresh or heat-treated, has no specific amoebicidal effect, but produces only the osmotic effect. Definite amoebicidal effect was seen with serum from a patient who had died with the amoebic disease, but only at a dilution of 1 in 2; the significance of this observation is uncertain since storage of the serum for 2yr at -15°C may well have reduced the amoebicidal factor.