

**Primary Isolation of *Donovania granulomatis* by Chick Brain Inoculation,
and Induction of Cerebral Miliary Granulomata. (18848)**

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D. granulomatis was cultivated by Anderson(1) in the yolk sac of developing chick embryos. The characteristic lesion of granuloma inguinale has not been reproduced by this organism, although Greenblatt(2), by inoculation of a human volunteer with a yolk sac culture, produced a nodule which after 60 days showed typical Donovan bodies. For yolk sac inoculation, an inoculum free of contaminants must be obtained. This may be accomplished by fragmenting the tissue and incubating it on solid media(1), using for inoculation the fragments which are found to be uncontaminated, or, if possible, by rendering contaminated material sterile by the use of antibiotics to which *D. granulomatis* is resistant.

On the presumption that following direct inoculation of contaminated material containing Donovan bodies into the brains of newly hatched chicks contaminants would not survive, but that *D. granulomatis* might persist, the following experiments were undertaken.

From each of 2 human cases of granuloma inguinale, a 0.5 g piece of granulation tissue, rich in Donovan bodies, was ground and suspended in 5 cc of sterile saline. Routine cultures, consisting of blood agar plates and thio-glycolate broth, showed the suspension to be grossly contaminated with Gram positive cocci, Gram negative rods, and diphtheroids. For each case a series of 6 one-day-old chicks were inoculated intracerebrally with .05 cc of the contaminated suspension. After 2 weeks, routine cultures of a brain from each series were sterile, although Donovan bodies persisted on smear. A .5 g piece of brain from a chick of each series was ground and suspended in 10 cc of sterile saline, and .5 cc quantities of this suspension were inoculated into the

yolk of a series of 12 five-day-old developing chick embryos. From each strain, growth of a small Gram-negative rod occurred which exhibited bipolar staining and morphological resemblance to *D. granulomatis*. The yolk sac cultures were passed successfully for 3 and 8 generations respectively, and were discontinued; no growth ever occurred on ordinary media.

The remaining 9 chicks in these series were sacrificed at from 14 to 24 weeks, and their brains were examined by smears and by histologic sections. In all of these, smears showed small numbers of organisms morphologically resembling *D. granulomatis*. In 2 examined at 14 weeks, and in one examined at 17 weeks, miliary granulomata occurred in the cerebral hemispheres near the site of inoculation.

It was of interest to reproduce, if possible, these lesions with yolk sac cultures of *D. granulomatis*. Accordingly, a series of chicks were inoculated intracerebrally, using .05 cc quantities of yolk sac cultures from each of the 2 strains above, and also of a culture of a strain previously isolated by the method described by Anderson(1) from a third human case of granuloma inguinale. The brains of 29 chicks were studied by smears and by histologic sections of formalin-fixed material at from 8 to 36 weeks after inoculation. In 6 of these, at from 10 to 27 weeks, miliary granulomata occurred which were similar in all respects to those obtained after inoculation with fresh human material. Organisms morphologically resembling *D. granulomatis* persisted in smears for the full 36 weeks. From three brains so inoculated, at one, 5, and 10 weeks after inoculation, yolk sac cultures were made, yielding organisms morphologically resembling *D. granulomatis*. These were successfully passed for one, 3, and 6 generations, respectively, before being discontinued. Routine cultures were always negative.

1. Anderson, K., *Science*, 1943, v97, 560.

2. Greenblatt, R. B., Dienst, R. B., Kupperman, H. S., and Reinstein, B. S., *J. Ven. Dis. Information*, USPH Service, 1947, v28, 183.

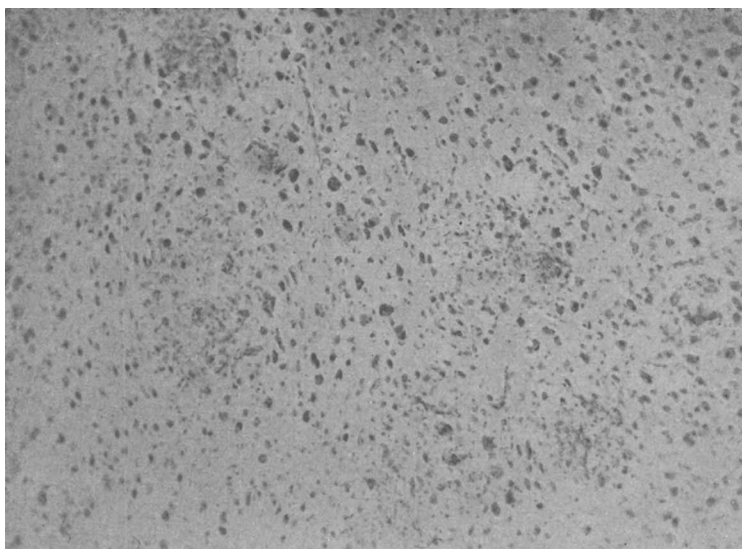


FIG. 1. Section through cerebral hemisphere of chick showing miliary granulomata 17 weeks after inoculation with yolk culture of *D. granulomatis*. H & E stain. $\times 90$.

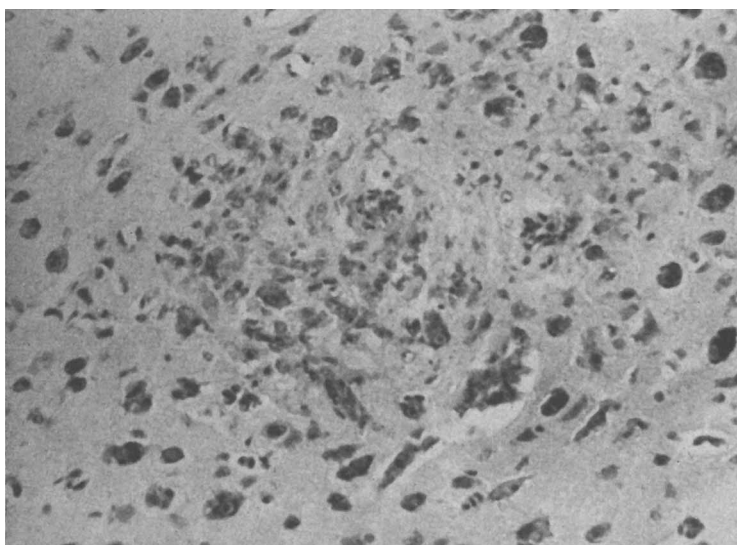


FIG. 2. Section through cerebral hemisphere of chick showing miliary granulomata 17 weeks after inoculation with yolk culture of *D. granulomatis*. H & E stain. $\times 320$.

Gross lesions were never seen in any of the brains, and signs of central nervous system involvement were never observed. Organisms could not be demonstrated histologically in any of the granulomata. A control series of 6 chicks were inoculated with sterile yolk, and no lesions were induced, nor were they induced in a series of 8 suckling mice inoculated intracerebrally with a yolk sac culture of *D. granulomatis*.

Summary and conclusions. 1. *D. granulomatis* was shown to persist for at least 9 months in the chick brain, while contaminating organisms rapidly died out. This finding was utilized in the primary isolation of the organism from contaminated material. 2. Miliary granulomata were induced in the brains of chicks by intracerebral inoculation of material containing *D. granulomatis*.